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ORIGINAL ARTICLE

# Serum ferritin level is associated with liver steatosis and fibrosis in Korean general population

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## Abstract

**Background** Elevation of serum ferritin levels is frequently observed in non-alcoholic fatty liver disease (NAFLD) patients. Our study aims to examine the association between serum ferritin levels and NAFLD in Korean population.

**Methods and results** A total of 25,597 participants were selected from Korean National Health and Nutritional Examination Surveys 2007–2012. The NAFLD liver fat score (NLFS) was used to define NAFLD. Elevation of ALT levels was defined as ALT level > 40 IU/L for male and ALT level > 31 IU/L for female. Multiple logistic regression was used to examine the association of serum ferritin levels and NAFLD by sex. After adjusting for multiple covariates, the ORs (95% CI) of the elevated ALT levels were 1.56 (95% CI: 1.17–2.07), 1.84 (95% CI: 1.39–2.45), and 4.08 (95% CI: 3.08–5.40) for the second, third and fourth serum ferritin quartiles in male ($p$ for trend < 0.01), 1.67 (95% CI: 1.24–2.23), 2.23 (95% CI: 1.68–2.96), and 5.72 (95% CI: 4.32–7.60) for the second, third and fourth serum ferritin quartiles in female ($p$ for trend < 0.01). Serum ferritin levels were also significantly associated with NAFLD and liver fibrosis both in male and female.

**Conclusions** Elevation of serum ferritin level is significantly associated with NAFLD and blood ALT elevation in Korean general population.

**Keywords** Iron · Alanine aminotransferase · Non-alcoholic fatty liver disease

## Abbreviations

| | |
|---|---|
| ALT | Alanine aminotransferase |
| AST | Aspartate aminotransferase |
| BMI | Body mass index |
| HSI | Hepatic steatosis index |
| KNHANES | Korean National Health and Nutritional Examination Survey |
| NAFLD | Non-alcoholic fatty liver disease |
| NLFS | Non-alcoholic fatty liver disease liver fat score |
| WC | Waist circumference |

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## Introduction

Non-alcoholic fatty liver disease (NAFLD) is the common liver disease which is characterized by the macrovesicular fat accumulation in the liver cells, not caused by alcohol drinking [1]. The NAFLD increased the risk of liver cirrhosis and hepatic cellular carcinoma [2] and led to increased mortality risk from liver disease [3]. In addition, NAFLD is associated with the diabetes mellitus and carotid artery sclerosis [4, 5] and eventually increased the cardiovascular risk [6]. Besides, the prevalence of the NAFLD is much higher compared with those of hepatitis B or hepatitis C. When the abdominal ultrasonography examination was conducted in the third National Health and Nutrition Examination Survey, the prevalence of hepatic steatosis was 21.4% and the prevalence of NAFLD was 19.0% in the United States [7]. In Korea, the prevalence of NAFLD which was found on the abdominal sonography was 18.7% in male and 16.1% in female, respectively [8]. The more worrying is the increasing trends of NAFLD accompanied with growing obesity and diabetes mellitus over the world [1, 9]. Indeed, the prevalence rate of NAFLD increased more than two times from 5.5% in 1988–1994 to 11.0% in 2005–2008 in the United States [10].

In recent years, accumulating studies reported that hepatic iron overload is associated with both in the progression and fibrosis of NAFLD. HFE mutation, which is closely related with hemochromatosis, is often observed in the NAFLD patients [11, 12]. Moreover, serum ferritin level, which is the surrogate indicator for the body iron stores, is associated with development, fibrosis and severity of NAFLD [13–15]. However, it is still not clear until now whether serum ferritin by itself is independently associated with NAFLD.

Therefore, we analyzed data from Korean National Health and Nutritional Examination Survey (KNHANES), which is of a large scale, nationally representative cross-sectional study ($\geq$ 25,000 participants) and investigated the association between serum ferritin level and NAFLD among Korean general population using non-invasive scoring system.

## Patients and methods

### Data sources

The Korean Center for Chronic Disease has conducted the nationwide representative repeated cross-sectional survey, the so-called Korean National Health and Nutritional Examination Survey (KNHANES) since 1998. The KNHANES was conducted using multi-staged sampling methods and includes variables about participants' demographic and social characteristics, anthropometric measurement, biochemical and laboratory examination, health survey and nutritional examinations. Study design and data profile for the KNHANES have been described elsewhere in detail [16]. In this study, we used the fourth and fifth KNHANES data from 2007 to 2012, because serum ferritin level was not measured before 2007 or after 2012.

### Study participants

A total of 50,405 participants enrolled in the 4th and 5th KNHANES between 2007 and 2012. Of these, we initially excluded 12,801 individuals aged less than 20 years. In addition, 3795 people who did not have information on blood glucose levels were excluded. Therefore, 33,809 participants were eligible for this study. Of 33,809 eligible participants, 8212 people was excluded for the following reasons: 986 had a past history of malignancy; 101 had a past history of chronic kidney disease; 1305 had a past history of cardiovascular disease; 1223 had a hepatitis B or hepatitis C; 37 had an exceptionally high serum ferritin levels more than 1000 ng/mL; 3422 were an excessive alcohol drinkers; 1138 did not had the information about

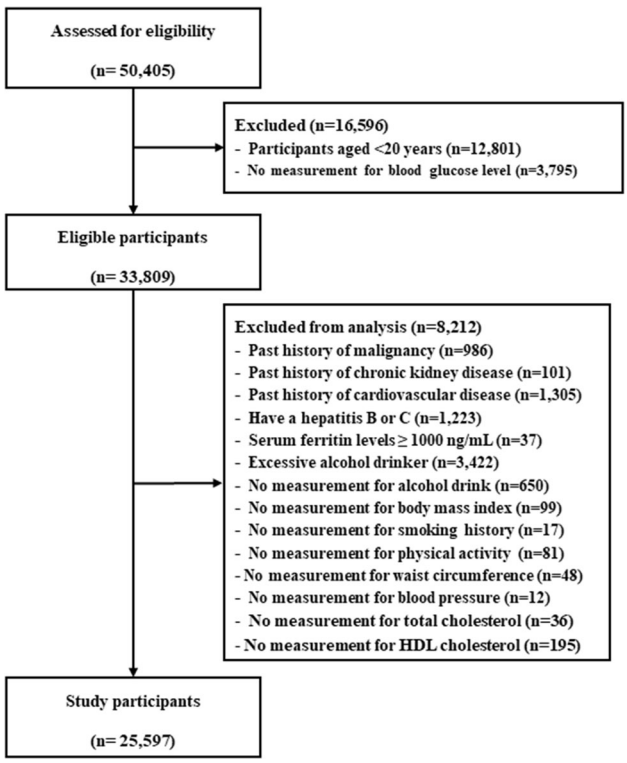


**Fig. 1** Flow chart for study participant selection

covariates. Finally, 25,597 individuals were included in the final analysis (Fig. 1).

