FACTORS INFLUENCING SERUM FERRITIN IN AN AUSTRALIAN ADULT POPULATION: IMPLICATIONS FOR CLINICAL FOLLOW-UP

Short Title: Ferritin reference range levels in adults

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Abbreviations: Body mass index (BMI), γ-glutamyltransferase (GGT)

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Abstract

Background & Aims: Serum ferritin levels are commonly used for assessment of iron stores but are influenced by other factors including obesity and chronic disease. Published reference ranges have remained unchanged for decades and upper limits are progressively being exceeded by greater numbers of individuals, prompting evaluation for potential iron overload.

Methods: Ferritin levels in 1188 Australian adults of the 2005 Busselton Population Survey were compared with those from the 1995 survey. Parametric regression assessed impact of body weight and biochemical parameters on serum ferritin to derive contemporary population-appropriate reference ranges.

Results: Age-adjusted ferritin levels were 21% (males, $P<0.0001$) and 10% (females, $P=0.01$) higher than determined in 1995; 31% of males exceeded levels of 300 µg/L compared with 23% previously. Body mass index (BMI) ≥25 kg/m^2 was associated with higher ferritin levels amongst males ≥35 years and postmenopausal females ($P\leq0.002$). Serum γ-glutamyltransferase (GGT) was the principal biomarker correlating with serum ferritin ($P<0.0001$). The estimated 95th centile for males varied from 353 to 495 µg/L (< 35 years), 350 to 511 µg/L (≥ 35 years, BMI < 25 kg/m^2), and 413 to 696 µg/L (≥35 years, BMI ≥25 kg/m^2) over GGT of 10-75 IU/L; for females, this centile varied from 106 to 235 µg/L (premenopausal), 222 to 323 µg/L (postmenopausal, BMI <25 kg/m^2) and 249 to 422 µg/L (postmenopausal, BMI ≥25 kg/m^2) over GGT of 8-45 IU/L.

Conclusion: Serum ferritin levels have significantly increased in recent years. Reference ranges which accommodate demographic and biomedical variation will assist clinicians in the correct identification of individuals requiring further evaluation for iron overload.

Keywords: hyperferritinemia, obesity, gamma glutamyltransferase

Introduction

Measurements of serum ferritin levels provide important information regarding body iron stores. Serum ferritin levels ≤20 µg/L are present in about 12% of women and 2% of men in the adult population and are suggestive of iron depletion,[1,2] requiring assessment of physiological or pathological causality.[3] Elevation of serum ferritin levels to values ≥300 µg/L is also commonplace, affecting 4% of women and 23% of men in an Australian population surveyed in 1995.[4]

Increasing ascertainment of adults with elevated serum ferritin levels has resulted in escalating rates of referral for evaluation or treatment of possible iron overload. Indeed, 36% of all referrals to the Australian Red Cross Blood Service for therapeutic phlebotomy in Australia are for such individuals.[5,6] However, serum ferritin levels are strongly influenced by dietary intake of iron containing products, alcohol consumption and body mass index (BMI),[2] and hyperferritinemia is observed not only in iron overload syndromes but also in subjects with other chronic liver diseases and non-hepatic inflammatory disorders.[7-10] The predictive value of elevated serum ferritin levels for iron overload is highest in those individuals with either a history of exposure to exogenously administered iron or blood products, or individuals of northern European descent who are homozygous for the C282Y mutation in the HFE gene.[11] In such individuals, serum ferritin levels >1000 µg/L indicate an increased risk of disease affecting the liver, joints and other organs.[12,13,14] It has been clearly demonstrated that hyperferritinemia outside of these clinical settings is associated with either normal or minimal elevation of hepatic iron concentration to levels not usually associated with iron-overload disease.[11,14] While interest exists in iron reduction therapy for amelioration of cancer

risk, improvement of insulin sensitivity in the metabolic syndrome and management of fatty liver disease, at the current time phlebotomy therapy is of no proven value in this group of individuals.[15-18]

