Reticulocyte hemoglobin content in a large sample of the general Dutch population and its relation to conventional iron status parameters

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Abstract

Background: No full consensus exists on which iron status parameters to use for iron status assessment. In this study, we assessed the usefulness of measurement of the hemoglobin content of reticulocytes (CHr) in the general population.

Methods: The following iron status parameters were assessed in 1024 adults: CHr, reticulocytes, hemoglobin (Hb), ferritin, serum iron, transferrin, transferrin saturation and mean corpuscular volume (MCV). Mean parameter values and correlation coefficients for CHr and other parameters were calculated. In addition, mean CHr levels in subgroups based on low and normal values of other iron status parameters were compared.

Results: Mean CHr values in men were 31.81 (SD=1.50) pg and in women 31.32 (SD=1.51) pg. A positive correlation was observed between CHr and Hb, ferritin, serum iron, transferrin saturation and MCV; a negative correlation was observed between CHr and transferrin. CHr levels were lower in subjects with low values of Hb, ferritin, serum iron and MCV compared to subjects with normal values for these parameters.

Conclusion: Mean CHr values in this population were comparable to values reported in small healthy control groups. Associations with other parameters were in agreement with associations reported in literature. CHr measurement might have additional value in iron status assessment.

200 woorden (max 200)

Keywords: iron status, reticulocyte hemoglobin content, hemoglobin, ferritin

1. Introduction

Iron deficiency can be diagnosed by measurement of several iron status parameters. No full international consensus exists on which tests to use for iron status assessment. Difficulties in the diagnosis of iron deficiency are that different parameters become affected during different stages in the development of iron deficiency [1], the published sensitivity and specificity of the different tests are highly variable, and some parameters are influenced by pathological conditions. Ferritin for example, is an acute phase reactant and its diagnostic utility is therefore limited in patients with inflammatory conditions.

In the last two decades, automated hematology analyzers that measure parameters which are reported to be uninfluenced by inflammation have been developed. One of these tests is the mean cellular hemoglobin content of reticulocytes (CHr), provided by ADVIA analyzers. This parameter was approved for clinical use by the Federal Drug Administration in 1997. Since May 2005, an equivalent parameter, reticulocyte hemoglobin equivalent (RetHe), is available on Sysmex analyzers. Good agreement between CHr and RetHe has been reported [2]. In the current study, we will further use the term CHr for this parameter.

Besides the advantage of not being influenced by inflammation, another advantage of CHr is that this parameter becomes affected in a relatively early stage in the development of iron deficiency. Reticulocytes are immature erythrocytes. They mature for 1-3 days within the bone marrow and then they are released into the blood. Here they circulate for 1-2 days before they become mature erythrocytes. Because reticulocytes are young erythrocytes, CHr provides an indirect measure of iron available for erythropoiesis over the previous 3-4 days. In contrast, measures of mature erythrocytes are not sensitive for early iron deficient erythropoiesis because the turnover of erythrocytes is approximately 120 days.

Although CHr measurements are not often used in clinical practice, studies have demonstrated that CHr is a useful parameter for diagnosis of iron deficiency in adults [3], children [4,5], pregnant women [6], elderly patients [7,8] and blood donors [9,10]. In addition, it is a useful test for monitoring the erythropoietic response to iron replacement therapy [11,12] and detecting iron-restricted erythropoiesis in patients receiving erythropoietin therapy [13].

Most studies have been performed in patients or other specific populations with conditions that may influence iron metabolism, for example inflammatory conditions or an increased erythrocyte turnover after blood donation. Results in these studies could therefore be influenced by these specific conditions. Although in some studies a small control group of healthy subjects was used, little is known about the significance of CHr measurement in the general population. The aim of the current study is therefore to investigate the usefulness of CHr measurement in iron status assessment in the general population by measuring CHr values and to investigate its relation with conventional iron status parameters in a large sample of the general Dutch population. Hereto, data from participants of the Nijmegen Exercise Study (NES) [14] were used. Results of this study will extend the knowledge on the use of CHr measurements.