## Health survey examinations and laboratory measurements

The information about smoking history, daily alcohol intake, physical activity and past medical histories such as past history of malignancy or liver cirrhosis was acquired by health interview questionnaire. Smoking history was divided into never-smoker, past smoker and current smoker. Daily alcohol intake (g/day) was calculated using the frequency of drinking and amount of alcohol per drink. The excessive alcohol drinker was defined as people who had $\geq 7$ drinks for male or $\geq 5$ drinks for female on a single occasion and had two or more drinks of alcohol per week [17]. Physical activity was defined as performing the moderate-intensity physical activity at least 30 min per day for more than 5 days each week or vigorous intensity physical activity at least 20 min per day for more than 3 days each week [18]. Height (cm) was measured with shoes removed (Seca 225; Seca, Germany). Weight (kg) was measured after more than 8 h of fasting (GL-6000-20; G-tech, Korea). Body mass index (BMI) was calculated as weight (kg) divided by the square of height (meters). Waist circumference (WC) was measured while standing at the level of umbilicus. Blood pressure was measured using a standard mercury sphygmomanometer at the same height as the heart in sitting position after 5 min of rest. Hypertension was defined as $\geq$ 140/90 mmHg for systolic blood pressure/diastolic blood pressure or taking a medication for blood pressure control.

Blood samples were drawn after more than 8 h of fasting. Hepatitis B antigen was measured using electrochemiluminescence immunoassays (Roche Diagnostics, Basel, Switzerland). The fasting blood glucose was measured using the high-performance liquid chromatography (Hitachi Automatic analyzer, Hitachi, Japan). Total cholesterol, high-density lipoprotein (HDL) and triglyceride were measured using enzymatic colorimetric tests (Hitachi, Tokyo, Japan). Insulin levels were measured with immunoradiometric assays (PerkinElmer, Turku, Filand). The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) were measured using an autoanlyzer (Hitach, Tokyo, Japan). Diabetes mellitus was defined as having fasting blood glucose $\geq$ 126 mg/dL or diagnosed with diabetes mellitus or taking medication for diabetes mellitus.

## Serum ferritin and transferrin saturation

Serum ferritin was measured using immunoradiometric assay (PerkinElmer, Turku, Filand) and serum iron was examined using bathophenanthroline direct method (Hitachi Automatic analyzer, Hitachi, Japan). Transferrin saturation was calculated as per the following formula:

$$\text{Transferrin saturation}\,(\%) = (\text{Serum iron level} \times 100)/\text{Total iron binding capacity}$$

The transferrin saturation status was divided into three categories: low transferrin saturation $< 20\%$, normal transferrin saturation = $20 \sim 50\%$, high transferrin saturation $> 50\%$ [19]. Although serum ferritin levels are known as the surrogate marker for body iron storage, serum ferritin levels are also affected by the inflammatory status. Furthermore, serum ferritin is accumulated in the liver and the elevated serum ferritin may only reflect the leaked product from damaged hepatocytes which were not associated with iron overload. It is very hard to distinguish that elevated serum ferritin suggests increased body iron storage or just reflects liver damage or inflammatory status. Therefore, we examined the association between serum ferritin level and ALT elevation by transferrin saturation status in the sensitivity analysis [19].

## Definition of NAFLD

To define NAFLD, we used the previously qualified scoring model for NAFLD. The revised National Cholesterol Education Program criteria were used to define the metabolic syndrome as more than three of the following five criteria: hyperglycemia, visceral obesity, hypertriglycemia, low HLD dyslipidemia and hypertension [20]. Of these five criteria, visceral obesity followed the adopted definition by the Korean Society of the Study of Obesity (waist circumference $\geq$ 90 cm for male and $\geq$ 85 cm for female) [21]. NAFLD liver fat score (NLFS score) and Hepatic steatosis index (HSI score) were selected for the scoring model for NAFLD, because there were too many missing variables to apply the other scoring model for NAFLD. The formula for NLFS score is as follows:

$$\begin{aligned}\text{NLFS score} = &-2.89 + 1.18 \times \text{metabolic syndrome}\,(\text{yes} = 1/\text{no} = 0)\\ &+ 0.45 \times \text{type 2 diabetes mellitus}\,(\text{yes} = 2/\text{no} = 0)\\ &+ 0.15 \times \text{fasting serum insulin}\,(\text{mU/L})\\ &+ 0.04 \times \text{AST}\,(\text{U/L}) - 0.94 \times \text{AST/ALT ratio}\,(\text{U/L})\end{aligned}$$

[22]. NLFS score was calculated and the cutoff value for NAFLD was set as $\geq -0.640$ [22]. We used another index—Hepatic steatosis index (HSI score)—to conduct the sensitivity analysis [23]. The formula for HSI score is as follows:

$$\text{HSI score} = 8 \times \text{ALT/AST ratio (U/L)} + \text{BMI (kg/m}^2\text{)}(+2, \text{if DM}),\ (+2 \text{ if women})$$

[23]. The cutoff value for HSI score was set as $\geq$ 36 [24]. Elevation of ALT levels was also evaluated as the surrogate marker for NAFLD. Elevation of ALT levels was defined as ALT level $>$ 40 IU/L for male and ALT level $>$ 31 IU/L for female [7].

In addition, we used Fiborsis-4 (FIB-4) score to assess the association serum ferritin levels and liver fibrosis. The formula for FIB-4 score is as follows:

$$\text{FIB} - 4 \text{ score} = \text{age (years)} \times \text{AST (U/L)} / \left[\text{platelets}(10^9/\text{L}) \times \sqrt{\text{ALT(U/L)}}\right]$$

[24]. The cutoff value of FIB-4 score for liver fibrosis was set as $>$ 2.67 [25].

