

# The Association between the Levels of Serum Ferritin and Sex Hormones in a Large Scale of Chinese Male Population

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## Abstract

**Background:** The ferritin is an important participant of iron-storage but its regulation and related factors were not well defined. The present objective was to explore the potential association between serum ferritin levels and sex hormones.

**Methods:** 1999 Chinese men in the Fangchenggang Area Male Health and Examination Survey (FAMHES) were recruited in this cross-sectional study. Levels of serum ferritin, total testosterone (free testosterone was calculated from the total one), estradiol and sex hormone-binding protein were detected in venous blood samples. The effects of age, BMI, smoking as well as alcohol consumption were analyzed on ferritin levels, respectively, and then the Pearson's correlation analysis was used to evaluate the association between ferritin levels and sex hormones adjusting for the above factors.

**Results:** The age, BMI and alcohol consumption significantly affected serum ferritin levels, but there was no significant difference between smokers and nonsmokers. Ferritin levels were significantly and negatively associated with total testosterone ( $R = -0.205$ ,  $P < 0.001$ ), sex hormone-binding protein ( $R = -0.161$ ,  $P < 0.001$ ) and free testosterone ( $R = -0.097$ ,  $P < 0.001$ ). After age and alcohol consumption were adjusted, the above associations were still significant ( $R = -0.200$ ,  $-0.181$  and  $-0.083$ , respectively, all  $P < 0.001$ ). However, there was only borderline negative association between ferritin levels and estradiol (adjusted  $R = -0.039$ ,  $P = 0.083$ ).

**Conclusion:** The large scale of epidemic results showed the significantly negative associations between serum ferritin levels and sex hormones, which may provide more clues to explore the potential regulation and biological mechanism of ferritin.

**Citation:** Liu Z, Ye F, Zhang H, Gao Y, Tan A, et al. (2013) The Association between the Levels of Serum Ferritin and Sex Hormones in a Large Scale of Chinese Male Population. PLoS ONE 8(10): e75908. doi:10.1371/journal.pone.0075908

**Editor:** Robert K. Hills, Cardiff University, United Kingdom

**Received:** May 5, 2013; **Accepted:** August 19, 2013; **Published:** October 11, 2013

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**Funding:** The study was supported by grants from the Guangxi Science Fund for Distinguished Young Scholars (2012jjFA40011), National Key Basic Research Program grant 973 (2012CB518300 and 2012CB518303), National Natural Science Foundation of China (81160072, 81060234), and Guangxi Natural Science Foundation (2010GXNSFB013064). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

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## Introduction

Ferritin is a protein that contributes to the iron-storage mainly in our body and distributes widely in all tissues, especially in some organs such as the liver, spleen and bone marrow [1], and it is also an inflammatory serum biomarker [2]. Some previous studies have demonstrated that levels of ferritin were related to several disease states. As we knew, it was associated with metabolic syndrome [3], hematopoiesis [4,5], renal disease [6,7], liver disease [8], autoimmunity [9], infection [10], the prognosis of cancer [11] and the mortality of liver transplantation [12].

It was reported that the two subunits of ferritin was synthesized under the control of different genes in chromosomes 11 and 19,

respectively [13,14]. The level of ferritin was affected by the iron metabolism in humans body and cytokines, growth factor as well as oxidants contributed to the regulation [15]. Especially, it should be noted that the hormones also may be a participant in the regulation, for example the thyroid hormone [15]. When it came to the sex hormones, they were well known as important participants in improving sexual organs, and also related to metabolic syndrome [16], anemia [17], inflammation [18] and the mortality in the elderly [19–21].

In view of common diseases and the regulation mechanism of ferritin that were mentioned above, sex hormones may potentially take part in the regulation of ferritin, and then emerge some evidences of correlations between them and serum ferritin levels.

However, few studies did describe the relationships between serum ferritin levels and sex hormones [22,23], and we were the first to perform a large scale of epidemiological investigation to directly explore the clues of their correlation.

## Subjects and Methods

### Study design and subjects recruitment

The Fangchenggang Area Male Health and Examination Survey (FAMHES) was designed to investigate the effects that environmental and genetic factors and their interaction had on the development of age-related chronic diseases and the people who participated in this investigation was non-institutionalized men from 17 to 88 years old in Guangxi, China. A total number of 4303 continuous participants in a large-scale physical examination in the Medical Center of Fangchenggang First People's Hospital from September 2009 to December 2009 were under a comprehensive demographic and health survey. All trained interviewers used this questionnaire to collect epidemiological data through face-to-face interview following the structured guidelines in condition of obtaining written informed consent and the Ethics and Human Subjects Committee of the Guangxi Medical University did approve of this study. Of the 4303 participants, 3136 was self-reported pure Han nationality for three generations and Fangchenggang residents. We excluded the participants with the self-reported information of neoplastic condition, diagnosis of chronic disease, infection with acute infectious diseases, use of any drug with a known effect on the endocrine system or who was considered as prescribed in the past two weeks and people whose data was incomplete about smoking and alcohol consumption were also excluded. Thus, 1999 men was finally recruited and then 10.0 ml venous blood samples was collected from everyone [24].