2. Methods

2.1 Study population

The study population for the current study consisted of NES participants [14]. These NES participants were recruited among participants of the Nijmegen Four Days Marches, an annual walking event in the Netherlands. A total of 1105 NES participants (639 men, 466 women) who volunteered to participate in blood tests were included in the current study. Subjects with CRP levels at or above 5.0 mg/L (n=81) were excluded to prevent an influence on the results due to inflammation. Finally, a total of 1024 NES participants (594 men, 430 women) were included in the current study. This study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Medical Ethical Committee of the Radboud University (file number: 2011/193; approval number: NL36743.091.11).

2.2 Laboratory analyses

Venous blood samples were collected one or two days before the first day of the Nijmegen Four Days Marches2015 (July 22), between 10:00 and 17:00 o'clock. In these samples blood parameters were measured on the same day. CHr, reticulocytes, Hb and MCV were measured on the ADVIA 2120i hematology analyzer (Siemens Healthineers, Erlangen, Germany). Ferritin, serum iron, transferrin and CRP were measured on the Vista Dimension 1500 (Siemens Healthineers, Erlangen, Germany). Transferrin saturation was calculated as the serum iron concentration divided by the total iron binding capacity. The latter was calculated from the transferrin concentration multiplied by 25.136. All measurements were performed in the Clinical Chemistry and Haematology Laboratory at the Gelderse Vallei Hospital using standard operating procedures and equipment.

2.3 Statistical analyses

Descriptive analyses were performed separately for men and women. Distributions of blood parameters were checked for normality with the Shapiro Wilk test; Levene's test was used to check for homogeneity of variances.

Mean values and standard deviations (SD) of the measured parameters were calculated. Mean parameter values in men and women were compared with a Mann-Whitney U test. This test was used because the distribution of parameters was not normal. To study the association between CHr and the other iron status parameters, Pearson's correlation coefficients were calculated. To assess a possible difference in correlation coefficients between men and women a Fisher's *r* to *z* transformation was performed first. The *z* scores were then compared and analyzed for significant difference between men and women.

Next, mean CHr values in subjects with values below and above the lower reference limit of the other iron status parameters were calculated. Mean CHr values in subjects with low and normal levels of other iron status parameters were compared with a Mann-Whitney U test.

Subsequently, additional analyses that focus specifically on CHr and two commonly used parameters to assess the iron status, Hb and ferritin, were performed. Subjects were divided into five different groups based on their Hb level. The five Hb level categories were as follows (in mmol/L): <7.5, 7.5-8.4, 8.5-9.4, 9.5-10.4 and ≥10.5. For each Hb level category group mean values of CHr and ferritin were calculated. Mean CHr and ferritin values in the different Hb level category groups were compared with a Welch test. When the Welch test was significant ($p<0.05$), a Games-Howell post hoc test was performed to investigate which of the five groups differed from each other. These tests were used because the assumption of homogeneity of variances was violated. Finally, subjects were divided into four different groups based on combinations of Hb and ferritin levels below and above the lower reference

limit. Mean CHr values were calculated for these Hb-ferritin category groups and compared with a Welch test and Games-Howell test.

Statistical analyses were performed with SPSS, Version 24, SPSS, Inc., Chicago, IL.

3. Results

Population characteristics and parameter values are presented in Table 1. The mean age in men was 64 (SD=9) years and in women 57 (SD=11) years. Mean CHr values were significantly higher in men compared to women (31.81 (SD=1.50) pg vs 31.32 (SD=1.51) pg). The range of CHr values in men was 23.36 to 35.77 pg and in women 25.13 to 37.22 pg. Mean values of reticulocytes, Hb, ferritin and transferrin saturation were also significantly higher in men, whereas transferrin levels were significantly lower.