Published reference ranges for serum ferritin levels were often derived many years ago and the upper limits of normal, which are mostly in the vicinity of 300-400 μg/L (Supplementary Table 1), are progressively being exceeded by greater numbers of individuals, prompting evaluation for potential iron overload. Differences in published reference ranges are most evident in the upper limit, and it is not clear if ranges are based on values of historical or clinical relevance, or determined from observed population central ranges. Given the increasing rates of obesity and the metabolic syndrome in most western populations,[19] it is quite likely that the previously determined reference ranges for serum ferritin within our population have changed over time.[2,4,20] The aims of this study were to (1) characterize how serum ferritin levels have changed over time, (2) define the upper limit of a population-appropriate reference range for serum ferritin, and (3) determine the effect of body weight and biochemical parameters including glucose, insulin, lipid and liver biochemistry on the reference range in a community-based study of adults.

Materials and Methods

Population and subjects. Busselton is a town in the southwest of Western Australia that has been prospectively surveyed since 1966 and is in many respects similar to the Framingham population.[21] Approximately 95% of the adult Busselton population is Caucasian, predominantly of Anglo-Celtic ancestry, and 1.5% Indigenous Australian (2006 census). Of

residents born overseas (15.2%) nearly three-quarters have emigrated from Europe, mostly the United Kingdom and Western Europe. In 2005, 6544 adults aged 20 to 79 years were randomly selected from the electoral roll and invited to participate, and data was collected from 2549 (39.0%). Of these participants, 81% were born in Australia, 16% in Europe and 1% in New Zealand. For the study presented here the cohort was stratified by age, with 100 men and 100 women of the 2549 adults randomly selected from each decade between 20 and 80 years, with the exception of males aged 20-29 where only 89 participants were available. Comparisons are made with data from 2991 randomly selected participants (51% male) of a 1995 study conducted as a follow-up to earlier surveys; 87% of this cohort were born in Australia and 11% in Europe. Permission was granted for this study by the Busselton Population Medical Research Foundation and The Committee for Human Rights at The University of Western Australia.

Biochemical and clinical measurements. The conduct of the Busselton health surveys has been described previously.[2,4,22,23] In brief, survey participants were asked to complete a comprehensive health and lifestyle questionnaire and to undergo various clinical measurements and tests. Anthropometric measures (waist circumference, weight and height - from which BMI was calculated) were obtained by trained survey staff using standardized protocols.

All blood samples were obtained from fasting participants in the early morning. Serum alkaline phosphatase, alanine aminotransferase and γ-glutamyltransferase (GGT) enzyme activities, total bilirubin and C-reactive protein, cholesterol and high-density cholesterol concentrations were determined at the time of the survey using a Hitachi 917 automated biochemical analyzer (Hitachi, Tokyo, Japan). Serum ferritin concentrations of the 2005 cohort were determined in 2012 on sera retrieved from cryofacility storage at -80 °C using a chemiluminescent microparticle immunoassay technology on an Abbott Architect 16200 automated analyzer (Abbott, Abbott Park, IL). Ferritin assay performance was assessed by calculating between assay analytical variations (CV_A) from the mean and SD of results for three quality control sera assayed daily over a three-month period. The CV_A values for ferritin were 5.3%, 3.3% and 4.4% at concentrations of 7, 59 and 440 µg/L, respectively. Ferritin analysis of the 1995 dataset used as a comparator had been performed in 1998 by a Chiron ACS-180 analyser (Ciba-Corning, Medfield, MA), having CV_A values for ferritin of 4.2%, 9.5% and 5.2% at concentrations of 9, 78 and 327 µg/L, respectively. Internal laboratory assessment of between-machine variation detected a downward bias in readings obtained from the later Architect machine, with readings estimated to be approximately 20% lower than if analysed by the ACS machine used earlier.