### Statistical analysis

All analysis was performed by sex. The serum ferritin levels were divided into four quartiles because of its right skewed distribution. The baseline characteristics of participants were described by serum ferritin quartiles for male and female, respectively. Continuous variables were expressed as the mean (standard error) and categorical variables were expressed as number (percentage) using the sampling weights based on the complex KNHANES survey design—stratification and cluster (Tables 1, 2). Linear regression models for continuous variables were used test linear trends across the serum ferritin quartiles, considering serum ferritin quartiles as continuous one (Tables 1, 2). Rao-Scott Chi-square test was used to compare the baseline characteristics across serum ferritin quartiles (Tables 1, 2). To examine the association between serum ferritin levels and ALT elevations, NAFLD, multiple logistic regression models were used after adjusting for age, sex, systolic blood pressure, BMI, fasting blood glucose, diabetes mellitus, total cholesterol level, daily alcohol intake, smoking history and physical activity (Tables 3, 4, 5). The ferritin level was used as both continuous covariates and categorical covariates (four quartiles) in the multiple logistic regression models (Tables 3, 4, 5). When ferritin was used as categorical variables (four quartiles) in the multiple logistic regression models, likelihood ratio tests were used to assess trends of odds ratios for ordinal variables (four quartiles of ferritin levels) [26]. Finally, the generalized additive model was used to examine the non-linear dose–response relationship between serum ferritin levels and blood ALT levels (Supplementary Fig. 1). The generalized additive model fits a linear additive model with a continuous serum ALT against serum ferritin level after adjusting for all covariates.

$p$ values $<$ 0.05 were considered to be statistically significant. All statistical analyses were performed using SAS (version 9.3, SAS Institute, Cary, NC, USA) and R 3.1.1 software.

## Results

Baseline characteristics for the study participants by serum ferritin quartiles were shown by sex in Tables 1 and 2. Serum iron levels also increased with increasing serum ferritin quartile groups ($p < 0.01$). Mean age, ALT level, AST levels and γ-GTP also showed positive relationships to serum ferritin quartile groups. The 4th serum ferritin quartile group had worse metabolic components and life styles except for the alcohol intake and physical activity compared to the 1st serum ferritin quartile group. The proportions of participants who had the elevated ALT levels were 24.8% and 15.9% for the 4th serum ferritin quartile male and female, whereas the proportions of the elevated ALT levels were only 5.9% and 2.6% in the 1st serum ferritin quartile male and female, respectively. With increasing serum ferritin quartiles groups, the proportion of NAFLD defined by both HSI score and NLFS score also increased both in male and female. In addition, the proportion of FIB-4 score $\geq$ 2.67 in 4th serum ferritin group was the highest.

Table 3 shows the ORs and 95% CIs for the elevated ALT level according to the serum ferritin quartiles. After adjusting for age, systolic blood pressure, BMI, fasting blood glucose, total cholesterol level, daily alcohol intake, smoking history and physical activity, the ORs (95% CI) for the elevated ALT levels were 1.07 (1.06–1.08) and 1.13 (1.11–1.15) per serum ferritin 10 ng/mL increase in male and female, respectively. When serum ferritin was divided into quartiles, the ORs (95% CI) for the elevated ALT levels were 1.56 (1.17–2.07), 1.84 (1.39–2.45), and 4.08 (3.08–5.40) for the second, third and fourth serum ferritin quartiles after adjusting for multiple covariates in male ($p$ for trend $< 0.01$). For female, after adjusting for multiple covariates, the ORs (95% CI) for the elevated ALT levels were 1.67 (1.24–2.23), 2.23 (1.68–2.96), and 5.72 (4.32–7.60) for the second, third and fourth serum ferritin quartiles ($p$ for trend $< 0.01$).

Table 4 represents the ORs and 95% CIs for the NAFLD by HSI score and NLFS score according to the serum ferritin quartiles. After adjusting for age, systolic blood pressure, BMI, fasting blood glucose, diabetes mellitus, total cholesterol level, daily alcohol intake, smoking history and physical activity, the ORs (95% CI) for the NAFLD defined by NLFS score were 1.05 (1.04–1.06) and 1.10 (1.08–1.12) per serum ferritin 10 ng/mL increase in male and female, respectively. After adjusting all

