

WestminsterResearch

<http://www.westminster.ac.uk/westminsterresearch>

**Measurement of liver iron by magnetic resonance imaging in the
UK Biobank population**

**McKay, A., Wilman, H., Dennis, A., Kelly, M., Gyngell, M.L.,
Neubauer, S., Bell, J.D., Banerjee, R. and Thomas, E.L.**

Copyright © 2018 Henry Wilman et al. This is an open access article distributed under the Creative Commons Attribution License (CC-BY) (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

It is available from the publisher at:

<https://dx.doi.org/10.1371/journal.pone.0209340>

The WestminsterResearch online digital archive at the University of Westminster aims to make the research output of the University available to a wider audience. Copyright and Moral Rights remain with the authors and/or copyright owners.

Whilst further distribution of specific materials from within this archive is forbidden, you may freely distribute the URL of WestminsterResearch: (<http://westminsterresearch.wmin.ac.uk/>).

In case of abuse or copyright appearing without permission e-mail repository@westminster.ac.uk

RESEARCH ARTICLE

Measurement of liver iron by magnetic resonance imaging in the UK Biobank population

Andy McKay^{1*}, Henry R. Wilman^{1,2*}, Andrea Dennis¹, Matt Kelly¹, Michael L. Gyngell¹, Stefan Neubauer^{1,3}, Jimmy D. Bell², Rajarshi Banerjee¹, E. Louise Thomas^{1D,2*}

1 Perspectum Diagnostics, Oxford, United Kingdom, **2** Research Centre for Optimal Health, School of Life Sciences, University of Westminster, London, United Kingdom, **3** Oxford Centre for Clinical Magnetic Resonance Research (OCMR), Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, United Kingdom

* These authors contributed equally to this work.

* L.Thomas3@westminster.ac.uk



OPEN ACCESS

Citation: McKay A, Wilman HR, Dennis A, Kelly M, Gyngell ML, Neubauer S, et al. (2018)

Measurement of liver iron by magnetic resonance imaging in the UK Biobank population. PLoS ONE 13(12): e0209340. <https://doi.org/10.1371/journal.pone.0209340>

Editor: David Meyre, McMaster University, CANADA

Received: September 26, 2018

Accepted: December 4, 2018

Published: December 21, 2018

Copyright: © 2018 McKay et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: The data in this study are owned by a third party, the UK Biobank (www.ukbiobank.ac.uk), and legal constraints do not permit public sharing of the data. The UK Biobank, however, is open to all bona fide researchers anywhere in the world. Thus, the data used in this study can be easily and directly accessed by applying for access through the UK Biobank Access Management System (www.ukbiobank.ac.uk/register-apply). After obtaining an account, a researcher can list which data fields they would like to use. The codes to access the minimal data set

Abstract

The burden of liver disease continues to increase in the UK, with liver cirrhosis reported to be the third most common cause of premature death. Iron overload, a condition that impacts liver health, was traditionally associated with genetic disorders such as hereditary haemochromatosis, however, it is now increasingly associated with obesity, type-2 diabetes and non-alcoholic fatty liver disease. The aim of this study was to assess the prevalence of elevated levels of liver iron within the UK Biobank imaging study in a cohort of 9108 individuals. Magnetic resonance imaging (MRI) was undertaken at the UK Biobank imaging centre, acquiring a multi-echo spoiled gradient-echo single-breath-hold MRI sequence from the liver. All images were analysed for liver iron and fat (expressed as proton density fat fraction or PDFF) content using LiverMultiScan. Liver iron was measured in 97.3% of the cohort. The mean liver iron content was 1.32 ± 0.32 mg/g while the median was 1.25 mg/g (min: 0.85 max: 6.44 mg/g). Overall 4.82% of the population were defined as having elevated liver iron, above commonly accepted 1.8 mg/g threshold based on biochemical iron measurements in liver specimens obtained by biopsy. Further analysis using univariate models showed elevated liver iron to be related to male sex ($p < 10^{-16}$, $r^2 = 0.008$), increasing age ($p < 10^{-16}$, $r^2 = 0.013$), and red meat intake ($p < 10^{-16}$, $r^2 = 0.008$). Elevated liver fat (>5.6% PDFF) was associated with a slight increase in prevalence of elevated liver iron (4.4% vs 6.3%, $p = 0.0007$). This study shows that population studies including measurement of liver iron concentration are feasible, which may in future be used to better inform patient stratification and treatment.

Introduction

The burden of chronic liver disease and its impact on morbidity in terms of liver failure, fibrosis and end-stage cirrhosis and, ultimately, mortality is increasing, reportedly at much higher

used in this paper are as follows: 20203 - Liver Dicoms; 1369 - Beef intake, 31 - Sex; 21001 - BMI; 21000 - Ethnic background; 21003 - Age when attended assessment centre. The authors confirm that these data will allow interested readers to replicate the study exactly. Additionally, the secondary analysis performed in this study will soon be made accessible by the UKBiobank.

Funding: Support was provided by Innovate UK 010271 [<https://www.gov.uk/government/organisations/innovate-uk>]. Perspectum Diagnostics provided support in the form of salaries for authors AM, AD, MK and RB, but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. SN acknowledges support from the Oxford NIHR Biomedical Research Centre.

Competing interests: The authors of this manuscript have the following competing interests: Andy McKay, Andrea Dennis, Matt Kelly, Michael Gyngell and Rajarshi Banerjee are employees of a commercial funder: Perspectum Diagnostics. Andy McKay, Andrea Dennis, Matt Kelly, Michael Gyngell, Stefan Neubauer, Rajarshi Banerjee, Henry Wilman are also stockholders of Perspectum Diagnostics. Stefan Neubauer has a paid consultancy with Perspectum Diagnostics, and also holds the position of non-executive director for the company. Jimmy Bell and E. Louise Thomas hold a Knowledge Transfer Partnership award from Innovate UK in collaboration with Perspectum Diagnostics, which funds Henry Wilman's employment at the University of Westminster. Jimmy Bell and E. Louise Thomas have also received funds for a contract research project from Perspectum Diagnostics to deliver image analysis for a project undertaken by Perspectum Diagnostics. Employment and consultancy of these authors does not alter our adherence to PLOS ONE policies on sharing data and materials.

rates in the UK than other western European countries [1,2]. Whilst the aetiology of liver disease varies, common causes include viruses (e.g. Hepatitis C), autoimmune conditions, as well as lifestyle related factors including obesity and alcohol. The consequence of an increasingly obese population has a significant impact both on the prevalence and severity of non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), as well as the progression of both Hepatitis C and alcohol related liver diseases [3,4].

