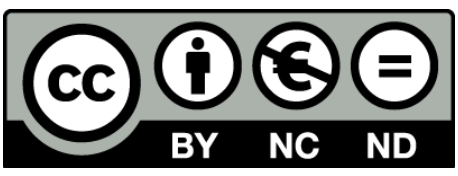

This is the text version of 'Plasma ferritin concentrations are not associated with AAA diagnosis, size or growth' – accepted for publication in Atherosclerosis 11-5-2016

Plasma ferritin concentrations are not associated with abdominal aortic aneurysm diagnosis, size or growth

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Abstract

Background and aims: Experimental studies using a rodent model have suggested that iron overload may contribute to abdominal aortic aneurysm (AAA) pathogenesis.

Methods: We assessed the association of total body iron, as measured by plasma ferritin, with AAA diagnosis, size and growth in 4024 community-dwelling older men screened for AAA, using logistic regression and linear mixed effects models.

Results: Plasma ferritin concentrations were similar in men who did (n=293) and did not (n=3731) have an AAA (median [inter-quartile range] concentrations 115.4 [63.0-203.1] and 128.5 [66.1-229.1] ng/mL respectively, *p*=0.124). There was no association between plasma ferritin concentration and AAA diagnosis in unadjusted logistic regression (odds ratio (OR) for a 1 standard deviation increase: 0.880 [95%CI: 0.764-1.015]; *p*=0.078), or when adjusting for AAA risk factors and factors known to influence circulating ferritin (OR for a 1 standard deviation increase: 0.898 [95% CI: 0.778-1.035]; *p*=0.138). Iron overload prevalence (plasma ferritin concentrations >200ng/mL) was lower in men with an AAA (25.3%) than those without (30.8%; *p*=0.048), but was not associated with AAA diagnosis after adjusting as above (OR: 0.781 [95% CI:0.589-1.035]; *p*=0.086). The association of iron overload with AAA growth was investigated in 265 men with small AAAs who received at least 1 repeat ultrasound scan in the 3 years following screening. We saw no difference in AAA growth between men who did and did not have iron overload (n=65 and 185 respectively, *p*=0.164).

Conclusions: Our data suggest that iron overload is unlikely to be important in AAA pathogenesis.

Short title: Ferritin, iron overload and AAA

Key words: Abdominal aortic aneurysm; Ferritin; Iron overload; Biomarker

Abbreviations: AAA: Abdominal aortic aneurysm; IQR: inter-quartile range; 95% CI: 95% confidence interval.

Introduction

Abdominal aortic aneurysm (AAA) is an abnormal progressive dilatation of the infra-renal aorta diagnosed by aortic diameter ≥30mm (1). AAA affects ~2% of men, and 1% of women aged over 65 years and is a leading cause of death in the elderly through aortic rupture and associated cardiovascular events (1-3). Recent data suggest that global mortality rates due to AAA increased by ~10% over the last 20 years (4). No currently available medications have been shown to slow AAA progression, and surgery is the only current treatment (1, 5). Several randomised control trials have demonstrated that surgery does not improve outcomes for patients with small (<55 mm) AAAs (6-9), and patients with small AAAs are managed conservatively via repeated imaging assessments to monitor AAA growth (1, 5). Approximately 60% of small AAAs ultimately expand to a size where surgical intervention is recommended (6, 7). Thus, there remains an unmet need to identify an effective non-surgical therapy to improve management of patients with small AAAs.

A large body of data suggest that aortic inflammation, oxidative stress, vascular smooth muscle cell death and extracellular matrix degradation are important in AAA pathogenesis (1, 5, 10). Several separate studies have identified iron deposits within infra-renal aortic biopsies recovered from patients with large AAAs (11-14). More recently, Sawada and colleagues reported that an iron-restricted diet reduced the development of AAA within a mouse model and posed the hypothesis that iron overload may be important in AAA pathogenesis (13). If this hypothesis was confirmed then iron restriction could be a treatment for AAA. This hypothesis has not yet been tested in large populations (13). Plasma ferritin concentration has been demonstrated to be a robust marker to assess body iron stores in older people (reviewed by Fairweather-Tait *et al.*(15)). In the current study we investigated the association of iron overload (as assessed by plasma ferritin concentration) with AAA diagnosis, size and growth in a cohort of 4024 community-dwelling men screened for AAA.

Materials and methods

Participants and definitions used:

This study included 4024 participants of the Health In Men Study (HIMS) for whom blood samples were available (16). The cohort characteristics and protocols of the HIMS have been previously described (16). HIMS developed from a randomized trial designed to examine if screening older men for AAA reduced mortality. Clinical information collected from each HIMS participant at recruitment included age, medical history and smoking status. Anthropomorphic measurements including height, weight and hip and waist circumference were also recorded. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in metres^2. Participants provided written informed consent, and this study was conducted under institutional ethics approval.