Table 2 presents Pearson's correlation coefficients for CHr and other iron status parameters. In both men and women a significant positive correlation was observed between CHr and the following parameters: Hb, ferritin, serum iron, transferrin saturation and MCV. A significant negative correlation was observed between CHr and transferrin. No correlation was observed between CHr and reticulocyte count. Most observed correlations were however weak to moderate, ranging from 0.231 for ferritin in men to 0.453 for transferrin saturation in women. Only the correlation between CHr and MCV was strong (r=0.732 in men and r=0.755 in women). For most correlations, the correlation coefficient was comparable in men and women, except for CHr and Hb, which was significantly higher in women (r=0.431) than in men (r=0.293).

Table 3 presents mean CHr levels in subjects with values below and above the lower reference limit of iron status parameters. Both in men and women, CHr levels were significantly lower in subjects with low values of Hb, ferritin, serum iron and MCV.

Table 4 presents mean CHr levels and mean ferritin levels in groups based on different Hb level categories. These results are graphically presented in Figure 1.

In men, subjects with Hb levels of 8.5-9.4 mmol/L had significantly lower CHr levels than subjects with Hb levels of 9.5-10.4 and ≥10.5 mmol/L. In women, subjects with Hb levels

<7.5 mmol/L had significantly lower CHr levels than subjects with Hb levels of 7.5-8.4, 8.5-9.4 and 9.5-10.4 mmol/L. Also, women with Hb levels 7.5-8.4 mmol/L had significantly lower CHr levels than women with Hb levels of 8.5-9.4 and 9.5-10.4 mmol/L.

Ferritin levels in male subgroups with Hb levels of <7.5, 7.5-8.4, 8.5-9.4 and 9.5-10.4 mmol/L differed significantly from each other; the lower the Hb level, the lower the ferritin level. Women with Hb levels of 7.5-8.4 mmol/L had significantly lower ferritin levels than women with Hb levels of 8.5-9.4, 9.5-10.4 and ≥10.5 mmol/L.

Table 5 presents mean CHr values in four different groups based on combinations of Hb and ferritin levels below and above the lower reference limit. Both in men and women, subjects with normal Hb and normal ferritin levels had significantly higher CHr levels compared to subjects with low Hb and low ferritin levels and subjects with normal Hb and low ferritin levels. Additionally, men with low Hb and normal ferritin levels had significantly higher CHr levels compared to men with low Hb and low ferritin levels.

4. Discussion

A relatively new test for iron status assessment is measurement of CHr values. This study aimed to investigate the usefulness of CHr measurement in iron status assessment in a large sample of the general Dutch population by measuring CHr values and to investigate its relation with conventional iron status parameters.

Mean CHr values in men were 31.81 (SD=1.50) pg and in women 31.32 (SD=1.51) pg. In other studies in which CHr values were measured on ADVIA analyzers in small control groups (n=34 to n=126) consisting of both male and female healthy subjects, the following mean values (in pg) were reported: 28.2 (SD=1.7) [15], 30.16 (SD=1.82) [2], 30.8 (SD=0.90) [3], 30.9 (SD=1.3) [16], and 32.5 (SD=0.7) [17]. Relatively large variation in mean values among these studies exists, and our results fall within the same range. The variation could be explained by the small sample sizes of the other studies.

In both men and women a significant positive correlation was observed between CHr and the following parameters: Hb, ferritin, serum iron, transferrin saturation and MCV; a significant negative correlation was observed between CHr and transferrin. This is in agreement with a study in female patients with iron deficiency, in which significant positive correlations were observed between CHr and Hb, serum iron, transferrin saturation and MCV, and a significant negative correlation was observed between CHr and transferrin [18]. Results are also in agreement with another study in patients with iron deficiency and with chronic renal failure, in which positive associations were found between CHr and Hb, ferritin, serum iron, transferrin saturation and MCV, and in which a negative association was found between CHr and total iron binding capacity, which is proportional to the transferrin concentration [16]. Results from these studies and from the current study might suggest that CHr measurement

can be used in both healthy persons as well as in patients for the assessment of iron deficiency.