**Table 1** Baseline characteristics of the male participants by serum ferritin level

| Characteristics | Overall ($n$ = 9615) | Serum ferritin quartiles | | | | $p$ value* |
|---|---|---|---|---|---|---|
| | | Quartile 1 ($n$ = 2403) | Quartile 2 ($n$ = 2404) | Quartile 3 ($n$ = 2403) | Quartile 4 ($n$ = 2405) | |
| Continuous variables (mean, s.e.) | | | | | | |
| Serum ferritin level (ng/mL) | 116.2 (1.1) | 0.01 to < 62.38 | 62.38 to < 96.98 | 96.98 to < 147.12 | 147.12 to 966.4 | |
| Serum iron (μg/dL) ($n$ = 4895) | 126.7 (1.0) | 118.9 (1.9) | 128.4 (1.8) | 128.0 (1.8) | 131.3 (1.7) | < 0.01 |
| Total iron binding capacity (μg/dL) ($n$ = 4895) | 307.8 (0.8) | 325.0 (1.7) | 306.2 (1.3) | 301.2 (1.2) | 299.7 (1.3) | < 0.01 |
| Transferrin saturation (%) ($n$ = 4895) | 41.6 (0.3) | 37.2 (0.6) | 42.2 (0.6) | 42.7 (0.6) | 44.2 (0.6) | < 0.01 |
| Age (years) | 43.6 (0.2) | 44.8 (0.4) | 42.9 (0.4) | 42.5 (0.4) | 44.2 (0.4) | 0.19 |
| ALT (IU/L) | 26.4 (0.3) | 21.1 (0.3) | 23.5 (0.3) | 26.4 (0.5) | 34.6 (0.9) | < 0.01 |
| AST (IU/L) | 23.4 (0.2) | 21.2 (0.3) | 21.8 (0.2) | 23.2 (0.3) | 27.6 (0.4) | < 0.01 |
| GGT (IU/L) ($n$ = 3380) | 40.4 (1.0) | 30.9 (1.6) | 34.9 (1.6) | 38.3 (1.7) | 56.8 (2.6) | < 0.01 |
| Systolic blood pressure (mmHg) | 119.0 (0.2) | 119.5 (0.4) | 118.2 (0.4) | 118.8 (0.4) | 119.8 (0.4) | 0.33 |
| Diastolic blood pressure (mmHg) | 78.7 (0.2) | 78.2 (0.3) | 78.3 (0.3) | 78.7 (0.3) | 79.7 (0.3) | < 0.01 |
| BMI (kg/m$^2$) | 24.0 (0.04) | 23.4 (0.08) | 23.6 (0.07) | 24.1 (0.08) | 24.7 (0.09) | < 0.01 |
| Waist circumference (cm) | 83.7 (0.1) | 82.2 (0.2) | 82.8 (0.2) | 84.0 (0.2) | 85.9 (0.3) | < 0.01 |
| Fasting glucose level (mg/dL) | 97.3 (0.3) | 84.9 (0.4) | 96.1 (0.5) | 96.5 (0.4) | 101.8 (0.8) | < 0.01 |
| Insulin (μIU/mL) ($n$ = 6456) | 10.1 (0.1) | 9.7 (0.2) | 9.7 (0.2) | 10.2 (0.2) | 10.9 (0.2) | < 0.01 |
| Total cholesterol (mg/dL) | 186.9 (0.5) | 182.7 (0.9) | 185.5 (0.9) | 188.0 (0.9) | 191.4 (0.9) | < 0.01 |
| HDL cholesterol (mg/dL) | 45.6 (0.1) | 46.5 (0.3) | 45.8 (0.3) | 45.5 (0.3) | 44.6 (0.3) | < 0.01 |
| Triglyceride (mg/dL) | 146.4 (1.4) | 129.7 (2.2) | 136.6 (2.3) | 144.1 (2.8) | 176.2 (3.6) | < 0.01 |
| Alcohol intake (g/day) | 6.2 (0.08) | 4.9 (0.1) | 5.9 (0.2) | 6.5 (0.2) | 7.5 (0.2) | < 0.01 |
| Categorical variables ($n$, %) | | | | | | |
| Smoking history | | | | | | |
| Never-smoker | 2371 (27.0) | 635 (29.3) | 594 (26.1) | 584 (27.6) | 558 (25.0) | 0.06 |
| Past smoker | 3506 (30.9) | 907 (31.6) | 859 (40.0) | 852 (29.6) | 888 (31.3) | |
| Current smoker | 3738 (42.1) | 861 (39.1) | 951 (42.9) | 967 (42.8) | 959 (43.7) | |
| Physical activity† | | | | | | |
| Yes | 2349 (24.5) | 611 (26.3) | 621 (25.5) | 583 (24.7) | 534 (21.6) | 0.10 |
| No | 7266 (75.5) | 1792 (73.7) | 1783 (74.5) | 1820 (75.3) | 1871 (78.4) | |
| Hypertension | | | | | | |
| Yes | 3091 (26.1) | 775 (25.7) | 716 (24.3) | 729 (24.7) | 871 (30.0) | < 0.01 |
| No | 6524 (73.9) | 1628 (74.3) | 1688 (75.7) | 1674 (75.3) | 1534 (70.0) | |
| Hypercholesterolemia ($n$ = 9373) | | | | | | |
| Yes | 949 (9.2) | 216 (7.5) | 202 (7.9) | 245 (9.9) | 286 (11.5) | < 0.01 |
| No | 8424 (90.8) | 2122 (92.5) | 2142 (92.1) | 2100 (90.1) | 2060 (88.5) | |
| Diabetes mellitus | | | | | | |
| Yes | 1017 (8.3) | 249 (7.4) | 194 (6.7) | 229 (7.6) | 345 (11.5) | < 0.01 |
| No | 8598 (91.7) | 2154 (92.6) | 2210 (93.3) | 2174 (92.4) | 2060 (88.5) | |
| Metabolic syndrome | | | | | | |
| Yes | 2475 (23.4) | 468 (17.6) | 532 (19.9) | 640 (23.5) | 835 (32.8) | < 0.01 |
| No | 7140 (76.6) | 1935 (82.4) | 1872 (80.1) | 1763 (76.5) | 1570 (67.2) | |
| Transferrin saturation status ($n$ = 4895) | | | | | | |
| Low (< 20%) | 311 (7.1) | 159 (12.9) | 64 (7.3) | 54 (5.1) | 34 (3.3) | < 0.01 |
| Normal (20–50%) | 3268 (65.9) | 826 (67.9) | 790 (64.9) | 824 (65.9) | 828 (64.9) | |
| High (> 50%) | 1316 (27.0) | 266 (19.2) | 315 (27.8) | 361 (29.0) | 414 (31.8) | |
| Elevated ALT levels | 1124 (13.1) | 115 (5.9) | 199 (9.2) | 274 (12.7) | 536 (24.8) | < 0.01 |
| FIB-4 score ($n$ = 6518) | | | | | | |

**Table 1** (continued)

| Characteristics | Overall ($n$ = 9615) | Serum ferritin quartiles | | | | $p$ value* |
|---|---|---|---|---|---|---|
| | | Quartile 1 ($n$ = 2403) | Quartile 2 ($n$ = 2404) | Quartile 3 ($n$ = 2403) | Quartile 4 ($n$ = 2405) | |
| < 1.30 | 4.601 (81.8) | 1137 (80.3) | 1187 (84.4) | 1176 (83.7) | 1101 (79.0) | < 0.01 |
| 1.30–2.67 | 1741 (16.7) | 492 (18.4) | 393 (14.4) | 394 (15.5) | 462 (18.4) | |
| > 2.67 | 176 (1.5) | 45 (1.3) | 39 (1.2) | 27 (0.8) | 65 (2.6) | |
| NAFLD by NLFS score ($n$ = 6265) | 1.894 (29.5) | 314 (19.0) | 402 (23.7) | 494 (32.0) | 684 (44.1) | < 0.01 |
| NAFLD by HSI score | 2183 (25.4) | 319 (15.8) | 475 (21.7) | 609 (27.6) | 780 (36.6) | < 0.01 |

Continuous variables were expressed as means (standard errors) and categorical variables were expressed as number (percentages) using the sampling weights to reflect complex KNHANES sampling design

*NAFLD* non-alcoholic fatty liver disease, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma glutamyl transferase, *BMI* body mass index, *FIB-4* fibrosis-4, *NLFS* non-alcoholic fatty liver disease liver fat score, *HSI* hepatic steatosis index

*Linear regressions for complex sampling survey design were used to test trend for continuous variables and Rao-Scott Chi-square for complex sampling survey design was used to test differences for categorical variables

†Physical activity was defined as moderate to vigorous intensity physical activity at least 30 min per day of moderate to vigorous intensity aerobic activity at least 5 days per week

covariates, the ORs (95% CI) for the NAFLD defined by HSI score were 1.03 (1.02–1.05) and 1.06 (1.04–1.08) per serum ferritin 10 ng/mL increase in male and female, respectively. When serum ferritin was divided into quartiles, the ORs (95% CI) for the NAFLD defined by HSI score and NAFLD defined by NLFS score were linearly associated with increasing serum ferritin quartile levels both in male and female ($p$ for trend < 0.01, respectively).