AAA was defined as a maximum infra-renal aortic diameter ≥30mm on ultrasound. Coronary heart disease was defined as prior history of myocardial infarction, angina or coronary artery revascularisation. Diabetes was defined as prior diagnosis or treatment for hyperglycaemia. Hypertension was defined by treatment for, or previous diagnosis of high blood pressure. Men found to have AAA were offered a follow up ultrasound scan at 6-monthly intervals if aortic diameter at screening was ≥40 mm, or at 12 month intervals if aortic diameter was 30-39 mm, as described previously (17). Previous data have demonstrated that ultrasound measurement of AAA diameter for these patients has excellent reproducibility (95% confidence interval <3mm, detailed in (17)). Circulating concentrations of C-reactive protein, low- and high-density lipoprotein cholesterol, creatinine and homocysteine were measured using reproducible assays as previously described (17-19).

Measurement of plasma ferritin:

Plasma ferritin was measured using a commercially available enzyme-linked immunosorbent assay (ELISA; #2825-300, Monobind Inc. Lake Forest, CA, USA), as directed by the manufacturer. The inter assay coefficient of variation was 12.9%; intra assay coefficient of

variation was 4.5%. Plasma ferritin concentrations >200 µg/L (or >200 ng/mL) were considered indicative of iron overload, in line with World Health Organisation guidelines (20).

Statistical analyses:

Data were analysed using the SPSS v 22, and R statistical software packages. Continuous data are reported as median and inter-quartile range (IQR). Continuous variables were compared between 2 groups using the Mann-Whitney U test, and between 3 groups using the Kruskal-Wallis test. Correlation between continuous variables was assessed using the Spearman-rho test. Nominal data are shown as count and percent, and were compared using the chi-squared test.

The association of plasma ferritin concentration with AAA diagnosis was further assessed using univariate binary logistic regression and multivariate regression adjusted for i) AAA risk factors (age, hypertension, diabetes, coronary heart disease, smoking and BMI), and ii) AAA risk factors and dietary/non dietary factors known to influence circulating ferritin (aspirin use, meat consumption and frequency of vigorous and non-vigorous exercise) (21). For the purposes of this study meat consumption was categorised as i) not eating meat on a weekly basis, ii) eating meat up to 2 times a week and, iii) eating meat at least 3 times a week. Frequency of vigorous and non-vigorous exercise was categorised as i) no hours per week, ii) fewer than 4 hours per week, and iii) at least 4 hours per week. For nominal variables, men who had the risk factor were compared to those who did not. To assess the effect of exercise and meat consumption, men who did not perform the activity were used as the reference group. Odds ratios (OR) and 95% confidence intervals are reported for all covariates in each model.

Yearly AAA growth rate for men in the growth analysis was calculated as a percentage of the size of the AAA in the preceding year (e.g. AAA growth rate between screening and year 1 rescan = 100*((Year 1 AAA diameter/AAA diameter at screening) – 1). Using this approach,

AAA growth rate could only be completed for men with consecutive scans. Accordingly, men were removed from the analysis at the time of their last consecutive AAA measurement. Yearly AAA growth was used as the response variable in a linear mixed effects model to investigate the association of iron overload with AAA growth using the R nlme and car packages. A random-intercept model was fitted using Iron overload, time (years, labelled as interval in Supplementary file 2) and initial AAA diameter as fixed effects. Variation between individual patients was treated as a random effect. More complex formulations of the random effects structure to allow random slopes did not improve the model as evaluated by the AIC or BIC criteria. Similarly, incorporation of interaction terms between the fixed effects did not improve the model. The distribution of residuals was examined via QQ-normal plots and scatter plots of fitted values vs standardised residuals and did not indicate problems with residual distributions.

For all analyses, p-values <0.05 were considered to be significant.

Results

Plasma ferritin concentrations were not associated with AAA diagnosis:

Plasma ferritin concentrations were compared between men who did and did not have an AAA (n=293 and 3731, respectively). Demographic characteristics of all participants are shown in Table 1. Men with AAA were older, and had a higher prevalence of hypertension, ever smoking and coronary heart disease. Serum C-reactive protein (hsCRP) concentration was significantly higher in men that had AAAs than those who did not (median hsCRP [IQR] 2.5 [1.4-5.1] and1.8 [1.0-3.7] mg/L respectively, p <0.001). Serum hsCRP concentrations were not correlated with plasma ferritin concentrations (Spearman r = 0.023; p=0.147). Median ferritin concentrations were 115.4 [IQR: 63.0-203.1] ng/mL and 128.5 [IQR: 66.1-229.1] ng/mL in men who did and did not have an AAA, respectively (p=0.124). No significant association of plasma ferritin with AAA diagnosis was observed in univariate binary regression (OR for an increase in plasma ferritin of 175 ng/mL, i.e. ~1 std deviation, 0.880, 95% confidence intervals [95% CI] 0.764-1.015, p=0.078). Similarly, no significant relationship between plasma ferritin concentrations and AAA diagnosis was observed in regression models adjusted for AAA risk factors (OR: 0.871 (95% CI: 0.774-1.026); p=0.110), or AAA risk factors plus dietary and non-dietary factors known to influence circulating ferritin concentration (OR: 0.898 (95% CI: 0.778-1.035); p=0.138; Supplementary Table 1) (21). The prevalence of iron overload (circulating ferritin concentrations >200 ng/mL) was significantly lower in men that had an AAA (25.3%) than those who did not (30.8%, p=0.048), however this association was not significant after adjusting for AAA risk factors and factors known to influence circulating ferritin concentrations (OR: 0.781 (95% CI:0.589-1.035); p=0.086; Table 2).

Plasma ferritin concentrations were not correlated with aortic diameter:

No correlation between plasma ferritin concentration and aortic diameter was seen when assessing all men included in this analysis (n=4024; Spearman r= -0.018; p=0.246), those without AAA only (n=3731; Spearman r=-0.009; p=0.603), or those with AAA only

(Spearman r = -0.006; p=0.919, Supplementary Table 2). Men that had an AAA were grouped into tertiles according to their infra-renal aortic diameter (Supplementary Table 3). Median plasma ferritin concentrations were 113.4 [IQR: 60.8-219.8], 106.1 [IQR: 59.9-207.1] and 121.2 [IQR: 66.3-195.5] ng/mL in the first, second and third tertiles, respectively (*p*=0.996). No difference in the prevalence of iron overload was observed between the tertiles (*p*=0.811). Finally, no difference in circulating ferritin concentration was observed when comparing men who had large AAAs (>50 mm, n=18), with those who did not have an AAA (n=3731; median serum ferritin concentration 141.0 [63.5-218.3] and 128.5 [66.1-229.1] ng/mL respectively; *p*=0.911).

Iron overload was not associated with AAA growth:

The influence of iron overload on AAA growth was assessed in a sub-group of 250 men with small AAAs (median AAA diameter 33.2 [IQR 31.2-36.5] mm) who underwent at least one additional ultrasound scan to measure AAA diameter in the 12-36 months after initial screening. Sixty five men with iron overload, and 185 men without iron overload were included in this growth analysis (Table 3). Two hundred and 13 (85.2%) men received 3 additional scans after recruitment, 20 (8.0%) were rescanned twice after recruitment, and 17 (6.8%) received 1 scan after recruitment. AAA growth rate was calculated for each year and compared between groups by linear mixed effects models. No difference in % annual increase in AAA diameter was observed between men who did or did not have iron overload (Table 3, and Supplementary File 1).

Discussion

Recent observations of i) iron deposits within the AAA wall; ii) co-localisation of aortic iron deposits with regions of oxidative stress and macrophage infiltration and iii), protection against AAA formation in experimental mice maintained on a low-iron diet (12, 13) have collectively stimulated the hypothesis that iron overload may be involved in AAA pathogenesis. To test this theory we investigated the association of iron overload with AAA diagnosis, size and growth, by measuring plasma ferritin concentrations in a large group of community-dwelling men screened for AAA. Plasma ferritin concentrations were not associated with AAA diagnosis or diameter, contrary to the assessed hypothesis. Moreover AAA growth was similar in men that did or did not have iron overload. These results collectively suggest that total body iron overload is not a major driver of AAA pathogenesis. This supposition is further supported by recent data from a small cohort of European patients with AAA which demonstrated no robust association of circulating concentrations of ferritin or hepcidin (the master regulator of iron retention) with AAA diagnosis (12).

Our results, however, do not rule out a pathological role for localised iron deposition in AAA. AAA is commonly accompanied by the formation of a large non-occlusive intraluminal thrombus (ILT) within the aneurysm sac, which has been suggested to be a major source of the iron deposited within the aortic wall (1, 12, 22, 23). The ILT remains in contact with the flowing blood and is continually remodelled allowing it to increase in volume in parallel with AAA expansion (24). Eventually the ILT forms a laminated neo-tissue comprising a blood-rich layer at the bloodstream interface and an acellular fibrinolysed region in contact with the AAA wall (25, 26). ILT remodelling results in the retention of circulating proteins, leukocytes and red blood cells (12, 22, 23, 27, 28). Relevant to the current study, lysis of red blood cells trapped within the ILT releases haem iron which is conveyed to the AAA wall (12, 22). This local aortic iron deposition could contribute to reactive oxidative species generation, which has been implicated in AAA (10, 12, 13).