Iron overload is traditionally associated with genetic disorders such as hereditary haemochromatosis (HH) or as a result of repeated blood transfusions. However elevated levels of liver iron are now often associated with metabolic diseases including insulin resistance, type-2 diabetes and NAFLD [5–7]. This has been referred to as insulin resistance-associated hepatic iron overload [8,9] or as it is now more commonly known dysmetabolic iron overload syndrome (DIOS). Unlike HH where liver iron concentrations are severely raised, with DIOS mild increases in iron stores are more common [6]. It has been suggested that one third of patients with NAFLD have DIOS [6], with others reporting even greater prevalence [7].

Irrespective of its causes, identifying individuals storing excess liver iron and assessing its prevalence within the population is paramount since elevated liver iron is associated with the development of fibrosis and cirrhosis, and thought to be involved in the progression from fibrosis to hepatocellular carcinoma [10]. There is however ongoing debate as to whether this progression is driven by the mild to moderate levels of liver iron typically observed in subjects with NAFLD or the high levels of serum ferritin that are often observed [11]. To fully understand the health implications of elevated levels of liver iron content, an assessment of its prevalence within the population is necessary as well as developing population appropriate methods for its measurement which will allow early identification and monitoring of response to interventions, prior to the onset of organ failure.

Liver biopsy is the gold standard for assessment of liver iron, with a normal levels of liver iron reported as <1.8 mg/g dry weight [12,13]. However, liver biopsy is not clinically recommended except in cases where advanced fibrosis is suspected [14,15]. The need to regularly measure liver iron content commonly relies on indirect blood-based biomarkers such as serum ferritin. However, ferritin levels can be influenced by liver damage and inflammation, and in many cases do not correlate well with liver iron stores [16]. This has necessitated the development of robust non-invasive techniques for the measurement of liver iron based on magnetic resonance imaging (MRI) [17,18].

MRI measurements of liver iron are based on the principle that the rate of MR signal decay is influenced by the levels of iron in the tissue; more iron present results in a faster signal decay. Multiple calibration studies have been published, comparing measurements of liver iron by MRI and tissue biopsy, establishing MRI as a suitable method for the assessment of liver iron in both healthy and iron-overload subjects [17,19–24]. Despite numerous studies utilising MRI to measure liver iron, these have generally been conducted in relatively small and well defined patient populations. Few studies have characterised the distribution of liver iron within the general population. In the present study, we describe the measurement of liver iron in a population >9,000 individuals within the UK Biobank Cohort, and assess its distribution in relation to age, sex, body habitus and liver fat content.

Patients and methods

Study design

The UK Biobank (UKB) imaging study is a large prospective study of people aged between 40–69 years (at the time of initial recruitment in 2006–2010), with a planned 100,000 subjects to be recruited from the wider 500,000-strong general UK Biobank Cohort. This study assesses

the first 9108 subjects from the UKB imaging enhancement protocol, acquired between 2014 and 2016, with patient meta-data obtained through UK Biobank Access Application number 9914. The UK Biobank has approval from the North West Multi-Centre Research Ethics Committee (MREC), and obtained written informed consent from all participants prior to the study.

We performed a cross-sectional study in UK Biobank (52.4% female, mean age 61.4 [44–73] years, 96.7% white), to determine how liver iron varies according to sex, age, body mass index (BMI) and liver fat.

Imaging protocol

Participants were scanned at the UK Biobank imaging centre in Cheadle (UK) using a Siemens 1.5T Magnetom Aera. A multi-echo spoiled gradient-echo single-breath-hold MRI sequence was acquired as a single transverse slice captured through the centre of the liver superior to the porta hepatis. This sequence is part of the LiverMultiScan protocol from Perspectum Diagnostics (UK) which forms part of the UK Biobank abdominal imaging protocol [25,26].

Image analysis

The data was analysed using the LiverMultiScan Discover software by a team of trained analysts, blinded to any subject variables. Analysts selected three 15mm diameter circular regions of interest (ROIs), to cover a representative sample of the liver parenchyma, avoiding vessels, bile ducts and other organs. Mean T2*, liver iron, and PDFF were calculated. The repeatability and reproducibility of the image analysis is described in the [S1 Appendix](#).

Presentation of liver iron

There is little consensus within the literature regarding the best protocol for the measurement of liver iron by MRI. Liver iron measures obtained by MRI are sometimes reported as T2* and/or R2* or converted to iron in mg/g, utilising a variety of formulas ([S1 Table](#)) based on regression analysis in studies where both MRI and biopsy data were available [17,19–23,27]. The impact on the final results arising from the use of these formulas can be seen in [S2 Table](#). In the present study values are presented as mg/g as described by Wood et al [19], with a cut-off of <1.8 mg/g dry weight for healthy liver iron [12,13], which equates to a R2* cut-off of 62.7 sec⁻¹. Wherever appropriate, T2* and R2* values are also shown ([S2 Table](#)).

Statistical methodology

Summary data are presented as means, medians and quartiles. Assessment of difference of prevalence of elevated liver iron levels by stratified groups was completed using chi-squared tests. Subjects were stratified into age (40–49, 50–59, 60–69 and 70–79 years) and BMI groups (<20 kg/m², 20–24.9 kg/m² (normal), 25–29.9 kg/m² (overweight), 30–34.9 kg/m² (obese), and >35 kg/m² (very obese)). Repeatability and reproducibility were assessed with intra-class correlation (ICC) [28] and Bland-Altman analyses [29] (presented in [S1 Appendix](#)). Linear models were fitted using R. Liver fat values were log transformed for the purposes of the models, since this transformation resulted in an improved fit, with more normal residuals. Categorical variables were modelled as fixed effects. Iron values were capped at 3mg/g in plots but left untransformed for all other analysis. For beef intake, the results were recoded to give groups with sufficient numbers. The "2–4 times a week", "5–6 times a week", and "Once or more daily" labels were combined into a single level of "More than once a week". The "Do not know" and "Prefer not to answer" levels were set to missing values. The remaining three levels, "Never",

"Less than once a week", and "Once a week", were left unchanged. Statistical analyses were undertaken using Python 3.6 (Python Software) and R 3.4.4 (R Core Team) [30, 31].

Results

Liver iron was measured in 8865 (97.33%) of the 9108 MRI datasets available. Of the 243 (2.67%) cases not deemed of sufficient quality for analysis; 170 had artefacts, in 41 cases the slice was incorrectly positioned and did not include usable liver, 15 could not be linked to their metafiles and 10 were rejected during quality control processing due to missing or corrupted files. A further 7 were rejected as their T2* values were below the limits of detection.

The demographics of the population included in the final analysis are presented in [Table 1](#).

The mean and median liver iron for this cohort was 1.32 ± 0.32 mg/g and 1.25 mg/g (min: 0.85 max: 6.44 mg/g) respectively ([Table 2](#)). The distribution of liver iron within the population is presented in [Fig 1](#) (and also shown as a T2* distribution in [S1 Fig](#)). Overall 4.82% of the population were defined as having elevated liver iron, above the commonly accepted 1.8mg/g threshold [12, 13].