To examine the association between serum ferritin level and liver fibrosis, we defined the FIB-4 score ≥ 2.67 as liver fibrosis (Table 5). After adjusting for age, systolic blood pressure, BMI, fasting blood glucose, diabetes mellitus, total cholesterol level, daily alcohol intake, smoking history and physical activity, the ORs (95% CI) for the liver fibrosis were 1.27 (0.75–2.14), 0.89 (0.49–1.60), and 2.47 (1.50–4.08) for the second, third and fourth serum ferritin quartiles in male ($p$ for trend < 0.01), and the ORs (95% CI) for the liver fibrosis were 4.10 (1.31–12.80), 7.18 (2.26–22.86), and 9.67 (3.28–28.57) for the second, third and fourth serum ferritin quartiles in female ($p$ for trend < 0.01),.

In addition, there were positive dose–response relationships between serum ferritin level and elevated ALT levels regardless of transferrin saturation except for female with low transferrin saturation ($p$ for trend < 0.01, Supplementary Table 1). In the generalized additive models, serum ferritin levels had positive associations with blood ALT levels after adjusting for multiple covariates both in male and female (Supplementary Figs. 1, 2).

Although serum ferritin alone was not good predictor for ALT elevation (C-statistics: 0.668 for male, 0.681 for female) or NAFLD (C-statistics: 0.619 for male, 0.665 for female), serum ferritin after adjusting for age predicted ALT elevation or liver fibrosis as well as BMI or fasting blood glucose (Supplementary Table 2).

## Discussion

In this study, serum ferritin level had clear dose–response relationships with NAFLD and serum ALT levels. Although there is a considerable amount of data linking high serum ferritin to the development of NAFLD [13–15], our result showed this association in a large-scale, nationally representative population. The present study not only provides additional information for diagnosing NALFD in the general population but may also help to understand the pathogenesis of NALFD.

Although we could not know the exact mechanism why the elevated serum ferritin levels are associated with hepatic fibrosis or steatosis, one of the possible explanation is insulin resistance. Previous studies suggest the close association between serum ferritin, insulin resistance and non-alcoholic fatty liver disease [13, 14, 27]. Increased serum ferritin level is closely correlated with insulin resistance, and impaired glucose tolerance [28] and insulin resistance play a crucial role in the development of NAFLD [29]. Our study result also showed that fasting glucose and insulin level, which is related with insulin

**Table 2** Baseline characteristics of the female participants by serum ferritin level

| Characteristics | Overall ($n$ = 15,982) | Serum ferritin quartiles | | | | $p$ value* |
|---|---|---|---|---|---|---|
| | | Quartile 1 ($n$ = 3992) | Quartile 2 ($n$ = 3998) | Quartile 3 ($n$ = 3995) | Quartile 4 ($n$ = 3997) | |
| Continuous variables (mean, s.e.) | | | | | | |
| Serum ferritin level (ng/mL) | 45.1 (0.5) | 0.01 to < 18.88 | 18.88 to < 37.96 | 37.96 to < 64.51 | 64.51 to 966.4 | |
| Serum iron (μg/dL) ($n$ = 8074) | 100.9 (0.6) | 81.9 (1.4) | 108.1 (1.1) | 107.4 (1.2) | 109.2 (1.0) | < 0.01 |
| Total iron binding capacity (μg/dL) ($n$ = 8074) | 323.9 (0.7) | 363.9 (1.4) | 318.0 (1.1) | 306.1 (1.0) | 300.4 (1.0) | < 0.01 |
| Transferrin saturation (%) ($n$ = 8074) | 32.1 (0.2) | 23.4 (0.4) | 34.3 (0.4) | 35.3 (0.4) | 36.6 (0.3) | < 0.01 |
| Age (years) | 45.5 (0.2) | 38.6 (0.2) | 42.7 (0.3) | 47.8 (0.3) | 55.2 (0.3) | < 0.01 |
| ALT (IU/L) | 17.3 (0.1) | 14.0 (0.1) | 15.8 (0.2) | 17.6 (0.2) | 22.8 (0.4) | < 0.01 |
| AST (IU/L) | 19.7 (0.1) | 17.7 (0.1) | 18.7 (0.1) | 19.8 (0.1) | 23.4 (0.2) | < 0.01 |
| GGT (IU/L) ($n$ = 5487) | 20.2 (0.3) | 16.0 (0.3) | 18.7 (0.4) | 20.5 (0.5) | 26.7 (0.8) | < 0.01 |
| Systolic blood pressure (mmHg) | 114.3 (0.2) | 109.9 (0.3) | 112.3 (0.3) | 115.8 (0.4) | 120.7 (0.4) | 0.33 |
| Diastolic blood pressure (mmHg) | 73.5 (0.1) | 71.9 (0.2) | 72.8 (0.2) | 74.1 (0.2) | 75.6 (0.2) | < 0.01 |
| BMI (kg/m$^2$) | 23.2 (0.04) | 22.6 (0.07) | 22.9 (0.07) | 23.4 (0.07) | 24.2 (0.08) | < 0.01 |
| Waist circumference (cm) | 77.9 (0.1) | 75.5 (0.2) | 76.8 (0.2) | 78.6 (0.2) | 81.5 (0.2) | < 0.01 |
| Fasting glucose level (mg/dL) | 94.7 (0.2) | 91.0 (0.3) | 92.5 (0.3) | 95.1 (0.4) | 101.6 (0.6) | < 0.01 |
| Insulin (μIU/mL) ($n$ = 10,652) | 10.1 (0.1) | 9.9 (0.1) | 9.9 (0.2) | 9.9 (0.1) | 11.0 (0.2) | < 0.01 |
| Total cholesterol (mg/dL) | 188.0 (0.4) | 182.4 (0.7) | 183.8 (0.7) | 190.0 (0.7) | 197.8 (0.8) | < 0.01 |
| HDL cholesterol (mg/dL) | 51.4 (0.1) | 53.5 (0.2) | 51.8 (0.2) | 51.1 (0.2) | 48.7 (0.2) | < 0.01 |
| Triglyceride (mg/dL) | 110.8 (0.8) | 96.7 (1.2) | 104.4 (1.3) | 113.1 (1.5) | 133.8 (1.8) | < 0.01 |
| Alcohol intake (g/day) | 1.6 (0.03) | 1.7 (0.06) | 1.8 (0.06) | 1.6 (0.06) | 1.4 (0.06) | < 0.01 |
| Categorical variables ($n$, %) | | | | | | |
| Smoking history | | | | | | |
| Never-smoker | 14,686 (90.4) | 3705 (92.1) | 3640 (89.4) | 3676 (90.2) | 3665 (89.8) | < 0.01 |
| Past smoker | 566 (4.1) | 126 (3.4) | 165 (5.1) | 128 (3.4) | 147 (4.3) | |
| Current smoker | 730 (5.5) | 161 (4.5) | 193 (5.5) | 191 (6.4) | 185 (5.9) | |
| Physical activity[†] | | | | | | |
| Yes | 3181 (19.4) | 789 (19.7) | 792 (18.6) | 813 (20.1) | 787 (19.4) | 0.59 |
| No | 12,801 (80.6) | 3203 (80.3) | 3206 (81.4) | 3182 (79.9) | 3210 (80.6) | |
| Hypertension | | | | | | |
| Yes | 4195 (21.6) | 531 (11.2) | 878 (18.0) | 1178 (24.6) | 1608 (35.9) | < 0.01 |
| No | 11,787 (78.4) | 3461 (88.8) | 3120 (82.0) | 2817 (75.4) | 2389 (64.1) | |
| Hypercholesterolemia ($n$ = 15,681) | | | | | | |
| Yes | 2182 (11.9) | 300 (6.8) | 438 (9.2) | 636 (13.7) | 808 (19.8) | < 0.01 |
| No | 13,499 (88.1) | 3617 (93.2) | 3478 (90.8) | 3294 (86.3) | 3110 (80.2) | |
| Diabetes mellitus | | | | | | |
| Yes | 1277 (6.9) | 125 (2.7) | 250 (5.3) | 338 (7.3) | 564 (13.8) | < 0.01 |
| No | 14,705 (93.1) | 3867 (97.3) | 3748 (94.7) | 3657 (92.7) | 3433 (86.2) | |
| Metabolic syndrome | | | | | | |
| Yes | 3652 (19.8) | 428 (9.6) | 727 (15.8) | 1022 (22.6) | 1475 (34.3) | < 0.01 |
| No | 12,330 (80.2) | 3564 (90.4) | 3271 (84.2) | 2973 (77.4) | 2522 (65.7) | |
| Transferrin saturation status ($n$ = 7908) | | | | | | |