The observation that iron reduced feed protected experimental mice against AAA, led Sawada and colleagues to suggest that reduction of total body iron through dietary intervention may have therapeutic potential for AAA by limiting the extent of aortic iron deposition (13). The current study suggests that total body iron overload is not present in patients with AAA. Furthermore, several studies have identified iron deficiency anaemia as a significant predictor for mortality in AAA patients (12, 29-32). It is therefore unlikely that iron restriction would be beneficial (and may even be harmful) for AAA patients. Our findings also highlight the need to carefully assess the relevance of rodent model findings to human AAA. It is likely that iron deposits within AAA are a result of remodelling of the large ILT not total body iron overload. It is plausible that anti-thrombotic therapy may provide a targeted means to reduce aortic iron accumulation in AAA patients. Moreover, as the ILT is also suggested to contribute to AAA pathogenesis by i) inducing hypoxia in ILT-covered regions of the aorta (33); and ii) providing a site for leukocyte and protease accumulation adjacent to the aneurysm sac (25, 26, 28, 34-36); anti-thrombotic therapy may provide additional benefits to AAA patients beyond reducing aortic iron deposition. This theory is supported by findings of studies demonstrating that experimental animals receiving platelet inhibitors show significant reductions in AAA diameter or rupture incidence coincident with lowered matrix metallo-proteinase activity and aortic inflammation, when compared to controls (37-39). It has also been reported in one surveillance study that patients with 40-49mm AAAs had slower AAA growth when prescribed aspirin (40). However, many AAA surveillance studies have reported no association between anti-platelet medication prescription and AAA growth although a current randomised trial is examining the benefit of ticagrelor in limiting AAA growth (41).

Findings of the current study should be considered in light of its limitations. First, total body iron was estimated by measuring plasma ferritin, which is known to rise under inflammatory conditions (15). AAA is an inflammatory disease (1), however, we observed no correlation between circulating hsCRP and ferritin concentrations, and inflammation appears an unlikely

confounder for this study. Moreover, circulating ferritin concentration is a well-accepted marker of iron status since it directly correlates with total body iron (15). Thus we believed that this protein was the most appropriate biomarker to include in the current study. Other markers of iron metabolism including circulating hepcidin, iron and haemoglobin concentration have been previously assessed in smaller groups of AAA patients, and have not been shown to be robustly associated with AAA (12). Accordingly, these other markers of iron metabolism were not measured in the current study. Second, the current study assessed community-dwelling men rather than a hospital cohort, and detected AAAs were mostly small (median AAA diameter 33.7 mm). In contrast, prior studies reporting an association of iron-related proteins with AAA were conducted in patients with large (≥55mm) AAAs (12, 13). It is possible that ferritin concentrations may differ between patients with large AAAs and non-aneurysmal controls, although we observed no correlation between AAA diameter and plasma ferritin, no difference in circulating ferritin concentrations between men with large AAAs >50mm and non-aneurysmal controls, and no association of iron overload with AAA growth. It is also important to note that our findings are based on the analysis of a single blood sample and it is possible that assessment of repeated blood samples may have shown different results. Finally, the samples assessed in the current study were collected from men aged over 65 years, and the possibility that iron overload earlier in life or in women may promote AAA formation cannot be discounted.

Acknowledgements

We thank Australian Institute of Tropical Health and Medicine for providing research infrastructure.

Declarations of interest: None

Funding

This work is supported by grants from James Cook University, the Australian National Health and Medical Research Council (NHMRC 1079193, 1079369, 1022752, 1021416, 1000967), The Townsville Hospital Private Practice Trust Fund and the Queensland Government. JG holds a Practitioner Fellowship from the NHMRC, Australia and a Senior Clinical Research Fellowship from the Queensland Government.

Author contributions

Participant recruitment, screening and collection of biological samples and clinical data: PEN, LF, OPA, GJH, BBY and JG. Study design: JG, PEN, JVM. Laboratory assessment of plasma ferritin: PC. Statistical analysis and interpretation of data: JVM, REJ, JG. Manuscript preparation: JVM, JG. All authors provided critical evaluation of the final manuscript and agree with its contents and publication.

