From the Institute for Community Medicine, Department SHIP/ Clinical-Epidemiological Research

Director: Prof. Dr. Henry Völzke

Of the Faculty of Medicine, University Medicine Greifswald

Associations of Liver Dysfunction with Glucose Intolerance and All-Cause Mortality in a General Population

Inaugural– Dissertation

To

Attainment of Academic Grades

in

Doktor der Wissenschaften in der Medizin

(Dr.rer.med)

from

Faculty of Medicine

of

University Medicine Greifswald

2022

Presented by: Muhammad Naeem
Born on: 11 April 1987
in: Malakand, Pakistan

Dean: Prof. Dr. Karl-Hans Endlich

1**. Expert:**	Prof. Dr. Henry Völzke
2. Expert:	Prof. Dr. med. Ali Aghdassi
3. Expert:	PD. Dr. Till Ittermann

City, Room:	Greifswald, SR Klinik für Innere Medizin A (Raum 7.0.15/17)
Day of Defense:	21.02.2023

Table of Contents

List of Tables

List of Figures

List of abbreviation

ADA	American Diabetes Association
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BMI	Body mass index
CI	Confidence interval
FLI	Fatty liver index
FIB-4	Fibrosis-4 score
GGT	γ-glutamyl transpeptidase
HOMA-IR	Homeostasis model assessment-insulin resistance score
i-IFG	Isolated impaired fasting glucose
i-IGT	isolated impaired glucose tolerance
MRI	Magnetic resonance imaging
PDFF	proton-density fat fraction
R2*	Transverse relaxation rate
SHIP	Study of Health in Pomerania
T2DM	Type 2 diabetes mellitus

1. Introduction

1.1 Epidemiology of Hepatic Steatosis and Iron Overload

The prevalence and incidence of chronic liver diseases are increasing globally and were reported to be associated with morbidity and mortality risks [1]. Approximately two million deaths per year are a consequence of liver diseases [2]. Hepatic steatosis is the most common liver disorder in the western population affecting one quarter of the adult world population [3]. Associations of genetic and environmental factors with hepatic steatosis were widely studied in population-based studies from Australia, South America, Asia and Africa [4]. A previous meta-analysis and systematic review showed that the risk of hepatic steatosis is lower among women than men, but the chance of progression to severe liver disease is more common in women than in men older than 50 years [5]. Hepatic steatosis is also common in metabolically unhealthy lean individuals that have potentially the same risk for all-cause mortality than obese individuals [4, 6].

Iron is an important micronutrient that is mostly stored in the liver. The liver is the organ affected by iron overload earlier than other organs. Most of the iron is stored in the liver as ferritin [7]. Deposition of excess iron in hepatocytes induces the production of free radicals, leading to injury and inflammation of the hepatocytes [8]. It is estimated that about one-third of the subjects with hepatic steatosis experiences iron overload [9]. In the population-based Study of Health in Pomerania (SHIP), 9% of the individuals have hepatic steatosis and iron overload in parallel [10].

1.2 Associations of Hepatic Steatosis and Iron Overload with Prediabetes

Previous longitudinal studies found positive associations between hepatic steatosis defined by ultrasound with prediabetes in selected or general populations [11-13], whereas other studies did not find evidence for such an association [14]. One cross-sectional study observed positive associations between hepatic steatosis defined by fatty liver index (FLI) and prediabetes in a general population [15]. In contrast, another population-based study failed to show significant associations between ultrasound-based hepatic steatosis and prediabetes categories [16].

Regarding iron overload, cross-sectional studies, using different iron metabolism biomarkers (ferritin, transferrin, transferrin saturation), found significant associations with prediabetes in

selected [17-19] or large population-based samples [20, 21]. On the other hand, a small cross-sectional study did not report any significant association between serum ferritin levels and prediabetes in males [22].

1.3 Associations of Hepatic Markers with All-cause Mortality

A simulation study suggested that hepatic steatosis will double mortality rates and the risk of advanced liver diseases in the aged populations of countries including China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States period between 2016 and 2030 [23].

Previous population-based and clinical studies demonstrated that hepatic steatosis, as measured by different scores based on ultrasonography, biopsy or elevated liver enzymes, was associated with an increased all-cause mortality after median follow-up times of 7 to 23 years [24-28]. A meta-analysis showed positive associations between hepatic steatosis mostly with preexisting conditions and all-cause mortality. However, in subgroup analysis, these associations were mostly significant for steatohepatitis but not for simple hepatic steatosis [29]. Similar positive associations were reported for the association between hepatic steatosis assessed by ultrasound and all-cause mortality in individuals with obesity or metabolic syndrome after a median follow-up of 22.8 years [30]. Another previous study, however, failed to find independent associations between hepatic steatosis defined by ultrasound and all-cause mortality after adjusting for confounding after a median follow-up of 23 years [31]. Furthermore, previous prospective studies reported positive associations between mild-to-moderate elevations of serum liver enzymes and the risk of all-cause mortality [32-40].

1.4 Limitations of Previous Research

Most of the previous studies on the association between hepatic steatosis and prediabetes lack to diagnose different prediabetes categories (isolated impaired fasting glucose (i-IFG), isolated impaired glucose tolerance (i-IGT), combined IFG and IGT (IFG + IGT) by using oral glucose tolerance test (OGTT) as proposed by American Diabetes Association (ADA) [41]. There is only one study, which reported associations between hepatic steatosis and different glucose intolerance states by using OGTT. However, in that study hepatic steatosis was defined by a FLI [15]. There is a sex and race difference for FLI cutoff values, which makes it difficult to validate this score for different groups of the population [42]. Similarly, the FLI includes γ-glutamyl

transpeptidase (GGT) and triglyceride levels, which might not be elevated due to hepatic steatosis and, therefore, the FLI is not considered to be a good marker for quantification and detection of liver fat [43]. Most widely used markers for iron overload are elevated levels of serum ferritin [44]. However, ferritin is not only elevated in iron overload, but might be also elevated due to inflammation and malignancies [45].

Previously mentioned studies for the associations between hepatic steatosis and all-cause mortality showed conflicting results. For instance, the associations between ultrasound-assessed hepatic steatosis and all-cause mortality were not observed independently of known metabolic risk factors (obesity, hypertension, hyperlipidemia and diabetes) [30, 46]. The risk of all-cause mortality is only pronounced in individuals with higher grades of hepatic steatosis proven histologically by biopsy [47].

1.5 How can we Overcome these Limitations?

Due to the heterogeneous clinical nature of hepatic steatosis there are challenges in diagnosis of subjects having higher chances of progression to severe liver diseases. The diagnosis of hepatic steatosis depends upon most reliable and accurate markers. Most widely used biomarkers are liver enzymes and ultrasound. However, liver enzymes are found to be normal in many cases of hepatic steatosis [48]. Similarly, ultrasound has limited sensitivity in individuals having less than 20% liver fat or have mild obesity [49]. In our study, we used MRI, which is the most accurate and sensitive non-invasive and non-radiation-based method to determine hepatic steatosis and iron overload [50]. MRI is able to quantify liver fat as well as hepatic iron overload in a single procedure [51]. Compared to ultrasound, the diagnostic validity of MRI is not affected by obesity, operator independent and has lower sample variability [52]. MRI is highly reproducible and fast for the quantification of the entire liver fat [53].

1.6 Aims of our Study

To our knowledge, there is no population-based study, which investigated associations of MRI-diagnosed hepatic steatosis, iron overload and liver volume with prediabetes and all-cause mortality. Prediabetes was categorized by OGGT data into IFG, IGT (alone or in combination) or previously unknown type 2 diabetes mellitus (T2DM), as proposed by ADA.

Therefore, the purpose of our research was to investigate associations of quantitative and qualitative hepatic markers with different glucose intolerance states and all-cause mortality in the general population.

2. Methodology

2.1 Study Population

SHIP is a population-based project conducted in Northeast Germany. It consists of two independent cohorts, SHIP-START and SHIP-TREND. For SHIP-START-0, individuals aged between 20-79 years were selected from population registers using a two-stage cluster sampling method. From a net sample of 6265 eligible subjects, 4308 (response 68.8%) participated between 1997 and 2001. SHIP-START-2 is the second follow up of SHIP-START, in which 2,333 individuals were examined between 2008 and 2012.

In parallel to SHIP-START-2, a second stratified random sample of 8826 adults aged 20-79 years was drawn for SHIP-TREND-0, of which 4420 subjects participated between 2008 and 2012 (response 50.1%). Random sample selection into the age and sex-strata was facilitated by centralization of local population registries in the German Federal State of Mecklenburg/West Pomerania. All participants gave written informed consent. The study conformed to the ethical guidelines of the Declaration of Helsinki as reflected in a priori approval by the local ethics committee of the University of Greifswald.

For our first two papers that investigate association of hepatic steatosis and iron overload with prediabetes we used OGTT data, which was only available from baseline data of the second cohort (SHIP-TREND-0). We excluded individuals who had a self-reported prior liver disorder (cirrhosis, chronic liver disease or hepatic steatosis; n= 46) or pre-existing T2DM (n= 461). Furthermore, we excluded individuals without MRI examination (n= 2130) and those with missing data in any of the variables of interest (n= 37). The final study population consists of 1,746 subjects (913 women) aged between 21 and 80 years. From analysis regarding the prediabetes status, we further excluded individuals, who did not undergo an OGTT (n=124), resulting in a study population of 1,622 (840 women).

For our third paper on the association of hepatic markers with all-cause of mortality, we used data from participants of SHIP-START-2 and SHIP-TREND-0 cohorts. From the 6753 adult individuals participating in SHIP-START-2 and SHIP-TREND-0 we excluded 3966 who did not have any measurements of hepatic fat content or liver volume. Furthermore, we also excluded 19 individuals with established liver cirrhosis, resulting in a final study population of 2769 individuals.

2.2 General Measurements

Socio-demographic characteristics and medical histories were assessed by computer assisted face-to-face interviews. Height and weight were measured for the calculation of the body mass index (BMI = weight [kg]/height² [m²]). Alcohol drinking habits were evaluated as beverage-specific alcohol consumption (beer, wine, distilled spirits) on the last weekend and last weekday preceding the examination, and the mean daily alcohol consumption was calculated using beverage specific pure ethanol volume proportions [54].

2.3 Liver Ultrasonography

Liver ultrasound examination were performed by examiners using a transportable B-mode ultrasound device (Vivid I; GE-Healthcare, Waukesha, WI, USA) with a 2.5 MHz ultrasonic transducer. The examiners used a 2-point scale to assess the presence of hepatic steatosis: (0) no steatosis, and (1) steatosis. Hepatic steatosis was defined as a hyperechogenic liver pattern in comparison to the renal cortex [55].

2.4 Magnetic Resonance Imaging

MRI examination was performed by using a 1.5-Tesla MR imaging system (Magnetom Avanto, software version VB15; Siemens Healthineers Erlangen, Germany) with a 12-channel phased-array surface coil [20]. Three-dimensional chemical shift encoded gradient-echo data with three echoes and flyback readout gradient were acquired from an axial slab during a single 19-second breath hold. Imaging parameters included repetition time, 11 ms; echo times, 2.4, 4.8, and 9.6 ms; flip angle, 10˚; number of signals acquired, one; bandwidth, ± 1065 Hertz per pixel; matrix, 224 × 168 × 64; field of view, 410 × 308 mm; parallel imaging effective acceleration factor, 1.8; and section thickness, 3.0 mm. Offline reconstructions of a proton-density fat fraction (PDFF) map (including correction for T1 bias and T2* decay) and a transverse relaxation rate (R2*) map

(based on T2* decay measurement of PDFF) were performed [21]. Fat and water ambiguities were resolved by using the phase of the acquired data [21]. Parametric maps of PDFF and R2* were used for further analyses.

One radiologist reviewed PDFF and R2* maps. Mean PDFF and R2* values were determined at operator-defined regions of interest placed at the center of the liver, by using Osirix (v3.8.1; Pixmec Sarl, Bernex, Switzerland). Care was taken when the regions of interest were placed to avoid blood vessels and regions that were obviously contaminated by partial volume effects and motion artifacts [20]. Hepatic steatosis was defined as PDFF ≥5.1%, while liver iron overload was defined as R2* ≥43.9 s-1 [21].

2.5 Assessment of Liver Volume

Assessment of liver volume was performed by a calculation of MRI-based volume indices. For this, measurement of liver diameters was performed using the software Osirix (version 4.6; Pixameo, Bernex, Switzerland) by a trained observer. In each participant, liver diameters were measured in mid-clavicular line in the following three orientations: anterior-posterior; lateral-medial; and cranio-caudal. Thereafter, we calculated the volume index and estimated liver volume using the following formula*: liver volume = (A x B x C) / 2.6[56]*.

2.6 Ascertainment of Diabetes and Metabolic Syndrome

Participants were classified as having T2DM if they reported physician's diagnosis of T2DM in the interview or took any glucose-lowering medications (Anatomical Therapeutic Chemical (ATC) classification system code A10). The metabolic syndrome was defined by the presence of at least three out of the following five characteristics: 1) waist circumference ≥94 cm in men and ≥80 cm in women; 2) high-density lipoprotein (HDL)-cholesterol <1.03 mmol/l in men and <1.29 mmol/l in women; 3) blood pressure ≥130/85 mmHg or anti-hypertensive treatment (ATC code C02); 4) random plasma glucose ≥8 mmol/l or glucose -lowering medication (ATC code A10); and 5) non-fasting triglycerides ≥2.3 mmol/l or lipid-lowering treatment (ATC code C10AB or C10AD). It was defined according to the modified AHA/NHLBI and IDF criteria based on non-fasting blood values [57].

2.7 Laboratory Data

In SHIP-TREND-0 but not in SHIP-2 we requested the individuals not to eat, smoke or consume caffeine containing drinks and to avoid sports for ≥8 hours before the examination, which was completed during the morning hours. Blood was collected by trained examiners following a standardized protocol, refrigerated to 4–8 °C and shipped on refrigerant packaging within four to a maximum of six hours to the laboratory. Measurements of fasting and 2-hour glucose concentrations were based on plasma samples [58]. All assays were performed according to the manufacturers' recommendations by skilled technical personnel. The study laboratory participated in official quarterly German external proficiency testing programs [59].

Fasting and 2-hour glucose levels were measured using a hexokinase method (Dimension Vista, Siemens Healthcare Diagnostics, Eschborn, Germany) [58]. Serum insulin concentrations were measured by commercially available chemiluminescence immunoassay kits (Immulite 2000 Xpi, Siemens Healthcare Diagnostics, Eschborn, Germany). Fasting and 2-hour insulin levels were expressed as µU/ml. The homeostasis model assessment-insulin resistance score (HOMA-IR) was calculated using the following equation: fasting insulin [µU/ml] X fasting glucose [mmol/l] / 22.5. Serum liver enzyme concentrations (alanine aminotransferase (ALT), aspartate aminotransferase (AST) and GGT were measured photometrically (Hitachi 704; Roche, Mannheim, Germany). Serum ALT, AST, and GGT concentrations were expressed as µkatal/l. The FIB-4 score was calculated using the formula: age (years) AST [U/l]/ (platelets [10^9/l] × (ALT [U/l) [60].

2.8 Ascertainment of Glucose Intolerance States

For OGTT testing, fasting glucose was sampled, and 75 grams of anhydrous glucose (Dextro OGT; Boehringer Mannheim, Mannheim, Germany) was orally given to all study participants. Following the ADA diagnostic criteria [41], we classified participants as having normal glucose tolerance if they had fasting plasma glucose values <5.6 mmol/l and 2-hour glucose values <7.8 mmol/l. We classified participants as having prediabetes if their fasting glucose values were between 5.6 mmol/l and 6.9 mmol/l (IFG) and/or 2-hour glucose values were between 7.8 and 11.0 mmol/l (IGT). Accordingly, we defined three different groups of individuals with prediabetes: those with isolated (i-IFG), those with isolated (i-IGT), and those with combined

IFG and IGT (IFG + IGT), respectively. Previously unknown diabetes was defined as fasting glucose values ≥7.0 mmol/l and/or 2-hour glucose values ≥11.1 mmol/l [58].

2.9 Mortality Follow-up Data+

Information on vital status for all study participants was requested from local health authorities at the place of death at regular intervals from time of enrolment into the study through March 31, 2019. Subjects were censored as dead or lost to follow-up. The number of years between baseline examination and censoring was used as the follow-up length. The median duration of follow-up was 8.9 years (25th, 18.6; 75th, 20.5), resulting in a mortality rate of 5.4 cases per 1000 person-years. During 23,898 person-years of follow –up, a total 129 individuals (89 men) were died.

2.10 Statistical Analysis

Continuous data are expressed as medians (with 25^{th} and 75^{th} percentiles) and categorical variables as absolute and relative percentages. For analyzing the associations between MRI-assessed hepatic steatosis and hepatic iron overload with continuous markers of glucose metabolism multivariable linear regression analyses were used by calculating β coefficients and 95% confidence intervals (95% CI). For the association between hepatic steatosis and iron overload with different glucose intolerance states (i-IFG, i-IGT, IFG+IGT or previously unknown diabetes), multinomial logistic regression analyses were used by calculating relative risk ratios and 95% CI. All the aforementioned regression models were adjusted for age, sex, BMI and daily alcohol consumption. Cox proportional hazard regression analyses were undertaken to examine the associations between various quantitative and qualitative markers of hepatic steatosis (including also MRI-assessed liver volume and diameters), and risk of all-cause mortality. Cox regression models were adjusted for age, sex, BMI, daily alcohol consumption, food frequency score, and education level. A value of $p<0.05$ was considered to be statistically significant. All statistical analyses were performed using STATA 14.1 software (Stata Corporation College Station, TX, USA).

3. Results

3.1 Associations of Hepatic Steatosis and Iron Overload with Prediabetes

Among the study population 1746 individuals (913 women) aged 21 to 80 years, had hepatic steatosis 37% by using MRI and 36% by using ultrasound. A proportion of 13% of individuals had MRI-assessed hepatic iron overload.

Table 1. Adjusted associations between MRI-assessed hepatic steatosis, hepatic iron overload and continuous markers of glucose metabolism.

	Hepatic steatosis (Ultrasound)	**Hepatic steatosis (MRI)**	**MRI-PDFF %**	**Hepatic iron overload (MRI)**	**Liver iron content on MRI (sec^{-1})**
Outcome variables	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Fasting glucose (mmol/l)	0.18 (0.11; 0.24)*	0.24 (0.17; 0.32)*	0.03 (0·02; 0·03)*	0.07 (-0.02; 0.16)	-0.00 (-0.00; 0.00)
2-hour glucose (mmol/l)	0.52 (0.31; 0.73)*	0.75 (0.53; 0.97)*	0.03 (0.02; 0.03)*	0.32 (0.04; 0.60)*	0.00 (-0.00; 0.01)
Fasting insulin (µU/ml)	4.18 (3.17; 5.20)*	4.86 (3.80; 5.94)*	0.50 (0.42; 0.59)*	-0.12 (-1.48; 1.24)	-0.02 (-.05; 0.01)
2-hour insulin (µU/ml)	22.3 (16.4; 28.2)*	36.9 (30.8; 43.0)*	3.49 (3.02; 3.97)*	7.1 (-0.80; 15.0)	0.07 (-0.13; 0.26)
HOMA-IR	1.21 (0.90; 1.52)*	1.44 (1.11; 1.77)*	0.15 (0.13; 0.18)*	0.00 (-0.41; 0.41)	-0.01 (-0.02; 0.00)

***** ($p< 0.05$)

β coefficients, derived from multivariable linear regression models, were adjusted for age, sex, BMI and daily alcohol consumption; 95% CI, adjusted 95% confidence intervals.. Hepatic iron overload was defined as MRI-assessed R2* $\geq$43.9 s^{-1}. Hepatic steatosis was defined as MRI-PDFF $\geq$5.1%.

Multivariable linear regressions models showed the association between hepatic steatosis and iron overload with continuous markers of glucose metabolism. We found significant associations between hepatic steatosis defined by either MRI or ultrasound with levels of fasting glucose β (95% CI) MRI; 0.24 (0.17; 0.32), ultrasound; 0.18 (0.11; 0.24), 2-h glucose MRI; 0.75 (0.53;

0.97); ultrasound; 0.52 (0.31; 0.73) , fasting insulin MRI;4.9 (3.8; 5.9); ultrasound; 4.2 (3.2; 5.2), 2-h insulin MRI; 36.9 (30.8; 43.0); ultrasound; 22.3 (16.4; 28.2) and HOMA-IR MRI; 1.4 (1.1; 1.8); ultrasound 1.2 (0.9; 1.5) **(Table1).** The mean level of 2-h glucose increased over the amount of fat in the liver **(Fig. 1)**. No significant associations were found between hepatic iron overload and other continuous markers of glucose metabolism except for association between hepatic iron with 2-h glucose 0.32 (0.04; 0.60) **(Table 1)**.