For a 'reference' population, defined as those individuals with $\text{BMI} < 25 \text{ kg/m}^2$, and $\text{PDFF} < 5\%$, the 95% range was 0.98–2.06 mg/g dry weight (this corresponds to a T2* range of 32.2–13.6 ms, and a R2* range of 31.02–74.87 s⁻¹). This population had a very similar average age (61.37 years) to the whole cohort (61.10 years).

Table 1. General population statistics.

	Subjects (n)	Subjects (%)
Total population	8865	
Sex		
Male	4219	47.6
Female	4646	52.4
Age (years)		
40–49 years	551	6.2
50–59 years	2651	29.9
60–69 years	4161	46.9
70–79 years	1093	12.3
Age not available	409	4.6
BMI (kg/m²)		
<20 kg/m ²	294	3.3
20–24.9 kg/m ²	3189	36.0
25–29.9 kg/m ²	3725	42.0
30–34.9 kg/m ²	1222	13.8
>35 kg/m ²	405	4.63
BMI not available	30	0.3
Ethnicity		
White	8512	96.0
Mixed	47	0.5
Asian	107	1.2
Black	51	0.6
Chinese	29	0.3
Other ethnicity	37	0.4
Ethnicity not available or subject preferred not to answer	82	0.9

<https://doi.org/10.1371/journal.pone.0209340.t001>

Table 2. Summary statistics for liver iron.

	liver iron mg/g (Wood [19])
Mean	1.32
St. dev	0.32
5 th Percentile	1.02
25 th Percentile	1.14
Median	1.25
75 th Percentile	1.40
95 th percentile	1.78

All data expressed as mg/g dry weight

<https://doi.org/10.1371/journal.pone.0209340.t002>

Relationship with proton density fat fraction (PDFF)

Subjects were further stratified according to their liver fat content (PDFF), derived from MRI. Overall 20.6% (1826) of participants had elevated PDFF ($>5.6\%$ [32]). There was a weak but significant relationship between liver iron and log(PDFF) ($r^2 = 0.0496$, $p < 10^{-16}$, Spearman's $r^2 = 0.0279$ Pearson's, $p < 10^{-16}$), as shown in Fig 2. The estimated regression coefficient was 0.0595 mg/g/log(%), indicating that a doubling of liver fat corresponds, on average to an increase of 0.0412 mg/g liver iron. This means that an individual with a PDFF of 1% and another with a PDFF of 20% (roughly the extremes of the data) would have an average difference in liver iron content of 0.18mg/g.

Age and sex

Age was positively correlated with liver iron ($p < 10^{-16}$, $r^2 = 0.013$), increasing 0.00512mg/g/year (Fig 3). Males had, on average, 0.0586 mg/g more liver iron than females ($p < 10^{-16}$, $r^2 = 0.008$). Prevalence of elevated liver iron content was significantly more common in male compared with female subjects (6.38% vs 3.4%, $p < 0.0001$).

Relationship with body mass index

The impact of BMI on liver iron was also investigated; in this cohort 39.3% of the population had a normal BMI, 42.0% were overweight and 18.4% were categorised as obese. There was a small yet significant correlation between BMI and liver iron ($r = 0.05$, $P < 0.0001$). The variation of liver iron against BMI is shown in Fig 4.

Ethnicity

While the cohort was predominantly Caucasian, ($n = 8512$) there were some Asian ($n = 107$), Black ($n = 51$) and mixed-race individuals represented to enable preliminary analysis. This showed a statistically significant difference ($p < 0.05$) in liver iron between Caucasian (median 1.32 mg/g, range 1.14–1.41 mg/g) and Asian (median 1.26 mg/g, range 1.09–1.33 mg/g) subjects, with no significant differences between the other ethnic groups.

Diet

There were significant differences in liver iron in relation to self-reported frequency of beef intake. A fixed-effects model relating liver iron to this variable (recoded into four groups), with the “Never” group as the baseline. The three other groups, “less than once a week”, “once a week”, and “more than once a week”, had significantly more iron ($p < 10^{-16}$ in all cases), with 0.080, 0.108 and 0.122 mg/g higher liver iron respectively.

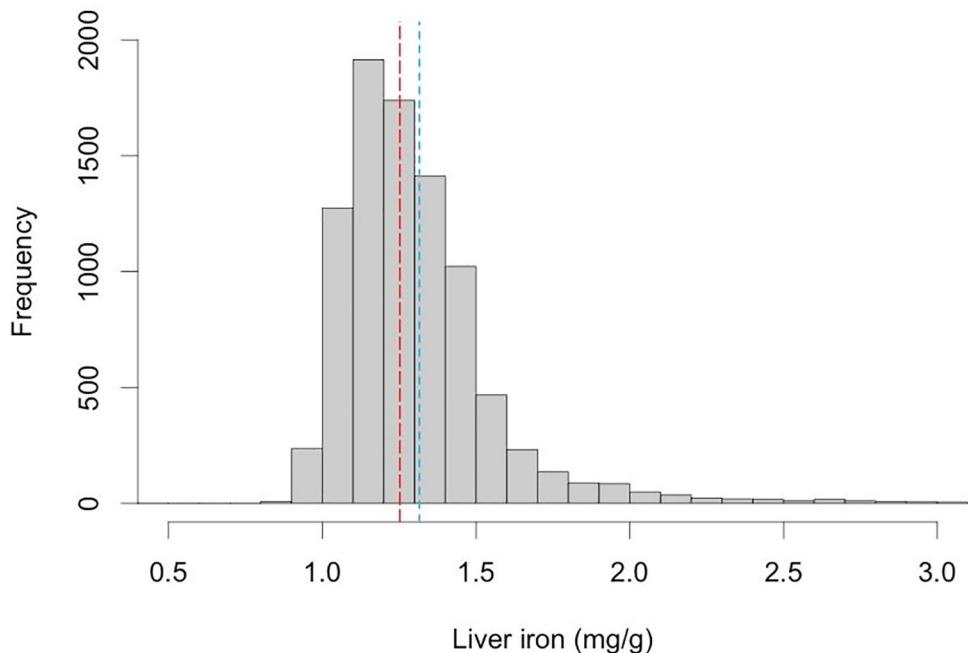


Fig 1. Distribution of liver iron concentration (liver iron) within the UK Biobank population. Median value (1.25 mg/g) shown in red, mean (1.32 mg/g) in blue. There were 52 individuals with liver iron > 3mg/g, who are not shown in this plot.