Clinical perspective

There is no current medical treatment to slow abdominal aortic aneurysm (AAA) growth. Recent animal data have suggested that an iron-reduced diet protects against AAA formation, suggesting that i) total body iron overload may be involved in AAA pathogenesis, and ii) reduction of iron may be a treatment for AAA. Here, we assessed whether total body iron overload as assessed by circulating ferritin concentration was associated with AAA diagnosis, size and growth in a large cohort of community-screened men. Our data demonstrate no relationship between serum ferritin and AAA and do not support iron reduction as a therapy for AAA.

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133 **Table 1:** Characteristics of 4024 HIMS participants included in the current study.

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Characteristic	Whole cohort (n=4024)	No AAA (n=3731)	AAA (n=293)	*p* value[a]
Abdominal aortic diameter (mm)	21.6 (20.2-23.8)	21.4 (20.0-23.1)	33.7 (31.5-39.3)	<0.001
AAA risk factors and co-morbidities				
Age	70.0 (67.0-73.0)	70.0 (67.0-73.0)	71.0 (68.0-74.0)	<0.001
Hypertension	1715 (42.6%)	1564 (41.9%)	151 (51.5%)	0.001
Diabetes	332 (8.3%)	302 (8.1%)	30 (10.2%)	0.199
Ever smoking	2597 (64.5%)	2344 (62.8%)	253 (86.3%)	<0.001
CHD	1237 (30.7%)	1102 (29.5%)	135 (46.1%)	<0.001
BMI	26.5 (24.4-28.7)[b]	26.5 (24.4-28.7)[b]	27.3 (25.1-29.5)	<0.001
Circulating concentrations of				
LDL-C (mmol/L)	2.8 (2.3-3.4)[c]	2.9 (2.3-3.4)[c]	2.6 (2.1-3.3)	<0.001
HDL-C (mmol/L)	1.3 (1.1-1.6)	1.4 (1.1-1.6)	1.2 (1.0-1.5)	<0.001

Creatinine (µmol/L)	89.0 (78.0-102.0)	88.0 (78.0-101.0)	96.0 (83.0-115.5)	<0.001
hsCRP (mg/L)	1.9 (1.0-3.8)[d]	1.8 (1.0-3.7)[d]	2.5 (1.4-5.1)	<0.001
Homocysteine (mol/L)	12.4 (10.3-15.1)[d]	12.3 (10.2-14.9)[d]	13.9 (11.4-17.5)	<0.001
Ferritin ng/mL	128.0 (65.7-227.3)	128.5 (66.1-229.1)	115.4 (63.0-203.1)	0.124
Iron overload[e]	1222 (30.4%)	1148 (30.8%)	74 (25.3%)	0.048

CHD: Coronary heart disease; BMI: Body mass index; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; hsCRP: C-reactive protein.

[a]Comparing men with and without AAA. Nominal variables are reported as count and %, and compared using the Chi-squared test. Continuous variables are reported as median and IQR, and compared via Mann-Whitney U test.; [b] 3 missing values; [c] 2 missing values; [d] 1 missing value; [e] Defined as circulating ferritin concentrations >200 ng/mL.

142 **Table 2:** Binary logistic regression testing the association of iron overload (plasma ferritin
143 >200ng/mL) with AAA adjusting for AAA risk factors and factors known to influence
144 circulating ferritin concentration in 4024 community screened men.

Covariate	Odds Ratio	95% CI	*p* value
Model 1: Unadjusted analysis			
Iron overload	0.760	0.579-0.998	0.049
Model 2: Adjusted for AAA and AAA risk factors			
Iron overload	0.774	0.586-1.022	0.071
Ever smoking	3.514	2.496-4.949	<0.001
History of hypertension	1.145	0.889-1.474	0.293
History of diabetes	1.066	0.708-1.603	0.760
History of CHD	1.861	1.449-2.390	<0.001
Older age[a]	1.375	1.189-1.591	<0.001
Body mass index [3][b]	1.291	1.144-1.456	<0.001
Model 3: Adjusted for AAA risk factors and factors affecting circulating ferritin[c]			
Iron overload	0.781	0.589-1.035	0.086
Ever smoking	3.516	2.493-4.958	<0.001
History of hypertension	1.302	1.008-1.681	0.043
History of diabetes	0.984	0.651-1.486	0.939
History of CHD	2.161	1.653-2.827	<0.001
Older age[a]	1.360	1.172-1.579	<0.001
Body mass index [3][b]	1.242	1.099-1.404	0.001
Aspirin prescription [184]	1.157	0.883-1.516	0.292
Hours non-vigorous exercise per week			

0		Reference	
1-4	0.701	0.520-0.946	0.020
>4	0.684	0.506-0.925	0.014
Hours vigorous exercise per week			
0		Reference	
1-4	0.853	0.619-1.177	0.334
>4	0.739	0.478-1.143	0.174
Weekly meat consumption [184]			
None		Reference	
1-2 servings	0.984	0.285-3.403	0.980
3 or more servings	1.261	0.373-4.260	0.709

Abbreviations: 95% CI: 95% confidence interval. CHD: Coronary heart disease.