Statistical analysis. Cohort summaries utilized nonparametric statistics, including group medians and interquartile ranges, and Spearman correlations. Within-sex differences between biomarker values grouped according to age (</≥ 50 years) were assessed by Mann-

Whitney tests, and differences between proportions with BMI ≥25 or 30 kg/m^2 by application of Pearson's chi-squared test. More formal analyses of serum ferritin levels were undertaken within a parametric regression framework, assuming an underlying Weibull distribution (see Supplementary information).

Modeling excluded extreme ferritin values of >1000 µg/L for males (n=2) and >800 µg/L for females (n=1), and values less than 20 µg/L were left-censored at 20 (n=4 males, n=44 females) to minimize influence of individuals with possible iron deficiency. Parametric estimation of reference limits was conducted over the central 90% range of observed GGT, the principal correlate of serum ferritin (males: 10-75 IU/L; females: 8-45 IU/L) utilizing models that included both linear and squared terms of log-transformed GGT. Women less than 45 years of age were considered as premenopausal, and those older than 55 years as postmenopausal. Analyses were undertaken using TIBCO Spotfire S+ 8.2 for Windows (TIBCO Software Inc., Palo Alto, CA).

Results

Baseline characteristics. Baseline study characteristics of the entire cohort and with stratification by age and gender are presented in Table 1. Males had significantly higher values for all parameters compared with females, excepting total cholesterol and insulin. Males aged 50 years and over had significantly higher BMI, weight, GGT, bilirubin, c-reactive protein, glucose, insulin and HOMA-IR values, but lower alanine aminotransferase, than

males aged less than 50 years. Females aged 50 years and over had higher values for all parameters, excepting bilirubin, compared with those aged less than 50 years.

Changes in serum ferritin values over time. Serum ferritin measures amongst the 2005 cohort were observed to be higher than those amongst individuals surveyed in 1995 (Figure 1). The effect was consistent across most age groups and for both males (21% increase, $P < 0.0001$ in age-adjusted regression model) and females (10% increase, $P = 0.01$), and translated to a notable increase in the proportions of individuals with levels elevated above the historical limit of 300 µg/L (males: 31% *vs* 23%; females: 5% *vs* 4%). Higher BMI values were also observed amongst participants of the later survey ($P < 0.0001$, Figure 1). Whilst the correlations between serum ferritin and BMI were higher for the 2005 cohort (1995: males r = 0.15, females r = 0.17; 2005: males r = 0.25, females r = 0.22), the cohort differences in ferritin values remained even after taking account of the association with BMI, particularly for the males; following adjustment for BMI and age, median values for the 2005 cohort were approximately 20% higher amongst males ($P < 0.0001$ compared with 1995 cohort) and 8% higher amongst females ($P = 0.05$ compared with 1995 cohort).

Variables influencing serum ferritin levels. Associations of ferritin with age, BMI and available biomarkers for the 2005 cohort are presented in Table 2. Of the biomarkers considered, ferritin levels were observed to correlate most strongly with GGT, an association that was evident for both sexes (males: r = 0.33, $P < 0.0001$; females: r = 0.35, $P < 0.0001$). BMI and waist circumference correlations with serum ferritin were quite similar, and also similar for both males and females ($0.23 \leq r \leq 0.25$, all $P < 0.0001$). A weak association

between alcohol intake and GGT was observed amongst males, but alcohol intake did not correlate with serum ferritin for either sex. Notably, for most biomarkers the correlation with GGT was greater than with the serum ferritin. Scattergram data plots (Supplementary Fig 1) reveal that the observed correlations reflect general associations with serum ferritin levels rather than the ability of any particular parameter to predict elevation above some threshold value. Skewing in distribution of both serum ferritin and GGT is consistent with previous reports (Supplementary Fig 2).