<https://doi.org/10.1371/journal.pone.0209340.g001>

Multivariate analysis

Age, sex, BMI, liver fat, and beef intake were used to create a combined model ([S3 Table](#)). Interaction terms were included in the initial model fit, and then backward selection was used

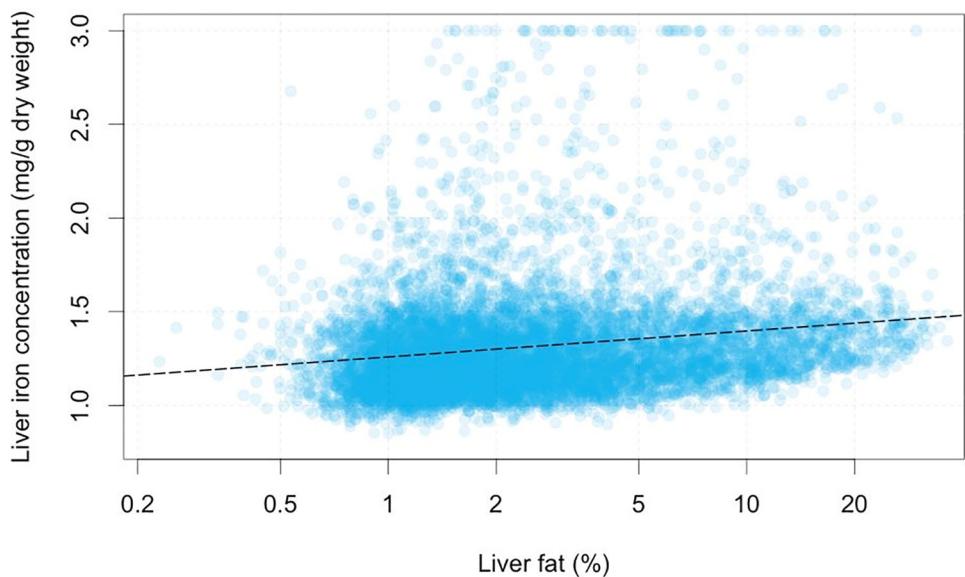


Fig 2. Correlation of liver iron concentration and liver fat (MRI proton density fat fraction) in the UK Biobank population.

<https://doi.org/10.1371/journal.pone.0209340.g002>

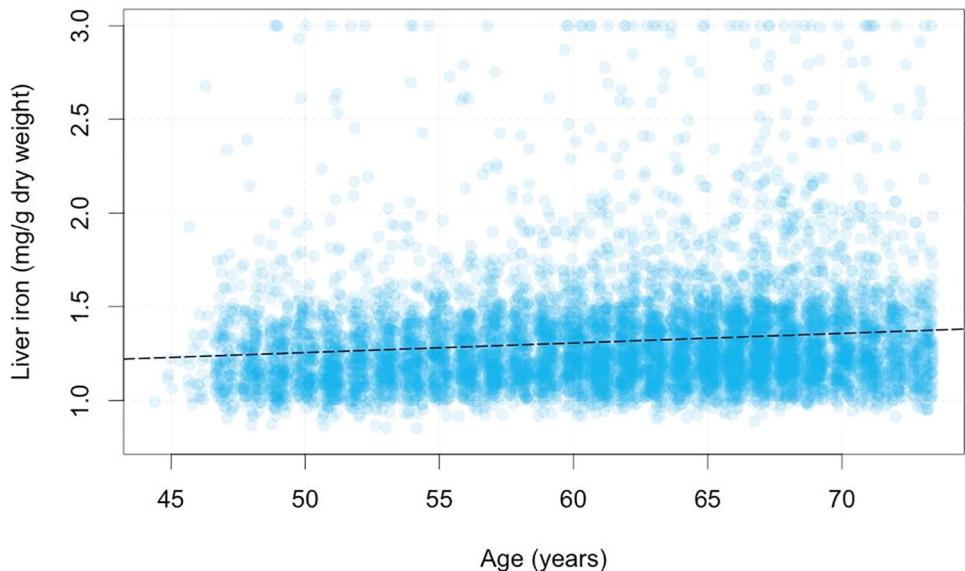


Fig 3. Correlation of liver iron concentration (mg/g dry weight) with age. Iron values above 3mg/g have been capped to 3mg/g in the plot, but not as part of the linear fit. Dashed black line shows line of best fit in univariate linear model.

<https://doi.org/10.1371/journal.pone.0209340.g003>

to build the final model. In the final model, no interaction term was included, but each of age, sex, BMI, log(PDFF), and beef intake were included. These were all found to be significantly correlated ($p < 10^{-7}$ in all cases). The coefficients from the model are shown in [S3 Table](#). The r^2 for the model was 0.053. In the multivariate model, the coefficient for log(PDFF) was larger than the univariate fit, but all other variables, except BMI, had lower coefficients in the

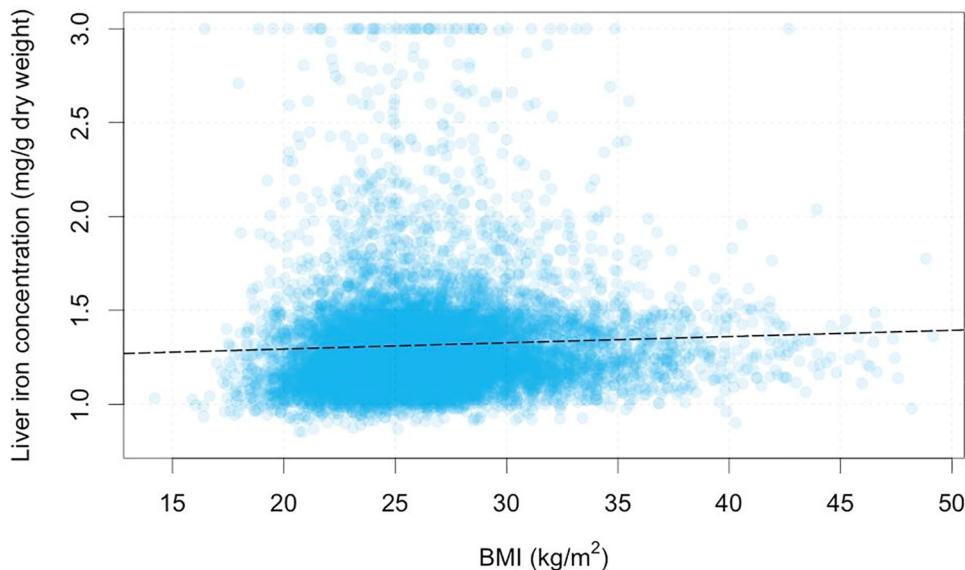


Fig 4. Graph showing distribution of BMI (kg/m²) in the UK Biobank population against liver iron (mg/g). Iron values above 3mg/g have been capped to 3mg/g in the plot, but not as part of the linear fit. Dashed black line shows line of best fit in univariate linear model.