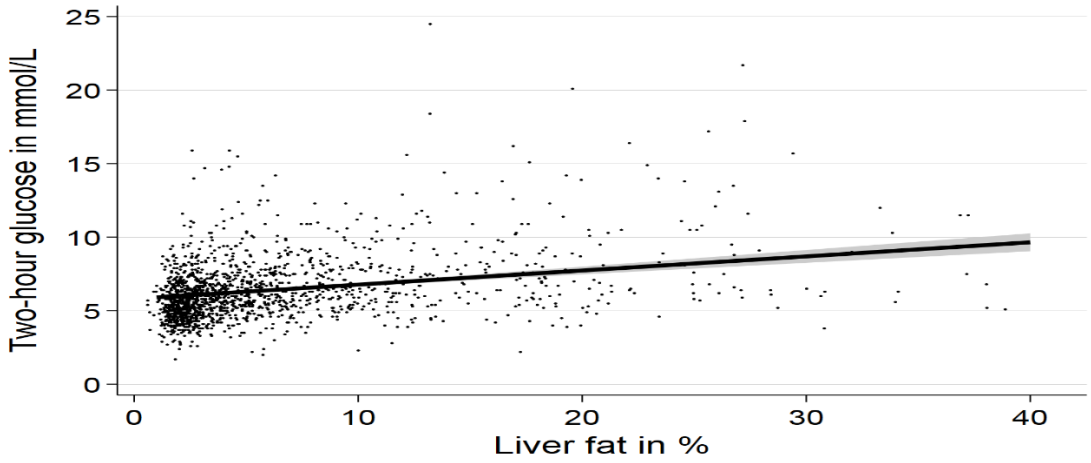

Figure 1. Association between liver fat fraction derived from quantitative MRI and two-hour plasma glucose levels based on linear regression adjusted for age, sex and body mass index.

Multinomial regression models were used to associate hepatic steatosis, hepatic iron and fat contents with different glucose intolerance states. We found that individuals with hepatic steatosis either defined by MRI or ultrasound had a higher relative risk ratio to different glucose tolerance states (i-IFG, i-IGT, IFG + IGT or previously unknown T2DM) than individuals without hepatic steatosis. Hepatic iron overload was significantly associated only with the combined IFG + IGT category. We observed a positive association between liver fat as assessed by MRI and glucose intolerance states (i-IFG, i-IGT, IFG + IGT or previously unknown T2DM) (**Fig 2)**.

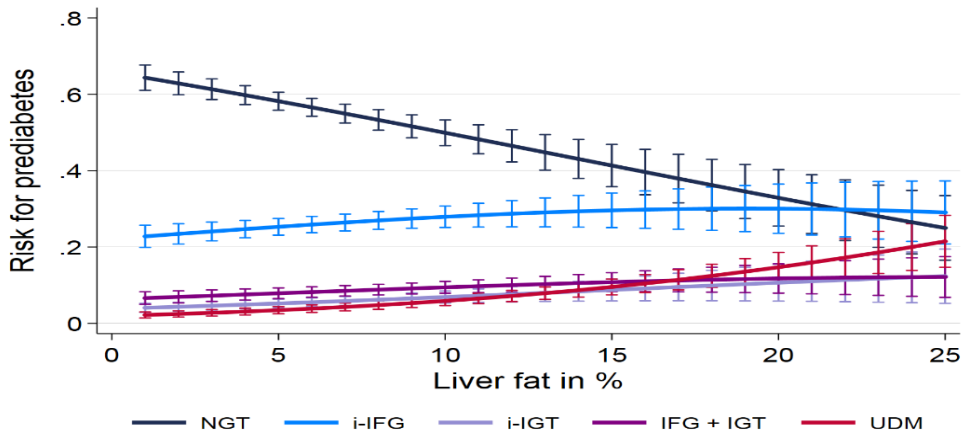

Figure 2. Associations between liver fat and different glucose tolerance states and expressed as absolute risks based on multinomial regression after adjustment for age, sex and body mass index.

Legend:6 NGT, normal glucose tolerance test; i-IFG, isolated impaired fasting glucose; i-IGT, isolated impaired glucose tolerance; UDM; unknown T2DM.

3.2 Associations of Hepatic Markers with Mortality

Cox proportional models were used for the associations between hepatic steatosis markers and all-cause mortality over a median period of 8.9 years. Results showed that, compared to other hepatic steatosis markers, a larger liver volume as assessed by MRI was significantly associated with a nearly 3-fold increased risk of all-cause mortality in the whole cohort and in both sexes **(Fig 3)**. These associations were consistent in the presence or absence of a metabolic syndrome or T2DM. Similarly, no significant associations were found between hepatic steatosis and all-cause mortality.

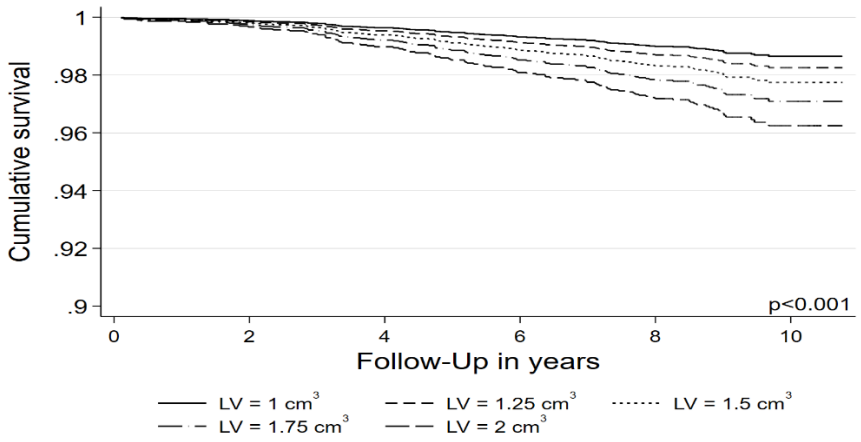

Figure 3. Survival curves for specific levels of liver volume based on Cox regression adjusted for confounding.

4. Discussion

In the present study, we investigated the associations between MRI-assessed hepatic markers with different glucose intolerance states (i-IFG, i-IGT, alone or combined and previously unknown T2DM) and all-cause mortality in the general adult population. Hepatic steatosis was more strongly associated with glucose intolerance states than iron overload. We found that a larger liver volume was significantly associated with all-cause mortality independent of known metabolic risk factors.

Previous studies found associations of hepatic steatosis and iron overload with glucose intolerance states [11-14, 16, 17, 21, 61]. In partial agreement with these studies we found significant associations between hepatic steatosis and glucose intolerance states, while hepatic iron overload was only associated with 2-hour plasma glucose levels and the IFG + IGT category. Compared to hepatic steatosis the associations between hepatic iron overload and glucose intolerance were less pronounced and not consistently observed.

The reason might be the low prevalence (13%) of hepatic iron overload in our study population compared to hepatic steatosis. Similar to our results previous studies also reported associations between iron overload and glucose intolerance states [17, 20, 21, 61]. However, these previous studies used laboratory markers like serum ferritin and transferrin for the definition of iron overload. Instead, we used MRI for the quantification of hepatic iron overload and hepatic steatosis, which is much more accurate and sensible marker than any of the laboratory markers

[62]. Similarly, we investigated specifically hepatic iron overload, while previous studies used only whole body iron overload. This might also partly explain the differences in results [17-19, 61].

In line with previous studies [25, 26, 31, 63-65], we did not find any significant associations between hepatic steatosis and all-cause mortality. Most of these previous studies defined hepatic steatosis either by scores or serum liver enzymes [25, 26, 31, 63-65]. In good agreement with these studies, we also found significant associations of the FIB-4 score and serum liver enzymes with all-cause mortality. However, we assessed hepatic steatosis by ultrasound and MRI, which is more accurate and sensitive marker for hepatic steatosis than the FIB-4 score and liver enzymes. Our study, however, lack information about different stages of hepatic steatosis and the association between hepatic steatosis and all-cause mortality may only be evident in individuals having fibrosis stages of hepatic steatosis [66].

As for as our knowledge concerned, we were the first having examined the association between MRI-assessed total liver volume and all-cause mortality in a population-based study. This association was independent of age, sex, BMI, nutrition, daily alcohol consumption, and education level. Furthermore, this association was consistent in all subgroups considered (men vs. women; presence or absence of metabolic syndrome or T2DM at baseline). We found that a larger liver volume, as assessed by MRI, is a better risk marker for all-cause mortality than hepatic fat content. This could be explained by the key role of hepatic inflammation in the association between liver volume and all-cause mortality.

4.1 Strengths and Limitations

Strengths of our study include its population-based study design. Further, we used MRI for assessing hepatic steatosis, hepatic iron overload and total liver volume, which is far more accurate and sensitive for this purpose than ultrasound or laboratory markers [67]. The glucose tolerance status was categorized by using standard OGTT testing according to ADA criteria as state-of-the art method.

One of the limitations of our study is that the associations between hepatic steatosis and iron overload with glucose intolerance were investigated cross-sectionally. Thus, we cannot draw casual inference. Additionally, we do not have any data on vibration-controlled transient

elastography nor histological confirmation of the severity of hepatic steatosis severity (i.e. grading of hepatic steatosis and necro-inflammation, as well as liver fibrosis stage).

5. Conclusion

In our population-based cohort of German adult individuals, we found significant associations between hepatic steatosis and different glucose intolerance states (i-IFG, i-IGT, IFG + IGT or previously unknown T2DM). Hepatic iron overload, however, was inconsistently associated with different glucose intolerance states. Associations were more pronounced for hepatic steatosis derived from MRI compared to ultrasound.

Our study showed a strong impact of hepatic steatosis but a weak effect of hepatic iron overloads on different glucose intolerance states in the general population. A larger liver volume was significantly associated with a nearly three-fold increase in the long-term risk of all-cause mortality. These findings imply that a larger liver volume *per se* might be particularly harmful, independent of the coexistence of other metabolic conditions. However, further prospective studies are needed to confirm these findings in independent samples and to uncover mechanistic models to explain the path from liver enlargement to mortality risks. Our results confirm that MRI is a more sensitive and accurate method for determining hepatic steatosis and total liver volume than ultrasound or any laboratory markers to investigate related disease risks.

Name: Muhammad Naeem

Thema: Associations of liver dysfunction with glucose intolerance and all-cause mortality in a general population

Summary

Liver dysfunctions are commonly associated with diabetes and mortality in the general population. However, previous studies lack to define these disorders with hepatic markers from MRI, which have been shown to be more accurate and sensitive than hepatic ultrasound and laboratory markers. Further, previous studies defining different categories of prediabetes by oral glucose tolerance states revealed controversial findings. Hence, this dissertation contributed to understand the associations of liver dysfunctions with glucose intolerance states and all-cause mortality in the general population.

In the first part of the dissertation, the associations of MRI-related hepatic steatosis and hepatic iron overload with prediabetes were investigated. Prediabetes was categorized into IFG, IGT, (alone or in combination) or previously unknown type 2 diabetes mellitus using OGGT data, as suggested by the ADA. For analyses, we included 1632 subjects with MRI who participated in an OGTT and reported no type 2 diabetes mellitus. We found that hepatic steatosis was positively associated with continuous markers of glucose metabolism. Similarly, subjects with hepatic steatosis as defined by MRI had a higher relative risk ratio to be in the prediabetes groups (i-IFG, i-IGT and IFG + IGT) or having undiagnosed diabetes than individuals without this condition. The observed associations were more obvious for MRI-derived hepatic steatosis compared to ultrasound. In comparison to hepatic steatosis, we found that MRI-assessed hepatic iron overload was positively associated only with both 2-hour plasma glucose and the combined IFG + IGT category. There were no significant associations between hepatic iron overload and other glucose tolerance states or biomarkers of glucose metabolism, regardless of possible confounding factors.

In the second part, the associations of liver volume and other markers of hepatic steatosis with all-cause mortality in the general population were investigated. We included 2769 middle-aged German subjects with a median follow-up of 8.9 years (23,898 person-years). Serum liver enzymes and FIB-4 score were used as quantitative markers, while MRI measurements of liver

fat content and total liver volume included as qualitative markers of hepatic steatosis. Compared to other markers of hepatic steatosis, larger liver volumes were significantly associated with a nearly three-fold increase in the long-term risk of all-cause mortality. Furthermore, this association was consistent across all subgroups considered (men vs. women; presence or absence of metabolic syndrome or type 2 diabetes at baseline). A positive association between FIB-4 score and all-cause mortality was found both in the entire cohort and in women. Likewise, positive associations of higher serum AST and GGT levels with all-cause mortality were found in the entire cohort and in men.

To conclude, this dissertation acknowledges the fact that prevention and early intervention of liver dysfunction has major impact to reduce the burden of public health problems. Thus, our findings suggest that hepatic markers contributes to an increased risk of prediabetes and all-cause mortality, which might be helpful to identify high risk groups who need closer attention with respect to prevention of liver disorders and diabetes.

6. References

1. Marcellin, P. and B.K. Kutala, *Liver diseases: A major, neglected global public health problem requiring urgent actions and large-scale screening.* Liver International, 2018. **38**: p. 2-6.
2. Asrani, S.K., et al., *Burden of liver diseases in the world.* Journal of hepatology, 2019. **70**(1): p. 151-171.
3. Younossi, Z.M., et al., *Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes.* Hepatology, 2016. **64**(1): p. 73-84.
4. Younossi, Z., et al., *Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention.* Nature reviews Gastroenterology & hepatology, 2018. **15**(1): p. 11-20.
5. Balakrishnan, M., et al., *Women have a lower risk of nonalcoholic fatty liver disease but a higher risk of progression vs men: a systematic review and meta-analysis.* Clinical Gastroenterology and Hepatology, 2021. **19**(1): p. 61-71. e15.
6. Kumar, R. and S. Mohan, *Non-alcoholic Fatty Liver Disease in Lean Subjects: Characteristics and Implications.* Journal of clinical and translational hepatology, 2017. **5**(3): p. 216-223.
7. Labranche, R., et al., *Liver iron quantification with MR imaging: a primer for radiologists.* Radiographics, 2018. **38**(2): p. 392-412.
8. Miyanishi, K., et al., *The role of iron in hepatic inflammation and hepatocellular carcinoma.* Free Radical Biology and Medicine, 2019. **133**: p. 200-205.
9. Datz, C., E. Müller, and E. Aigner, *Iron overload and non-alcoholic fatty liver disease.* Minerva Endocrinol, 2017. **42**(2): p. 173-183.
10. Kühn, J.-P., et al., *Prevalence of fatty liver disease and hepatic iron overload in a northeastern German population by using quantitative MR imaging.* Radiology, 2017. **284**(3): p. 706-716.
11. Liu, M., et al., *Association of NAFLD With Diabetes and the Impact of BMI Changes: A 5-Year Cohort Study Based on 18,507 Elderly.* J Clin Endocrinol Metab, 2017. **102**(4): p. 1309-1316.
12. Yamada, T., et al., *Fatty liver predicts impaired fasting glucose and type 2 diabetes mellitus in Japanese undergoing a health checkup.* J Gastroenterol Hepatol, 2010. **25**(2): p. 352-6.
13. Zelber-Sagi, S., et al., *Non-alcoholic fatty liver disease independently predicts prediabetes during a 7-year prospective follow-up.* Liver Int, 2013. **33**(9): p. 1406-12.
14. Ming, J., et al., *Non-alcoholic fatty liver disease predicts type 2 diabetes mellitus, but not prediabetes, in Xi'an, China: a five-year cohort study.* Liver Int, 2015. **35**(11): p. 2401-7.
15. Ruckert, I.M., et al., *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**(8): p. e22932.

16. Mohan, V., et al., *Prevalence of non-alcoholic fatty liver disease in urban south Indians in relation to different grades of glucose intolerance and metabolic syndrome.* Diabetes Research and Clinical Practice, 2009. **84**(1): p. 84-91.

17. Kim, C.H., et al., *Association of elevated serum ferritin concentration with insulin resistance and impaired glucose metabolism in Korean men and women*. Metabolism, 2011. **60**(3): p. 414-20.

18. Sharifi, F., N.M. Nasab, and H.J. Zadeh, *Elevated serum ferritin concentrations in prediabetic subjects.* Diab Vasc Dis Res, 2008. **5**(1): p. 15-8.

19. Hughes, E.A., et al., *Ferritin as a Risk Factor for Glucose Intolerance amongst Men and Women Originating from the Indian Subcontinent.* International journal of endocrinology, 2015. **2015**: p. 924387-924387.

20. Huth, C., et al., *Biomarkers of iron metabolism are independently associated with impaired glucose metabolism and type 2 diabetes: the KORA F4 study.* Eur J Endocrinol, 2015. **173**(5): p. 643-53.

21. Cheung, C.L., et al., *High ferritin and low transferrin saturation are associated with pre-diabetes among a national representative sample of U.S. adults.* Clin Nutr, 2013. **32**(6): p. 1055-60.

22. Aguirre, L.G., et al., *High serum ferritin levels are associated with insulin resistance but not with impaired glucose tolerance in a healthy people population.* Diabetes Metab Syndr, 2017. **11 Suppl 2**: p. S983-s988.

23. Estes, C., et al., *Modeling nafld disease burden in china, france, germany, italy, japan, spain, united kingdom, and united states for the period 2016–2030.* Journal of hepatology, 2018. **69**(4): p. 896-904.

24. Önnerhag, K., et al., *Non-invasive fibrosis scoring systems can predict future metabolic complications and overall mortality in non-alcoholic fatty liver disease (NAFLD).* Scand J Gastroenterol, 2019. **54**(3): p. 328-334.

25. Cheung, C.L., et al., *Non-invasive score identifies ultrasonography-diagnosed non-alcoholic fatty liver disease and predicts mortality in the USA.* BMC Med, 2014. **12**: p. 154.

26. Dunn, W., et al., *Suspected nonalcoholic fatty liver disease and mortality risk in a population-based cohort study.* Am J Gastroenterol, 2008. **103**(9): p. 2263-71.

27. Calori, G., et al., *Fatty liver index and mortality: the Cremona study in the 15th year of follow-up.* Hepatology, 2011. **54**(1): p. 145-52.

28. Haring, R., et al., *Ultrasonographic hepatic steatosis increases prediction of mortality risk from elevated serum gamma-glutamyl transpeptidase levels.* Hepatology, 2009. **50**(5): p. 1403-11.

29. Liu, Y., et al., *Nonalcoholic fatty liver disease and mortality from all causes, cardiovascular disease, and cancer: a meta-analysis.* Scientific reports, 2019. **9**(1): p. 11124-11124.

30. Huang, Q., et al., *NAFLD or MAFLD: Which Has Closer Association With All-Cause and Cause-Specific Mortality?—Results From NHANES III.* Frontiers in Medicine, 2021. **8**.

31. Unalp-Arida, A. and C.E. Ruhl, *Noninvasive fatty liver markers predict liver disease mortality in the U.S. population.* Hepatology, 2016. **63**(4): p. 1170-83.

32. Choi, K.M., et al., *Implication of liver enzymes on incident cardiovascular diseases and mortality: A nationwide population-based cohort study.* Scientific Reports, 2018. **8**.

33. Karaphillis, E., et al., *Serum alanine aminotransferase levels and all-cause mortality.* Eur J Gastroenterol Hepatol, 2017. **29**(3): p. 284-288.

34. Katzke, V., et al., *Circulating liver enzymes and risks of chronic diseases and mortality in the prospective EPIC-Heidelberg case-cohort study.* BMJ Open, 2020. **10**(3): p. e033532.

35. Kim, K.N., et al., *Associations of serum liver enzyme levels and their changes over time with all-cause and cause-specific mortality in the general population: a large-scale national health screening cohort study.* BMJ Open, 2019. **9**(5): p. e026965.

36. Koehler, E., et al., *Serum liver enzymes are associated with all-cause mortality in an elderly population.* Liver international : official journal of the International Association for the Study of the Liver, 2013. **34**.

37. Lee, H., et al., *Association Between Change in Serum Aminotransferase and Mortality: A Nationwide Cohort Study in Korea.* Medicine, 2016. **95**(12): p. e3158-e3158.

38. Mahady, S.E., et al., *Elevated Liver Enzymes and Mortality in Older Individuals: A Prospective Cohort Study.* J Clin Gastroenterol, 2017. **51**(5): p. 439-445.

39. Oh, C.M., et al., *Alanine aminotransferase and gamma-glutamyl transferase have different dose-response relationships with risk of mortality by age.* Liver Int, 2016. **36**(1): p. 126-35.

40. Schmilovitz-Weiss, H., et al., *Risk of mortality and level of serum alanine aminotransferase among community-dwelling elderly in Israel.* Eur J Gastroenterol Hepatol, 2018. **30**(12): p. 1428-1433.

41. *2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018.* Diabetes Care, 2018. **41**(Suppl 1): p. S13-s27.

42. Takahashi, S., et al., *Prediction and validation of nonalcoholic fatty liver disease by fatty liver index in a Japanese population.* Endocrine Journal, 2021. **advpub**.

43. Borman, M.A., et al., *The Fatty Liver Index has limited utility for the detection and quantification of hepatic steatosis in obese patients.* Hepatol Int, 2013. **7**(2): p. 592-9.

44. Garcia-Casal, M.N., et al., *Serum or plasma ferritin concentration as an index of iron deficiency and overload.* Cochrane Database Syst Rev, 2021. **5**(5): p. Cd011817.

45. Wang, W., et al., *Serum ferritin: Past, present and future.* Biochim Biophys Acta, 2010. **1800**(8): p. 760-9.

46. Kim, D., et al., *Metabolic dysfunction-associated fatty liver disease is associated with increased all-cause mortality in the United States.* J Hepatol, 2021. **75**(6): p. 1284-1291.

47. Wattacheril, J. and N. Chalasani, *Nonalcoholic fatty liver disease (NAFLD): is it really a serious condition?* Hepatology, 2012. **56**(4): p. 1580-1584.

48. Maximos, M., et al., *The role of liver fat and insulin resistance as determinants of plasma aminotransferase elevation in nonalcoholic fatty liver disease.* Hepatology, 2015. **61**(1): p. 153-160.

49. Zhou, J.-H., et al., *Noninvasive evaluation of nonalcoholic fatty liver disease: Current evidence and practice.* World journal of gastroenterology, 2019. **25**(11): p. 1307.

50. Starekova, J. and S.B. Reeder, *Liver fat quantification: where do we stand?* 2020. **45**(11): p. 3386-3399.

51. Yoshizawa, E. and A. Yamada, *MRI-derived proton density fat fraction.* Journal of Medical Ultrasonics, 2021. **48**(4): p. 497-506.