Derivation of serum ferritin reference range. To guide the derivation of clinically useful reference ranges, exploratory parametric analyses focused on GGT, the principal biomarker correlating with serum ferritin levels, together with age and BMI, which are readily categorized in a clinical setting. The effect of these variables was considered both singly and jointly in a series of sex-specific models (see supplementary information for details). The analyses found BMI greater than 25 kg/m^2 to be associated with significantly higher ferritin levels amongst older men (≥ 35 years, $P < 0.001$) and postmenopausal women ($P = 0.002$), but not amongst younger individuals. Values for those considered obese (BMI ≥ 30 kg/m^2) were similar to those only overweight (BMI: 25-30 kg/m^2). A consistent correlation of serum ferritin with GGT was observed across all age groups with the associations remaining significant over and above any effect of higher BMI.

Consideration of the findings of the exploratory analyses indicated that reference limits could be derived according to appropriate categorization of age and BMI, with further adjustment to reflect increasing limits with increasing GGT levels. Unadjusted reference limits obtained from

observed sub-group 5th and 95th ferritin centiles are presented in Table 3. Further refinements to these limits are then displayed in the plots of Figure 2, where the corresponding ranges have been estimated over GGT levels spanning approximately 90% of the gender-specific values (males: 10-75 IU/L; females: 8-45 IU/L). As observed effects of BMI and GGT were similar for middle-aged and males ≥60 years in the exploratory analyses, we considered men as either younger than 35 years or ≥35 years, with those in the older age bracket dichotomized as normal BMI (BMI <25 kg/m^2) or overweight/obese (BMI ≥25 kg/m^2). Unadjusted 5-95th ferritin centiles ranged from 54-561 µg/L amongst all males combined, with corresponding sub-group ranges of 50-409 µg/L (males <35 years), 45-476 µg/L (≥35 years, BMI <25 kg/m^2) and 68-585 µg/L (≥35 years, BMI ≥25 kg/m^2). For those younger than 35 years the ferritin levels were observed to increase by approximately 140% over the range of GGT levels, with the estimated reference range increasing from 36-353 to 51-495 µg/L (Figure 2). Ferritin levels of older males of normal weight increased similarly over the range of GGT levels. In contrast, the estimates for older males who were either overweight or obese increased by nearly 170% over the same range of GGT levels, yielding a maximum reference range of 71-696 µg/L corresponding to GGT of 75 IU/L. Limits for women are presented for those aged <45 years (premenopausal), 45-54 years, and ≥55 years of age (postmenopausal), with the older sub-groups again dichotomized on the basis of their BMI. Amongst premenopausal women, the unadjusted 5-95th centiles ranged from 13-157 µg/L, but in a reflection of the strong effect observed in both univariate and multivariate analyses, estimated centile values increased by over 200% over the range of GGT levels yielding a maximum upper limit of 235 µg/L corresponding to GGT of 45 IU/L. Whilst postmenopausal women had higher serum ferritin levels on average (unadjusted reference range of 25-346

μg/L), more modest increases were observed over the same GGT range; those of normal weight had estimated limits ranging from 18-222 to 26-323 μg/L, and from 20-249 μg/L increasing to 34-422 μg/L for those whose BMI exceeded 25 kg/m^2. We note an increased variability in ferritin levels for women in the 45-54 years age group (Table 3; Supplementary Figure 1) accompanied by a strong correlation with GGT levels (Supplementary Table 2). For most women menopause occurs between 45-54 years; hence, the greater ferritin variability within this age bracket reflects a mix of data from women before and after cessation of menstrual blood loss.

Discussion

This study demonstrates an increase over time in population levels of serum ferritin, with levels affected by biochemical and clinical parameters which we contend should be accommodated in revised reference ranges. The ability to determine if observed serum ferritin levels are either within or above a “normal” reference range requires definition of ranges appropriate to the patient characteristics and we demonstrate the utility of simple categorizations based on gender, stage of life and body mass. The incorporation of adjustments for GGT levels provides further refinement to the categorical ranges which enables assessment tailored to the individual.