### Survey of BMI and living habits

In the present study, age, body mass index (BMI), smoking, alcohol consumption were considered as potential confounding predictors for ferritin and/or sex hormones [25,26]. Height and weight were obtained with participants with light clothing and without shoes, and in this study the value of 25 was treated as the threshold for BMI. Conditions of smoking and alcohol consumption were self-reported, participants who had never smoked or had stopped smoked for 6 months as least to the time for interview were considered as 'nonsmoker', while the people who had smoked for 6 months or longer and still smoked to the time for interview or had stopped smoked less than 6 months were 'smokers'. For alcohol consumption, participants who had ever consumed three or more drinks (beer, wine, and hard liquor) weekly and done so for six consecutive months were marked with 'yes' as described elsewhere [27].

### Detection of serum ferritin and sex hormones

Ferritin, total testosterone, sex hormone-binding protein (SHBG) and estradiol were measured with electrochemiluminescence immunoassay on COBAS 6000 system E601 (Elecsys module) immunoassay analyzers (Roche Diagnostics, IN, Germany) with the same batch of reagents. The interassay coefficients of variation were 3.4%, 3.6%, 4.4% and 3.4%, respectively. The free testosterone (FT) and free testosterone index (FTI) were calculated from the detected total testosterone by the described procedure [28].

## Statistical analyses

Basic characteristics and information of variables were shown by Mean±Standard Deviation (SD), data of serum total testosterone, free testosterone, estradiol and SHBG were normally distributed. Additionally, data of serum ferritin was shown by median and interquartile range, and it was normally distributed after logarithmic transformation (log-ferritin). The One-way ANOVA was used to make comparison in subgroups of age, BMI, smoking and alcohol consumption for log-ferritin. Especially, the multiple comparisons between subgroups were carried under Fisher's LSD method. The analysis of Pearson's correlation coefficient and Partial correlation coefficient were performed to explore the correlations between log-ferritin and other variables respectively, and the latter was in condition of age and alcohol consumption were adjusted because BMI was correlated with ferritin and age. Besides, the quartile of serum total testosterone, free testosterone and SHBG was obtained and the correlation between them and relevant log-ferritin was explored respectively. All the analysis was performed by SPSS 17.0.

## Results

### General information of the 1999 Chinese men

Basic characteristics and information of variables were shown in Table 1. A total number of 1999 men were recruited in the present study with the age of  $37.54 \pm 11.10$  years (aged 20–69 years) and the BMI of  $23.30 \pm 3.35$  kg/m<sup>2</sup>. Data of serum ferritin was not normally distributed with the result of 318.00 (221.40, 459.90) ng/ml and then it was under the logarithmic transformation for further analysis ( $2.49 \pm 0.27$  ng/ml, 95%CI: 1.25–2.89). The levels of serum free testosterone ( $0.42 \pm 0.12$  nM, 95%CI: 0.14–0.62) and free testosterone index ( $58.28 \pm 22.58\%$ ) were calculated from the data of detected total testosterone ( $6.27 \pm 1.91$  ng/ml, 95%CI: 2.20–9.70). In addition, 590 subjects (29.5%) were considered as "overweight", and 1021 subjects (51.1%) were considered as "smoker" and 1651 participants (82.6%) were marked with "yes" in view of alcohol consumption.

### Serum ferritin in subgroups based on general information

Comparisons for levels of serum ferritin in different subgroups were performed based on age, BMI, smoking and alcohol consumption (Table 2). Our results showed that age was significantly associated with levels of serum ferritin with

**Table 1.** Characteristics and variables of the 1999 men who conformed well this study.

Variable	Mean ± SD
Age (year)	37.54 ± 11.10
BMI (kg/m <sup>2</sup> )	23.30 ± 3.35
Ferritin <sup>a</sup> (ng/ml)	318.00 (221.40, 459.90)
Estradiol (pg/ml)	34.44 ± 9.99
SHBG <sup>b</sup> (nM)	42.21 ± 20.56
Total testosterone (ng/ml)	6.27 ± 1.91
Free testosterone (nM)	0.42 ± 0.12
Free testosterone index (%)	58.28 ± 22.58

Ferritin<sup>a</sup>: the data was shown by median and interquartile range;

SHBG<sup>b</sup>: sex hormone-binding globulin.