52. Zhou, J.H., et al., *Noninvasive evaluation of nonalcoholic fatty liver disease: Current evidence and practice.* World J Gastroenterol, 2019. **25**(11): p. 1307-1326.

53. Stern, C. and L. Castera, *Non-invasive diagnosis of hepatic steatosis.* Hepatol Int, 2017. **11**(1): p. 70-78.

54. Alte, D., et al., *Distribution and dose response of laboratory markers to alcohol consumption in a general population: results of the study of health in Pomerania (SHIP).* Journal of Studies on Alcohol, 2003. **64**(1): p. 75-82.

55. Volzke, H., et al., *Cohort profile: the study of health in Pomerania.* Int J Epidemiol, 2011. **40**(2): p. 294-307.

56. Kromrey, M.L., et al., *Reference values of liver volume in Caucasian population and factors influencing liver size.* European Journal of Radiology, 2018. **106**: p. 32-37.

57. Pitchika, A., et al., *Associations of iron markers with type 2 diabetes mellitus and metabolic syndrome: Results from the prospective SHIP study.* Diabetes Res Clin Pract, 2020. **163**: p. 108149.

58. Tamayo, T., et al., *Regional differences of undiagnosed type 2 diabetes and prediabetes prevalence are not explained by known risk factors.* PLoS One, 2014. **9**(11): p. e113154.

59. Krebs, A., et al., *Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20–79.* European Heart Journal, 2010. **31**(12): p. 1494-1501.

60. Vallet-Pichard, A., et al., *FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest.* Hepatology, 2007. **46**(1): p. 32-6.

61. Park, R.J. and J.D. Moon, *Low transferrin saturation is associated with impaired fasting glucose and insulin resistance in the South Korean adults: the 2010 Korean National Health and Nutrition Examination Survey.* Diabet Med, 2015. **32**(5): p. 673-8.

62. Horng, D.E., D. Hernando, and S.B. Reeder, *Quantification of liver fat in the presence of iron overload.* Journal of magnetic resonance imaging : JMRI, 2017. **45**(2): p. 428-439.

63. Mao, X., et al., *Non-invasive fibrosis markers are associated with mortality risk in both general populations and non-alcoholic fatty liver disease patients.* 2021. **51**(1): p. 90-101.

64. Ong, J.P., A. Pitts, and Z.M. Younossi, *Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease.* J Hepatol, 2008. **49**(4): p. 608-12.

65. Unalp-Arida, A. and C.E. Ruhl, *Liver fibrosis scores predict liver disease mortality in the United States population.* Hepatology, 2017. **66**(1): p. 84-95.

66. Wattacheril, J. and N. Chalasani, *Nonalcoholic fatty liver disease (NAFLD): is it really a serious condition?* Hepatology (Baltimore, Md.), 2012. **56**(4): p. 1580-1584.

67. Wells, S.A., *Quantification of hepatic fat and iron with magnetic resonance imaging.* Magn Reson Imaging Clin N Am, 2014. **22**(3): p. 397-416.

7. Scientific papers

The present dissertation is based on the following three scientific papers arranged in the given order.

1. Naeem M, Bülow R, Schipf S, *et al.* Association of hepatic steatosis derived from ultrasound and quantitative MRI with prediabetes in the general population. *Sci Rep* 11, 13276 (2021)
2. Naeem M, Schipf S, Bülow R, *et al.* Association between hepatic iron overload assessed by magnetic resonance imaging and glucose intolerance states in the general population. Nutrition, Metabolism and Cardiovascular Diseases, 2022 jun;32(6):1470-1476
3. Naeem M, Markus MRP, Mousa M, *et al*. Associations of liver volume and other markers of hepatic steatosis with all-cause mortality in the general population. Liver Int. 42(3):575-584 (2022)

7.1 Naeem M, Schipf S, Bülow R, Werner N, Dörr M, Lerch MM, Kühn JP, Rathmann W, Nauck M, Markus MRP, Ittermann T, Völzke H

Association of hepatic steatosis derived from ultrasound and quantitative MRI with prediabetes in the general population

scientific reports

Check for updates

OPEN

Association of hepatic steatosis derived from ultrasound and quantitative MRI with prediabetes in the general population

Muhammad Naeem[1,10]✉, Robin Bülow[4,6], Sabine Schipf[1], Nicole Werner[1], Marcus Dörr[2,6], Markus M. Lerch[3], Jens-Peter Kühn[8], Wolfgang Rathmann[7,9], Matthias Nauck[5], Marcello Ricardo Paulista Markus[2,6], Till Ittermann[1,11] & Henry Völzke[1,6,7,11]

The aim of our study was to investigate the association of hepatic steatosis derived from quantitative ultrasound and magnetic resonance imaging (MRI) with prediabetes in a large population-based study conducted in Northeast Germany. Hepatic steatosis was assessed through transabdominal ultrasound and quantitative MRI. For analysis we included 1622 subjects with MRI who participated in an oral glucose tolerance test and reported no known type 2 diabetes mellitus (T2DM). We classified participants as proposed by the American Diabetes Association: isolated impaired fasting glucose (i-IFG), isolated impaired glucose tolerance (i-IGT), combined IFG and IGT (IFG + IGT), and undiagnosed T2DM. Regression models were adjusted for age, sex body mass index and alcohol consumption. We observed positive associations of hepatic steatosis with glycated hemoglobin, fasting glucose and insulin, 2-h glucose and insulin, as well as homeostasis model assessment-insulin resistance index. Similarly, individuals having hepatic steatosis as defined by MRI had a higher relative risk ratio (RR) to be in the prediabetes groups i-IFG (RR = 1.6; 95% confidence interval (CI) 1.2; 2.2), i-IGT (RR = 3.3, 95% CI 2.0; 5.6) and IFG + IGT (RR = 2.5, 95% CI 1.6; 3.9) or to have undiagnosed T2DM (RR = 4.8, 95% CI 2.6; 9.0). All associations were attenuated when defining hepatic steatosis by ultrasound. Hepatic steatosis is associated with prediabetes and undiagnosed T2DM in the general population. Quantitative liver MRI revealed stronger associations with prediabetes and undiagnosed T2DM compared to ultrasound, which indicates the higher sensitivity and specificity of MRI to determine hepatic steatosis.

Hepatic steatosis is defined as an excessive fat deposition (> 5%) in the liver in the absence of competing liver disease or hepatocellular carcinoma[1]. Hepatic steatosis is highly prevalent affecting 25% of the world population[2] and up to 70% of patients with type 2 diabetes mellitus[3,4]. The prevalence of ultrasound-determined hepatic steatosis is highest in the Middle East (32%) and South America (30%), lower in Europe (24%), and lowest in Africa (13%)[2].

[1]Institute for Community Medicine, University Medicine Greifswald, Walther Rathenau Str. 48, 17475 Greifswald, Germany. [2]Department of Internal Medicine B—Cardiology, Intensive Care, Pulmonary Medicine and Infectious Diseases, University Medicine Greifswald, Greifswald, Germany. [3]Department of Gastroenterology, University Medicine Greifswald, Greifswald, Germany. [4]Institute of Diagnostic Radiology and Neuroradiology, University Medicine Greifswald, Greifswald, Germany. [5]Institute for Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany. [6]DZHK (German Center for Cardiovascular Research), partner site Greifswald, Germany. [7]DZD (German Center for Diabetes Research), Dresden, Germany. [8]Institute and Policlinic for Diagnostic and Interventional Radiology, University Hospital, Carl Gustav Carus University, TU Dresden, Dresden, Germany. [9]Institute for Biometrics and Epidemiology, German Diabetes Center (DDZ), Leibniz Center for Diabetes Research At Heinrich Heine University Düsseldorf, Düsseldorf, Germany. [10]Department of Zoology, University of Malakand, Chakdara 18800, Pakistan. [11]These authors contributed equally: Till Itterman and Henry Völzke. ✉email: muhammad.naeem@stud.uni-greifswald.de

Hepatic steatosis occurs usually when lipid storage is increased through hepatic uptake and de novo lipogenesis through fatty acid oxidation and export of lipid in very low density lipoprotein[5]. Hepatic steatosis is strongly associated with insulin resistance[6] and postprandial hyperinsulinemia indicating its possible role in the pathogenesis of type 2 diabetes mellitus[7]. Furthermore, the association between hepatic steatosis and type 2 diabetes mellitus may be bidirectional as suggested from some studies[8–10].

Population-based studies defining hepatic steatosis by computed tomography showed significant associations with type 2 diabetes mellitus[8,11]. Likewise, several previous studies demonstrated associations between sonographically determined hepatic steatosis and type 2 diabetes mellitus[9,12–17]. Although being easy to use and non-radiation-based and therefore a suitable method for population-based research, ultrasound has a low sensitivity for detecting mild steatosis, and limitations in the examination of obese individuals[18,19].

While there is strong evidence that hepatic steatosis is associated with type 2 diabetes mellitus, data regarding the association between hepatic steatosis and prediabetes is inconsistent. Previous cohort studies demonstrated associations between hepatic steatosis defined by ultrasound and prediabetes defined by fasting glucose and 2-h glucose or glycated hemoglobin (HbA1c)[20–23]. One cross-sectional study found an association between hepatic steatosis defined by fatty liver index and prediabetes categories according to American Diabetes Association (ADA) criteria[24], whereas others did not[16,21].

To the best of our knowledge there is no population-based study, which investigated the association of hepatic steatosis as defined by quantitative magnetic resonance imaging (MRI) with prediabetes and undiagnosed type 2 diabetes mellitus. From MRI, the proton density fat fraction (PDFF) can be calculated, which is a quantitative marker for liver fat, more accurate than similar markers taken from ultrasound or computed tomography[25]. In addition, MRI is able to differentiate between liver fat and iron[26] as well as between focal, regional and general steatosis in a single procedure.

Against this background, the aim of our study is to clarify the association of hepatic steatosis assessed through ultrasound and MRI with prediabetes and undiagnosed type 2 diabetes mellitus defined the by oral glucose tolerance test (OGTT) in a large population-based sample.

Materials and methods

Study sample. The Study of Health in Pomerania (SHIP) is a population-based project conducted in Northeast Germany. It consists of the two independent cohorts SHIP and SHIP-Trend. For the present study we used baseline data from the second cohort (SHIP-Trend-0). A stratified random sample of 8826 adults aged between 20 and 79 years was drawn, of which 4420 subjects participated between 2008 and 2012 (response 50.1%). Random sample selection into age and sex-strata was facilitated by centralization of local population registries in the German Federal State of Mecklenburg/West Pomerania[27].

All participants gave written informed consent. The study conformed to the ethical guidelines of the Declaration of Helsinki as reflected in a priori approval by the local ethics committee of the University of Greifswald.

We excluded individuals without MRI examination (n = 2130), those who reported known liver cirrhosis or hepatitis (n = 46), known type 2 diabetes mellitus (n = 461), and participants with missing data in any of the considered variables (n = 37). The final study population consisted of 1746 (913 women) subjects aged 21 to 82 years. From the analysis regarding prediabetes we further excluded all individuals without OGTT (n = 124) resulting in data from 1,622 (840 women) available for analysis of prediabetes.

General measurements. Sociodemographic characteristics and medical histories were assessed by computer-assisted face-to-face interviews. Height and weight were measured for calculating the body mass index (BMI = weight [kg]/height^2 [m^2]). Alcohol intake was evaluated as beverage-specific alcohol consumption (beer, wine, distilled spirits) on the last weekend and last weekday preceding the examination. The mean daily alcohol consumption was calculated using beverage-specific pure ethanol volume proportions[28].

Ultrasound. Transabdominal ultrasound of the liver was performed by examiners using a transportable B-mode ultrasound device (vivid I; GE-Healthcare, Waukesha, WI, USA) with a 2.5 MHz ultrasonic transducer. The examiners used a 2-point scale to assess the presence of hepatic steatosis: (0) no steatosis and (1) steatosis. Hepatic steatosis was defined as a hyperechogenic liver pattern in comparison to the renal cortex[27].

Magnetic resonance imaging (MRI). MRI was performed by using a 1.5-Tesla MRI system (Magnetom Avanto, software version VB15; Siemens Healthineers Erlangen, Germany) with a 12-channel phased-array surface coil[29]. Three-dimensional chemical shift encoded gradient-echo data with three echoes and flyback readout gradient were acquired from an axial slab during a single 19-s breath hold. Imaging parameters included repetition time, 11 ms; echo times, 2.4, 4.8, and 9.6 ms; flip angle, 10°; number of signals acquired, one; bandwidth, ± 1065 Hz per pixel; matrix, 224 × 168 × 64; field of view, 410 × 308 mm; parallel imaging effective acceleration factor, 1.8; and section thickness, 3.0 mm.

Offline reconstructions of a PDFF map (including correction for T1 bias and T2* decay) and a transverse relaxation rate (R2*) map (based on T2* decay measurement of PDFF) were performed. Fat and water ambiguities were resolved by using the phase of the acquired data[30]. Parametric maps of PDFF were used for further analyses.

One trained radiologist reviewed the PDFF. Mean fat fraction values were determined at operator-defined regions of interest placed at the center of the liver by using Osirix (v3.8.1; Pixmec Sarl, Bernex, Switzerland). Regions of interest were placed carefully to avoid blood vessels and regions that were obviously contaminated by partial volume effects and motion artifacts[29]. Hepatic steatosis was defined as PDFF > 5%[30].

Laboratory measurements. We requested the participants not to eat, smoke or consume caffeine-containing drinks and to avoid sports for ≥ 8 h before the examination, which was completed during the morning hours. Blood was collected by a trained examiner following a standardized protocol, refrigerated to 4–8 °C and shipped on refrigerant packaging within 4 to a maximum of 6 h to the laboratory. Measurements of fasting glucose and 2-h glucose were based on plasma samples[31]. All assays were performed according to the manufacturers' recommendations by skilled technical personnel. The study laboratory participated in official quarterly German external proficiency testing programs[32].

Fasting glucose and 2-h glucose levels were measured using a hexokinase method (Dimension Vista, Siemens Healthcare Diagnostics, Eschborn, Germany)[31]. HbA1c was determined by high-performance liquid chromatography (Diamat, Bio-Rad Laboratories, Munich, Germany). Insulin serum values were measured by a chemiluminescence immunoassay (Immulite 2000 Xpi, Siemens Healthcare Diagnostics, Eschborn, Germany). Fasting insulin and 2-h insulin are expressed as µU/ml. The homeostasis model assessment-insulin resistance index (HOMA-IR) was calculated as (fasting insulin [µU/ml] x fasting glucose [mmol/l] / 22.5)[33]. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyl transpeptidase (GGT) concentrations were measured photometrically (Hitachi 704; Roche, Mannheim, Germany). ALT, AST, and GGT concentrations are expressed as µkatal/l.

Ascertainment of diabetes and prediabetes. Participants were classified as having type 2 diabetes mellitus if they reported physician's diagnosis of type 2 diabetes mellitus in the interview or took glucose-lowering medication (Anatomical Therapeutic Chemical (ATC) classification system code A10). For the OGTT, fasting glucose was sampled, and 75 g of anhydrous glucose (Dextro OGT, Boehringer Mannheim, Mannheim, Germany) was given to the participants without diabetes and glucose-lowering agents. Following the criteria of the ADA[34], we classified individuals as having normal glucose tolerance when they had fasting glucose values < 5.6 mmol/l and 2-h glucose < 7.8 mmol/l. We classified participants as having prediabetes if fasting glucose values were between 5.6 and 6.9 mmol/l (impaired fasting glucose: IFG) and/or 2-h glucose values were between 7.8 and 11.0 mmol/l (impaired glucose tolerance: IGT). We defined three groups of prediabetes: isolated impaired fasting glucose (i-IFG), isolated impaired glucose tolerance (i-IGT), and combined IFG and IGT (IFG + IGT). Undiagnosed type 2 diabetes mellitus was defined as fasting glucose values ≥ 7.0 mmol/l or 2-h glucose ≥ 11.1 mmol/l[31,33].

Statistical analysis. Continuous data are reported as median (with 25th and 75th percentiles) and categorical variables as absolute numbers and percentages. Difference between the subjects with and without hepatic steatosis were tested by Wilcoxon rank-sum test for continous data and chi-square test for categorical data. For analyzing the association between hepatic steatosis and continuous markers of glucose metabolism linear regression models were used by calculating β coefficients and 95% confidence intervals (95% CI). For investigating the association between hepatic steatosis and prediabetes groups, multinomial logistic regression was run by calculating relative risk ratios and 95% CI. All models were adjusted for age, sex BMI and alcohol consumption. A value of $p < 0.05$ was considered statistically significant in all calculations. All statistical analyses were performed by Stata 14.1 (Stata Corporation, College Station, TX, USA).

Results

Among the study population consisting of 1,746 individuals (913 women) aged 21 to 80 years the prevalence of hepatic steatosis was 37% (95% CI 34%; 39%) by using MRI and 36% (95% CI 33%; 38%) by using ultrasound. Four-hundred-sixty-seven individuals (73%) with hepatic steatosis identified by ultrasound also had hepatic steatosis derived from MRI (Table 1).

We observed that individuals having hepatic steatosis derived from MRI were older, comprised more males, had a higher BMI as well as higher levels of HbA1c, fasting glucose, 2-h glucose, fasting insulin, 2-h insulin, and HOMA-IR compared to those without hepatic steatosis. Individuals with hepatic steatosis through MRI had slightly higher levels of ALT, AST, and GGT compared to those without hepatic steatosis. Similarly, individuals with MRI-based definition of hepatic steatosis had more often prediabetes (i-IGT, i-IFG, IFG + IGT) or undiagnosed type 2 diabetes mellitus than individuals without hepatic steatosis (Table 1).

Linear regression models adjusted for age, sex, BMI and alcohol consumption revealed significantly positive associations between hepatic steatosis defined either by ultrasound or MRI. Levels of HbA1c, fasting glucose, 2-h glucose, fasting insulin, 2-h insulin and HOMA-IR were also associated with hepatic steatosis derived from ultrasound or MRI (Table 2). The mean level of 2-h glucose increased over the amount of fat in the liver (Fig. 1).

Table 3 shows the associations between hepatic steatosis and prediabetes and undiagnosed type 2 diabetes mellitus using multinomial logistic regression models adjusted for age, sex, BMI and alcohol consumption. Individuals with hepatic steatosis defined either by ultrasound or MRI had a higher relative risk ratio to be in one of the prediabetes groups (i-IFG, i-IGT, IFG + IGT) or to have undiagnosed type 2 diabetes mellitus than individuals without hepatic steatosis. All associations were stronger when hepatic steatosis was defined by MRI compared to the definition from ultrasound. We observed a positive continuous association between the liver fat as assessed by MRI with prediabetes (Fig. 2).

To investigate a potential effect modification by sex on our associations we tested the interaction term of hepatic steatosis defined by MRI or ultrasound with sex on all outcomes. In none of these analyses, we observed any significant interactions.

Variables	Number of individuals	Hepatic steatosis derived from MRI		P value
		No (n = 1,106)	Yes (n = 640)	
Age (years)	1746	46 (37; 57)	55 (47, 64)	< 0.001
Male	833	459 (42%)	374 (58%)	< 0.001
Female	913	647 (58%)	266 (42%)	< 0.001
BMI (kg/m^2)	1746	25 (23; 28)	30 (27; 32)	< 0.001
Alcohol consumption (g/day)	1732	4 (1; 9)	5 (1; 14)	< 0.001
Hepatic steatosis ultrasound				< 0.001
Negative	1119	949 (86%)	170 (27%)	
Positive	619	152 (14%)	467 (73%)	
Liver fat (MRI %)	1746	2.6 (2; 3.5)	9.4 (6.7; 15.2)	< 0.001
ALT (µkatal/l)	1745	0.33 (0.25; 0.43)	0.49 (0.36; 0.67)	< 0.001
AST (µkatal/l)	1743	0.27 (0.21; 0.33)	0.32 (0.26; 0.40)	< 0.001
GGT (µkatal/l)	1745	0.43 (0.35; 0.56)	0.64 (0.48; 0.93)	< 0.001
HbA1c %	1745	5.1 (4.8; 5.4)	5.3 (5; 5.6)	< 0.001
Glucose (mmol/l)				
Fasting	1746	5.2 (4.9; 5.6)	5.7 (5.3; 6.1)	< 0.001
2-h	1622	5.6 (4.8; 6.6)	6.6 (5.6; 8.1)	< 0.001
Insulin (µU/ml)				
Fasting	1617	7.5 (5.5; 10.5)	13.8 (9.6; 20.1)	< 0.001
2-h	1619	39 (25; 58)	72 (47; 145)	< 0.001
HOMA-IR	1617	1.8 (1.2; 2.5)	3.5 (2.3; 5.3)	< 0.001
OGTT				< 0.001
NGT	918	710 (69%)	208 (35%)	
i-IFG	404	208 (20%)	196 (33%)	
i-IGT	87	39 (4%)	48 (8%)	
IFG + IGT	133	49 (5%)	84 (14%)	
Undiagnosed T2DM	80	17 (2%)	63 (10%)	

Table 1. Characteristics of the study population stratified by hepatic steatosis (MRI). Data are given as absolute number and percentage for categorical data and as median (25th and 75th percentiles) for continuous data. *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* γ-glutamyl transpeptidase, *HbA1c* Glycated Hemoglobin, *HOMA-IR* Homeostasis model assessment-insulin resistance index, *NGT* normal glucose tolerance, *i-IFG* isolated impaired fasting glucose, *i-IGT* isolated impaired glucose tolerance, *OGTT* oral glucose tolerance test, *T2DM* type 2 diabetes mellitus. To calculate p value chi-square tests were used for categorical variables and Wilcoxon rank-sum tests for continuous variables.