The upper limits of the ranges derived in the present study reach values that are substantially higher than most currently published reference ranges (Supplementary Table 1). The implications of our observations are profound - clearly, current published reference ranges for adult serum ferritin levels result in excessive numbers of “elevated values” being reported and

triggering investigation which is likely to be expensive and of limited value in terms of yielding subjects with clinically relevant iron overload. Indeed, the number of males who now exceed the value of 300 µg/L, an historical upper limit of normal that is still commonly used, has risen from 23% to 31% over the last 10 years. We believe that the adoption of population-based reference ranges such as presented here would facilitate more targeted identification of individuals for potential further investigation.

The principal clinical use of serum ferritin levels in the community reflects its role as a biomarker of iron status. Ferritin performs most reliably in iron deficiency where levels less than 20 µg/L are known to reflect true iron store depletion.[1] Elevation of the upper limit of the ferritin reference range may raise potential concern regarding possible under-diagnosis of clinically relevant iron overload at an early stage in those individuals who have a serum ferritin level greater than the current upper limit of normal but below that which we propose. However, this is unlikely to present as a clinical problem. Outside of genetically defined Hereditary Haemochromatosis and secondary iron overload syndromes, serum ferritin levels do not correlate with body iron stores.[10] Furthermore, even in the presence of an iron loading disorder, ferritin levels below 1000 µg/L have not been shown to increase the risk of adverse clinical outcomes.[11,12,24-26]

The correlation of ferritin levels with GGT was not only the strongest of the biomarkers we examined, it was evident for both sexes, remained consistent across all age groups and persisted when adjusted for BMI. The observed associations of both ferritin and GGT with BMI, liver enzymes, lipids and indices of insulin resistance most likely reflect a complex

interplay between these parameters, with GGT in particular serving as a useful marker of metabolic syndrome and relevant across all ages. In the current context, the value of considering the effect of biomarkers on ferritin levels lies in understanding that higher levels accompany metabolic syndrome components without necessarily implying iron overload, and this should be taken into account when considering the need for further follow-up.

Potential limitations of our study include its reference group being Australians of predominantly NorthernEuropean descent. Differences in dietary and lifestyle factors, such as red meat and alcohol consumption, may influence suitability of the reference ranges presented here for other populations, even those with similar ancestry. However, we believe that incorporation of the biomarkers BMI and GGT enhances general applicability of these ranges and will minimize the effects of such differences. It is also likely that other populations may have similar qualitative but different quantitative parameters related to serum ferritin levels and the factors which influence ferritin. Evidence of differences between ethnic groups has been presented in the HEIRS study, which reported that the prevalence of elevated ferritin at several different cut-offs varied considerably according to ethnicity.[27] For example, 19.0% of Asian subjects exceeded a cut-off of 400 μg/L for men and 300 μg/L for women, compared to 5.9% of Caucasian and 5.3% of Hispanic subjects. A further limitation is that our community-based study does not have the capacity to differentiate between levels at the lower end of a “normal” range and those indicative of true iron deficiency; to assist in clinical ascertainment of iron depletion, further exploration of a known healthy population could focus on similarly refining the lower limits of targeted reference ranges. We have not sought to define potential clinical utility for the increased serum ferritin levels observed in the general

population in the current study. We believe the principal application of our study to clinical practice relates to more objective interpretation of the significance of ferritin levels and the levels above which it should be further evaluated. Interpretation is enhanced by understanding that there is a component of the observed variation in ferritin levels that is explained by concomitant variation in biological parameters, variation which is over and above differences attributable to the standard demographic variables of sex and age. More particularly, given that higher serum ferritin values may reflect components of nonalcoholic fatty liver disease, metabolic syndrome or simple obesity, taken at face value the simple observation of a ferritin level greater than an old historical cut-off may not mandate further follow up for iron overload in the primary care or hepatology setting.