<https://doi.org/10.1371/journal.pone.0209340.g004>

multivariate model than in their respective univariate models. The BMI coefficient was changed in sign with respect to the univariate model.

Discussion

The findings from this cross-sectional study into liver iron concentration in the UK Biobank revealed that 4.82% of the subjects have elevated liver iron ($>1.8 \text{ mg/g}$) and that age, sex, ethnicity, dietary intake of beef, BMI, and liver fat, had a significant impact.

The use of MRI based methodology to assess liver iron has enabled measurement in populations where biopsy may not have been appropriate. Whilst previous studies of liver iron have generally focussed on small well-defined patient cohorts, there has recently been a similar population based study of liver iron in a German cohort ($n = 2561$) [33]. That study reported a prevalence of elevated levels of liver iron of 17.4%, considerably higher than that reported in the present study (<5%). The reason(s) underlying this difference are unclear but may relate to the nature of the cohort under study. For example, prevalence of fatty liver disease within the UK Biobank population was lower (20%) [26], than that reported by Kuhn et al (42.2%) [33]. Given that in the present study we observed a higher proportion of elevated liver iron levels in subjects with high PDFF, a far greater prevalence of elevated liver iron would also be expected within the German cohort. However methodological differences in MR acquisition and analysis, as well as the thresholds used for defining elevated levels of liver iron cannot be discounted, as they could potentially contribute significantly to these differences. Kuhn's study reports a considerably lower median $R2^*$ (34.4 s^{-1}) and hence lower median liver iron, than measured in our study (41.4 s^{-1}). Moreover, reanalysis of our dataset using their stated cut-off for elevated levels of liver iron, defined as $R2^* > 41 \text{ sec}^{-1}$ [34], would suggest that 51.5% of the subjects in the UK Biobank would be deemed as having elevated levels of liver iron. Interestingly, others have suggested a quite different cut-off for the normal $R2^*$ threshold, (see S4 Table for a summary of this data). Applying 57 s^{-1} cut-off suggested for multi-echo Dixon sequences gives an UKBB prevalence of elevated liver iron levels of 7.5% [17]. Using 65 s^{-1} as suggested by Paisant et al [27] would give a prevalence of elevated levels of liver iron within the UK Biobank of 4.2%.

Moreover, using the $<70 \text{ s}^{-1}$ suggested by Henninger et al [20] would result in a prevalence of elevated levels of liver iron of 3.0%. However, sequence dependence for cut-offs are known, with 3D multi-echo Dixon sequences showing lower $R2^*$ values than 2D ME GRE sequences [17]. Clearly choice of threshold to define elevated levels of liver iron will have considerable impact on reported prevalence in population studies. Whilst these published thresholds have been determined from extensive calibration studies combining MRI and biopsy data, many are still dependent on several factors including choice of sequence, field strength and cohort. Our overall finding of a prevalence of elevated levels of liver iron of just under 5%, based on biopsy related cut-off, are in line with previously published studies of explant pathology reports which found mildly elevated levels of liver iron in 5.6% of patients studied [35].

Correlations were observed between liver iron and several variables tested here. The effect sizes were consistent between the univariate models and the combined model, except for the BMI effect. The r^2 was 0.05, indicating that these variables only explain a small amount of the population variability, so it is likely that there are more drivers of liver iron concentration than those discussed here. It is possible that the factors we have investigated in this model may be surrogates for factors that affecting iron absorption or liver iron deposition and not causative factors, further longitudinal and/or interventional studies will be required to understand this further. The change in sign of the BMI effect between the univariate and combined models, is

likely a result of the linear regression using correlated variables (BMI correlates with log (PDFF), $r^2 = 0.31$), and the resulting impact of this on the effect estimates of the linear model.

In the present study we report that liver iron increased significantly with age; 0.005 mg/g/year in the univariate model, and 0.004 mg/g/year in the multivariate model. We did not observe a different age-related trend in the male and female participants. An age-related increase in liver iron has previously been reported [33], the hepatic iron index is based on the concept that liver iron increases with age in HH [13]. Some of these have suggested that liver iron increases until the age of 50 years and then declines [34, 36]. However there have also been conflicting studies that have reported no age association between liver iron and HH [37, 38]. Further work may be necessary to fully understand this association, however there are relatively few individuals with diagnosed HH within the UKBiobank study; 123 in the overall cohort of 500,000, equating to approximately two individuals within the imaging cohort included in the present study. The very small numbers of HH individuals appears to support the view that the aetiology of the elevated levels of liver iron observed within the UKBB cohort is more likely associated with DIOS, undiagnosed HH, or other factors such as thalassemia. Currently, it is unclear whether this relatively small increase in liver iron can be regarded as clinically relevant. However, several studies have suggested a link between liver iron and development of fibrosis in subjects with NAFLD, although the underlying mechanisms may depend on whether the liver iron accumulation was hepatocellular or parenchymal [39, 40]. Consequently, it may be that in subjects with NAFLD even relatively small increases in liver iron may be significant and warrant further investigation.

Liver iron was significantly higher in male compared with female subjects in the UK Biobank cohort, although in absolute terms this difference was small. Prevalence of elevated liver iron was also significantly more common in male subjects compared with female subjects. This confirms the findings of previous studies which have reported similar sex differences in liver iron levels and prevalence of elevated levels of liver iron [33, 40]. This is further supported by studies showing more frequent prevalence of DIOS in males [8]. It has been shown that iron levels in females are general low until the menopause when they increase considerably [41], one suggestion is the gradual increases in body iron storage that occurs throughout aging in men are ameliorated by the effects of menstruation [42], although other studies suggest that this change may be accounted for by changes in dietary habits [43].

Ethnicity appeared to have a small yet significant impact of liver iron levels, which were significantly elevated in Caucasian compared with Asian subjects. Given the differences in the median values are very small, this may not be clinically significant, however reports of hemochromatosis within Asian populations are low [44], therefore the impact of ethnicity of liver iron levels may warrant further investigation.

There was a very weak relationship between liver iron and BMI, with prevalence of elevated liver iron levels being less common in subjects with a BMI less than 20 kg/m². Relatively few studies have explored the relationship between BMI and elevated liver iron levels and available data are conflicting. Zheng et al reported a positive association between liver iron and BMI [45]; however, this is at odds with the findings of Nelson et al who reported lower BMI to be associated with increased liver iron [40]. These differences may be accounted for by the fact that our cohort was comprised from relatively healthy subjects from the general population whereas previous studies had a disease positive NASH cohort.

A relationship between self-reported frequency of beef consumption of liver iron was observed. It is established that there is a significant relationship between red meat consumption and serum ferritin levels [46], and that overfeeding animals with dietary iron causes an increase in liver iron [47]. Furthermore, there is a positive association between red meat intake and liver cancer and chronic liver disease [48, 49]. Although the results presented here are

preliminary, the UK Biobank data will allow further investigation of the impact of other dietary factors which have been suggested to have an influence on liver iron and liver health outcomes.