doi:10.1371/journal.pone.0075908.t001

$P < 0.001$ , especially, and those male subjects in the age subgroup of 40–49 years had the highest serum ferritin levels ( $2.53 \pm 0.25$  ng/ml), but no significant trend was found with the increasing age groups,  $P > 0.05$ . When the value of  $25 \text{ kg/m}^2$  was considered as the threshold on the BMI stratification, a significant difference was also shown between two subgroups ( $2.45 \pm 0.28$  vs  $2.59 \pm 0.23$  ng/ml) with  $P < 0.001$ . In addition, the alcohol consumption was also significantly associated with levels of serum ferritin and those subjects who were marked with “yes” had higher ferritin levels than those marked with “no” ( $2.50 \pm 0.27$  vs  $2.46 \pm 0.28$  ng/ml,  $P = 0.011$ ), however, no significant difference was found between smokers and nonsmokers ( $2.50 \pm 0.27$  vs  $2.49 \pm 0.27$  ng/ml,  $P = 0.447$ ).

**Correlation between serum ferritin and sex hormone/sex hormone-binding protein**

We firstly investigated the direct correlation between ferritin and sex hormone levels without any adjustment of potential confounding factors. Our data showed that the correlation between serum log-ferritin and all sex hormones were significant and negative (Table 3), including total testosterone, free testosterone and SHBG, all  $P < 0.001$  ( $R = -0.205$ ,  $-0.097$  and  $-0.161$ , respectively), and the correlation between serum ferritin and estradiol levels was also significant ( $R = -0.048$ ,  $P = 0.034$ ). Furthermore, the analysis of covariate correlation was used to explore the association between them after the adjustment of potential confounding factors (age and alcohol consumption), and similarly significant results were obtained (Table 3), the correlations between ferritin and total testosterone, free testosterone and SHBG were still significant ( $R = -0.200$ ,  $-0.083$  and  $-0.182$ , respectively), all  $P < 0.001$ . However, the correlation was not significant any longer between ferritin and estradiol level ( $R = -0.039$ ,  $P = 0.083$ ).

Additionally, we stratified all subjects into four subgroups according to the quartiles of sex hormone levels, and those subjects

**Table 2.** Comparisons about the concentration of serum ferritin based on general information that stratified by subgroups.

Variable	Number (%)	Serum ferritin <sup>a</sup> (ng/ml)	P-value
Age (year)			<0.001
20–29	556 (27.81)	2.44 ± 0.24	
30–39	682 (34.12)	2.52 ± 0.26	
40–49	474 (23.71)	2.53 ± 0.25	
50–59	172 (8.60)	2.47 ± 0.34	
60–69	115 (5.75)	2.47 ± 0.36	
BMI (kg/m <sup>2</sup> )			<0.001
<25	1409 (70.49)	2.45 ± 0.28	
≥25	590 (29.51)	2.59 ± 0.23	
Smoking			0.447
yes	1021 (51.08)	2.50 ± 0.27	
no	978 (48.92)	2.49 ± 0.27	
Alcohol consumption			0.011
yes	1651 (82.59)	2.50 ± 0.27	
no	348 (17.41)	2.46 ± 0.28	

Serum ferritin<sup>a</sup>: the data of serum ferritin was log-transformed and the results were present by Mean ± SD.  
doi:10.1371/journal.pone.0075908.t002

**Table 3.** The correlations between serum log-ferritin (ng/ml) and other detected variables.

Variables	R <sub>1</sub> <sup>a</sup>	P-value	R <sub>2</sub> <sup>b</sup>	P-value
Total testosterone (ng/ml)	-0.205	<0.001	-0.200	<0.001
Free testosterone (nM/L)	-0.097	<0.001	-0.083	<0.001
SHBG <sup>c</sup> (nM/L)	-0.161	<0.001	-0.182	<0.001
Estradiol (pg/ml)	-0.048	0.034	-0.039	0.083

R<sub>1</sub><sup>a</sup>: Pearson’s correlation coefficient in condition of none was adjusted; R<sub>2</sub><sup>b</sup>: Partial correlation coefficient in condition of age and alcohol consumption was adjusted; SHBG<sup>c</sup>: sex hormone-binding globulin;  
doi:10.1371/journal.pone.0075908.t003

with the free testosterone levels from 0.42 to 0.47 nM had higher ferritin levels than those with levels from 0.35–0.41 nM,  $P < 0.001$ . Especially, we also found a significant decrease trend of ferritin levels with the rise of total testosterone ( $R = -0.997$ ,  $P < 0.001$ ), free testosterone ( $R = -0.838$ ,  $P < 0.001$ ) and SHBG ( $R = -0.983$ ,  $P < 0.001$ ).