In conclusion, we have observed a significant increase in recent years in serum ferritin levels in an Australian Caucasian adult population. Previous estimates of applicable “reference ranges” in adult populations require revision to assist clinicians correctly identify individuals requiring further evaluation for iron overload. Serum ferritin levels, which are significantly influenced by gender, age and menopausal status also correlate independently with BMI and GGT levels in adults, and we have produced a simple, easy to use reference chart for interpretation of observed values.

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Table 1. Baseline characteristics of the study cohort stratified by sex and age.

	Male				Female			
	ALL	**<50 years (N=288)**	**≥50 years (N=300)**	***P*†**	**ALL**	**<50 years (N=300)**	**≥50 years (N=300)**	***P*†**
Age (years)	51 [37-68]	37 [28-42]	67 [58-72]	-	50 [35-67]	35 [28-41]	67 [57-72]	-
Serum ferritin (µg/L)	219 [133-349]	209 [125-319]	240 [139-373]	NS	74 [40-131]	51 [31-80]	115 [70-177]	***
Body mass index (kg/m^2)	27.1 [24.7-29.7]	26.4 [24.0-29.3]	27.4 [25.1-30.0]	**	25.4 [22.7-29.6]	24.3 [21.9-28.3]	26.8 [23.5-30.1]	***
≥ 25 kg/m^2	72%	69%	76%	*	54%	44%	63%	***
≥ 30 kg/m^2	23%	20%	26%	NS	23%	20%	26%	NS
Waist (cm)	96 [88-104]	94 [84-100]	99 [92-106]	***	84 [76-95]	80 [74-91]	88 [80-97]	***
Alcohol intake (standard drinks per day)	3 [2-5]	4 [3-5]	3 [2-4]	***	2 [2-3]	3 [2-3]	2 [1-3]	***
γ-glutamyltransferase (IU/L)	25 [18-39]	24 [17-36]	28 [18-43]	*	16 [12-24]	14 [10-19]	18 [14-25]	***
Alanine transferase (IU/L)	14 [10-20]	16 [11-23]	14 [10-19]	*	10 [7-14]	9 [6-13]	11 [8-15]	***
Alkaline phosphatase (IU/L)	68 [55-79]	67 [55-77]	69 [56-81]	NS	65 [52-78]	56 [46-69]	72 [60-88]	***
Total bilirubin (µmol/L)	10 [8-13]	10 7-13]	11 8-14]	*	8 [6-10]	8 [6-10]	8 [6-11]	NS
C-reactive protein (mg/L)	1.3 [0.6-2.4]	1.0 [0.5-2]	1.6 [0.7-3.1]	***	1.7 [0.6-3.8]	1.3 [0.5-3.3]	1.9 [0.8-4.2]	*
Total cholesterol (mmol/L)	5.1 [4.6-5.9]	5.1 [4.6-5.8]	5.2 [4.6-5.9]	NS	5.2 [4.6-5.9]	4.8 [4.4-5.4]	5.6 [4.9-6.3]	***
Triglycerides (mmol/L)	1.2 [0.9-1.8]	1.1 [0.9-1.8]	1.3 [1-1.8]	NS	1 [0.8-1.5]	0.9 [0.7-1.3]	1.2 [0.9-1.7]	***
High-density lipoprotein (mmol/L)	1.3 [1.1-1.5]	1.3 [1.1-1.5]	1.3 [1.1-1.5]	NS	1.6 [1.3-2]	1.6 [1.3-1.8]	1.7 [1.4-2]	**
Glucose (mmol/L)	5.2 [4.9-5.6]	5.1 [4.9-5.4]	5.3 [5-5.7]	***	4.9 [4.6-5.3]	4.7 [4.5-5]	5.1 [4.8-5.5]	***
Insulin (mU/L)	7 [4-10]	6 [4-9]	7 [5-11]	***	6 [4-9]	5 [4-8]	7 [5-10]	***
HOMA-IR index#	1.6 [1.0-2.4]	1.3 [0.9-2.2]	1.8 [1.1-2.7]	***	1.4 [0.9-2.1]	1.2 [0.8-1.8]	1.6 [1.1-2.5]	***

Values are median [interquartile range] or % of group.