Limitations

There are several limitations that may impact on the extrapolation of the data obtained from this UK Biobank population to the wider population. It has been shown that the UK Biobank population are somewhat ‘healthier’ than the general UK population, furthermore the age range is limited and there is relatively little ethnic diversity [50].

A further limitation of the current study is the paucity of serum ferritin measurements. Given their general use in assessing iron status in subjects with NAFLD and their potential to add to differentiation between individuals with DIOS and other forms of iron overload [11], they would have been a valuable addition to this population study. However, this marker has not yet been made available by the UKBiobank. In future it is envisaged that blood and other biomarker will be used in combination with the MR measurements described in this paper to aid the development of less complex and less expensive non-invasive tools for liver iron assessment.

MRI measurement of liver iron based on acquisition of T2* or R2*, whilst having the advantage of being fast and non-invasive, can struggle to obtain reliable measurements in very high levels of liver iron content, since the MR signal decays very rapidly. Ideally multi-echo sequences with sufficient echoes, and a short initial echo time need to be employed, but logistic and technical limitations do not make this approach feasible for “large data” studies.

A further confounding factor which may influence the accurate assessment of liver iron relates to the presence of fat infiltration in the liver, since fat-water signal cancellation effects [18] are a particular problem for the type of T2*-Dixon sequence employed in the present study [18, 51, 52]. This issue may be overcome by the use of the IDEAL sequence [53], which addresses these effects by separating out water and fat signals.

Values here are derived from ROIs placed by trained analysts. While more automated approaches are in development, currently human interaction is required to determine appropriate parts of the image to use. However, intra- and inter-rater reliability is excellent for this analysis, as demonstrated by the ICC and Bland-Altman analysis.

Conclusions

In this paper we show that population studies measuring liver iron are not only feasible, but can now become part of the ever expanding “big data” consortium. As more data is released from the UK Biobank, future studies on liver iron will enable more in-depth investigation on gene-environment interaction, as well as the impact of various lifestyle factors. Moreover, it will provide unique insights to better inform future patient stratification and treatments for elevated liver iron levels. This study has been able to uniquely assess the combined burden of fatty liver and elevated liver iron levels within the UK Biobank population. We have reported that a significant proportion of this population has fatty liver (20.7%) and elevated liver iron levels (4.8%); with 1.3% of the total population having both elevated levels of fat and iron. It has been cautioned in recent years that the UK health system is not prepared for the current liver disease epidemic, with the Lancet Commission for Liver Disease making a series of recommendations in its 2014 report [54]. The numbers of individuals with abnormal levels for two key liver disease risk factors are significant, and potentially represent millions in the wider population. Therefore both liver fat and iron are noteworthy within the liver field’s future research priorities and following public health response.

Supporting information

S1 Appendix. Describes the assessment of repeatability and reproducibility of liver iron measurements included in the manuscript.

(DOCX)

S1 Table. Describes the published formulas used in previous publications for converting R2* at 1.5T to concentration of liver iron in mg/g.

(DOCX)

S2 Table. Shows the summary statistics for measurement of liver iron within the UK Biobank cohort expressed T2* (1.5T), R2* (1.5T) and as mg/g. The impact of applying the various formula described in [S1 Table](#) on the measurement of liver iron in mg/g are also detailed.

(DOCX)

S3 Table. Correlation coefficients for variables. In the univariate models, a model was fitted for each variable, with liver iron as the dependent variable, and only the variable of choice as the independent variable. In the multivariate model, a single model was fitted, with liver iron as the dependent variable, and all variables in the table as independent variables.

(DOCX)

S4 Table. Impact of using different R2* cut-offs to indicate iron overload on incidence of the condition within the UK Biobank population.

(DOCX)

S1 Fig. Describes the distribution of T2* within the UK Biobank population. Median value (24.18 ms) shown in red, mean (23.96 ms) in blue. Values < 15.9ms represent overloaded individuals.

(DOCX)

Acknowledgments

This research has been conducted using the UK Biobank Resource under application 9914.

Author Contributions

Conceptualization: Henry R. Wilman, Matt Kelly, Stefan Neubauer, Jimmy D. Bell, Rajarshi Banerjee, E. Louise Thomas.

Data curation: Henry R. Wilman.

Funding acquisition: Jimmy D. Bell, Rajarshi Banerjee, E. Louise Thomas.

Investigation: Andy McKay, Henry R. Wilman, Andrea Dennis.

Methodology: Michael L. Gyngell.

Resources: Rajarshi Banerjee.

Supervision: E. Louise Thomas.

Writing – original draft: Andy McKay, Henry R. Wilman, Matt Kelly, E. Louise Thomas.

Writing – review & editing: Andy McKay, Henry R. Wilman, Matt Kelly, Michael L. Gyngell, Stefan Neubauer, Jimmy D. Bell, Rajarshi Banerjee, E. Louise Thomas.