**Table 2** (continued)

| Characteristics | Overall ($n$ = 15,982) | Serum ferritin quartiles | | | | $p$ value* |
|---|---|---|---|---|---|---|
| | | Quartile 1 ($n$ = 3992) | Quartile 2 ($n$ = 3998) | Quartile 3 ($n$ = 3995) | Quartile 4 ($n$ = 3997) | |
| Low (< 20%) | 1426 (19.5) | 874 (46.0) | 244 (13.0) | 184 (8.7) | 124 (6.2) | < 0.01 |
| Normal (20-50%) | 5852 (70.3) | 982 (50.0) | 1572 (75.5) | 1600 (79.0) | 1698 (80.1) | |
| High (> 50%) | 796 (10.2) | 81 (4.0) | 219 (11.5) | 246 (12.3) | 250 (13.7) | |
| Elevated ALT levels | 1120 (6.8) | 110 (2.6) | 172 (4.3) | 252 (6.3) | 586 (15.9) | < 0.01 |
| FIB-4 score ($n$ = 10,906) | | | | | | |
| < 1.30 | 8409 (82.8) | 2513 (94.8) | 2264 (86.8) | 1974 (78.4) | 1658 (67.4) | < 0.01 |
| 1.30–2.67 | 2344 (16.2) | 186 (5.1) | 470 (12.7) | 709 (20.3) | 979 (30.0) | |
| > 2.67 | 153 (1.0) | 5 (0.1) | 26 (0.5) | 38 (1.3) | 84 (2.6) | |
| NAFLD by NLFS score ($n$ = 10,429) | 2218 (19.5) | 297(10.9) | 407 (14.2) | 575 (20.0) | 939 (36.5) | < 0.01 |
| NAFLD by HSI score | 3000 (18.3) | 476 (11.9) | 611 (15.6) | 813 (19.9) | 1100 (27.8) | < 0.01 |

Continuous variables were expressed as means (standard errors) and categorical variables were expressed as number (percentages) using the sampling weights to reflect complex KNHANES sampling design

*NAFLD* non-alcoholic fatty liver disease, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *GGT* gamma glutamyl transferase, *BMI* body mass index, *FIB-4* fibrosis-4, *NLFS* non-alcoholic fatty liver disease liver fat score, *HSI* hepatic steatosis index

*Linear regressions for complex sampling survey design were used to test trend for continuous variables and Rao-Scott Chi-square for complex sampling survey design was used to test differences for categorical variables

†Physical activity was defined as moderate to vigorous intensity physical activity at least 30 min per day of moderate to vigorous intensity aerobic activity at least 5 days per week