**Discussion**

In our population-based cross-sectional study, data showed that levels of serum ferritin were negatively associated with sex hormones. For serum total testosterone, free testosterone and SHBG, the associations were significant whether those potential confounding factors were adjusted or not.

The small quantity of human serum ferritin was regulated by several factors and associated with several diseases [3,4,15,29]. Previous reports revealed that Tumor necrosis factor [30], inflammation [31], thyroid hormone [32], B-boxing-factor [33] and insulin [34] may regulate the ferritin levels in different sides. Besides, abnormal functions of serum ferritin would lead to the iron deficiency anemia [4,5], age-related cataract [29] and cardiovascular diseases [3]. Above all, the regulation of serum ferritin is divers. Meanwhile, it may be an important multiple-perspective regulator in humans through many biochemical and molecular mechanisms that remain to explore. For testosterone, as we all knew it was one of the most active androgens and mainly bounded to SHBG and albumin. However, only free testosterone was bioactive [35,36]. So far we know that testosterone was with important functions of regulatory [37] and also related to many diseases, such as anemia, metabolic syndrome and the iron stage [18,36]. Furthermore, previous studies had also showed that the levels of total testosterone and SHBG were negatively associated with metabolic syndrome [16] while the levels of serum ferritin was in a positive association with metabolic syndrome [3]. In prostate cancer, lower testosterone or higher ferritin levels was related with poorer diagnosis or estimation relatively [38–40]. Besides, serum ferritin was considered as a risk factor [3] while the sex hormone may benefit for women in cardiovascular diseases [22]. And the mentioned above was consistent with our results.

Interestingly, the correlation between serum ferritin and sex hormones was value of a deeper exploration in term of hematopoiesis. For erythrocytosis, it was significantly related with sex hormones, especially the testosterone, because the iron was stimulated into erythrocyte by testosterone [41–44] and the process was under a strong regulation of hepcidin [45–47]. Reports also showed that humans were with a gender difference in lower limits of haemoglobin, serum ferritin, and red blood cell count [18] and the difference in haemoglobin at puberty might be owing to testosterone increased the upper limit of haemoglobin in

humans [41]. However, testosterone was suppressed by hepcidin and it would lead to more serum free iron while estradiol could suppress the hepcidin which usually led to the increasing iron uptake [7,48]. On the other hand, ferritin and transferrin were in a significant relation with iron metabolism and they were under the control of hepcidin [48] due to hepcidin was positively associated with levels of serum ferritin and transferrin receptor [45]. Nevertheless, in order to gain an object and convictive cognize of the relation between ferritin and sex hormones we should be paid more attention to the micro parts.

To our knowledge, there was no direct investigation to explore relationships between sex hormones [18] and iron storage before and our data showed that those associations were negative. In addition, it was well known that the ferritin L and H genes were located in 11q12 and 19q13 respectively [13,14]. Moreover, the genes *GSTP1* and *SLCO2B1* in 11q13 as well as the genes *LHB* and *KLK3* in 19q13 were reported to involve in sex hormones metabolic pathway, though the previous study showed that the latter two genes were not so significant for risk factor in prostate cancer [49]. It should be noted that the gene *GSTP1* was in an association with ferritin H gene [50], gene *LHB* was significantly with testosterone and estradiol levels [51] and special *CYP3A/KLK3* genotypes increased metastatic disease while the expression of *CYP3A* in prostate cancer was regulated by androgen [52,53]. Considering the diseases with abnormal levels of serum testosterone and ferritin, adding those genes mentioned above were in such a near location, it seems that the potential interact effect between serum ferritin levels and sex hormones is probable. Exceptionally, some reports revealed that the liver iron overload related with higher concentration of SHBG [54] and the Fe-deficient diet did not

affect the testosterone in adult male rat in a relatively short time [23].

Some study limitations needed to be addressed when interpreting these results. Firstly, this present study was a cross-sectional design so that the direction and convincingness of association should be validated by some experiments *in vitro*. Secondly, the female subjects were not included in the participants so that we could not make a comparison in gender and the results may lack of enough persuasion. Thirdly, there was no enough data about iron metabolism obtained in the present study so that we could not make more comprehensive analyses of the association between the serum ferritin and sex hormones.

## Conclusion

In conclusion, our data showed significantly negative associations between levels of serum ferritin and sex hormones, and these results may be helpful to the further exploration about the regulation or mechanisms of the ferritin.

## Acknowledgments

We thank the local research teams from Fangchenggang First People's Hospital, Fangchenggang, China, for their contribution to the survey sincerely.

## Author Contributions

Conceived and designed the experiments: ZNM XBY ZFL HYZ FHY SJZ. Performed the experiments: YG AHT XQ HYZ CLW ZL YJZ ML. Wrote the paper: ZFL FHY QX BZ LLH BBY.

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