References

1. Institute for Health Metrics and Evaluation (IHME). GBD compare data visualization. Seattle, WA: IHME, University of Washington, 2016. <http://vizhub.healthdata.org/gbd-compare>. Accessed: 19/01/2017.
2. Ratib S, West J and Fleming KM. Liver cirrhosis in England—an observational study: are we measuring its burden occurrence correctly? *BMJ Open* 2017; 7: e013752 <https://doi.org/10.1136/bmjopen-2016-013752> PMID: 28710203
3. Pais R, Charlotte F, Fedchuk L, Bedossa P, Lebray P, Poynard T, et al. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. *J Hepatol*. 2013; 59: 550–6. <https://doi.org/10.1016/j.jhep.2013.04.027> PMID: 23665288
4. Hu KQ, Kyulo NL, Esrailian E, Thompson K, Chase R, Hillebrand DJ, et al. Overweight and obesity, hepatic steatosis, and progression of chronic hepatitis C: a retrospective study on a large cohort of patients in the United States. *J Hepatol*. 2004; 40: 147–54. [https://doi.org/10.1016/S0168-8278\(03\)00479-3](https://doi.org/10.1016/S0168-8278(03)00479-3) PMID: 14672626
5. Deugnier Y, Bardou-Jacquet É, Lainé F. Dysmetabolic iron overload syndrome (DIOS). *Presse Med*. 2017; 36: e-306–311. <https://doi.org/10.1016/j.lpm.2017.05.036> PMID: 29169710
6. Dongiovanni P, Fracanzani AL, Fargion S, Valentini L. Iron in fatty liver and in the metabolic syndrome: A promising therapeutic target. *J Hepatol*. 2011; 55: 920–932. <https://doi.org/10.1016/j.jhep.2011.05.008> PMID: 21718726
7. Turlin B, Mendlér MH, Moirand R, Guyader D, Guillygomarc'h A, Deugnier Y. Histologic features of the liver in insulin resistance-associated iron overload. A study of 139 patients. *Am J Clin Pathol*. 2001; 116: 263–70. <https://doi.org/10.1309/WWNE-KW2C-4KTW-PTJ5> PMID: 11488074
8. Mendlér MH, Turlin B, Moirand R, Jouanolle AM, Sapey T, Guyader D, et al. Insulin resistance-associated hepatic iron overload. *Gastroenterology*. 1999; 117: 1155–63. PMID: 10535879
9. Moirand R, Mortaji AM, Loréal O, Paillard F, Brissot P, Deugnier Y. A new syndrome of liver iron overload with normal transferrin saturation. *Lancet*. 1997; 349: 95–7. [https://doi.org/10.1016/S0140-6736\(96\)06034-5](https://doi.org/10.1016/S0140-6736(96)06034-5) PMID: 8996422
10. Sorrentino P, D'Angelo S, Ferbo U, Micheli P, Bracigliano A, Vecchione R. Liver iron excess in patients with hepatocellular carcinoma developed on non-alcoholic steatohepatitis. *J Hepatol*. 2009; 50: 351–7. <https://doi.org/10.1016/j.jhep.2008.09.011> PMID: 19070395
11. Datz C, Müller E, Aigner E. Iron overload and non-alcoholic fatty liver disease. *Minerva Endocrinol*. 2017; 42: 173–183. <https://doi.org/10.23736/S0391-1977.16.02565-7> PMID: 27834478
12. Nuttall KL, Palaty J, Lockitch G. Reference limits for copper and iron in liver biopsies. *Ann Clin Lab Sci*. 2003; 33: 443–50. PMID: 14584759
13. Bassett ML, Halliday JW, Powell LW. Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. *Hepatology*. 1986; 6: 24–9. <https://doi.org/10.1002/hep.1840060106> PMID: 3943787
14. European Association for the Study of the Liver. EASL clinical practice guidelines for HFE haemochromatosis. *J Hepatology*. 2010; 53: 3–22. <https://doi.org/10.1016/j.jhep.2010.03.001> PMID: 20471131
15. Bacon BR, Adams PC, Kowdley KV, Powell LW, Tavill AS, American Association for the Study of Liver Diseases. Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology*. 2011; 54: 328–343. <https://doi.org/10.1002/hep.24330> PMID: 21452290
16. Nielsen P, Günther U, Dürken M, Fischer R, Düllmann J. Serum ferritin iron in iron overload and liver damage: correlation to body iron stores and diagnostic relevance. *J Lab Clin Med*. 2000; 135: 413–8. <https://doi.org/10.1067/mlc.2000.106456> PMID: 10811057
17. Henninger B, Zoller H, Kannengiesser S, Zhong X, Jaschke W, Kremser C. 3D multiecho Dixon for the evaluation of hepatic iron and fat in a clinical setting. *J Magn Reson Imaging*. 2017; 46: 793–800. <https://doi.org/10.1002/jmri.25630> PMID: 28225576
18. Hernando D, Levin YS, Sirlin CB, Reeder S. Quantification of liver iron with MRI: state of the art and remaining challenges. *J Magn Reson Imaging*. 2014; 40: 1003–21. <https://doi.org/10.1002/jmri.24584> PMID: 24585403
19. Wood JC, Enriquez C, Ghugre N, Tyzka JM, Carson S, Nelson MD, et al. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. *Blood* 2005; 106: 1460–5. <https://doi.org/10.1182/blood-2004-10-3982> PMID: 15860670
20. Henninger B, Zoller H, Rauch S, Finkenstedt A, Schocke M, Jaschke W, et al. R2* relaxometry for the quantification of hepatic iron overload: biopsy-based calibration and comparison with the literature. *Rofo*. 2015; 187: 472–9. <https://doi.org/10.1055/s-0034-1399318> PMID: 25877992

21. Anderson LJ, Holden S, Davis B, Prescott E, Charrier CC, Bunce NH, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J.* 2001; 22: 2171–9. <https://doi.org/10.1053/euhj.2001.2822> PMID: 11913479
22. Hankins JS, McCarville MB, Loeffler RB, Smeltzer MP, Onciu M, Hoffer FA, et al. R2* magnetic resonance imaging of the liver in patients with iron overload. *Blood.* 2009; 113: 4853–5. <https://doi.org/10.1182/blood-2008-12-191643> PMID: 19264677
23. Garbowski MW, Carpenter JP, Smith G, Roughton M, Alam MH, He T, et al. Biopsy-based calibration of T2* magnetic resonance for estimation of liver iron concentration and comparison with R2 Ferriscan. *J Cardiovasc Magn Reson.* 2014; 16: 40. <https://doi.org/10.1186/1532-429X-16-40> PMID: 24915987
24. Virtanen JM, Komu ME, Parkkola RK. Quantitative liver iron measurement by magnetic resonance imaging: in vitro and in vivo assessment of the liver to muscle signal intensity and the R2* methods. *Magn Reson Imaging.* 2008; 26: 1175–82. <https://doi.org/10.1016/j.mri.2008.01.028> PMID: 18524528
25. Perspectum Diagnostics. LiverMultiscan Discover [Internet]. 2016. <http://perspectum-diagnostics.com/services/livermultiscan-discover/>
26. Wilman HR, Kelly M, Garratt S, Matthews PM, Milanesi M, Herlihy A, et al. Characterisation of liver fat in the UK Biobank cohort. *PLoS One.* 2017; 12: e0172921. <https://doi.org/10.1371/journal.pone.0172921> PMID: 28241076
27. Paisant A, d'Assignies G, Bannier E, Bardou-Jacquet E, Gandon Y. MRI for the measurement of liver iron content, and for the diagnosis and follow-up of iron overload disorders. *Presse Med.* 2017; 46: e279–e287. <https://doi.org/10.1016/j.lpm.2017.10.008> PMID: 29133084
28. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychol Bull.* 1979; 86: 420–8. PMID: 18839484
29. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986; 327: 307–10. [https://doi.org/10.1016/S0140-6736\(86\)90837-8](https://doi.org/10.1016/S0140-6736(86)90837-8)
30. Python Software Foundation. Python 3.6. <https://www.python.org/pf/>
31. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
32. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab.* 2005; 288: E462–8. <https://doi.org/10.1152/ajpendo.00064.2004> PMID: 15339742
33. Kühn JP, Meffert P, Heske C, Kromrey ML, Schmidt CO, Mensel B, 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. <https://doi.org/10.1148/radiol.2017161228> PMID: 28481195
34. Kühn JP, Hernando D, Muñoz del Rio A, Evert M, Kannengiesser S, Völzke H, et al. Effect of multipex spectral modeling of fat for liver iron and fat quantification: correlation of biopsy with MR imaging results. *Radiology* 2012; 265: 133–42. <https://doi.org/10.1148/radiol.12112520> PMID: 22923718
35. Ko C, Siddaiah N, Berger J, Gish R, Brandhagen D, Sterling RK, et al. Prevalence of hepatic iron overload and association with hepatocellular cancer in end-stage liver disease: results from the National Hemochromatosis Transplant Registry. *Liver Int.* 2007; 27: 1394–401 <https://doi.org/10.1111/j.1478-3231.2007.01596.x> PMID: 17927713
36. Nomura H, Hayashi J, Kajiyama W, Kashiwagi S. Prevalence of liver abnormalities in the general population of Okinawa, Japan. *Fukuoka Igaku Zasshi.* 1997; 88: 345–51. PMID: 9430951
37. Adams PC, Deugnier Y, Moirand R, Brissot P. The relationship between iron overload, clinical symptoms, and age in 410 patients with genetic hemochromatosis. *Hepatology.* 1997; 25: 162–6. <https://doi.org/10.1002/hep.510250130> PMID: 8985284
38. St Pierre TG, Clark PR, Chua-Anusorn W, Fleming AJ, Jeffrey GP, Olynyk JK, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood.* 2005; 105: 855–61. <https://doi.org/10.1182/blood-2004-01-0177> PMID: 15256427
39. Valenti L, Fracanzani AL, Bugianesi E, Dongiovanni P, Galmozzi E, Vanni E, et al. HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. *Gastroenterology.* 2010; 138: 905–12. <https://doi.org/10.1053/j.gastro.2009.11.013> PMID: 19931264
40. Nelson JE, Wilson L, Brunt EM, Yeh MM, Kleiner DE, Unalp-Arida A, et al. Relationship between the pattern of hepatic iron deposition and histological severity in nonalcoholic fatty liver disease. *Hepatology.* 2011; 53: 448–57. <https://doi.org/10.1002/hep.24038> PMID: 21274866
41. Zacharski LR, Ornstein DL, Woloshin S, Schwartz LM. Association of age, sex, and race with body iron stores in adults: analysis of NHANES III data. *Am Heart J.* 2000; 140: 98–104. <https://doi.org/10.1067/mhj.2000.106646> PMID: 10874269