Outcome variables	Hepatic steatosis (Ultrasound)		Hepatic steatosis (MRI)		MRI-PDFF %	
	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value
HbA1c %	0·07 (0·01; 0·12)	0.012	0·09 (0·03; 0·14)	0.004	0·01 (0·01; 0·01)	< 0.001
Fasting glucose (mmol/l)	0.18 (0.11; 0.24)	< 0.001	0.24 (0.17; 0.32)	< 0.001	0.03 (0·02; 0·03)	< 0.001
2-h glucose (mmol/l)	0.52 (0.31; 0.73)	< 0.001	0.75 (0.53; 0.97)	< 0.001	0.03 (0.02; 0.03)	< 0.001
Fasting insulin (µU/ml)	4.2 (3.2; 5.2)	< 0.001	4.9 (3.8; 5.9)	< 0.001	0.5 (0.4; 0.6)	< 0.001
2-h insulin (µU/ml)	22.3 (16.4; 28.2)	< 0.001	36.9 (30.8; 43.0)	< 0.001	3.5 (3.0; 4.0)	< 0.001
HOMA-IR	1.2 (0.9; 1.5)	< 0.001	1.4 (1.1; 1.8)	< 0.001	0.1 (0.1; 0.2)	< 0.001

Table 2. Associations between hepatic steatosis derived from ultrasound and PDFF-MRI with continuous markers for glucose metabolism adjusted for age, sex, BMI and alcohol consumption. β, derived from linear regression adjusted for age, sex, BMI and alcohol consumption; *95% CI* Adjusted 95% confidence interval, *BMI* body mass index, *HOMA-IR* homeostasis model assessment-insulin resistance index, *PDFF* proton density fat fraction.

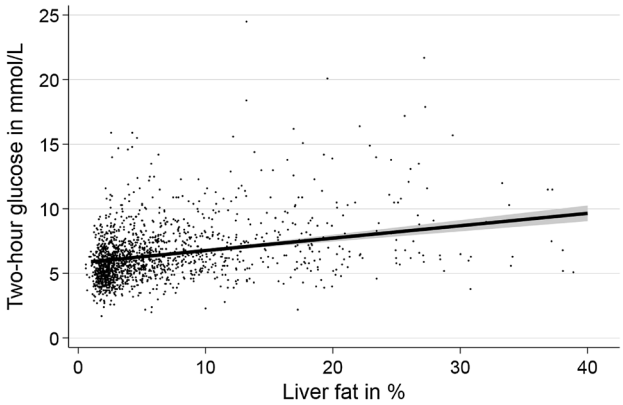

Figure 1. Association between liver fat fraction derived from quantitative MRI and two-hour glucose based on linear regression adjusted for age, sex, body mass index and alcohol consumption.

	Hepatic steatosis (Ultrasound)		Hepatic steatosis (MRI)		MRI-PDFF %	
Outcome variables	RRR 95% CI	P value	RRR 95% CI	P value	RRR 95% CI	P value
i-IFG	1.5 (1.2; 2.0)	0.002	1.6 (1.2; 2.2)	0.001	1.1 (1.0; 1.1)	< 0.001
i-IGT	1.7 (1.1; 2.8)	0.029	3.3 (2.0; 5.6)	< 0.001	1.1 (1.0; 1.1)	< 0.001
IFG + IGT	2.1 (1.4; 3.3)	< 0.001	2.5 (1.6; 3.9)	< 0.001	1.1 (1.0; 1.1)	< 0.001
Undiagnosed T2DM	2.8 (1.6; 4.8)	< 0.001	4.8 (2.6; 9.0)	< 0.001	1.2 (1.1; 1.2)	< 0.001

Table 3. Associations of hepatic steatosis with categories of prediabetes and undiagnosed type 2 diabetes mellitus. Multinomial regression with normal glucose tolerance (NGT) as base outcome adjusted for age, sex, BMI and alcohol consumption. *RRR* relative risk ratio, 95% confidence interval (CI), adjusted 95% confidence interval; *BMI* body mass index, *i-IFG* isolated impaired fasting glucose, *i-IGT* isolated impaired glucose tolerance, *T2DM* type 2 diabetes mellitus, *PDFF* proton density fat fraction.

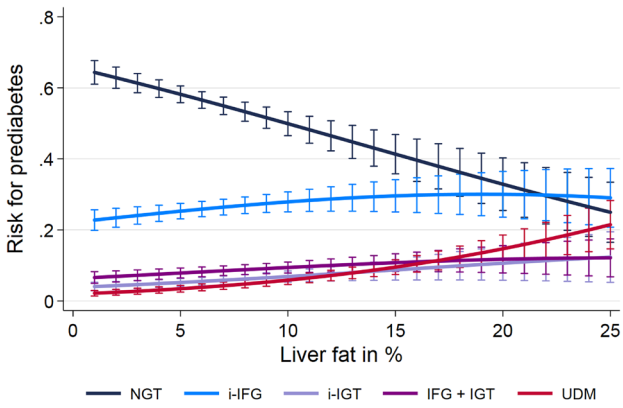

Figure 2. Association between liver fat and prediabetes and expressed as absolute risks based on multinomial regression after adjustment for age, sex, body mass index and alcohol consumption. *NGT* normal glucose tolerance test, *i-IFG* isolated impaired fasting glucose, *i-IGT* isolated impaired glucose tolerance, *UDM* undiagnosed type 2 diabetes.

Discussion

In the present study, we investigated the association of hepatic steatosis derived from transabdominal ultrasound and MRI with prediabetes and undiagnosed type 2 diabetes mellitus in the general adult population. We demonstrated positive associations of hepatic steatosis with markers of glucose metabolism including HbA1c, fasting glucose, 2-h glucose, fasting insulin, 2-h insulin, and HOMA-IR. Similarly, we observed that individuals with hepatic steatosis had a higher risk of prediabetes or undiagnosed type 2 diabetes mellitus than individuals

without hepatic steatosis. Associations were consistently stronger for hepatic steatosis derived from MRI compared to the ultrasound-based assessment.

Previous literature demonstrated associations between hepatic steatosis and type 2 diabetes mellitus[9,12–17], but only few studies investigated the association between sonographically assessed hepatic steatosis and prediabetes in general populations[16,20–23]. A large occupational cohort of Chinese men showed that hepatic steatosis was a risk factor for prediabetes ascertained by OGTT after a follow-up of 5 years[20]. Similar results were observed in a longitudinal study with Japanese health-checkup participants defining IFG by fasting glucose levels[22]. Another study with a relatively small sample size (n = 213) demonstrated an association between hepatic steatosis and incident prediabetes defined by fasting glucose or HbA1c after a follow-up of 7 years[23].

In line with our finding, data from the cross-sectional German KORA F4 study showed that subjects having hepatic steatosis as derived from fatty liver index (as calculated from BMI, waist circumference, GGT and triglycerides)[35] had an increased chance to be in one of the prediabetes groups as defined by the ADA criteria[24]. In contrast to our results, a cohort study in 508 healthy subjects with a follow-up of five years failed to demonstrate a significant association of hepatic steatosis with incident prediabetes as defined by OGTT[16]. The discrepant finding may be explained by differences in study design and over-adjustment in the previous study[16]. For example, smoking or blood pressure are not considered as co-variables for the investigated association, because they do not confound the association between hepatic steatosis and metabolic endpoints.

Also a cross-sectional study from India[21] did not find any association of hepatic steatosis with prediabetes categories as defined by the ADA criteria. Although that study adjusted for similar confounders (age, gender and waist circumference) as we did, probably no association was found due to the relatively small sample size of (n = 541) participants in that study[21].

In our study we assessed hepatic steatosis by both ultrasound and MRI. We observed that the effect sizes for the association of hepatic steatosis with markers of prediabetes and undiagnosed type 2 diabetes mellitus were consistently larger when defining hepatic steatosis by MRI. This can be explained by the fact that MRI is a more sensitive and specific than ultrasound to detect liver fat[25]. Similarly, compared to ultrasound MRI is operator independent and has a lower sample variability[36]. MRI is highly reproducible and need less time for the examination of the entire liver[25]. Further, liver fat assessment by MRI is less confounded by body fat than liver fat measurement by ultrasound[37].

It has been proposed that excessive lipid metabolites like diacylglycerol and ceramides within the liver cause insulin resistance by reducing phosphorylation of insulin receptor substrate 1 and 2 and activation of proinflammatory receptors[38]. An experimental study in mice suggested that diacylglycerol promotes insulin resistance in liver steatosis[39]. As a consequence, insulin is unable to suppress intrahepatic gluconeogenesis and lipolysis in adipose tissue, while promoting de novo hepatic lipogenesis[40]. In hepatic steatosis, endoplasmic reticulum stress and mitochondrial dysfunction may induce oxidative stress, which leads to production of reactive oxygen species[41]. As a result, β-cells of the pancreas are unable to compensate for the oxidative stress, which may lead to type 2 diabetes mellitus[42,43]. Recently, it has been investigated that various types of hepatokines such as fetuin A and B secreted by hepatocytes are increased in hepatic steatosis resulting in decreased insulin signaling, inflammation, lipolysis and insulin resistance[44].

The association of hepatic steatosis with prediabetes and undiagnosed type 2 diabetes mellitus may be bidirectional as suggested from studies in patients with type 2 diabetes mellitus[8–10]. Similarly, there are hereditary factors to cause hepatic steatosis, which is then accompanied by insulin resistance and type 2 diabetes mellitus suggesting that liver fat may be a consequence rather than a cause of insulin resistance and type 2 diabetes mellitus[45].

One strength of our study is the large population-based sample. Further, we defined hepatic steatosis according to sophisticated MRI analysis, which is more sensitive and specific than ultrasound[46], because the threshold for detecting fat is lower and liver fat can be differentiated from liver iron[26]. Prediabetes was derived from the ADA criteria. Besides OGTT, we included further markers of glucose metabolism including HbA1c, fasting insulin, 2-h insulin and HOMA-IR.

A limitation of our study is that associations were only investigated cross-sectionally. Thus, we cannot draw causal inference. However, previous genomic studies using mendelian randomization demonstrated a causal relationship between hepatic steatosis and type 2 diabetes mellitus[47,48]. Although we adjusted our analysis for confounding, we cannot exclude residual confounding. Similarly, due to ethical constrains in our population of volunteers we did not use biopsy, which is the gold standard method to determine hepatic steatosis, or computed tomography as a radiation-based examination method[49].

Conclusions

Hepatic steatosis is associated with prediabetes and undiagnosed type 2 diabetes mellitus in the general population. The PDFF derived from liver MRI seems to be the more sensitive and specific method to determine hepatic steatosis than ultrasound, because it revealed stronger associations between hepatic steatosis and prediabetes.

Received: 7 January 2021; Accepted: 11 June 2021
Published online: 24 June 2021

References

1. Chalasani, N. *et al.* The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the american association for the study of liver diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology (Baltimore, MD)* **55**(6), 2005–2023 (2012).
2. Younossi, Z. M. *et al.* Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology (Baltimore, MD)* **64**(1), 73–84 (2016).

3. Bril, F. & Cusi, K. Management of nonalcoholic fatty liver disease in patients with type 2 diabetes: A call to action. *Diabetes Care* **40**(3), 419–430 (2017).
4. Hazlehurst, J. M., Woods, C., Marjot, T., Cobbold, J. F. & Tomlinson, J. W. Non-alcoholic fatty liver disease and diabetes. *Metabolism* **65**(8), 1096–1108 (2016).
5. Ipsen, D. H., Lykkesfeldt, J. & Tveden-Nyborg, P. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. *Cell. Mol. Life Sci.* **75**(18), 3313–3327 (2018).
6. Lonardo, A., Ballestri, S., Marchesini, G., Angulo, P. & Loria, P. Nonalcoholic fatty liver disease: A precursor of the metabolic syndrome. *Dig. Liver Dis.* **47**(3), 181–190 (2015).
7. Manchanayake, J., Chitturi, S., Nolan, C. & Farrell, G. C. Postprandial hyperinsulinemia is universal in non-diabetic patients with nonalcoholic fatty liver disease. *J. Gastroenterol. Hepatol.* **26**(3), 510–516 (2011).
8. Ma, J. *et al.* Bi-directional analysis between fatty liver and cardiovascular disease risk factors. *J. Hepatol.* **66**(2), 390–397 (2017).
9. Li, Y. *et al.* Bidirectional association between nonalcoholic fatty liver disease and type 2 diabetes in Chinese population: Evidence from the Dongfeng-Tongji cohort study. *PLoS ONE* **12**(3), e0174291 (2017).
10. Lonardo, A. & Lugari, S. A round trip from nonalcoholic fatty liver disease to diabetes: molecular targets to the rescue?. *Acta Diabetol.* **56**(4), 385–396 (2019).
11. Shah, R. V. *et al.* Liver fat, statin use, and incident diabetes: The multi-ethnic study of atherosclerosis. *Atherosclerosis* **242**(1), 211–217 (2015).
12. Chen, G.-Y. *et al.* New risk-scoring system including non-alcoholic fatty liver disease for predicting incident type 2 diabetes in East China: Shanghai Baosteel Cohort. *J. Diabet. Investig.* **7**(2), 206–211 (2016).
13. Chen, S. C. *et al.* Liver fat, hepatic enzymes, alkaline phosphatase and the risk of incident type 2 diabetes: A prospective study of 132,377 adults. *Sci. Rep.* **7**(1), 4649 (2017).
14. Chang, Y. *et al.* Cohort study of non-alcoholic fatty liver disease, NAFLD fibrosis score, and the risk of incident diabetes in a Korean population. *Am. J. Gastroenterol.* **108**(12), 1861–1868 (2013).
15. Tokita, Y. *et al.* Non-alcoholic fatty liver disease is a risk factor for type 2 diabetes in middle-aged Japanese men and women. *Internal Med. (Tokyo, Japan).* **56**(7), 763–771 (2017).
16. Ming, J. *et al.* Non-alcoholic fatty liver disease predicts type 2 diabetes mellitus, but not prediabetes, in Xi'an, China: A five-year cohort study. *Liver Int.* **35**(11), 2401–2407 (2015).
17. Shibata, M., Kihara, Y., Taguchi, M., Tashiro, M. & Otsuki, M. Nonalcoholic fatty liver disease is a risk factor for type 2 diabetes in middle-aged Japanese men. *Diabetes Care* **30**(11), 2940–2944 (2007).
18. AlShaalan, R., Aljiffry, M., Al-Busafi, S., Metrakos, P. & Hassanain, M. Nonalcoholic fatty liver disease: Noninvasive methods of diagnosing hepatic steatosis. *Saudi J. Gastroenterol.* **21**(2), 64–70 (2015).
19. Bohte, A. E., van Werven, J. R., Bipat, S. & Stoker, J. The diagnostic accuracy of US, CT, MRI and 1H-MRS for the evaluation of hepatic steatosis compared with liver biopsy: A meta-analysis. *Eur. Radiol.* **21**(1), 87–97 (2011).
20. Liu, M., Wang, J., Zeng, J., Cao, X. & He, Y. Association of NAFLD with diabetes and the impact of BMI changes: A 5-year cohort study based on 18,507 elderly. *J. Clin. Endocrinol. Metab.* **102**(4), 1309–1316 (2017).
21. Mohan, V., Farooq, S., Deepa, M., Ravikumar, R. & Pitchumoni, C. S. Prevalence of non-alcoholic fatty liver disease in urban south Indians in relation to different grades of glucose intolerance and metabolic syndrome. *Diabetes Res. Clin. Pract.* **84**(1), 84–91 (2009).
22. Yamada, T. *et al.* Fatty liver predicts impaired fasting glucose and type 2 diabetes mellitus in Japanese undergoing a health checkup. *J. Gastroenterol. Hepatol.* **25**(2), 352–356 (2010).
23. Zelber-Sagi, S. *et al.* Non-alcoholic fatty liver disease independently predicts prediabetes during a 7-year prospective follow-up. *Liver Int.* **33**(9), 1406–1412 (2013).
24. Ruckert, I. M. *et al.* 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* **6**(8), e22932 (2011).
25. Stern, C. & Castera, L. Non-invasive diagnosis of hepatic steatosis. *Hep. Intl.* **11**(1), 70–78 (2017).
26. Kinner, S., Reeder, S. B. & Yokoo, T. Quantitative imaging biomarkers of NAFLD. *Dig. Dis. Sci.* **61**(5), 1337–1347 (2016).
27. Volzke, H. *et al.* Cohort profile: The study of health in Pomerania. *Int. J. Epidemiol.* **40**(2), 294–307 (2011).
28. Alte, D. *et al.* Distribution and dose response of laboratory markers to alcohol consumption in a general population: results of the study of health in Pomerania (SHIP). *J. Stud. Alcohol* **64**(1), 75–82 (2003).
29. Kuhn, J. P. *et al.* Quantitative chemical shift-encoded MRI is an accurate method to quantify hepatic steatosis. *J. Magn. Reson. Imaging.* **39**(6), 1494–1501 (2014).
30. Kühn, J.-P. *et al.* Effect of multipeak spectral modeling of fat for liver iron and fat quantification: correlation of biopsy with MR imaging results. *Radiology* **265**(1), 133–142 (2012).
31. Tamayo, T. *et al.* Regional differences of undiagnosed type 2 diabetes and prediabetes prevalence are not explained by known risk factors. *PLoS ONE* **9**(11), e113154 (2014).
32. Krebs, A. *et al.* Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20–79. *Eur. Heart J.* **31**(12), 1494–1501 (2010).
33. Markus, M. R. P. *et al.* Prediabetes is associated with lower brain gray matter volume in the general population. The Study of Health in Pomerania (SHIP). *Nutr. Metab. Cardiovasc. Dis.* **27**(12), 1114–1122 (2017).
34. Classification and Diagnosis of Diabetes. Standards of Medical Care in Diabetes-2018. *Diabetes Care* **41**(Suppl 1), S13-s27 (2018).
35. Bedogni, G. *et al.* The Fatty Liver Index: A simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol.* **6**(1), 33 (2006).
36. Starekova, J. & Reeder, S. B. Liver fat quantification: Where do we stand?. *Abdominal Radiol. (New York).* **45**(11), 3386–3399 (2020).
37. Heba, E. R. *et al.* Accuracy and the effect of possible subject-based confounders of magnitude-based MRI for estimating hepatic proton density fat fraction in adults, using MR spectroscopy as reference. *J. Magn. Reson. Imaging JMRI.* **43**(2), 398–406 (2016).
38. Reccia, I. *et al.* Non-alcoholic fatty liver disease: A sign of systemic disease. *Metabolism* **72**, 94–108 (2017).
39. Petersen, M. C. *et al.* Insulin receptor Thr1160 phosphorylation mediates lipid-induced hepatic insulin resistance. *J. Clin. Investig.* **126**(11), 4361–4371 (2016).
40. Mikolasevic, I. *et al.* Nonalcoholic fatty liver disease—A multisystem disease?. *World J. Gastroenterol.* **22**(43), 9488–9505 (2016).
41. Masarone, M. *et al.* Role of oxidative stress in pathophysiology of nonalcoholic fatty liver disease. *Oxid. Med. Cell Longev.* **2018**, 9547613 (2018).
42. Tilg, H., Moschen, A. R. & Roden, M. NAFLD and diabetes mellitus. *Nat. Rev. Gastroenterol. Hepatol.* **14**(1), 32–42 (2017).
43. Yazıcı, D. & Sezer, H. Insulin Resistance, Obesity and Lipotoxicity. In *Obesity and Lipotoxicity* (eds Engin, A. B. & Engin, A.) 277–304 (Springer International Publishing, 2017).
44. Meex, R. C. R. & Watt, M. J. Hepatokines: Linking nonalcoholic fatty liver disease and insulin resistance. *Nat. Rev. Endocrinol.* **13**(9), 509–520 (2017).
45. Valenti, L., Bugianesi, E., Pajvani, U. & Targher, G. Nonalcoholic fatty liver disease: Cause or consequence of type 2 diabetes?. *Liver Int.* **36**(11), 1563–1579 (2016).
46. Qu, Y., Li, M., Hamilton, G., Zhang, Y. N. & Song, B. Diagnostic accuracy of hepatic proton density fat fraction measured by magnetic resonance imaging for the evaluation of liver steatosis with histology as reference standard: a meta-analysis. *Eur. Radiol.* **29**(10), 5180–5189 (2019).

47. Liu, Z. *et al.* Causal relationships between NAFLD, T2D and obesity have implications for disease subphenotyping. *J. Hepatol.* **73**(2), 263–276 (2020).
48. Dongiovanni, P. *et al.* Causal relationship of hepatic fat with liver damage and insulin resistance in nonalcoholic fatty liver. *J. Intern. Med.* **283**(4), 356–370 (2018).
49. Sumida, Y., Nakajima, A. & Itoh, Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J. Gastroenterol.* **20**(2), 475–485 (2014).

Author contributions

M.N.: data analysis, manuscript writing. R.B.: design of the study, manuscript drafting. S.S.: manuscript drafting. N.W.: manuscript drafting. M.D.: design of the study, manuscript drafting. M.M.L.: design of the study, manuscript drafting. J-P.K: design of the study, manuscript drafting. W.R.: manuscript drafting. M.N.: design of the study, manuscript drafting. M.R.P.M.: manuscript drafting. T.I.: data analysis, manuscript writing. H.V.: design of the study, manuscript writing.

Funding

Open Access funding enabled and organized by Projekt DEAL. The work is part of the Community Medicine Research net (CMR) of the University of Greifswald, Germany, which is supported by the Federal State of Mecklenburg-West Pomerania. This study is supported by the German Center for Diabetes Research (DZD).