Square brackets indicate number of missing data points for that variable. For nominal variables, men who had the risk factor were compared to those who did not. [a] Refers to a 5 year increase in age. [b] Odds ratio for an increase of ~1 std deviation in BMI (3.4).

[c] Including dietary and non-dietary factors known to influence circulating ferritin concentrations (see (21)).

152 **Table 3:** Characteristics of 250 HIMS participants with and without iron overload in whom AAA growth was assessed.

Characteristic	**No iron overload (plasma ferritin <200 ng/mL)**	**Iron overload (plasma ferritin >200 ng/mL)**	***p* value**
Number	185	65	---
	AAA risk factors and co-morbidities:		
Age	71 (68-75)	71 (68-74)	0.357
Hypertension	94 (50.8%)	31 (47.7%)	0.665
Diabetes	20 (10.8%)	7 (10.8%)	0.993
Ever smoking	155 (83.8%)	60 (92.3%)	0.088
CHD	76 (41.2%)	31 (47.7%)	0.354
BMI[a]	27.2 (25.1-29.3)	27.2 (25.2-29.4)	0.722
AAA diameter at screening	33.2 (31.2-36.5)	33.1 (31.2-35.8)	0.446
	Circulating concentrations of:		
LDL-C (mmol/L)	2.6 (2.0-3.3)	2.7 (2.1-3.4)	0.940

HDL-C (mmol/L)	1.2 (1.0-1.4)	1.3 (1.0-1.5)	0.665
Creatinine (µmol/L)	95.0 (82.5-112.0)	90.0 (78.5-117.5)	0.411
hsCRP (mg/L)	2.3 (1.2-4.8)	2.7 (1.5-6.3)	0.338
Homocysteine (mol/L)	13.6 (11.4-17.9)	14.3 (11.3-17.2)	0.745
Ferritin (ng/mL)	85.4 (47.1-134.3)	312.5 (252.9-442.9)	<0.001
	Percent growth per year:[b]		0.164[c]
Screening to year 1	4.3 ± 0.4[183]	3.5 ± 0.6 [65]	
Years 1 to 2 (%)	3.4± 0.4[166]	2.7 ± 0.6 [62]	
Years 2 to 3 (%)	3.1± 0.4 [153]	2.9 ± 0.6 [62]	

CHD: Coronary heart disease; BMI: Body mass index; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; hsCRP: C-reactive protein. [a] Denotes 3 missing data points. [b] Reported as mean and standard error (unadjusted for random effects), square brackets denote number of patients included in each group for each time point. [c] *p* value comparing AAA growth rate between groups over the follow up period assessed via linear mixed effects modelling. Nominal variables are reported as count and %, and compared using the Chi-squared test. Continuous variables are reported as median and IQR and are compared via the Mann-Whitney U test.

Moxon *et al:* Plasma ferritin concentrations are not associated with abdominal aortic aneurysm diagnosis, size or growth. Supplementary material.

Supplementary Table 1: Binary logistic regression testing the association of circulating ferritin concentration with AAA in 4024 community screened men.

Supplementary Table 2: The correlation of plasma ferritin concentration with infra-renal aortic diameter and demographic and biochemical characteristics of: A) All participants of the current study (n=4,024); B) Men who did not have AAA (n=3,731); and C) Men who had an AAA (n=293).

Supplementary Table 3: Characteristics of 293 participants in the current study who had an AAA, stratified into tertiles according to AAA diameter.

Supplementary File 1: Statistical output of linear mixed effect model assessing the effect of iron overload on AAA growth.

Supplementary Table 1: Binary logistic regression testing the association of circulating ferritin concentration with AAA in 4024 community screened men.