42. Milliken JA, Brown TM. Hemochromatosis in a Menstruating Woman. *Can Med Assoc J.* 1963; 88: 1028–30. PMID: [20327538](#)
43. Milman N, Byg KE, Ovesen L, Kirchhoff M, Jürgensen KS. Iron status in Danish women, 1984–1994: a cohort comparison of changes in iron stores and the prevalence of iron deficiency and iron overload. *Eur J Haematol.* 2003; 71: 51–61. <https://doi.org/10.1034/j.1600-0609.2003.00090.x> PMID: [12801299](#)
44. Lok CY, Merryweather-Clarke AT, Viprakasit V, Chinthammitr Y, Srichairatanakool S, Limwongse C, et al. Iron overload in the Asian community. *Blood.* 2009; 114: 20–5. <https://doi.org/10.1182/blood-2009-01-199109> PMID: [19342478](#)
45. Zheng X, Jiang T, Wu H, Zhu D, Wang L, Qi R, et al. Hepatic iron stores are increased as assessed by magnetic resonance imaging in a Chinese population with altered glucose homeostasis. *Am J Clin Nutr.* 2011; 94: 1012–9. <https://doi.org/10.3945/ajcn.111.015743> PMID: [21880847](#)
46. Quintana Pacheco DA, Sookthai D, Wittenbecher C, Graf ME, Schübel R, Johnson T, et al. Red meat consumption and risk of cardiovascular diseases—is increased iron load a possible link? *Am J Clin Nutr.* 2018; 107: 113–119. <https://doi.org/10.1093/ajcn/nqx014> PMID: [29381787](#)
47. Brown KE, Dennery PA, Ridnour LA, Fimmel CJ, Kladney RD, Brunt EM, et al. Effect of iron overload and dietary fat on indices of oxidative stress and hepatic fibrogenesis in rats. *Liver Int.* 2003; 23: 232–42. <https://doi.org/10.1034/j.1600-0676.2003.00832.x> PMID: [12895262](#)
48. Cross AJ, Leitzmann MF, Gail MH, Hollenbeck AR, Schatzkin A, Sinha R. A prospective study of red and processed meat intake in relation to cancer risk. *PLoS Med.* 2007; 4: e325. <https://doi.org/10.1371/journal.pmed.0040325> PMID: [18076279](#)
49. Freedman ND, Cross AJ, McGlynn KA, Abnet CC, Park Y, Hollenbeck AR, et al. Association of meat and fat intake with liver disease and hepatocellular carcinoma in the NIH-AARP cohort. *J Natl Cancer Inst.* 2010; 102: 1354–65. <https://doi.org/10.1093/jnci/djq301> PMID: [20729477](#)
50. Fry A, Littlejohns TJ, Sudlow C, Doherty N, Adamska L, Sprosen T, et al. Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants with those of the General Population. *Am J Epidemiol.* 2017; 186: 1026–34. <https://doi.org/10.1093/aje/kwx246> PMID: [28641372](#)
51. Bydder M, Hamilton G, de Rochemont L, Desai A, Heba ER, Loomba R, et al. Sources of systematic error in proton density fat fraction (PDFF) quantification in the liver evaluated from magnitude images with different numbers of echoes. *NMR Biomed.* 2018; 31: e3843. <https://doi.org/10.1002/nbm.3843> PMID: [29130539](#)
52. Leporq B, Lambert SA, Ronot M, Vilgrain V, Van Beers BE. Simultaneous MR quantification of hepatic fat content, fatty acid composition, transverse relaxation time and magnetic susceptibility for the diagnosis of non-alcoholic steatohepatitis. *NMR Biomed.* 2017; 30: e3766. <https://doi.org/10.1002/nbm.3766> PMID: [28678410](#)
53. Reeder SB, McKenzie CA, Pineda AR, Yu H, Shimakawa A, Brau AC, et al. Water-fat separation with IDEAL gradient-echo imaging. *J Magn Reson Imaging.* 2007; 25: 644–52. <https://doi.org/10.1002/jmri.20831> PMID: [17326087](#)
54. Williams R, Aspinall R, Bellis M, Camps-Walsh G, Cramp M, Dhawan A, et al. Addressing liver disease in the UK: a blueprint for attaining excellence in health care and reducing premature mortality from lifestyle issues of excess consumption of alcohol, obesity, and viral hepatitis. *Lancet.* 2014; 384: 1953–97. [https://doi.org/10.1016/S0140-6736\(14\)61838-9](https://doi.org/10.1016/S0140-6736(14)61838-9) PMID: [25433429](#)