The observed positive and negative correlations can be explained by physiological mechanisms. An adequate or high iron status is associated with sufficient levels of serum iron and increased iron stores. The latter are reflected by high ferritin levels. There is more iron available for erythropoiesis and hemoglobin synthesis, resulting in increased levels of CHr, Hb and MCV. The negative correlation between transferrin and CHr indicates that in an iron deficient state, when CHr levels are decreased, transferrin levels are increased in order to increase the iron uptake from the blood stream into cells.

Within subgroups based on Hb level categories we observed increasing mean CHr levels with increasing Hb level categories. We observed more significant differences in mean CHr values among female subgroups compared to male subgroups, which is in agreement with the stronger correlation between CHr and Hb in women. Within the same subgroups we also observed increasing mean ferritin levels with increasing Hb level categories. Results of mean CHr values in four different groups based on combinations of Hb and ferritin levels below and above the lower reference limit correspond to these results: subjects with normal Hb and normal ferritin levels had significantly higher CHr levels compared to subjects with low Hb and low ferritin levels and subjects with normal Hb and low ferritin levels.

Also when subjects were categorized into two subgroups with values below and above the lower reference limit of the other iron status parameters, significantly higher mean CHr values were observed in subjects with iron status parameter values above the lower reference limit. Besides Hb and ferritin, this was true for serum iron and MCV. The difference in mean CHr value between subjects with low and normal values was largest for the parameter MCV. This observation is in agreement with the high correlation for CHr and MCV.

The range in mean CHr levels in subjects with iron status parameters above the lower reference limit goes from 31.81 to 31.94 pg in men and from 31.29 to 31.52 pg in women. The variation in mean CHr values in subjects with iron status parameters below the lower reference limit was larger: in men mean CHr values range from 27.87 to 31.92 pg and in women from 27.97 to 32.06 pg. The larger variation could be explained by the relatively small number of subjects with iron status parameters below the lower reference limit.

In literature, different lower reference limits of CHr values for the diagnosis of iron deficiency are reported. In studies in hemodialysis patients, limits of 26 pg [19] and 32 pg [20] are reported as appropriate for the assessment of iron deficiency. In another study in hospitalized patients, a cutoff value of 28 pg was reported for the diagnosis of iron deficiency [21]. For the diagnosis of iron deficiency anemia in hospitalized patients cutoff values of 29 pg [18] and 30.5 pg [7] were reported. In all these studies, CHr values were measured on ADVIA analyzers. Differences in study populations and criteria for the diagnosis of iron deficiency might explain the different lower reference limits reported in different studies. The study population of one study consisted of only women [18]; in the other studies no distinction was made between men and women, which might be necessary, based on our observations.

Universal reference ranges are not available for a large number of parameters. The assessment of reference ranges for iron status parameters is difficult because there are so many aspects that are iron related, like sex, age, nutritional status and disease states. Furthermore, measurement of several iron status parameters lack standardization, and therefore results differ between methodologies and laboratories. Finally, the use of different definitions of iron deficiency and the lack of a perfect gold standard makes it difficult to assess reference ranges. The large variation in reported cutoff levels for CHr between different studies confirm this difficulty.

We observed higher mean CHr levels in subjects with iron status parameter values above the lower reference limit than in subjects with iron status parameter values below the lower reference limit. However, it occurred that individual subjects with a high CHr level had a low value for another iron status parameter and vice versa. CHr measurement should therefore not be used as one single test for iron status assessment, but it might have additional value in combination with other tests. In another study it was also concluded that detection of iron restriction is not possible with a single test used in isolation [22]. A huge advantage of CHr is that it is not influenced by inflammation. Furthermore, CHr becomes affected in a relatively early stage in the development of iron deficiency before iron deficiency anemia and clinical symptoms become apparent. Therefore, this parameter might be useful to identify persons, healthy and diseased, at risk of developing iron deficiency anemia and subsequently to apply interventions in order to prevent further development of iron deficiency anemia. Future research using longitudinal data is necessary to study the value of CHr measurements in predicting the development of iron deficiency anemia in both healthy as well as diseased persons.