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to M.N.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

7.2 Naeem M, Schipf S, Bülow R, Werner N, Dörr M, Lerch MM, Kühn JP, Rathmann W, Nauck M, Markus MRP, Targher G, Ittermann T, Völzke H

Association between hepatic iron overload assessed by magnetic resonance imaging and glucose intolerance states in the general population.

Nutrition, Metabolism & Cardiovascular Diseases (2022) **32**, 1470–1476

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Association between hepatic iron overload assessed by magnetic resonance imaging and glucose intolerance states in the general population

Muhammad Naeem [a,j,*], Sabine Schipf [a,g], Robin Bülow [d,f], Nicole Werner [a], Marcus Dörr [b,f], Markus M. Lerch [c], Jens-Peter Kühn [h], Wolfgang Rathmann [g,i], Matthias Nauck [e], Marcello Ricardo Paulista Markus [b,f,g], Giovanni Targher [k], Till Ittermann [a,1], Henry Völzke [a,f,g,1]

[a] *Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany*
[b] *Department of Internal Medicine B – Cardiology, Intensive Care, Pulmonary Medicine and Infectious Diseases, University Medicine Greifswald, Greifswald, Germany*
[c] *Department of Gastroenterology, University Medicine Greifswald, Greifswald, Germany*
[d] *Institute of Diagnostic Radiology and Neuroradiology, University Medicine Greifswald, Greifswald, Germany*
[e] *Institute for Laboratory Medicine and Clinical Chemistry, University Medicine Greifswald, Greifswald, Germany*
[f] *German Centre for Cardiovascular Research (DZHK), Partner Site Greifswald, Germany*
[g] *German Center for Diabetes Research (DZD), Partner Site Greifswald, Germany*
[h] *Institute and Policlinic for Diagnostic and Interventional Radiology, University Hospital, Carl Gustav Carus University, TU Dresden, Dresden, Germany*
[i] *Institute for Biometrics and Epidemiology, German Diabetes Center (DDZ), Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany*
[j] *Department of Zoology, University of Malakand, 18800, Pakistan*
[k] *Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, University of Verona, Verona, Italy*

Received 4 June 2021; received in revised form 3 February 2022; accepted 18 February 2022
Handling Editor: L. Ghiadoni
Available online 24 February 2022

KEYWORDS
Hepatic iron overload;
MRI;
Prediabetes

Abstract *Background and aim:* While there is evidence that iron overload disorders are associated with type 2 diabetes, the relationship between hepatic iron overload and prediabetes remains unclear. We aimed to investigate the association between hepatic iron overload, as assessed by magnetic resonance imaging (MRI), and different glucose intolerance states in the population-based Study.

Methods and results: We included data from 1622 individuals with MRI data, who did not have known type 2 diabetes (T2DM). Using an oral glucose tolerance testing, participants were classified as having isolated impaired fasting glucose (i-IFG), isolated impaired glucose tolerance (i-IGT), combined IFG and IGT (IFG + IGT) or previously unknown T2DM. Hepatic iron and fat contents were assessed through quantitative MRI. We undertook linear and multinomial logistic regression models adjusted for potential confounders and MRI-assessed hepatic fat content to examine the association of hepatic iron overload with different glucose intolerance states or continuous markers of glucose metabolism.

MRI-assessed hepatic iron overload was positively associated only with both 2-h plasma glucose ($\beta = 0.32$; 95%CI 0.04–0.60) and the combined IFG + IGT category (relative risk ratio = 1.87; 95%CI 1.15–3.06). No significant associations were found between hepatic iron overload and other glucose intolerance states or biomarkers of glucose metabolism, independently of potential confounders.

* Corresponding author. Institute for Community Medicine, University Medicine Greifswald, Walther Rathenau Str. 48, D-17475 Greifswald, Germany.
E-mail address: muhammad.naeem@stud.uni-greifswald.de (M. Naeem).
[1] Contributed equally to the manuscript.

https://doi.org/10.1016/j.numecd.2022.02.013

Conclusions: MRI-assessed hepatic iron overload was associated with higher 2-h glucose concentrations and the combined IFG + IGT category, but not with other glucose intolerance states. Our findings suggest a weak adverse impact of hepatic iron overload on glucose metabolism, but further studies are needed to confirm these findings.

1. Introduction

Iron is a strong biological pro-oxidant and causes oxidative stress through formation of free radicals which can damage cellular machinery [1]. Iron overload disorders, like hereditary hemochromatosis, are associated with type 2 diabetes mellitus (T2DM), suggesting a possible role of iron overload in T2DM development [2]. Several longitudinal [3–8] and cross-sectional studies [9,10] also reported a significant association between iron overload, as defined by elevated levels of serum ferritin, and T2DM. However, serum ferritin is an acute phase protein and its circulating levels may be elevated not only due to iron overload, but also by underlying inflammation, infection or malignancy, which may adversely affect the endocrine/metabolic system [11].

However, most of the aforementioned longitudinal and cross-sectional studies diagnosed T2DM according to self-report or fasting glucose and glycated hemoglobin (HbA1c) levels [3,4,6–8]. Two studies diagnosed T2DM with oral glucose tolerance test (OGTT) results, but did not diagnose other different glucose intolerance states (impaired fasting glucose (IFG) and impaired glucose tolerance (IGT), as proposed by the American Diabetes Association (ADA) [5,9]. Similarly, most of these studies were conducted in Asian individuals undergoing routine health checkups and, thus, the results may be not representative for the general population from other different countries [3,4].

Regarding the prediabetes status, some previous cross-sectional studies reported an association between iron overload (defined by elevated levels of serum ferritin or transferrin saturation) and presence of prediabetes [9,12–14]. However, most of these studies considered prediabetes by fasting glucose or HbA1c levels, while only one study diagnosed prediabetes status according to OGTT data [9], but again without differentiating groups of individuals with different states of prediabetes [15].

The liver is considered as an important organ for iron storage and regulation, and iron overload of the liver is a frequent condition [16]. To our knowledge there are currently no population-based cohort studies investigating the associations of hepatic iron content with prediabetes and previously undiagnosed T2DM, based on OGTT data.

Therefore, the aim of our cross-sectional study was to evaluate the association between hepatic iron overload and presence of OGTT-defined IFG, IGT (alone or in combination) or previously unknown T2DM in a population-based cohort study of German adults without known T2DM. For the diagnosis of hepatic iron overload, we used the transverse relaxation rate ($R2^*$), as assessed by magnetic resonance imaging (MRI), which is the most accurate technique to non-invasively assess hepatic iron overload [17].

2. Methods

2.1. Population

The Study of Health in Pomerania (SHIP) is a population-based project conducted in Northeast Germany. It consists of the two independent cohorts, the SHIP and SHIP-TREND. For the present analysis, we used data from participants of the baseline SHIP-TREND examination [18]. A stratified random sample of 8826 individuals aged between 20 and 80 years was drawn, of which 4420 subjects participated between 2008 and 2012 (response 50.1%). Random sample selection into the age and sex-strata was facilitated by centralization of local population registries in the German Federal State of Mecklenburg/West Pomerania.

All participants gave written informed consent. The study conformed to the ethical guidelines of the Declaration of Helsinki as reflected in a priori approval by the local ethics committee of the University of Greifswald.

We excluded individuals who had a prior liver disorder (cirrhosis, chronic liver disease or hepatic steatosis; n = 46) or pre-existing T2DM (n = 461). Furthermore, we excluded individuals without MRI examination (n = 2130) and those with missing data in any of the variables of interest (n = 37). The final study population consists of 1746 subjects (913 women) aged between 21 and 80 years. From analysis regarding the prediabetes status, we further excluded individuals, who did not undergo a 75-g OGTT (n = 124), resulting in a study population of 1622 (840 women).

2.2. General measurements

Socio-demographic characteristics and medical histories were assessed by computer assisted face-to-face interviews. Height and weight were measured for the calculation of the body mass index (BMI = weight [kg]/height^2 [m^2]). Alcohol drinking habits were evaluated as beverage-specific alcohol consumption (beer, wine, distilled spirits) on the last weekend and last weekday preceding the examination, and the mean daily alcohol consumption was calculated using beverage specific pure ethanol volume proportions [19].

2.3. Magnetic resonance imaging

MRI examination was performed by using a 1.5-T MR imaging system (Magnetom Avanto, software version VB15; Siemens Healthineers Erlangen, Germany) with a 12-channel phased-array surface coil [20]. Three-dimensional chemical shift encoded gradient-echo data with three echoes and flyback readout gradient were acquired from an axial slab during a single 19-s breath hold. Imaging parameters included repetition time, 11 ms; echo times, 2.4, 4.8, and 9.6 ms; flip angle, 10°; number of signals acquired, one; bandwidth, ± 1065 Hz per pixel; matrix, 224 × 168 × 64; field of view, 410 × 308 mm; parallel imaging effective acceleration factor, 1.8; and section thickness, 3.0 mm. Offline reconstructions of a proton-density fat fraction (PDFF) map (including correction for T1 bias and T2* decay) and a transverse relaxation rate (R2*) map (based on T2* decay measurement of PDFF) were performed [21]. Fat and water ambiguities were resolved by using the phase of the acquired data [21]. Parametric maps of PDFF and R2* were used for further analyses.

One radiologist reviewed PDFF and R2* maps. Mean PDFF and R2* values were determined at operator-defined regions of interest placed at the center of the liver, by using Osirix (v3.8.1; Pixmec Sarl, Bernex, Switzerland). Care was taken when the regions of interest were placed to avoid blood vessels and regions that were obviously contaminated by partial volume effects and motion artifacts [20]. Hepatic steatosis was defined as PDFF ≥5.1%, while liver iron overload was defined as R2* ≥43.9 s^{-1} [21].

2.4. Laboratory data

We requested the individuals not to eat, smoke or consume caffeine containing drinks and to avoid sports for ≥8 h before the examination, which was completed during the morning hours. Blood was collected by trained examiners following a standardized protocol, refrigerated to 4–8 °C and shipped on refrigerant packaging within four to a maximum of six hours to the laboratory. Measurements of fasting and 2-h glucose concentrations were based on plasma samples [22]. All assays were performed according to the manufacturers' recommendations by skilled technical personnel. The study laboratory participated in official quarterly German external proficiency testing programs [23].

Fasting and 2-h glucose levels were measured using a hexokinase method (Dimension Vista, Siemens Healthcare Diagnostics, Eschborn, Germany) [22]. Serum insulin concentrations were measured by commercially available chemiluminescence immunoassay kits (Immulite 2000 Xpi, Siemens Healthcare Diagnostics, Eschborn, Germany). Fasting and 2-h insulin levels were expressed as μU/ml. The homeostasis model assessment-insulin resistance score (HOMA-IR) was calculated using the following equation: fasting insulin [μU/ml] X fasting glucose [mmol/l]/22.5. Serum levels of total cholesterol, HDL (high density lipoprotein)-cholesterol, LDL (low density lipoprotein)–cholesterol, triglycerides and liver enzymes were measured using the Dimension Vista 500 analytical system (Siemens Healthcare Diagnostics, Eschborn, Germany). Serum liver enzyme alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyl transpeptidase (GGT) were expressed as μkatal/l.

2.5. Ascertainment of glucose intolerance states

For OGTT testing, fasting glucose was sampled, and 75 g of anhydrous glucose (Dextro OGT; Boehringer Mannheim, Mannheim, Germany) was orally given to all study participants. Following the ADA diagnostic criteria [15], we classified participants as having normal glucose tolerance if they had fasting plasma glucose values < 5.6 mmol/l (<100 mg/dl) and 2-h glucose values < 7.8 mmol/l (<140 mg/dl). We classified participants as having prediabetes if their fasting glucose values were between 5.6 mmol/l (100 mg/dl) and 6.9 mmol/l (125 mg/dl) (i.e., impaired fasting glucose: IFG) and/or 2-h glucose values were between 7.8 (140 mg/dl) and 11.0 mmol/l (200 mg/dl) (i.e., impaired glucose tolerance: IGT). Accordingly, we defined three different groups of individuals with prediabetes: those with isolated impaired fasting glucose (i-IFG), those isolated impaired glucose tolerance (i-IGT), and those with combined IFG and IGT (IFG + IGT), respectively. Previously unknown diabetes was defined as fasting glucose values ≥ 7.0 mmol/l (≥126 mg/dl) and/or 2-h glucose values ≥ 11.1 mmol/l (≥200 mg/dl) [22].

2.6. Statistical analysis

Continuous data are expressed as medians (with 25th percentile and 75th percentile) and categorical variables as absolute numbers and percentages. Differences between the individuals with and without hepatic iron overload were tested by chi-square test for categorical data and the Kruskal-Wallis test for continuous data. For analyzing the association between MRI-assessed hepatic iron overload and continuous markers of glucose metabolism (fasting and 2-h plasma glucose, insulin levels and HOMA-IR), linear regression analyses were used by calculating β coefficients and 95% confidence intervals (95% CI). For investigating the association between hepatic iron overload and different glucose intolerance states (i-IFG, i-IGT, IFG + IGT or previously unknown diabetes), multinomial logistic regression analyses were used by calculating relative risk ratio and 95% CI. In these models, we also examined the individual and combined effects of hepatic iron overload and increased hepatic fat content on both continuous markers of glucose metabolism and different categories of glucose intolerance. All the aforementioned regression models were adjusted for age, sex, BMI and daily alcohol consumption. A value of $p < 0.05$ was considered to be statistically significant. All statistical analyses were performed by STATA 14.1 software (Stata Corporation, College Station, TX, USA).

3. Results

The study population consisted of 1746 adult individuals (913 women, 52%), aged 21–80 years, who had a prevalence of hepatic iron overload of 13% defined as MRI-assessed R2* ≥43.9 s-1, and a prevalence of hepatic steatosis of 37% defined as MRI-assessed PDFF ≥5.1%, respectively. In total 235 individuals with hepatic iron overload 128 (54%) had also hepatic steatosis. By study design, none of these individuals had previously known diabetes and prior chronic liver diseases.

As shown in Table 1, individuals with hepatic iron overload and hepatic steatosis were more likely to be male, older and to have higher levels of plasma glucose and insulin both at baseline and 2-h after glucose load, as well as higher HOMA-IR score, high dyslipidemia and serum liver enzyme levels compared to those without hepatic iron overload and hepatic steatosis, as assessed by MRI examination. Notably, individuals with hepatic iron overload and hepatic steatosis also had a greater proportion of both prediabetes (i-IFG, and IFG + IGT combined) and previously unknown T2DM compared to those without hepatic iron overload and hepatic steatosis.

As reported in Table 2, multivariable linear regression models adjusted for age, sex, BMI and daily alcohol consumption revealed a significant positive association between hepatic iron overload and 2-h plasma glucose levels. Individuals with hepatic iron overload but without coexisting hepatic steatosis did not have significantly higher levels of fasting glucose and 2-h glucose, insulin levels and HOMA-1R score compared to those without hepatic iron overload and hepatic steatosis. Conversely, individuals with both hepatic steatosis and iron overload had significantly higher levels of fasting glucose and 2-h glucose, insulin levels and HOMA-IR score than individuals without iron overload and hepatic steatosis.

As shown in Table 3, in multinomial regression models adjusted for age, sex, BMI and alcohol consumption, we observed a significant association of hepatic iron overload only with the combined IFG + IGT category. Also in such case, MRI-PDFF assessed hepatic steatosis was significantly associated with all categories of glucose intolerance, regardless of the presence or absence of hepatic iron overload.

To further investigate a potential effect modification by sex on our observed associations, we tested the interaction

Table 1 Characteristics of the study population stratified by different combinations of MRI-assessed hepatic fat and iron groups.

Variables	Numbers of individuals	Fat − Iron −	Fat + Iron −	Fat − Iron +	Fat + Iron +	*P*-value
Age (years)	1746	45 (36; 57)	54 (45; 62)	52 (45; 64)	60 (52; 67)	<0.001
Men (n, %)	833	378 (38%)	270 (53%)	81 (76%)	104 (81%)	0.000
Women (n, %)	913	621 (62%)	242 (47%)	26 (24%)	24 (19%)	
BMI (kg/m^2)	1746	25 (23; 28)	30 (27; 33)	26 (25; 28)	29 (27; 32)	<0.001
Alcohol consumption (g/day)	1732	3.3 (0.8; 8.5)	4.2 (1.0; 12.1)	7.9 (2.6; 14.4)	10.1 (3.2; 21.4)	<0.001
Liver iron content (MRI sec^{-1})	1746	33 (30; 36)	35 (32; 37)	50 (46; 59)	50 (46; 58)	<0.001
Liver fat content (MRI, %)	1746	2.5 (1.9; 3.4)	9.6 (6.7; 15.3)	3.2 (2.4; 4.0)	8.7 (6.5; 14.8)	<0.001
Cholesterol (mmol/l)	1746	5.3 (4.6; 6.1)	5.7 (5.0; 6.4)	5.4 (4.8; 6.1)	5.5 (5.0; 6.3)	<0.001
HDL-cholesterol (mmol/l)	1746	1.5 (1.3; 1.8)	1.3 (1.1; 1.6)	1.4 (1.1; 1.6)	1.3 (1.1; 1.6)	<0.001
LDL-cholesterol (mmol/l)	1746	3.2 (2.6; 3.8)	3.6 (3.1; 4.2)	3.4 (2.9; 4.0)	3.6 (3.0; 4.0)	<0.001
Triglycerides (mmol/l)	1746	1.1 (0.80; 1.4)	1.7 (1.2; 2.3)	1.2 (0.86; 2.0)	1.6 (1.2; 2.1)	<0.001
Lipid lowering medication (C10)						0.019
- No	1600	932 (93%)	460 (90%)	97 (91%)	111 (87%)	
- yes	146	67 (7%)	52 (10%)	10 (9%)	17 (13%)	
ALT (μkatal/l)	1745	0.31 (0.24; 0.43)	0.48 (0.36; 0.66)	0.38 (0.30; 0.48)	0.50 (0.42; 0.75)	<0.001
AST (μkatal/l)	1743	0.28 (0.22; 0.35)	0.32 (0.26; 0.40)	0.30 (0.24; 0.37)	0.34 (0.28; 0.42)	<0.001
GGT (μkatal/l)	1745	0.42 (0.35; 0.55)	0.62 (0.47; 0.90)	0.55 (0.42; 0.70)	0.70 (0.53; 1.0)	<0.001
Glucose (mmol/l)						
- Fasting	1746	5.2 (4.9; 5.5)	5.6 (5.2; 6.1)	5.5 (5.2; 5.9)	5.8 (5.3; 6.3)	<0.001
- 2-h	1622	5.6 (4.7; 6.5)	6.6 (5.6; 7.9)	6.0 (5.3; 7.0)	6.7 (5.7; 8.7)	<0.001
Insulin (μU/ml)						
- Fasting	1617	7.5 (5.5; 10.4)	14.1 (9.9; 20.5)	7.7 (5.7; 11.6)	13.1 (9.0; 18.9)	<0.001
- 2-h	1619	39 (25; 58)	72 (47; 143)	41 (26; 62)	73 (46; 152)	<0.001
HOMA-IR score	1617	1.7 (1.2; 2.4)	3.5 (2.3; 5.5)	1.9 (1.3; 2.9)	3.4 (2.2; 4.9)	<0.001
OGTT results (n, %)						<0.001
- NGT	918	657 (71%)	172 (36%)	53 (53%)	36 (30%)	
- i-IFG	404	176 (19%)	159 (33%)	32 (32%)	37 (31%)	
- i-IGT	87	37 (4%)	41 (9%)	2 (2%)	7 (6%)	
- IFG + IGT	133	39 (4%)	58 (12%)	10 (10%)	26 (21%)	
- Previously unknown diabetes	80	14 (2%)	48 (10%)	3 (3%)	15 (12%)	

Data are given as absolute numbers and percentages for categorical data and as medians (25th percentile and 75th percentile) for continuous data. Hepatic iron overload was defined as MRI-assessed R2* ≥43.9 s-1.

Abbreviations: BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; ALT, alanine aminotransferase; AST; aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; HOMA-IR, homeostasis model assessment-insulin resistance; NGT, normal glucose tolerance; i-IFG, isolated impaired fasting glucose; i-IGT, isolated impaired glucose tolerance. To calculate p-values for the inter-group differences, we used the chi-square test for categorical variables and the Kruskal-Wallis test for continuous variables.

Table 2 Adjusted associations between MRI-assessed hepatic iron overload and continuous markers of glucose metabolism.

Variables	Fasting glucose (mmol/l)	2-h glucose (mmol/l)	Fasting insulin (μU/ml)	2-h insulin (μU/ml)	HOMA-IR score
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Hepatic iron overload (MRI)	0.07 (−0.02; 0.16)	0.32 (0.04; 0.60)*	−0.12 (−1.5; 1.2)	7.1 (−0.80; 15.0)	0.00 (−0.41; 0.41)
Liver iron content on MRI (sec^{-1})	−0.00 (−0.00; 0.00)	0.00 (−0.00; 0.01)	−0.02 (−.05; 0.01)	0.07 (−0.13; 0.26)	−0.01 (−0.02; 0.00)
Hepatic steatosis and iron overload on MRI					
Fat + iron −	0.26 (0.18; 0.33)*	0.73 (0.50; 0.97)*	4.8 (3.7; 6.0)*	36.4 (29.8; 43.0)*	1.4 (1.1; 1.8)*
Fat − iron +	0.09 (−0.04; 0.21)	0.27 (−0.12; 0.66)	−0.59 (−2.4; 1.3)	3.9 (−6.9; 14.7)	−0.15 (−0.73; 0.42)
Fat + iron +	0.27 (0.15; 0.40)*	1.0 (0.63; 1.4)*	4.5 (2.7; 6.4)*	41.9 (31.3; 52.4)*	1.4 (0.83; 1.7)*

* ($p < 0.05$).
β coefficients, derived from multivariable linear regression models, were adjusted for age, sex, BMI and daily alcohol consumption; 95% CI, adjusted 95% confidence intervals. Hepatic iron overload was defined as MRI-assessed R2* ≥43.9 s-1. Hepatic steatosis was defined as MRI-PDFF ≥5.1%.
Abbreviations: BMI, body mass index. HOMA-IR, homeostasis model assessment-insulin resistance.