Covariate	Odds Ratio	95% CI	*p* value
Model 1: Unadjusted analysis			
Plasma ferritin[a]	0.880	0.764-1.015	0.078
Model 2: Adjusted for AAA risk factors			
Plasma ferritin[a]	0.891	0.774-1.026	0.110
Ever smoking	3.504	2.489-4.934	<0.001
History of hypertension	1.149	0.892-1.479	0.282
History of diabetes	1.065	0.708-1.603	0.761
History of CHD	1.866	1.453-2.396	<0.001
Older age[b]	1.374	1.188-1.590	<0.001
Body mass index [3][a]	1.291	1.145-1.456	<0.001
Model 3: Adjusted for AAA risk factors and potential confounders[c]			
Plasma ferritin[a]	0.898	0.778-1.035	0.138
Ever smoking	3.508	2.488-4.948	<0.001
History of hypertension	1.303	1.009-1.682	0.042
History of diabetes	0.986	0.653-1.489	0.948
History of CHD	2.159	1.651-2.823	<0.001
Older age[b]	1.359	1.171-1.577	<0.001
Body mass index [3][a]	1.242	1.099-1.403	0.001
Aspirin prescription [184]	1.165	0.889-1.526	0.267
Hours non-vigorous exercise per week			
0		Reference	
1-4	0.700	0.519-0.944	0.020
>4	0.683	0.505-0.923	0.013
Hours vigorous exercise per week			
0		Reference	
1-4	0.856	0.620-1.180	0.342
>4	0.735	0.476-1.137	0.167
Weekly meat consumption [184]			
None		Reference	
1-2 servings	0.999	0.289-3.456	0.999
3 or more servings	1.285	0.380-4.343	0.687

Abbreviations: 95% CI: 95% confidence interval. CHD: Coronary heart disease.

Square brackets indicate number of missing data points for that variable. For nominal variables, men who had the risk factor were compared to those who did not.

[a]Odds ratios refers to an increase of ~1 std deviation in plasma ferritin (175 ng/mL) and BMI (3.4).

[b] Refers to a 5 year increase in age.

[c] Includes dietary and non-dietary factors known to influence circulating ferritin concentrations (detailed by Liu *et al.* (2003), Am J Clin Nutr 78:1160-7).

Supplementary Table 2: The correlation of plasma ferritin concentration with infra-renal aortic diameter and demographic and biochemical characteristics of: A) All participants of the current study (n=4,024); B) Men who did not have AAA (n=3,731); and C) Men who had an AAA (n=293).

	Plasma ferritin (ng/mL) in:					
	A) All participating men (n=4,024)		**B) Men without AAA (n=3,731)**		**C) Men with AAA (n=293)**	
Characteristic	Spearman's rho	*p* value	Spearman's rho	*p* value	Spearman's rho	*p* value
Infra-renal aortic diameter (mm)	-0.18	0.246	-0.009	0.603	-0.006	0.919
Age (years)	-0.051	0.001	-0.044	0.007	-0.110	0.060
BMI	0.058 [3]	<0.0001	-0.063 [3]	<0.001	0.005	0.931
Circulating LDL-C (mMol/L)	0.042 [2]	0.008	0.047 [2]	0.004	-0.058	0.325
Circulating HDL-C (mMol/L)	-0.010	0.517	-0.016	0.322	0.026	0.657
Circulating homocysteine (Mol/L)	-0.077 [1]	<0.001	-0.079 [1]	<0.001	-0.026	0.663
Circulating creatinine (µMol/L)	-0.028	0.079	-0.024	0.135	-0.018	0.764
Circulating hsCRP (mg/L)	0.023 [1]	0.147	0.022 [1]	0.184	0.074	0.206

Abbreviations: LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol.

Numbers in square brackets denote the number of missing data-points for that characteristic.

Supplementary Table 3: Characteristics of 293 participants in the current study who had an AAA, stratified into tertiles according to AAA diameter.

Characteristic	Tertile 1 (n=106)	Tertile 2 (n=95)	Tertile 3 (n=92)	*p* value[a]
Abdominal aortic diameter (mm)	31.0 (30.5-31.7)	34.0 (33.2-35.2)	43.5 (40.0-47.5)	<0.001
AAA risk factors and co-morbidities				
Age (years)	72.0 (68.0-75.0)	71.0 (68.0-73.0)	72.0 (68.0-75.0)	0.390
Hypertension	49 (46.2%)	50 (52.6%)	52 (56.5%)	0.340
Diabetes	9 (8.5%)	11 (11.6%)	10 (10.9%)	0.749
Ever smoking	92 (8.7%)	80 (84.2%)	81 (88.0%)	0.737
CHD	48 (45.3%)	40 (42.1%)	47 (51.1%)	0.459
BMI	27.1 (25.1-29.4)	27.2 (25.4-29.4)	27.7 (24.3-29.9)	0.897
Circulating concentrations of				
LDL-C (mmol/L)	2.7 (2.0-3.4)	2.7 (2.2-3.3)	2.4 (1.9-3.0)	0.034
HDL-C (mmol/L)	1.3 (1.0-1.5)	1.3 (1.1-1.4)	1.2 (1.0-1.4)	0.232
Creatinine (µmol/L)	100.0 (82.0-116.5)	90.0 (80.0-104.0)	101.5 (89.3-136.8)	0.001
hsCRP (mg/L)	2.6 (1.6-4.8)	2.4 (1.2-5.0)	2.4 (1.4-5.6)	0.757
Homocysteine (mol/L)	13.9 (11.6-17.5)	13.7 (11.2-17.9)	13.9 (10.7-17.4)	0.784
Ferritin ng/mL	113.4 (60.8-219.8)	106.1 (59.9-207.1)	121.2 (66.3-195.5)	0.996
Iron overload[b]	28 (26.4%)	25 (26.3%)	21 (22.8%)	0.811

[a]Calculated using the Kruskal-Wallis test (continuous variables) or Chi-squared test (nominal variables).
[b] Defined as plasma ferritin concentrations >200 ng/mL.