Our study population consisted of a large random sample of the general Dutch population. As general populations do not consists of healthy people only, diseases or disease events were also prevalent among our study population. A total of 723 subjects (71%) reported to have (had) a disease or a disease event. Some examples of diseases or disease events that were reported are cancer, cardiovascular disease, heart attack, hypertension, diabetes, asthma, depression and allergy. We excluded subjects with high CRP levels and therefore an influence of inflammatory conditions associated with certain diseases can be rules out. Our study population, meant to be a reflection of the general Dutch population, consisted of participants of the Nijmegen Four Days Marches. It is likely that these participants are above average

physically active. On the other hand, our study population was relatively large, which is a strength of the study.

5. Conclusion

In this study we have confirmed in a large sample of the general Dutch population results from earlier studies in small control groups of healthy adults. Mean CHr values in this study population fall within the same range as mean CHr values reported in small or specific patient populations. Associations with other iron status parameters in this study population are also in agreement with associations reported in literature. As the iron status can be assessed more accurate by a combination of tests, it would be valuable to measure CHr in conjunction with conventional parameters such as Hb and ferritin to assess the iron status.

Highlights

- CHr values correlate well with conventional iron status parameters.
- CHr measurement might have additional value in iron status assessment.
- CHr measurement might be useful to identify persons at risk of developing anemia.

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Tables

Table 1 Study population characteristics*

Characteristic	Men (n=594)	Women (n=430)
Age, years	64 (9) †	57 (11) †
CHr, pg	31.81 (1.50) †	31.32 (1.51) †
Reticulocytes, 10^9/L	69 (17) †	60 (16) †
Hb, mmol/L‡	9.6 (0.6) †	8.8 (0.6) †
Ferritin, µg/L	128 (121) †	73 (65) †
Serum iron, µmol/L	18.2 (5.7) †	16.2 (5.2) †
Transferrin, g/L	2.7 (0.4) †	2.8 (0.4) †
Transferrin saturation, fraction	0.27 (0.09) †	0.24 (0.09) †
MCV, fL	88 (4)	87 (4)
CRP, mg/L	3.1 (0.3)	3.1 (0.3)

* Data are presented as mean (SD)

† Significant difference between men and women (p-value Mann Whitney U test <0.05)

‡ Conversion factor for g/dL: 1.61

Table 2 Pearson's correlation coefficients for CHr and other iron status parameters

Parameter	**Men (n=594)**	**Women (n=430)**
Reticulocytes	-0.028	-0.020
Hb	0.293 *†	0.431 *†
Ferritin	0.231 *	0.288 *
Serum iron	0.319 *	0.412 *
Transferrin	-0.289*	-0.351*
Transferrin saturation	0.387 *	0.453 *
MCV	0.732*	0.755*

* Significant correlation between CHr and parameter (p-value <0.05)

† Significant difference in correlation coefficient between men and women (p-value <0.05)

Table 3 CHr levels in subjects with other iron status parameters below and above lower reference limit