Table 3 Adjusted associations of hepatic iron overload by MRI with different glucose intolerance states (prediabetes and previously unknown diabetes).

Variables	Prediabetes						Unknown diabetes (n = 80)	
	i-IFG (n = 404)		i-IGT (n = 87)		IFG + IGT (n = 133)			
	RR	95% CI	RR	95% CI	RR	95% CI	RR	95% CI
Hepatic iron overload (MRI)	1.1	(0.76; 1.6)	1.1	(0.49; 2.3)	1.9	(1.2; 3.1)*	1.4	(0.77; 2.7)
Liver iron content on MRI (sec^{-1})	1.0	(0.99; 1.0)	1.0	(0.99; 1.0)	1.0	(0.99; 1.0)	1.0	(0.98; 1.0)
Hepatic steatosis and iron overload on MRI								
Fat + iron −	1.8	(1.3; 2.4)*	3.3	(1.9; 5.7)*	2.5	(1.5; 4.1)*	5.0	(2.6; 9.9)*
Fat − iron +	1.3	(0.81; 2.2)	0.8	(0.18; 3.4)	1.9	(0.87; 4.2)	1.5	(0.41; 5.6)
Fat + iron +	1.5	(0.88; 2.5)	3.2	(1.2; 8.5)*	3.9	(2.0; 7.6)*	5.7	(2.3; 13.6)*

* ($p < 0.05$).
Data derived from multinomial regression models adjusted for age, sex, BMI and daily alcohol consumption. Normal glucose tolerance was the reference category.
Abbreviations: RR, relative risk ratio; 95% confidence interval (CI), adjusted 95% confidence interval; BMI, body mass index; i-IFG, isolated impaired fasting glucose; i-IGT, isolated impaired glucose tolerance.

term of hepatic iron overload with sex on all outcome measures. In none of these analyses we observed any significant interaction ($p > 0.05$ in all cases).

In sensitive analysis, we analyzed the association of hepatic iron with continuous marker of glucose metabolism in subgroups of hepatic iron overload and sex. We found an inverse association between hepatic iron and fasting glucose in individuals with hepatic iron overload. While failed to report any further significant effect of these subgroups on associations between hepatic iron and continuous glucose markers.

4. Discussion

In the present cross-sectional study, we investigated the association between hepatic iron overload, as assessed by MRI, and the presence of different glucose intolerance states (i-IFG, i-IGT, IFG + IGT and previously unknown T2DM) in the German general adult population. We observed that hepatic iron overload, defined as MRI-assessed R2* ≥43.9 s-1, was positively associated only with higher 2-h plasma glucose concentrations and the combined IFG + IGT category. We failed to find any significant association between MRI-assessed hepatic iron overload and other glucose intolerance states or biomarkers of glucose metabolism (HOMA-IR score and fasting or 2-h insulin levels), independently of age, sex, BMI, daily alcohol consumption and hepatic steatosis, defined as MRI-PDFF ≥5.1%.

Previous studies reported a significant association between iron overload, as defined by elevated serum ferritin levels, and the presence of T2DM [3–10], but very few cross-sectional studies do exist that investigated the association between global iron overload and presence of prediabetes states. A prior study performed on an occupational cohort of South Korean individuals showed that iron overload (defined by increased serum ferritin levels) might be a risk factor for IFG and T2DM [13]. Another population-based Korean study reported an association between higher serum ferritin levels and IFG in men, but not in women. Furthermore, that study also reported an association between low transferrin saturation and IFG in both the sexes [14]. Likewise, the KORA (Cooperative Health Research in the Region of Augsburg, South Germany) F4 Study, a large population-based study from Southern Germany, showed that higher serum ferritin and transferrin levels were associated with a higher risk of prediabetes

defined through OGTT [9]. Similarly, data from the National Health and Nutrition Examination Survey (NHANES) showed that higher serum ferritin and low transferrin saturation were associated with a greater risk of prediabetes, as defined by fasting plasma glucose or HbA1c levels, in a population-based sample of US adults [12].

Similarly, a recent study from our group, using serum ferritin, showed positive association between ferritin and different glucose intolerance states (IFG + IGT or previously unknown T2DM) in total population as well as in individuals without hepatic iron overload. This association was more pronounced in presence of hepatic steatosis or hepatic overload defined by MRI, which might suggest the presences of hepatic steatosis or iron overload as a risk factor for different glucose intolerance states [24].

It has been reported that excess iron in the body is responsible for increased production of reactive oxygen species [25]. Further, it is known that pancreatic β cells have less antioxidant enzymes compared to other body organs, making these cells more susceptible to T2DM development in the presence of iron overload [26]. An experimental study in mice suggested that iron overload caused oxidative/nitrative stress in the liver and it might be implicated in T2DM development [27]. Similarly, mice models having mutant gene for heredity hemochromatosis documented an increased hepatic glucose production in these mice, which is characteristic feature of T2DM [28]. Further experimental evidence from animal studies suggested that lowering iron levels by chelation therapy or phlebotomy may improve insulin sensitivity [29].

In the present study, we found a significant association between hepatic iron overload and the combined (IFG + IGT) category only. Similarly, previous studies also found significant associations between some laboratory markers of iron overload and prediabetes [9,12–14]. However, only one study reported an association between serum ferritin levels and different prediabetes categories [30]. In that study, higher ferritin levels were associated with all prediabetes categories in women, but only with the combined IFG + IGT category in men [30]. Similar to our results, a randomized clinical trial also did not show any beneficial effect of iron reduction therapy on insulin sensitivity [31]. It is possible to hypothesize that the weak associations we observed in our study between hepatic iron overload and different prediabetes categories might be due to the population-based study design, which includes few individuals with markedly increased hepatic iron levels. Potentially, an association between hepatic iron overload and prediabetes states might be evident in individuals with very high hepatic iron levels. Similarly, we looked for specific liver iron storage, while previous studies used only laboratory biomarkers of iron overload, which may also, at least in part, explain the differences in results.

In addition, and most importantly, in this population-based study, we confirmed the existence of a strong association between MRI-PDFF assessed hepatic steatosis (mostly due to non-alcoholic fatty liver disease) and risk of having greater insulin resistance (as reflected by higher HOMA-IR and hyperinsulinemia), as well as unknown diabetes or other glucose intolerance states, irrespective of age, sex, daily alcohol consumption, adiposity measures and MRI-assessed hepatic iron overload [32].

A major strength of our study is the large number of individuals derived from the general adult population, who were categorized by different glucose intolerance status using standard OGTT testing, according to ADA criteria. In addition, we assessed hepatic iron overload by MRI, which is a more accurate and sensitive method for measuring iron overload than serum ferritin levels or other laboratory biochemical indexes [33].

The major limitation of our study is its cross-sectional design. Thus, we cannot draw any causal inference about the observed associations. Although we adjusted our results for age, sex, BMI, daily alcohol consumption and MRI-assessed hepatic fat content, we cannot exclude residual confounding. Finally, we do not have any histological confirmation of hepatic iron overload in these participants. However, we believe that it would be unethical to perform liver biopsies in our study participants who had fairly normal serum liver enzymes.

5. Conclusions

In this population-based cohort of German adult individuals, we found that hepatic iron overload, as assessed by MRI, was significantly associated with higher 2-h plasma glucose levels and presence of combined IFG + IGT, but not with other glucose intolerance states or other biomarkers of glucose metabolism. These findings may argue for a weak effect of hepatic iron overload on the development of glucose intolerance in the general population. However, large longitudinal studies are needed to further evaluate these findings.

Funding sources

The work is part of the Community Medicine Research net (CMR) of the University of Greifswald, Germany, which is supported by the Federal State of Mecklenburg-West Pomerania. This study is supported by the German Center for Diabetes Research (DZD).

Declaration of competing interest

The authors have nothing to disclose.

Acknowledgements

SHIP is part of the Community Medicine Research Network of the University Medicine Greifswald, which is supported by the German Federal State of Mecklenburg- West Pomerania.

References

[1] Meynard D, Babitt JL, Lin HY. The liver: conductor of systemic iron balance. Blood 2014;123:168.

[2] Barton JC, Acton RT. Diabetes in HFE hemochromatosis. J Diabetes Res 2017;2017:9826930.
[3] Akter S, Nanri A, Kuwahara K, et al. Circulating ferritin concentrations and risk of type 2 diabetes in Japanese individuals. J Diabetes Investig 2017;8:462–70.
[4] Jung CH, Lee MJ, Hwang JY, et al. Elevated serum ferritin level is associated with the incident type 2 diabetes in healthy Korean men: a 4 Year longitudinal study. PLoS One 2013;8:e75250.
[5] Zhou F, Zhao Z, Tian L, et al. Association of serum ferritin level with risk of incident abnormal glucose metabolism in southwestern China: a prospective cohort study. Biol Trace Elem Res 2016;169:27–33.
[6] Montonen J, Boeing H, Steffen A, et al. Body iron stores and risk of type 2 diabetes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. Diabetologia 2012;55:2613–21.
[7] Jehn ML, Guallar E, Clark JM, et al. A prospective study of plasma ferritin level and incident diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol 2007;165:1047–54.
[8] Podmore C, Meidtner K, Schulze MB, et al. Association of multiple biomarkers of iron metabolism and type 2 diabetes: the EPIC-InterAct study. Diabetes Care 2016;39:572–81.
[9] Huth C, Beuerle S, Zierer A, et al. Biomarkers of iron metabolism are independently associated with impaired glucose metabolism and type 2 diabetes: the KORA F4 study. Eur J Endocrinol 2015;173:643–53.
[10] Yeap BB, Divitini ML, Gunton JE, et al. Higher ferritin levels, but not serum iron or transferrin saturation, are associated with Type 2 diabetes mellitus in adult men and women free of genetic haemochromatosis. Clin Endocrinol (Oxf) 2015;82:525–32.
[11] Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. Serum ferritin: past, present and future. Biochim Biophys Acta 2010;1800:760–9.
[12] Cheung CL, Cheung TT, Lam KS, Cheung BM. High ferritin and low transferrin saturation are associated with pre-diabetes among a national representative sample of U.S. adults. Clin Nutr 2013;32:1055–60.
[13] Kim CH, Kim HK, Bae SJ, Park JY, Lee KU. Association of elevated serum ferritin concentration with insulin resistance and impaired glucose metabolism in Korean men and women. Metabolism 2011;60:414–20.
[14] Park RJ, Moon JD. Low transferrin saturation is associated with impaired fasting glucose and insulin resistance in the South Korean adults: the 2010 Korean National Health and Nutrition Examination Survey. Diabet Med 2015;32:673–8.
[15] 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2018. Diabetes Care 2018;41:S13–27.
[16] Brissot P, Loreal O. Iron metabolism and related genetic diseases: a cleared land, keeping mysteries. J Hepatol 2016;64:505–15.
[17] Wells SA. Quantification of hepatic fat and iron with magnetic resonance imaging. Magn Reson Imag Clin N Am 2014;22:397–416.
[18] Volzke H, Alte D, Schmidt CO, et al. Cohort profile: the study of health in Pomerania. Int J Epidemiol 2011;40:294–307.
[19] Alte D, Lüdemann J, Piek M, et al. Distribution and dose response of laboratory markers to alcohol consumption in a general population: results of the study of health in Pomerania (SHIP). J Stud Alcohol 2003;64:75–82.
[20] Kuhn JP, Meffert P, Heske C, et al. Prevalence of fatty liver disease and hepatic iron overload in a northeastern German population by using quantitative MR imaging. Radiology 2017;284:706–16.
[21] Kühn J-P, Hernando D, Muñoz del Rio A, et al. Effect of multipeak spectral modeling of fat for liver iron and fat quantification: correlation of biopsy with MR imaging results. Radiology 2012;265:133–42.
[22] Tamayo T, Schipf S, Meisinger C, et al. Regional differences of undiagnosed type 2 diabetes and prediabetes prevalence are not explained by known risk factors. PLoS One 2014;9:e113154.
[23] Krebs A, Wallaschofski H, Nauck M, et al. Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20–79. Eur Heart J 2010;31:1494–501.
[24] Pitchika A, Kühn JP, Schipf S, et al. Hepatic steatosis and hepatic iron overload modify the association of iron markers with glucose metabolism disorders and metabolic syndrome. Liver Int 2021;41:1841–52.
[25] Backe MB, Moen IW, Ellervik C, Hansen JB, Mandrup-Poulsen T. Iron regulation of pancreatic beta-cell functions and oxidative stress. Annu Rev Nutr 2016;36:241–73.
[26] Gerber PA, Rutter GA. The role of oxidative stress and hypoxia in pancreatic beta-cell dysfunction in diabetes mellitus. Antioxid Redox Signal 2017;26:501–18.
[27] Li X, Li H, Lu N, et al. Iron increases liver injury through oxidative/nitrative stress in diabetic rats: involvement of nitrotyrosination of glucokinase. Biochimie 2012;94:2620–7.
[28] Huang J, Jones D, Luo B, et al. Iron overload and diabetes risk: a shift from glucose to Fatty Acid oxidation and increased hepatic glucose production in a mouse model of hereditary hemochromatosis. Diabetes 2011;60:80–7.
[29] Hansen JB, Moen IW, Mandrup-Poulsen T. Iron: the hard player in diabetes pathophysiology. Acta Physiol (Oxf) 2014;210:717–32.
[30] Liu Y, Song J, Tian H, et al. Association of serum ferritin concentrations with prevalence of prediabetes, type 2 diabetes mellitus, and metabolic syndrome in a Chinese population from Sichuan. Int J Diabetes Dev Ctries 2015;35:522–8.
[31] Houschyar KS, Lüdtke R, Dobos GJ, et al. Effects of phlebotomy-induced reduction of body iron stores on metabolic syndrome: results from a randomized clinical trial. BMC Med 2012;10:54.
[32] Targher G, Corey KE, Byrne CD, Roden M. The complex link between NAFLD and type 2 diabetes mellitus — mechanisms and treatments. Nat Rev Gastroenterol Hepatol 2021;18:599–612.
[33] İdilman İS, Akata D, Özmen MN, Karçaaltıncaba M. Different forms of iron accumulation in the liver on MRI. Diagn Interventional Radiol 2016;22:22–8.

7.3 Naeem M, Markus MRP, Mousa M, Schipf S, Dörr M, Steveling A, Aghdassi A, Kühn JP, komrey ML, Nauck M, Targher G, Völzke H, Ittermann T

Associations of liver volume and other markers of hepatic steatosis with all-cause mortality in the general population

Received: 3 September 2021 | Revised: 2 December 2021 | Accepted: 3 December 2021

DOI: 10.1111/liv.15133

Liver INTERNATIONAL WILEY

ORIGINAL ARTICLE

Associations of liver volume and other markers of hepatic steatosis with all-cause mortality in the general population

Muhammad Naeem[1,2] | Marcello R. P. Markus[3,4] | Mohammed Mousa[1] | Sabine Schipf[1] | Marcus Dörr[3,4] | Antje Steveling[5] | Ali Aghdassi[5] | Jens-Peter Kühn[6] | Marie-Luise Kromrey[7] | Matthias Nauck[8] | Giovanni Targher[9] | Henry Völzke[1,4,10] | Till Ittermann[1,4]

[1]Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany

[2]Department of Zoology, University of Malakand, Chakdara, Pakistan

[3]Department of Internal Medicine B – Cardiology, Intensive Care, Pulmonary Medicine and Infectious Diseases, University Medicine Greifswald, Greifswald, Germany

[4]DZHK (German Center for Cardiovascular Research), Partner Site Greifswald, Greifswald, Germany

[5]Department of Medicine A, University Medicine Greifswald, Greifswald, Germany

[6]Institute and Policlinic for Diagnostic and Interventional Radiology, University Hospital, Carl Gustav Carus University, Dresden, Germany

[7]Institute of Diagnostic Radiology and Neuroradiology, University Medicine Greifswald, Greifswald, Germany

[8]Institute for Laboratory Medicine and Clinical Chemistry, University Medicine Greifswald, Greifswald, Germany

[9]Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, University of Verona, Verona, Italy

[10]DZD (German Center for Diabetes Research), Site Greifswald, Greifswald, Germany

Correspondence
Muhammad Naeem, Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany.
Email: muhammad.naeem@stud.uni-greifswald.de

Funding information
This research work was funded in part by grants from the German Federal Ministry for Education and Research, from the Ministry for Education, Research and Cultural Affairs, the Ministry for Social Affairs of the State Mecklenburg-West Pomerania.

Handling Editor: Luca Valenti

Abstract

Aims: We examined the associations between liver volume and other quantitative and qualitative markers of hepatic steatosis with all-cause mortality in the general population.

Methods: We included 2769 German middle-aged individuals with a median follow-up of 8.9 years (23,898 person-years). Quantitative markers used were serum liver enzymes and FIB-4 score, while qualitative markers of hepatic steatosis included magnetic resonance imaging (MRI) measurements of liver fat content and total liver volume. Cox proportional hazards models, adjusted for confounding factors, were undertaken to investigate the associations of liver volume and other markers of hepatic steatosis with all-cause mortality.

Results: A larger MRI-assessed liver volume was associated with a nearly three-fold increased risk of all-cause mortality (Hazard Ratio = 3.16; 95% confidence interval

Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ATC, Anatomical Therapeutic Chemical; BMI, Body mass index; CI, Confidence interval; FIB-4 score, Fibrosis-4 score; GGT, γ-glutamyl transpeptidase; HR, Hazard ratio; MRI, Magnetic resonance imaging; NAFLD, Non-alcoholic fatty liver disease; PDFF, Proton density fat fraction; R2*, Transverse relaxation rate; SHIP, Study of Health in Pomerania.

Henry Völzke and Till Ittermann equally contributed to this study.

1.88; 5.30), independent of age, sex, body mass index, food frequency score, alcohol consumption and education level. This association was consistent in all subgroups considered (men vs. women; presence or absence of overweight/obesity, metabolic syndrome or diabetes). Higher serum liver enzyme levels and FIB-4 score were also significantly associated with higher all-cause mortality in the total population and in all subgroups. No independent associations were found between other quantitative and qualitative markers of hepatic steatosis and the risk of all-cause mortality.

Conclusions: We showed for the first time that larger liver volume was associated with a three-fold increase in long-term risk of all-cause mortality. This association remained significant after adjustment for age, sex, alcohol consumption, obesity and other coexisting metabolic disorders.

KEYWORDS
all-cause mortality, hepatic markers, liver volume, MRI

1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent condition with a global prevalence estimated at around 25% in the general adult population.[1] Previous studies demonstrated that NAFLD contributes to the development of type 2 diabetes and may progress to advanced liver fibrosis and cirrhosis.[2,3] NAFLD is a risk factor for all-cause mortality.[1] In line with this, previous data from population-based studies showed that hepatic steatosis, as detected by ultrasonography, blood biomarkers or scores, was associated with increased all-cause mortality after 7–23 years.[4-9]

Beside transabdominal ultrasound, serum liver enzyme levels like alanine aminotransferase (ALT), aspartate aminotransferase (AST) or gamma-glutamyltransferase (GGT), are widely used to diagnose hepatic steatosis in routine clinical practice.[10] Previous prospective studies reported a significant association between mild-to-moderate elevations of serum liver enzymes and higher risk of all-cause mortality.[11-19] For example, a large case-cohort sample from an European population showed that higher serum ALT, AST and GGT levels (even within their normal range) were associated with increased all-cause mortality over a median follow-up of 15.6 years, independently of potentially confounding factors.[13]

However, to our knowledge, there are currently no population-based cohort studies that examined the associations between liver fat content and liver volume, as measured by magnetic resonance imaging (MRI), which is the most accurate technique to non-invasively quantify both hepatic fat infiltration and liver volume,[20] and the risk of all-cause mortality in the general population.

Therefore, the main aim of our prospective study was to assess the associations of a wide range of quantitative and qualitative markers of hepatic steatosis derived from laboratory, ultrasound and MRI examinations with the risk of all-cause mortality in a population-based cohort of German middle-aged individuals.

Key Points

- MRI-assessed liver volume is more sensible and accurate than other markers of hepatic for the associations with all-cause mortality.
- Liver volume was associated with a nearly three-fold increased risk of all-cause mortality in whole population.
- This association was consistent in all sub-groups considered (men vs women; presence or absence of overweight/obesity, metabolic syndrome or diabetes).

2 | MATERIALS AND METHODS

2.1 | Population

The Study of Health in Pomerania (SHIP) is a population-based project conducted in Northeast Germany. It consists of two independent cohorts, the SHIP-START and SHIP-TREND. For the SHIP-START-0, individuals aged between 20 and 79 years were selected from population registers using a two-stage cluster sampling method. From a net sample of 6265 eligible subjects, 4308 (response 68.8%) participated between 1997 and 2001. SHIP-START-2 is the second follow-up of SHIP-START, in which 2,333 individuals were examined between 2008 and 2012.

In parallel to the SHIP-START-2, a second stratified random sample of 8826 adults aged 20-79 years was drawn for SHIP-TREND-0, of which 4420 subjects participated between 2008 and 2012 (response 50.1%). Random sample selection into the age and sex-strata was facilitated by centralization of local population registries in the German Federal State of Mecklenburg/West Pomerania. For the present study, we used data from participants of the SHIP-START-2 and SHIP-TREND-0.