**Table 3** Association between serum ferritin level and elevated ALT level among general Korean population

| Variables | Unadjusted OR (95% CI) | Age-adjusted OR (95% CI) | Multivariable adjusted OR (95% CI) |
|---|---|---|---|
| Male | | | |
| Serum ferritin level (ng/mL) | | | |
| Per 10 ng/mL increase | 1.07 (1.06–1.08) | 1.08 (1.07–1.09) | 1.07 (1.06–1.08) |
| Quartile 1 (0.01–62.37) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Quartile 2 (62.38–96.97) | 1.62 (1.23–2.13) | 1.58 (1.20–2.08) | 1.56 (1.17–2.07) |
| Quartile 3 (96.98–147.11) | 2.32 (1.76–3.04) | 2.25 (1.71–2.95) | 1.84 (1.39–2.45) |
| Quartile 4 (147.12–966.4) | 5.26 (4.03–6.86) | 5.31 (4.06–6.94) | 4.08 (3.08–5.40) |
| $p$ for trend | < 0.01 | < 0.01 | < 0.01 |
| Female | | | |
| Serum ferritin level (ng/mL) | | | |
| Per 10 ng/mL increase | 1.15 (1.13–1.16) | 1.14 (1.12–1.16) | 1.13 (1.11–1.15) |
| Quartile 1 (0.01–18.87) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Quartile 2 (18.88–37.95) | 1.70 (1.28–2.26) | 1.67 (1.25–2.23) | 1.67 (1.24–2.23) |
| Quartile 3 (37.96–64.50) | 2.52 (1.93–3.29) | 2.43 (1.85–3.19) | 2.23 (1.68–2.96) |
| Quartile 4 (64.51–969.06) | 7.12 (5.52–9.17) | 6.67 (5.07–8.78) | 5.72 (4.32–7.60) |
| $p$ for trend | < 0.01 | < 0.01 | < 0.01 |

Logistic regression model for complex sampling survey design was used to examine the association between increase in serum ferritin levels per 10 ng/mL, serum ferritin quartiles and elevated ALT level

When ferritin was used as categorical variables (four quartiles) in the multiple logistic regression models, likelihood ratio tests were used to assess trends of odds ratios for ordinal variables ($p$ for trend)

Age-adjusted OR was adjusted for only age; Multivariable adjusted OR was adjusted for age, systolic blood pressure, BMI, fasting blood glucose, diabetes mellitus, total cholesterol, daily alcohol intake, smoking history and physical activity

**Table 4** Association between serum ferritin level and NAFLD among general Korean population

| NAFLD | Unadjusted OR (95% CI) | Age-adjusted OR (95% CI) | Multivariable adjusted OR (95% CI) |
|---|---|---|---|
| Male | | | |
| By NLFS score ($n$ = 6265) | | | |
| Serum ferritin level (ng/mL) | | | |
| Per 10 ng/mL increase | 1.06 (1.05–1.07) | 1.06 (1.05–1.07) | 1.05 (1.04–1.06) |
| Quartile 1 (0.01–62.37) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Quartile 2 (62.38–96.97) | 1.33 (1.10–1.60) | 1.35 (1.11–1.63) | 1.16 (0.93–1.46) |
| Quartile 3 (96.98–147.11) | 2.01 (1.65–2.45) | 2.07 (1.70–2.53) | 1.79 (1.41–2.26) |
| Quartile 4 (147.12–966.4) | 3.36 (2.77–4.08) | 3.40 (2.80–4.12) | 2.51 (2.00–3.15) |
| $p$ for trend | < 0.01 | < 0.01 | < 0.01 |
| By HSI score | | | |
| Serum ferritin level (ng/mL) | | | |
| Per 10 ng/mL increase | 1.04 (1.04–1.05) | 1.05 (1.04–1.05) | 1.03 (1.02–1.05) |
| Quartile 1 (0.01–18.87) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Quartile 2 (18.88–37.95) | 1.48 (1.24–1.75) | 1.44 (1.21–1.71) | 1.61 (1.27–2.05) |
| Quartile 3 (37.96–64.50) | 2.02 (1.69–2.42) | 1.97 (1.64–2.35) | 1.90 (1.48–2.43) |
| Quartile 4 (64.51–969.06) | 3.07 (2.57–3.68) | 3.10 (2.58–3.72) | 2.72 (2.13–3.48) |
| $p$ for trend | < 0.01 | < 0.01 | < 0.01 |
| Female | | | |
| By NLFS score ($n$ = 10,429) | | | |
| Serum ferritin level (ng/mL) | | | |
| Per 10 ng/mL increase | 1.15 (1.13–1.17) | 1.10 (1.08–1.11) | 1.10 (1.08–1.12) |
| Quartile 1 (0.01–62.37) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Quartile 2 (62.38–96.97) | 1.36 (1.14–1.62) | 1.15 (0.95–1.38) | 1.14 (0.89–1.45) |
| Quartile 3 (96.98–147.11) | 2.05 (1.72–2.44) | 1.44 (1.20–1.73) | 1.29 (1.03–1.63) |
| Quartile 4 (147.12–966.4) | 4.72 (3.97–5.60) | 2.62 (2.18–3.16) | 2.55 (2.03–3.19) |
| $p$ for trend | < 0.01 | < 0.01 | < 0.01 |
| By HSI score | | | |
| Serum ferritin level (ng/mL) | | | |
| Per 10 ng/mL increase | 1.07 (1.05–1.09) | 1.05 (1.04–1.07) | 1.06 (1.04–1.08) |
| Quartile 1 (0.01–18.87) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Quartile 2 (18.88–37.95) | 1.36 (1.17–1.59) | 1.31 (1.12–1.52) | 1.44 (1.16–1.79) |
| Quartile 3 (37.96–64.50) | 1.84 (1.59–2.13) | 1.67 (1.44–1.95) | 1.95 (1.54–2.46) |
| Quartile 4 (64.51–969.06) | 2.85 (2.47–3.29) | 2.41 (2.06–2.81) | 2.94 (2.25–3.85) |
| $p$ for trend | < 0.01 | < 0.01 | < 0.01 |

Logistic regression model for complex sampling survey design was used to examine the association between increase in serum ferritin levels per 10 ng/mL, serum ferritin quartiles and NAFLD

When ferritin was used as categorical variables (four quartiles) in the multiple logistic regression models, likelihood ratio tests were used to assess trends of odds ratios for ordinal variables (P for trend)

Age-adjusted OR was adjusted for only age; Multivariable adjusted OR was adjusted for age, systolic blood pressure, BMI, fasting blood glucose, diabetes mellitus, total cholesterol, daily alcohol intake, smoking history and physical activity

resistance [30, 31], is proportionally increased across ferritin quartile. Additionally, obesity is a potential mediator between serum ferritin and NAFLD. Recent study reported that increased BMI was associated with hyperferritinemia even in individuals with low levels of iron storage [32], and obesity is a well-known risk factor of NAFLD. In the present study, BMI and waist circumference increased proportionally with ferritin concentration, so it seems reasonable that the prevalence of NALFD increased in the population with elevated ferritin level. However, our study result also showed that the association between serum ferritin and NAFLD was consistently significant in BMI, fasting glucose and other possible covariate adjusted model. Moreover, Fracanzani et al. indicated that visceral obesity seems to be associated with initiation of the NAFLD, but not associated with the progression or fibrosis