† P-value for comparison across the two age groups by a Mann Whitney or Pearson's chi-squared test: *** ($P \leq 0.0001$), ** ($0.0001 < P \leq 0.001$), * ($0.001 < P \leq 0.05$).

Homeostasis model assessment-estimated insulin resistance index: fasting plasma glucose × serum insulin /22.5.

Table 2. Spearman correlations between age, body mass index and biochemical parameters and serum ferritin levels in adult males and females.

	Males				Females			
	Serum ferritin	**Age**	**BMI**	**GGT**	**Serum ferritin**	**Age**	**BMI**	**GGT**
Age (years)	0.08	-	0.13	0.11	0.43	-	0.19	0.30
Body mass index (kg/m^2)	0.25	0.13	-	0.38	0.22	0.19	-	0.32
Waist (cm)	0.24	0.31	0.87	0.41	0.23	0.25	0.88	0.35
Alcohol intake (drinks per day)	0.07	-0.31	-0.03	0.13	0.03	-0.24	-0.04	-0.05
γ-glutamyltransferase (IU/L)	0.32	0.11	0.38	-	0.35	0.30	0.32	-
Alanine transferase (IU/L)	0.25	-0.19	0.32	0.46	0.23	0.18	0.22	0.43
Alkaline phosphatase (IU/L)	0.00	0.07	0.09	0.24	0.20	0.36	0.34	0.36
Total bilirubin (µmol/L)	0.08	0.09	-0.06	-0.09	0.11	0.12	-0.21	0.01
C-reactive protein (mg/L)	0.10	0.22	0.30	0.22	0.15	0.08	0.46	0.28
Total cholesterol (mmol/L)	0.21	0.03	0.11	0.28	0.19	0.38	0.14	0.19
Triglycerides (mmol/L)	0.22	0.07	0.45	0.46	0.22	0.31	0.42	0.35
High-density lipo-protein (mmol/L)	-0.12	0.01	-0.37	-0.18	-0.02	0.14	-0.32	-0.07
Glucose (mmol/L)	0.16	0.23	0.27	0.28	0.24	0.47	0.38	0.28
Insulin (mU/L)	0.18	0.16	0.61	0.38	0.19	0.14	0.61	0.36
HOMA-IR index[#]	0.20	0.19	0.61	0.39	0.22	0.21	0.62	0.38

[#]Homeostasis model assessment-estimated insulin resistance index: fasting plasma glucose × fasting serum insulin /22.5.

Table 3. Reference limits for serum ferritin according to sex, stage of life and body mass index dichotomies, derived by observed 5-95th centiles.

	Stage of life	BMI (kg/m^2)	Serum ferritin (μg/L)
Male	<35 years age	unrestricted	50 - 409
	≥35 years age	<25	45 - 476
	≥35 years age	≥25	68 - 585
Female	<45 years (premenopausal)	unrestricted	†13 - 157
	45-54 years	<25	†8 - 211
	45-54 years	≥25	†13 - 348
	≥55 years age (postmenopausal)	<25	26 - 295
	≥55 years age (postmenopausal)	≥25	26 - 364

†Below the common clinical cut-off of 20 μg/L that is used as an indicator for iron depletion.

Figure 1. Age-stratified serum ferritin concentrations (μg/L) determined from Busselton Population Health Surveys in 1995 and 2005. The box-and-whisker plots show the median (heavy black line, interquartile range (box) and 5th-95th centiles (whiskers).