From the 6753 adult individuals participating in the SHIP-START-2 or SHIP-TREND-0, we excluded 3966 who did not have any measurements of hepatic fat content or liver volume. Furthermore, we also excluded 19 individuals with established liver cirrhosis, resulting in a final study population of 2769 individuals.

All participants gave their written informed consent. The study conformed to the ethical guidelines of the Declaration of Helsinki as reflected in an a priori approval by the local Ethics Committee of the University of Greifswald.

2.2 | General measurements

Socio-demographic characteristics and medical histories were assessed by computer-assisted face-to-face interviews. Height and weight were measured for calculating the body mass index (BMI = weight [kg]/height² [m²]). Normal weight was defined as a BMI <25 kg/m^2, overweight as a BMI ≤25 to <30 kg/m^2 and obesity as a BMI ≥30 kg/m^2.

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 subject standing comfortably with weight distributed evenly on both feet. Blood pressure was measured three times on the right arm in a sitting position after at least 5-min at rest, using an oscillometric device (OMRON HEM 705-CP). Alcohol intake was evaluated as beverage-specific alcohol consumption (beer, wine, distilled spirits) on the last weekend and last weekday preceding the examination. The mean daily alcohol consumption was calculated using beverage-specific pure ethanol volume proportions. Food categories were selected from a food-frequency questionnaire and the classifications were summarized to a dietary pattern score for each subject. Smoking habit was assessed by dividing into categories of current, former and never smokers. Education was categorized into three levels, that is low (<10 years), medium (10 years) or high (>10 years), based on the Eastern German three-level school system.[21]

2.3 | Liver ultrasonography

Liver ultrasound examination was performed by examiners using a transportable B-mode ultrasound device (Vivid I; GE-Healthcare, Waukesha, WI, USA) with a 2.5 MHz ultrasonic transducer. The examiners used a 2-point scale to assess the presence of hepatic steatosis: (0) no steatosis, and (1) steatosis. Hepatic steatosis was defined as a hyperechogenic liver pattern in comparison to the renal cortex.[22]

2.4 | Magnetic resonance imaging

Liver MRI examinations were performed by using a 1.5-Tesla MR imaging system (Magnetom Avanto, software version VB15; Siemens Healthineers Erlangen, Germany) with a 12-channel phased-array surface coil.[23] Three-dimensional chemical shift encoded gradient-echo data with three echoes and flyback readout gradient were acquired from an axial slab during a single 19-second breath hold. Imaging parameters included repetition time, 11 ms; echo times, 2.4, 4.8 and 9.6 ms; flip angle, 10˚; number of signals acquired, one; bandwidth, ±1065 Hertz per pixel; matrix, 224 × 168 × 64; field of view, 410 × 308 mm; parallel imaging effective acceleration factor, 1.8 and section thickness, 3.0 mm. Offline reconstructions of a proton-density fat fraction (PDFF) map (including correction for T1 bias and T2* decay) and a transverse relaxation rate (R2*) map (based on T2* decay measurement of PDFF) were performed.[24] Fat and water ambiguities were resolved by using the phase of the acquired data.[24] Parametric maps of PDFF and R2* were used for further analyses.

A single experienced radiologist reviewed PDFF and R2* maps. Mean PDFF and R2* values were determined at operator-defined regions of interest placed at the centre of the liver, by using Osirix (v3.8.1; Pixmec Sarl, Bernex, Switzerland). Care was taken when the regions of interest were placed to avoid blood vessels and regions that were obviously contaminated by partial volume effects and motion artefacts.[23] Hepatic steatosis was defined as MRI-PDFF >5%.[24]

2.5 | Assessment of liver volume

Assessment of liver volume was performed by a calculation of volume indices. For this reason, measurement of liver diameters was performed using the software Osirix (version 4.6; Pixameo, Bernex, Switzerland) by a trained observer. In each participant, MRI-assessed liver diameters were measured in mid-clavicular line in the following three orientations: anterior-posterior; lateral-medial and cranio-caudal. Thereafter, we calculated the volume index and estimated liver volume using the following formula: *liver volume* = $(A \times B \times C) / 2.6$.[25]

2.6 | Ascertainment of diabetes and metabolic syndrome

Participants were classified as having type 2 diabetes if they reported physician's diagnosis of disease in the interview or took any glucose-lowering medications (Anatomical Therapeutic Chemical (ATC) classification system code A10). Metabolic syndrome was defined by the presence of at least three out of the following five metabolic risk abnormalities: (a) waist circumference ≥94 cm in men and ≥80 cm in women; (b) high-density lipoprotein (HDL)-cholesterol <1.03 mmol/l in men and <1.29 mmol/l in women; (c) blood pressure ≥130/85 mmHg or anti-hypertensive treatment (ATC code C02); (d) random plasma glucose ≥8 mmol/l or glucose -lowering medication (ATC code A10) and (e) non-fasting triglycerides ≥2.3 mmol/l or lipid-lowering treatment (ATC code C10AB or C10AD). It was defined according to the modified AHA/NHLBI and IDF criteria based on non-fasting blood values.[26]

2.7 | Laboratory data

For laboratory examinations, non-fasting blood samples were drawn from the cubital vein in the supine position. Serum levels of total cholesterol, HDL-cholesterol, triglycerides, glucose and liver enzymes were measured using the Dimension Vista 500 analytical system (Siemens Healthcare Diagnostics, Eschborn, Germany). Serum ALT, AST and GGT concentrations were expressed as IU/L. The FIB-4 score was calculated using the formula: age (years) × AST [U/L]/ (platelets [10^9/L] × ($ALT^{1/2}$ [U/L]).[27]

2.8 | Mortality follow-up data

Information on vital status for all study participants was requested to local health authorities at the place of death at regular intervals from time of enrolment into the study through March 31, 2019. Subjects were censored as dead or lost to follow-up. The number of years between baseline examination and censoring was used as the follow-up length. The median duration of follow-up was 8.9 years (25th, 18.6; 75th, 20.5). The major causes for death were cancer and cardiovascular events (both 24.2% respectively). In 3.6% the major cause of death was according to liver disease (liver cancer or alcoholic cirrhosis).

2.9 | Statistical analysis

Continuous data are expressed as medians (with 25th percentile and 75th percentile) and categorical variables as absolute and relative percentages. Differences between the subjects alive and dead were tested by Wilcoxon rank-sum tests for continuous data and chi-square tests for categorical data. Cox proportional hazard regression analyses were undertaken to examine the associations between various quantitative and qualitative markers of hepatic steatosis (including also MRI-assessed liver volume and diameters) and risk of all-cause mortality. All these multivariable regression models were adjusted for age, sex, BMI, daily alcohol consumption, food frequency score and education level. For the discriminative ability of exposure markers Harrell's C statistics were calculated separately based on crude Cox regression models. Subgroup analyses were also performed for all-cause mortality in men and women, in patients with and without pre-existing type 2 diabetes, in those with and without metabolic syndrome, as well as in subjects stratified by different BMI categories. A value of $P < .05$ was considered to be statistically significant. All statistical analyses were performed using STATA 14.1 software (Stata Corporation College Station, TX, USA).

3 | RESULTS

During 23,898 person-years of follow-up, a total of 129 individuals (89 men) died, resulting in an overall rate of all-cause mortality of 5.4 cases per 1000 person-years.

At baseline individuals who died during the follow-up period were more likely to be male, older and had higher values of BMI, waist circumference, serum liver enzymes, as well as a higher prevalence of hepatic steatosis (detected by ultrasound or MRI), higher levels of liver fat content and greater total liver volume (as assessed by MRI) compared to individuals still alive (Table 1). Moreover, the former were also more likely to be former smokers, less educated and had a higher proportion of metabolic syndrome, and pre-existing type 2 diabetes than the latter.

Table 2 shows the results of the associations between various quantitative and qualitative markers of hepatic steatosis and risk of all-cause mortality both in the whole cohort and in men and women, separately. These associations were adjusted for age, sex, BMI, food frequency score, daily alcohol intake and education level. Notably, larger liver volume as assessed by MRI was significantly associated with a nearly three-fold increased risk of all-cause mortality in the whole cohort and in both sexes (Figure 1). The FIB-4 score was positively associated with all-cause mortality both in the whole cohort and in women. Similarly, higher serum AST and GGT levels were positively associated with all-cause mortality both in the whole cohort and in men. In contrast, no significant independent associations were observed between other quantitative and qualitative markers of hepatic steatosis (including presence/absence of hepatic steatosis on imaging techniques) and all-cause mortality risk (Table 2). We also adjusted the models additionally for diabetes, which did not change the results significantly.

As shown in Table 3, after stratifying individuals by BMI categories, there were significant positive associations between MRI-assessed total liver volume, FIB-4 score and serum liver enzyme levels (especially serum GGT levels) with the risk of all-cause mortality in subjects with overweight or obesity, whereas no such associations were observed in lean individuals, except for serum ALT levels.

When we stratified our study participants by presence/absence of pre-existing diabetes or metabolic syndrome at baseline, a larger total liver volume (as assessed by MRI) remained among the strongest and independent risk factors of all-cause mortality, regardless of the presence or absence of metabolic syndrome or diabetes (Table 4). FIB-4 score and serum liver enzymes were positively associated with risk of all-cause mortality only in those with metabolic syndrome or pre-existing type 2 diabetes.

For sensitive analysis, we calculated associations of liver volume with mortality in subjects with or without hepatic steatosis. Liver volume was significantly associated with mortality in both groups.

The C-statistics for mortality based on crude Cox regression models were 0.6122 for liver volume, 0.7379 for the FIB4-score, 0.5641 for ALT and 0.5960 for GGT. Thus, it can be seen that the C-statistic is higher for liver volume compared to the liver transaminases. The higher C-statistic for the FIB4-score may be related to the fact that age is included in its formula.

4 | DISCUSSION

In this prospective population-based study, we investigated the associations of MRI-assessed total liver volume and other quantitative

TABLE 1 Baseline characteristics of the study population stratified by all-cause mortality

Variables	Numbers of individuals	Alive, n = 2660	Dead, n = 129	*P* value
Age (years)	2769	52 (42; 62)	68 (60; 74)	<.001
Men (n, %)	1330	1241 (47%)	89 (69%)	<.001
Women (n, %)	1439	1399 (53%)	40 (31%)	
BMI (kg/m^2)	2769	27 (24; 30)	28 (26; 32)	.001
Normal weight (BMI <25 kg/m^2)	836	814 (31%)	22 (17%)	.003
Overweight (25 ≤ BMI <30 kg/m^2)	1156	1096 (41%)	60 (47%)	
Obesity (BMI ≥30 kg/m^2	777	730 (28%)	47 (36%)	
Waist circumference	2765	89 (79.5; 98.5)	97 (87.8; 104.7)	<.001
Metabolic syndrome				<.001
No	2099	2021 (78%)	78 (62%)	
Yes	603	555 (22%)	48 (38%)	
Prior diabetes	203	169 (6%)	33 (26%)	<.001
Alcohol consumption (g/day)	2752	4.2 (1.3; 11)	3.4 (0.7; 9.6)	.240
Hepatic steatosis (Ultrasound)				.005
Negative	1713	1649 (63%)	64 (50%)	
Positive	1041	978 (37%)	63 (50%)	
Hepatic steatosis (MRI-PDFF ≥5.1%)				.001
Negative	1663	1603 (61%)	60 (47%)	
Positive	1106	1037 (39%)	69 (53%)	
Liver volume (MRI, cm^3)	1940	1.4 (1.2; 1.7)	1.5 (1.3; 1.9)	.005
Liver volume calc. from diameters (MRI, cm^3)	2769	1.6 (1.4; 1.8)	1.7 (1.5; 2.1)	<.001
Liver fat content (MRI, %)	2769	4 (2.5; 8.2)	6.3 (3.2; 11.9)	<.001
FIB-4 score	2590	0.8 (0.6; 1.1)	1.3 (0.9; 1.8)	<.001
ALT (IU/L)	2727	23 (17; 32)	23 (18; 28)	.543
AST (IU/L)	2743	17 (14; 22)	19 (15; 25)	.011
GGT (IU/L)	2747	29 (23; 42)	34 (25; 54)	<.001
Food frequency score	2762	14 (12; 17)	14 (12; 16)	.733
Smoking status				.049
Never	1080	1041 (39%)	39 (30%)	
Former	1043	982 (37%)	61 (47%)	
Current	645	616 (23%)	29 (22%)	
Education level				<.001
< 10 years	478	426 (16%)	52 (41%)	
= 10 years	1461	1409 (54%)	52 (41%)	
> 10 years	780	756 (29%)	24 (19%)	

Note: Data are given as absolute numbers and percentages for categorical data and as medians (25th and 75th percentiles) for continuous data. To calculate p value Wilcoxon rank-sum tests were used for continuous variable and chi-square tests for categorical variables.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyltranspeptidase.

TABLE 2 Adjusted associations of qualitative and quantitative markers for hepatic steatosis with risk of all-cause mortality, stratified by sex

	All		Men		Women	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Hepatic steatosis (Ultrasound)	1.15 (0.74; 1.62)	.483	1.10 (0.69; 1.76)	.674	1.31 (0.66; 2.61)	.437
Hepatic steatosis (MRI-PDFF)	0.94 (0.64, 1.39)	.762	0.92 (0.57; 1.47)	.715	1.10 (0.54; 2.25)	.798
Liver fat content (MRI %)	1.01 (0.98; 1.04)	.372	1.01 (0.98; 1.05)	.537	1.02 (0.98; 1.07)	.339
Liver volume (cm^3)	3.16 (1.88; 5.30)	<.001	3.30 (1.69; 6.45)	<.001	3.40 (1.43; 8.09)	.006
Liver volume calc. from diameters (cm^3)	2.78 (1.76; 4.39)	<.001	3.46 (2.01; 5.97)	<.001	2.21 (0.90; 5.43)	.085
FIB-4 score	1.42 (1.12; 1.78)	.003	1.31 (0.98; 1.75)	.068	1.61 (1.13; 2.30)	.013
ALT (IU/L)	1.07 (0.95; 1.20)	.256	1.10 (0.95; 1.28)	.201	1.08 (0.89; 1.32)	.429
AST (IU/L)	1.20 (1.06; 1.37)	.004	1.25 (1.09; 1.45)	.002	1.15 (0.87; 1.52)	.331
GGT (IU/L)	1.02 (1.01; 1.03)	<.001	1.02 (1.01; 1.03)	<.001	1.05 (0.98; 1.13)	.165

Note: Hazard ratios (HR) and 95% confidence intervals (95% CI) were derived from multivariable Cox regression models adjusted for age, sex (included only for the analyses on the whole cohort), BMI, daily alcohol consumption, food frequency score and education level.
ALT, AST and GGT levels were compared as 1/10 units.

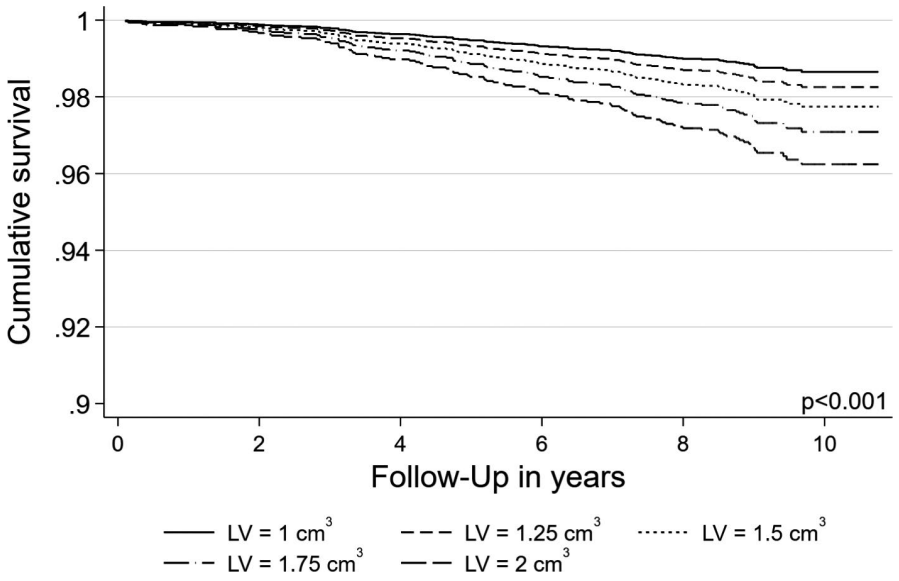

FIGURE 1 Survival curves for specific levels of liver volume based on Cox regression adjusted for confounding. Legend; LV; Liver Volume

and qualitative markers of hepatic steatosis with the long-term risk of all-cause mortality in German middle-aged individuals, over a median period of 8.9 years. We found that a 1 cm^3 increase in liver volume was significantly associated with a three-fold risk of all-cause mortality in the whole population (Figure 1). This association was independent of age, sex, BMI, food frequency score, daily alcohol consumption and education level. Furthermore, this association was consistent in all subgroups considered (men vs women; presence or absence of metabolic syndrome or type 2 diabetes at baseline). Similarly, higher FIB-4 score and higher serum liver enzyme levels (especially serum AST and GGT) were associated with all-cause mortality both in the total population and in different subgroups of individuals. However, we failed to observe any significant and independent associations between other quantitative and qualitative markers of hepatic steatosis (including the evidence of hepatic steatosis on ultrasonography or MRI) and the long-term risk of all-cause mortality in the whole population and in different subgroups of individuals with metabolic disorders.

To the best of our knowledge, this is the first prospective population-based study examining the association between MRI-assessed total liver volume and all-cause mortality risk. Previous studies showed that liver size can be affected by obesity, excessive alcohol consumption and metabolic syndrome,[25,28] which are also known risk factors for all-cause mortality. We could hypothesize that the components of metabolic syndrome may have worsened the metabolic risk profile of individuals, which may also affect liver size, independent of hepatic steatosis.[29] In our statistical analyses, we also adjusted for daily alcohol consumption. However, this variable might be affected by information bias, because it was derived from self-report of participants.

TABLE 3 Adjusted associations of qualitative and quantitative markers for hepatic steatosis with risk of all-cause mortality, stratified by body mass index

	Normal weight (BMI <25 kg/m^2)	*P* value	Overweight (25 ≤ BMI <30 kg/m^2)	*P* value	Obesity (BMI ≥30 kg/m^2)	*P* value
Hepatic steatosis (Ultrasound)	1.28 (0.42; 3.92)	.660	0.97 (0.57; 1.64)	.901	1.55 (0.78; 3.10)	.208
Hepatic steatosis (MRI)	0.99 (0.30; 3.28)	.990	0.92 (0.55; 1.54)	.758	1.02 (0.50; 2.09)	.943
Liver fat content (MRI %)	1.03 (0.90; 1.18)	.641	0.99 (0.94; 1.04)	.603	1.03 (1.00; 1.07)	.061
Liver volume (cm^3)	6.47 (0.41; 101)	.184	4.65 (1.55; 13.9)	.006	3.06 (1.77; 5.63)	.001
Liver volume calc. from diameters (cm^3)	3.44 (0.60; 19.88)	.167	3.11 (1.35; 7.18)	.008	3.38 (1.87; 6.11)	<.001
FIB-4 score	0.84 (0.37; 2.00)	.701	1.30 (0.90; 1.87)	.165	1.73 (1.24; 2.42)	.001
ALT (IU/L)	0.43 (0.19; 0.96)	.039	0.86 (0.66; 1.13)	.287	1.24 (1.11; 1.39)	<.001
AST (IU/L)	0.75 (0.35; 1.62)	.466	1.06 (0.80; 1.40)	.689	1.66 (1.38; 2.00)	<.001
GGT (IU/L)	1.01 (0.89; 1.15)	.868	1.02 (1.00; 1.03)	.003	1.07 (1.04; 1.10)	<.001

Note: Hazard ratios (HR) and 95% confidence intervals (95% CI) were derived from multivariable Cox regression models adjusted for age, sex, daily alcohol consumption, food frequency score and education.

ALT, AST and GGT levels were compared as 1/10 units.

TABLE 4 Associations of qualitative and quantitative markers for hepatic steatosis with risk of all-cause mortality stratified by presence of established type 2 diabetes or metabolic syndrome

	Known type 2 diabetes				Metabolic syndrome			
	Yes		No		Yes		No	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Hepatic steatosis (Ultrasound)	0.98 (0.47; 2.06)	.957	1.10 (0.69; 1.74)	.698	1.12 (0.60; 2.10)	.718	1.18 (0.71; 1.96)	.520
Hepatic steatosis (MRI)	1.27 (0.59; 2.71)	.546	0.78 (0.48; 1.25)	.295	0.80 (0.41; 1.60)	.538	0.91 (0.55; 1.52)	.730
Liver fat content (MRI %)	1.02 (0.98; 1.07)	.316	0.99 (0.95; 1.03)	.625	1.02 (0.98; 1.07)	.231	1.00 (0.95; 1.04)	.866
Liver volume (cm^3)	3.83 (1.68; 8.71)	.001	2.42 (1.13; 5.17)	.028	2.84 (1.42; 5.68)	.003	4.39 (1.49; 12.9)	.007
Liver volume calc. from diameters (cm^3)	3.33 (1.58; 7.02)	.002	2.03 (1.10; 3.73)	.020	3.41 (1.80; 6.47)	.000	2.55 (1.22; 5.34)	.013
FIB-4 score	1.82 (1.33; 2.49)	.000	1.12 (0.78; 1.61)	.513	1.95 (1.47; 2.57)	.000	0.89 (0.57; 1.39)	.643
ALT (IU/L)	1.13 (1.00; 1.28)	.049	0.91 (0.75; 1.12)	.395	1.24 (1.10; 1.39)	.000	0.77 (0.59; 1.01)	.057
AST (IU/L)	1.26 (1.09; 1.47)	.002	1.00 (0.77; 1.31)	.984	1.47 (1.26; 1.70)	.000	0.68 (0.45; 1.00)	.052
GGT (IU/L)	1.02 (1.01; 1.04)	.001	1.03 (0.98; 1.08)	.243	1.02 (1.01; 1.04)	.000	1.01 (0.95; 1.08)	.664

Note: Hazard ratios (HR) and 95% confidence intervals (95% CI) were derived from multivariable Cox regression models adjusted for age, sex, BMI, daily alcohol consumption, food frequency score and education.