Sex	Parameter	Reference range	Below lower reference limit		At or above lower reference limit	
			n (%)	CHr (pg)*	n (%)	CHr (pg)*
Men	Reticulocytes, 10^9/L	40 – 140	17 (2.9)	31.92 (1.19)	577 (97.1)	31.81 (1.51)
	Hb, mmol/L‡	8.5 – 11.0	23 (3.9)	30.39 (2.77) †	571 (96.1)	31.87 (1.40) †
	Ferritin, µg/L	15 – 200	40 (6.7)	29.96 (2.25) †	554 (93.3)	31.94 (1.34) †
	Serum iron, µmol/L	10.0 – 30.0	29 (4.9)	29.85 (2.34) †	565 (95.1)	31.91 (1.37) †
	Transferrin, g/L	2.0 – 3.6	3 (0.5)	31.52 (0.67)	591 (99.5)	31.81 (1.50)
	MCV, fL	80 – 100	19 (3.2)	27.87 (2.19) †	575 (96.8)	31.94 (1.28) †
Women	Reticulocytes, 10^9/L	40 – 140	30 (7.0)	31.78 (1.04)	400 (93.0)	31.29 (1.53)
	Hb, mmol/L‡	7.5 – 10.0	8 (1.9)	27.97 (1.52) †	422 (98.1)	31.39 (1.43) †
	Ferritin, µg/L	15 – 150	52 (12.1)	29.88 (1.98) †	378 (87.9)	31.52 (1.31) †
	Serum iron, µmol/L	10.0 – 30.0	40 (9.3)	29.63 (1.87) †	390 (90.7)	31.50 (1.35) †
	Transferrin, g/L	2.0 – 3.6	1 (0.2)	32.06 (-)	429 (99.8)	31.32 (1.51)
	MCV, fL	80 – 100	16 (3.7)	27.97 (1.60) †	414 (96.3)	31.45 (1.35) †

* Data are presented as mean (SD)

† Significant difference between subjects with values below and above the lower reference limit (p-value Mann Whitney U test <0.05)

‡ Conversion factor for g/dL: 1.61

Table 4 CHr and ferritin levels within different Hb level categories

Sex	**Hb level category (mmol/L[†])**	**n (%)**	**CHr (pg)***	**Ferritin (µg/L)***
Men	Hb< 7.5	2 (0.3)	25.14 (2.51)	5.13 (0.57)
	Hb≥ 7.5 and< 8.5	21 (3.5)	30.89 (2.25)	61.16 (61.63)
	Hb≥ 8.5 and< 9.5	232 (39.1)	31.58 (1.65)	106.04 (115.28)
	Hb≥ 9.5 and< 10.5	293 (49.3)	32.03 (1.19)	141.75 (116.97)
	Hb≥ 10.5	46 (7.7)	32.28 (0.94)	182.95 (156.35)
Women	Hb< 7.5	8 (1.9)	27.97 (1.52)	37.63 (49.71)
	Hb≥ 7.5 and< 8.5	107 (24.9)	30.67 (1.62)	48.15 (39.97)
	Hb≥ 8.5 and< 9.5	279 (64.9)	31.59 (1.30)	81.85 (71.13)
	Hb≥ 9.5 and< 10.5	34 (7.9)	31.90 (0.98)	87.38 (57.84)
	Hb≥ 10.5	2 (0.5)	32.87 (2.74)	89.17 (6.65)

* Data are presented as mean (SD)

[†] Conversion factor for g/dL: 1.61

Table 5 CHr levels within categories of combinations of low and normal Hb and ferritin levels

Sex	**Hb-ferritin level category**	**n (%)**	**CHr (pg)***
Men	Hb low (<8.5 mmol/L[†]), ferritin low (<15 µg/L)	7 (1.2)	27.41 (2.87)
	Hb low (<8.5 mmol/L), ferritin normal	16 (2.7)	31.70 (1.40)
	Hb normal, ferritin low(<15 µg/L)	33 (5.6)	30.50 (1.70)
	Hb normal, ferritin normal	538 (90.6)	31.95 (1.34)
Women	Hb low (<7.5 mmol/L), ferritin low (<15 µg/L)	5 (1.2)	27.97 (1.39)
	Hb low (<7.5 mmol/L), ferritin normal	3 (0.7)	27.98 (2.04)
	Hb normal, ferritin low(<15 µg/L)	47 (10.9)	30.08 (1.93)
	Hb normal, ferritin normal	375 (87.2)	31.55 (1.27)