CHD: Coronary heart disease; BMI: Body mass index; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; hsCRP: C-reactive protein.

Supplementary File 1: Statistical output of linear mixed effect model assessing the effect of iron overload on AAA growth.

This analysis was conducted using the freely available 'car', and 'nlme' R packages (1,2).

Linear mixed effects models were constructed to assess changes in AAA diameter over time between groups of patients with/without iron overload. Individual patients were treated as random effects. Fixed effects were Iron.overload, interval and startSize (assuming an interaction between Iron.overload and interval). Variables in this analysis were:

Rate: Growth rate of AAA for that period calculated as AAA diameter at start of period/AAA diameter at start of prior period

Sequence: Arbitrary participant ID

Iron Overload: Does the participant have iron overload evidenced by plasma ferritin concentration >200 ng/mL.(coded as 0=no, 1=yes)

Interval: Time period assessed (e.g. rate0.1 = time between screening and year 1 rescan)

startSize: AAA diameter at the start of the interval

Creating models

```
AAA.growth.lme<-lme(rate~Iron.overload*interval + startSize,
                    random=~1|Sequence, data=AAA.growth,
                  na.action='na.omit')

anova(AAA.growth.lme)
##                          numDF denDF F-value p-value
## (Intercept)                  1   436  308566  <.0001
## Iron.overload                1   246       2  0.1635
## interval                     2   436       3  0.0569
## startSize                    1   436       3  0.0710
## Iron.overload:interval       2   436       0  0.8275
```

Calculating estimates and standard errors of AAA growth per interval in patients with and without iron overload (no adjustment for random effects).

These data will be generated by fitting and summarizing linear models to assess AAA growth rates in subsets of men without iron overload (first model), and with iron overload.

```
1 – Model for men without iron overload

no.iron.overload.lm<-lm(rate~interval-1, data=AAA.growth,
                        subset=Iron.overload=='0', na.action=na.exclude)
summary(no.iron.overload.lm)
##
## Call:
## lm(formula = rate ~ interval - 1, data = AAA.growth, subset =
Iron.overload ==
##     "0", na.action = na.exclude)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
```

```
## -0.14605 -0.03412 -0.00694  0.02743  0.18405
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## intervalrate0.1  1.04275    0.00371     281   <2e-16 ***
## intervalrate1.2  1.03424    0.00390     265   <2e-16 ***
## intervalrate2.3  1.03091    0.00406     254   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.0502 on 499 degrees of freedom
##   (53 observations deleted due to missingness)
## Multiple R-squared:  0.998,  Adjusted R-squared:  0.998
## F-statistic: 7.12e+04 on 3 and 499 DF,  p-value: <2e-16


2 – Model for men with iron overload
iron.overload.lm<-lm(rate~interval-1, data=AAA.growth,
                     subset=Iron.overload=='1', na.action=na.exclude)
summary(iron.overload.lm)
##
## Call:
## lm(formula = rate ~ interval - 1, data = AAA.growth, subset =
Iron.overload ==
##     "1", na.action = na.exclude)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.16445 -0.02707 -0.00471  0.02435  0.11740
##
## Coefficients:
##                 Estimate Std. Error t value Pr(>|t|)
## intervalrate0.1  1.03496    0.00571     181   <2e-16 ***
## intervalrate1.2  1.02707    0.00576     178   <2e-16 ***
## intervalrate2.3  1.02931    0.00576     179   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.0454 on 184 degrees of freedom
##   (8 observations deleted due to missingness)
## Multiple R-squared:  0.998,  Adjusted R-squared:  0.998
## F-statistic: 3.22e+04 on 3 and 184 DF,  p-value: <2e-16

#Note, to convert coefficients to % growth, all estimates were treated as
(1-estimate)*100. Std errors were multiplied by 100.
```

References

1. Fox J and Weisberg S (2011). An R companion to applied regression, Second Edition. Thousand Oaks, CA: Sage.

2. Pinheiro J, Bates D, DebRoy S, Sarkar D and the R Development Core Team (2013). nlme: Linear and nonlinear mixed effects models. R package version 3.1-111.