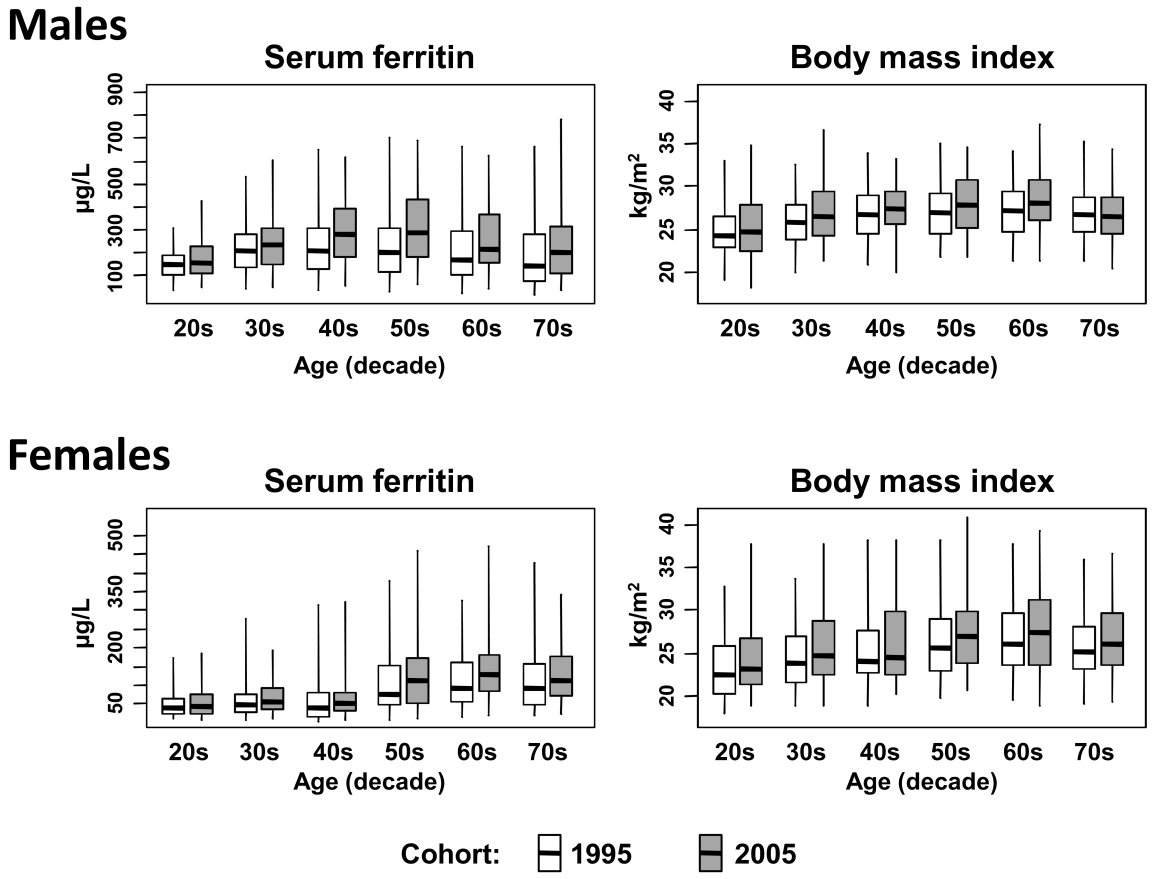

Figure 2. Reference ranges for serum ferritin levels in adult males and females with adjustments for age/menopausal status, body mass index (BMI) and γ-glutamyltransferase (GGT) as derived by parametric (Weibull) modeling. The limits are presented as the estimated 95th (heavy line) and 5th (dotted line) centiles over GGT levels ranging from ≤10 to ≥75 IU/L for males and ≤8 to ≥45 IU/L for females. Central 90% ranges for corresponding sub-groups without GGT adjustment, as calculated by observed 5th to 95th centiles, are denoted by the vertical arrows.