ALT, AST and GGT levels were compared as 1/10 units.

Thus, residual confounding by alcohol consumption cannot be definitely excluded.

Our findings are also in line with results from previously published studies showing a positive association between elevated serum liver enzyme levels and risk of all-cause mortality.[13,15-18] In accordance with two previous studies, we found that the association between serum liver enzyme levels and risk of all-cause mortality was mainly observed among individuals who had metabolic syndrome or known type 2 diabetes at baseline.[30,31] Similarly, some prospective studies also reported a significant association between

moderately higher levels of serum ALT and GGT and increased risk of incident type 2 diabetes.[32,33]

The underlying mechanisms for the associations between higher serum ALT and GGT levels and all-cause mortality we observed especially among individuals with metabolic syndrome or those with known type 2 diabetes are not completely understood. A possible mechanism may be that elevated serum liver enzymes (especially high serum GGT levels) act as markers for increased oxidative stress and low-grade inflammation,[34,35] which are two established risk factors for all-cause mortality.[36,37] In addition, serum liver enzyme levels are higher in people with type 2 diabetes[38,39] and are likewise associated with it.[40] Moreover, higher levels of serum liver enzymes are also associated with metabolic syndrome.[41,42]

Our results are similar to previous studies, which reported that FIB-4 score is a risk factor for all-cause mortality in a general population.[43,44] Similar to our results, one previous study also did not show any association between ultrasound-detected hepatic steatosis and all-cause mortality, but found a positive association between the FIB-4 score and all-cause mortality.[45] The only difference is that we calculated FIB-4 score in a population-based sample, whereas the latter study calculated this score only in individuals with hepatic steatosis.

However, most of previous studies identified the FIB-4 score as a risk factor for mortality in individuals with NAFLD.[46-48]

In the present population-based study, we found that larger liver volume, as assessed by MRI, is a better risk marker for all-cause mortality than hepatic fat content. Similarly, individuals with the same liver volume may have the same liver fat content but a different degree of inflammation in the liver that might also result in elevated serum liver enzymes. Furthermore, individuals with a large and small liver volume may have the same amount of liver fat, but obviously the subjects with the larger liver has also more total liver fat. We hypothesize that hepatic inflammation might play a key role explaining the association of liver volume with all-cause mortality.

In line with previous studies, we did not find any association between hepatic steatosis and all-cause mortality.[7,49] Similar to previous studies, that observed significant associations between hepatic steatosis (defined either by liver fat score or by serum liver enzymes) and all-cause mortality,[4-6,8] we also found significant associations between serum liver enzymes and all-cause mortality. However, we furthermore assessed hepatic steatosis by ultrasound and MRI and, thus, we used liver enzymes as independent markers for associations with all-cause mortality. Furthermore, the association between hepatic steatosis and all-cause mortality may only be evident in individuals with severe hepatic steatosis.[50] In our population-based study, most of individuals had only a mild or moderate hepatic steatosis, which may explain the lack of a significant finding. Similarly, in our study population there was only a weak correlation of 0.117 between the FIB-4 score and hepatic steatosis. This suggests that the FIB-4 score is not a good substitute for hepatic steatosis in population-based samples. However, this may be different in cohorts with a large number of individuals having severe hepatic steatosis. Likewise, FIB-4 score is somewhat better discriminating than the liver volume, but the FIB-4 score is a combination of the biomarkers ALT, platelets and age, which may explain the better discrimination.

The most important strengths of our study are its population-based design, the relatively long duration of follow-up (median of 8.9 years) and the use of a wide range of quantitative and qualitative markers of hepatic steatosis (including the MRI-assessment of liver fat content and liver volume). Among the limitations of the study, we should mention that MRI-assessed total liver volume and serum liver enzyme levels were only measured once. Intra-individual variations in serum liver enzyme levels over time have been previously reported,[51] which might have some impacts on our results. Additionally, we do not have any data on vibration-controlled transient elastography nor histological confirmation of the NAFLD severity (ie grading of hepatic steatosis and necro-inflammation, as well as liver fibrosis stage) in these participants.

5 | CONCLUSIONS

In this prospective population-based study of German adults, we found for the first time that larger liver volume was significantly associated with a nearly three-fold increase in the long-term risk of all-cause mortality. This significant association persisted even after adjustment for age, sex, BMI, food frequency score, daily alcohol consumption, education level, as well as the presence or absence of metabolic syndrome or type 2 diabetes. These findings imply that a larger liver volume per se might be particularly harmful, regardless of the coexistence of other metabolic conditions. However, further prospective studies are certainly needed to confirm these findings in independent samples.

ACKNOWLEDGEMENT

The Study of Health in Pomerania is part of the Community Medicine Research net of the University Medicine Greifswald, which is supported by the German Federal State of Mecklenburg-West Pomerania (http://www.community-medicine.de). Open access funding enabled and organized by ProjektDEAL.

CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTION

Muhammad Naeem and Till Ittermann carried out data analysis and manuscript writing. Mohammed Mousa, Sabine Schipf, Marcello Ricardo Paulista Markus and Giovanni Targher involved in manuscript drafting. Markus Doerr, Antje Steveling, Jens-Peter Kühn, Marie-Luise Kromrey and Matthias Nauck involved in design of the study, manuscript drafting. Henry Völzke carried out design of the study and manuscript writing.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Muhammad Naeem https://orcid.org/0000-0001-8455-6599
Giovanni Targher https://orcid.org/0000-0002-4325-3900
Till Ittermann https://orcid.org/0000-0002-0154-7353

REFERENCES

1. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology (Baltimore, MD)*. 2016;64:73-84.
2. Mantovani A, Petracca G, Beatrice G, et al. Non-alcoholic fatty liver disease and risk of incident diabetes mellitus: an updated meta-analysis of 501 022 adult individuals. *Gut*. 2021;70:962.
3. Mantovani A, Scorletti E, Mosca A, et al. Complications, morbidity and mortality of nonalcoholic fatty liver disease. *Metabolism*. 2020;111:154170.
4. Cheung CL, Lam KS, Wong IC, Cheung BM. Non-invasive score identifies ultrasonography-diagnosed non-alcoholic fatty liver disease and predicts mortality in the USA. *BMC Med*. 2014;12:154.
5. Dunn W, Xu R, Wingard DL, et al. Suspected nonalcoholic fatty liver disease and mortality risk in a population-based cohort study. *Am J Gastroenterol*. 2008;103:2263-2271.
6. Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. *J Hepatol*. 2008;49:608-612.
7. Unalp-Arida A, Ruhl CE. Noninvasive fatty liver markers predict liver disease mortality in the U.S. population. *Hepatology (Baltimore, MD)*. 2016;63:1170-1183.
8. Calori G, Lattuada G, Ragogna F, et al. Fatty liver index and mortality: the Cremona study in the 15th year of follow-up. *Hepatology (Baltimore, MD)*. 2011;54:145-152.
9. Haring R, Wallaschofski H, Nauck M, et al. Ultrasonographic hepatic steatosis increases prediction of mortality risk from elevated serum gamma-glutamyl transpeptidase levels. *Hepatology (Baltimore, MD)*. 2009;50:1403-1411.
10. Kwo PY, Cohen SM, Lim JK. ACG Clinical Guideline: evaluation of abnormal liver chemistries. *Am J Gastroenterol*. 2017;112:18-35.
11. Choi KM, Han K, Park S, et al. Implication of liver enzymes on incident cardiovascular diseases and mortality: a nationwide population-based cohort study. *Sci Rep*. 2018;8(1):3764.
12. Karaphillis E, Goldstein R, Murphy S, Qayyum R. Serum alanine aminotransferase levels and all-cause mortality. *Eur J Gastro Hepatol*. 2017;29:284-288.
13. Katzke V, Johnson T, Sookthai D, et al. Circulating liver enzymes and risks of chronic diseases and mortality in the prospective EPIC-Heidelberg case-cohort study. *BMJ Open*. 2020;10:e033532.
14. Kim K-N, Joo J, Sung HK, et al. Associations of serum liver enzyme levels and their changes over time with all-cause and cause-specific mortality in the general population: a large-scale national health screening cohort study. *BMJ Open*. 2019;9:e026965.
15. Koehler E, Sanna D, Hansen B, et al. Serum liver enzymes are associated with all-cause mortality in an elderly population. *Liver Int*. 2014;34(2):296-304
16. Lee H, Shin DW, Lee TH, et al. Association between change in serum aminotransferase and mortality: a nationwide cohort study in Korea. *Medicine (Baltimore)*. 2016;95:e3158.
17. Mahady SE, Wong G, Turner RM, et al. Elevated liver enzymes and mortality in older individuals: a prospective cohort study. *J Clin Gastroenterol*. 2017;51:439-445.
18. Oh C-M, Won Y-J, Cho H, et al. Alanine aminotransferase and gamma-glutamyl transferase have different dose-response relationships with risk of mortality by age. *Liver Int*. 2016;36:126-135.
19. Schmilovitz-Weiss H, Gingold-Belfer R, Boltin D, et al. Risk of mortality and level of serum alanine aminotransferase among community-dwelling elderly in Israel. *Eur J Gastro Hepatol*. 2018;30:1428-1433.
20. Starekova J, Reeder SB. Liver fat quantification: where do we stand? *Abdom Radiol*. 2020;45:3386-3399.
21. Völzke H, Baumeister SE, Alte D, et al. Independent risk factors for gallstone formation in a region with high cholelithiasis prevalence. *Digestion*. 2005;71:97-105.
22. Volzke H, Alte D, Schmidt CO, et al. Cohort profile: the study of health in Pomerania. *Int J Epidemiol*. 2011;40:294-307.
23. Kühn J-P, Meffert P, Heske C, et al. Prevalence of fatty liver disease and hepatic iron overload in a Northeastern German population by using quantitative mr imaging. *Radiology*. 2017;284:706-716.
24. Kühn J-P, Hernando D, Muñoz del Rio A, et al. Effect of multipeak spectral modeling of fat for liver iron and fat quantification: correlation of biopsy with MR imaging results. *Radiology*. 2012;265:133-142.
25. Kromrey ML, Ittermann T, vWahsen C, et al. Reference values of liver volume in Caucasian population and factors influencing liver size. *Eur J Radiol*. 2018;106:32-37.
26. Pitchika A, Schipf S, Nauck M, et al. Associations of iron markers with type 2 diabetes mellitus and metabolic syndrome: results from the prospective SHIP study. *Diabetes Res Clin Pract*. 2020;163:108149.
27. Vallet-Pichard A, Mallet V, Nalpas B, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and fibrotest. *Hepatology (Baltimore, MD)*. 2007;46:32-36.
28. Patzak M, Porzner M, Oeztuerk S, et al. Assessment of liver size by ultrasonography. *J Clin Ultrasound JCU*. 2014;42:399-404.
29. Ju S-Y, Lee J-Y, Kim D-H. Association of metabolic syndrome and its components with all-cause and cardiovascular mortality in the elderly: a meta-analysis of prospective cohort studies. *Medicine (Baltimore)*. 2017;96:e8491.
30. Williams KH, Sullivan DR, Nicholson GC, et al. Opposite associations between alanine aminotransferase and γ-glutamyl transferase levels and all-cause mortality in type 2 diabetes: analysis of the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. *Metab Clin Exp*. 2016;65:783-793.
31. Deetman PE, Alkhalaf A, Landman GWD, et al. Alanine aminotransferase and mortality in patients with type 2 diabetes (ZODIAC-38). *Eur J Clin Invest*. 2015;45:807-814.
32. Li Y, Wang J, Han XU, et al. Serum alanine transaminase levels predict type 2 diabetes risk among a middle-aged and elderly Chinese population. *Ann Hepatol*. 2019;18:298-303.
33. Gao F, Huang X-L, Jiang X-P, et al. Independent effect of alanine transaminase on the incidence of type 2 diabetes mellitus, stratified by age and gender: a secondary analysis based on a large cohort study in China. *Clin Chim Acta*. 2019;495:54-59.
34. Kunutsor SK. Gamma-glutamyltransferase-friend or foe within? *Liver Int*. 2016;36:1723-1734.
35. Contreras-Zentella ML, Hernández-Muñoz R. Is liver enzyme release really associated with cell necrosis induced by oxidant stress? *Oxid Med Cell Longev*. 2016;2016:3529149.
36. Damba T, Bourgonje AR, Abdulle AE, et al. Oxidative stress is associated with suspected non-alcoholic fatty liver disease and all-cause mortality in the general population. *Liver International*. 2020;40:2148-2159.
37. Maluf CB, Barreto SM, Giatti L, et al. Association between C reactive protein and all-cause mortality in the ELSA-Brasil cohort. *J Epidemiol Community Health*. 2020;74:421-427.
38. Mandal A, Bhattarai B, Kafle P, et al. Elevated liver enzymes in patients with type 2 diabetes mellitus and non-alcoholic fatty liver disease. *Cureus*. 2018;10:e3626.

39. Abro MUR, Butt A, Baqa K, et al. Association of serum liver enzyme Alanine Aminotransferase (ALT) in patients with type 2 diabetes. *Pak J Med Sci*. 2018;34:839-843.
40. Jeon CY, Roberts CK, Crespi CM, Zhang ZF. Elevated liver enzymes in individuals with undiagnosed diabetes in the U.S. *J Diabetes Complications*. 2013;27:333-339.
41. Chen Y-F, Lin Y-A, Yeh W-C, et al. The association between metabolic syndrome and elevated alanine aminotransferase levels in an indigenous population in Northern Taiwan: a community-based and cross-sectional study. *Evid-Based Complementary Altern Med*. 2020;2020:6612447.
42. Kondo T, Osugi S, Shimokata K, et al. Metabolic syndrome and all-cause mortality, cardiac events, and cardiovascular events: a follow-up study in 25,471 young- and middle-aged Japanese men. *Eur J Cardiol Prev Rehabil*. 2011;18:574-580.
43. Mao X, Liu Z, Shi O, et al. Non-invasive fibrosis markers are associated with mortality risk in both general populations and non-alcoholic fatty liver disease patients. *Hepatol Res*, 2021;51:90-101.
44. Unalp-Arida A, Ruhl CE. Liver fibrosis scores predict liver disease mortality in the United States population. *Hepatology (Baltimore, MD)*. 2017;66:84-95.
45. Kim D, Kim WR, Kim HJ, Therneau TM. Association between non-invasive fibrosis markers and mortality among adults with nonalcoholic fatty liver disease in the United States. *Hepatology (Baltimore, MD)*. 2013;57:1357-1365.
46. Hagström H, Nasr P, Ekstedt M, et al. Accuracy of noninvasive scoring systems in assessing risk of death and liver-related endpoints in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. 2019;17:1148-1156.e1144.
47. Tada T, Kumada T, Toyoda H, et al. Progression of liver fibrosis is associated with non-liver-related mortality in patients with nonalcoholic fatty liver disease. *Hepatol Commun*. 2017;1:899-910.
48. Xun Y-H, Guo J-C, Lou G-Q, et al. Non-alcoholic fatty liver disease (NAFLD) fibrosis score predicts 6.6-year overall mortality of Chinese patients with NAFLD. *Clin Exp Pharmacol Physiol*. 2014;41:643-649.
49. Lazo M, Hernaez R, Bonekamp S, et al. Non-alcoholic fatty liver disease and mortality among US adults: prospective cohort study. *BMJ*. 2011;343: d6891.
50. Wattacheril J, Chalasani N. Nonalcoholic fatty liver disease (NAFLD): is it really a serious condition? *Hepatology (Baltimore, MD)*. 2012;56:1580-1584.
51. Lazo M, Selvin E, Clark JM. Clinical implications of short-term variability in liver function test results. *Ann Intern Med*. 2008;148:348-352.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Naeem M, Markus MRP, Mousa M, et al. Associations of liver volume and other markers of hepatic steatosis with all-cause mortality in the general population. *Liver Int*. 2021;00:1–10. doi:10.1111/liv.15133

8. Appendix

8.1 Eidesstattliche Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbstständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet habe.

Die Dissertation ist bisher keiner anderen Fakultät vorgelegt worden

Ich erkläre, dass ich kein Promotionsverfahren erfolglos beendet habe und dass eine Anerkennung eines bereits erworbenen Doktorgrades nicht vorliegt

Greifswald, Date: Muhammad Naeem

8.2 Own Contributions

Explanation of Individual Shares in Joint Work

Data were collected from The Study of Health in Pomerania (SHIP) is part of the Community Medicine Research net of the University Medicine Greifswald, which is supported by the German Federal State of Mecklenburg-West Pomerania (http://www.commu nity-medicine.de).

The author performed the literature search, conceptual design of data analysis, explored the theoretical framework, performed the statistical analysis and drafted all the research articles and the thesis to their final version.

Muhammad Naeem Prof. Dr. Henry Völzke

The above information is confirmed:

Prof. Dr. Henry Völzke

Greifswald, Date:

8. 3 Curriculum Vitae

Education

2011-2014	MPhil. Zoology; Department of Zoology, Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan
2008-2010	MSc. Zoology; Department of Zoology, Kohat University of Science and Technology, Kohat, Khyber Pakhtunkhwa, Pakistan
2005-2007	BSc. Biology; Government College Dargai, Malakand University of Malakand, Khyber Pakhtunkhwa, Pakistan.

Professional Occupation

2014-2018	Biology Teacher (permanent); Govt High school Inzargai, Malakand, Khyber Pakhtunkhwa, Pakistan
2010-2011	Lecturer in Zoology (Contract); Government College Dargai, Malakand, Khyber Pakhtunkhwa, Pakistan

List of own scientific publications

Naeem M, Schipf S, Bülow R, *et al.* Association Between Hepatic Iron Overload Assessed by Magnetic Resonance Imaging and Glucose Intolerance States in the General Population. Nutrition, Metabolism and Cardiovascular Diseases, (2022)

Naeem M, Markus MRP, Mousa M, *et al*. Associations of liver volume and other markers of hepatic steatosis with all-cause mortality in the general population. Liver Int. 42(3):575-584 (2022)

Naeem M, Bülow R, Schipf S, *et al*. Association of hepatic steatosis derived from ultrasound and quantitative MRI with prediabetes in the general population. *Sci Rep* 11, 13276 (2021)

Naeem M, Khattak R M, Rehman M. and Khattak, M N K. The role of glycated hemoglobin (HbA1c) and serum lipid profile measurements to detect cardiovascular diseases in type 2 diabetic patients. *South East Asia Journal of Public Health*, *5*(2), 30–34 (2016).

List of presentations

Association of Hepatic Steatosis Derived from Ultrasound and Quantitative MRI with Prediabetes in the General Population [04– 06/11/2019]

Oral presentation at First Annual Academy at MPIDR, Rostock.

Association of liver dysfunction with prediabetes and all-cause mortality in a general population [11–12/06/2020]

Oral presentation at Graduate Workshop online at MPIDR, Rostock.

Association of quantitative and qualitative hepatic markers with all-cause mortality in subgroups of a population-based study [07-09/2020].

Oral presentation at Second Annual Academy at MPIDR, Rostock.

Associations of liver volume and other markers of hepatic steatosiswith all-cause mortality in the general population [10–11/06/2021]

Poster presentation at Graduate Workshop online at MPIDR, Rostock.

Associations of Liver Dysfunction with Glucose Intolerance and All-cause Mortality in a General Population [01-03/12/2021]

Poster presentation at Annual Academy online at MPIDR, Rostock.

Lower cardiorespiratory fitness and handgrip strength is associated with lower liver volume in a general population. [09–10/06/2022]

Poster presentation at Graduate Workshop online at MPIDR, Rostock.

8.4 Acknowledgements

Above all, I express my gratitude to almighty Allah for giving me the strength, energy and patience to carry out this research work. I would like to thank everyone who made this thesis possible. My heartiest thanks to Prof. Dr Henry Völzke for providing me with the opportunity to work under his supervision, and further increasing my research-related capabilities. I am obliged to him for providing intellectual support to the SHIP team and in the international scientific community.

My special thanks to PD Dr Till Ittemann for his assistance throughout my stay at Institute for Community Medicine. He has always been a beacon of light throughout the research I conducted at the Institute for Community Medicine, University Medicine Greifswald, Germany.

I would also thank my all colleagues in the SHIP team for their friendly behavior and for creating a pleasant environment, which makes my stay comfortable.

I extend my gratitude to my family, particularly my late father (Said Amin Shah), my mother, grandmother, wife, uncle Abdul Zamin Shah, kids (Anayiza Naeem, Irtaza Khan) and my brothers and sisters. Especially, I will remember my aunt who passed away in August 2021. She was always supportive of me all the way throughout my life. May Allah bless her Jannah. Ameen.

I would also like to thank all my friends, especially Muhammad Mousa, Dr Faruq Hussain, Husnain Alvi, Saiful Hassan Khan and Safiullah Achakzai for the wonderful times during my journey as a doctoral student.

I would particularly like to thank the Higher Education Commission (HEC) of Pakistan for providing funding for my stay in Germany for the completion of my doctoral degree. I would also like to thank the University of Malakand (Chartered University) and all our funding partners for the different projects.