**Table 5** Association between serum ferritin level and the degree of hepatic fibrosis using FIB-4 score among general Korean population

| Liver fibrosis (FIB-4 score > 2.67) | Unadjusted OR (95% CI) | Age-adjusted OR (95% CI) | Multivariable adjusted OR (95% CI) |
|---|---|---|---|
| Male | | | |
| By FIB-4 score ($n$ = 6518) | | | |
| Serum ferritin level (ng/mL) | | | |
| Per 10 ng/mL increase | 1.05 (1.04–1.07) | 1.05 (1.04–1.06) | 1.05 (1.03–1.06) |
| Quartile 1 (0.01–62.37) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Quartile 2 (62.38–96.97) | 0.90 (0.56–1.45) | 1.31 (0.78–2.18) | 1.27 (0.75–2.14) |
| Quartile 3 (96.98–147.11) | 0.61 (0.35–1.08) | 0.96 (0.54–1.71) | 0.89 (0.49–1.60) |
| Quartile 4 (147.12–966.4) | 1.99 (1.22–3.24) | 2.75 (1.65–4.60) | 2.47 (1.50– 4.08) |
| $p$ for trend | 0.02 | < 0.01 | < 0.01 |
| Female | | | |
| By FIB-4 score ($n$ = 10,906) | | | |
| Serum ferritin level (ng/mL) | | | |
| Per 10 ng/mL increase | 1.07 (1.02–1.12) | 1.05 (1.02–1.08) | 1.07 (1.04–1.09) |
| Quartile 1 (0.01–18.87) | 1.00 (reference) | 1.00 (reference) | 1.00 (reference) |
| Quartile 2 (18.88–37.95) | 7.24 (2.40–21.82) | 3.81 (1.24–11.68) | 4.10 (1.31–12.80) |
| Quartile 3 (37.96–64.50) | 16.30 (5.42–48.99) | 6.00 (1.94–18.51) | 7.18 (2.26–22.86) |
| Quartile 4 (64.51–969.06) | 33.93 (12.21–94.26) | 8.02 (2.79–23.00) | 9.67 (3.28–28.57) |
| $p$ for trend | < 0.01 | < 0.01 | < 0.01 |

Logistic regression model for complex sampling survey design was used to examine the association between increase in serum ferritin levels per 10 ng/mL, serum ferritin quartiles and liver fibrosis

When ferritin was used as categorical variables (four quartiles) in the multiple logistic regression models, likelihood ratio tests were used to assess trends of odds ratios for ordinal variables ($p$ for trend)

Age-adjusted OR was adjusted for only age; Multivariable adjusted OR was adjusted for age, systolic blood pressure, BMI, fasting blood glucose, diabetes mellitus, total cholesterol, daily alcohol intake, smoking history and physical activity

of the NAFLD [15]. They also showed that serum ferritin levels were independently associated with severe liver fibrosis among people with low visceral fat tissue after adjusting multiple covariates [15]. These findings suggest that elevated serum ferritin is independently related with NALFD even in subjects with obesity and increased insulin resistance.

Another possible explanation of the association between serum ferritin and NALFD is a chronic inflammation. Serum ferritin levels are known as the surrogate marker for body iron storage; however, serum ferritin levels are also affected by the inflammatory status. On the other hand, serum ferritin means the damage of liver parenchyme, not due to increased body iron or inflammation. Therefore, it is very hard to distinguish that elevated serum ferritin suggests increased liver iron storage or inflammatory status or reflects liver damage caused by other factors. The chronic inflammation appears to be an important mechanism to explain the association of ferritin concentration and NALFD, but follow-up study is needed to determine exactly how it relates.

The major strength of the present study is a large scale (25,597 participants) based on the nationwide representative sample. There was a low possibility of selection bias in the KNHANES. Therefore, our findings provide a solid association between serum ferritin level and NAFLD in general population. The prevalence of NAFLD in the highest serum ferritin quartile was profoundly increased (36.6–44.1% in male and 27.8–36.5% in female). The serum ferritin level check can be done by simple blood test in primary clinics and is essential when iron deficiency anemia is suspected. However, even in the absence of iron deficiency anemia, subjects with increased serum ferritin may have other metabolic diseases such as NAFLD. Our findings suggest that additional liver enzyme test and imaging studies may be needed in this case.

Nonetheless, the limitation of the study should be acknowledged. Although we used scoring system for NAFLD and ALT levels, the standard method to diagnose the NAFLD is imaging study and liver biopsy. Therefore, there may be a bias to define the NAFLD. However, our study is based on the national representative sampling survey and it is impossible to assess the participants using abdominal ultrasound or CT. Therefore, it was impossible to examine the predictive ability of serum ferritin levels with ALT levels to distinguish the NAFLD among general

population. Second, it is impossible to examine the temporal relationship between serum ferritin level and NAFLD, because our study design is based on the cross-sectional study. Third, we could not know the history of drug use; therefore, we could not exclude the effects of medication. Fourth, we could not investigate the cause of serum ferritin elevation. Further study is needed to investigate what causes the liver damage and increases the serum ferritin levels in the apparently healthy general population.

In summary, our finding showed that elevated ferritin level was a clinically significant marker for NAFLD in general Korean population. Second, serum ferritin levels had a significant dose–response relationship with blood ALT levels and liver fibrosis score, which is a surrogate marker for liver damage. Further follow-up studies are needed to clarify the underlying etiology of this finding.

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## Compliance with ethical standards

**Conflict of interest** Ju Young Jung, Jae Jun Shim, Sung Keun Park, Jae-Hong Ryoo, Joong-Myung Choi, In-Hwan Oh, Kyu-Won Jung, Hyunsoon Cho, Moran Ki, Young-Joo Won and Chang-Mo Oh have declared that no competing interests exist.

**Ethics approval** Ethics approval for the research protocol was obtained by the institutional review board (IRB) of the Kyung Hee University (IRB No: KHSIRB-17-073, Seoul, Korea).

**Informed consent** Written informed consents were obtained from study participants before the KNHANES survey started.

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