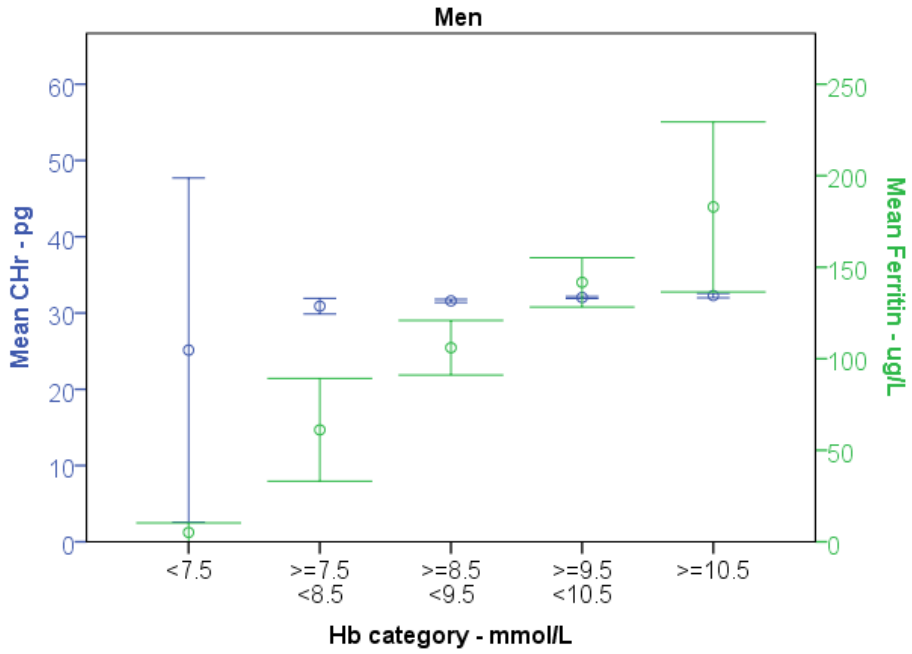
* Data are presented as mean (SD)

[†] Conversion factor for g/dL: 1.61

Figures

Figure 1 Mean CHr and ferritin levels within different Hb level categories in men (A) and women (B)

A



B

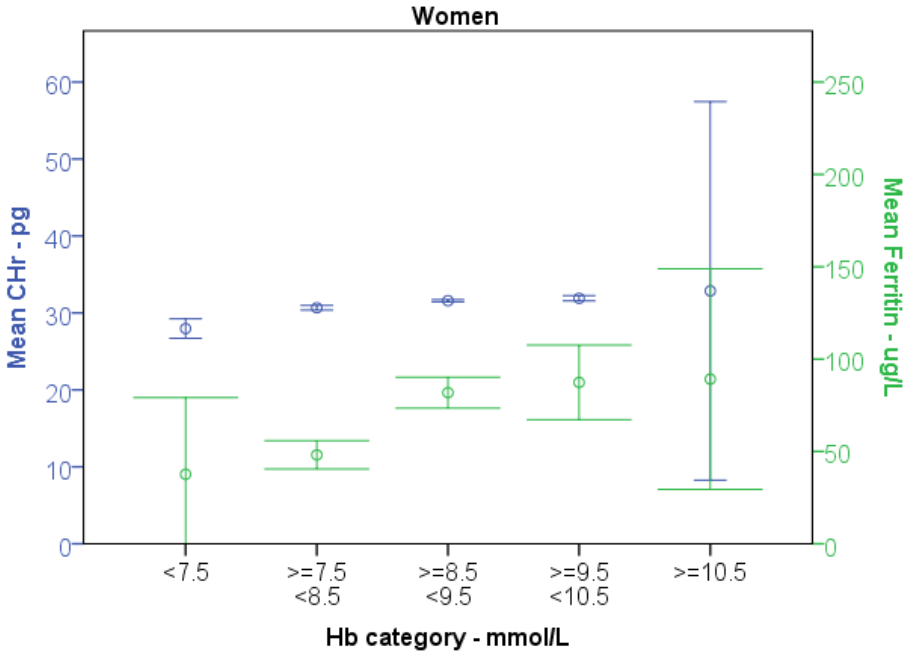


Figure 1 Mean CHr (blue) and ferritin (green) levels with 95% CI error bars within different Hb level categories in men (A) and women (B)