British Journal of Nutrition (2017), **117**, 1693–1701
doi:10.1017/S0007114517001696

Transferrin saturation concentrations associated with telomeric ageing: a population-based study

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(Submitted 1 December 2016 – Final revision received 10 June 2017 – Accepted 13 June 2017 – First published online 19 July 2017)

Abstract

There are limited data on the association between Fe overload and leucocyte telomere length (LTL), known as a useful biomarker of the replicative ageing of cells. The aim of the study was to evaluate associations between Fe-status biomarkers and LTL. A cross-sectional study included 1174 men and women aged 50–79 years who provided blood samples for assays of Fe-status biomarkers including ferritin, transferrin saturation (TSAT), total Fe-binding capacity (TIBC) and relative LTL. They were free of hepatitis, potential infection or Fe deficiency. In multiple linear regression analysis adjusted for potential confounding variables, log-transformed LTL was positively associated with TIBC (adjusted coefficient estimate for its highest quartile: 0·17 (SE 0·03), $P<0·001$) and inversely associated with TSAT (adjusted coefficient estimate for its third and fourth quartiles: −0·09 (SE 0·03), $P<0·01$). These associations were consistent after additional adjustment for serum concentrations of high-sensitivity C-reactive protein, alanine transaminase and aspartate transaminase. In particular, participants with not only abnormally high concentrations (>45 %) but also with high-normal concentrations (35–45 %) of TSAT had shorter LTL compared with those with low-normal concentrations (<30 %) ($P<0·05$). We also observed that less-active or obese persons with high TSAT concentrations had shorter LTL than others. Our findings that cellular ageing is influenced not only by Fe overload but also by high-normal concentrations of TSAT support the hypothesis regarding the detrimental effects of labile Fe, which has a potent pro-oxidant activity in the body.

Key words: Iron status: Transferrin saturation: Telomere length: Iron overload: General populations

Fe is one of the essential bioelements in the human body and is required for erythrocyte production, enzyme formation and function (e.g. cytochrome P450 complex is an Fe-containing enzyme and ribonucleotide reductase, which is necessary for DNA synthesis, is an Fe-dependent enzyme), the immune system and metabolic processes[1]. Thus, negative Fe balance or Fe deficiency can cause anaemia and further lead to defects in energy metabolism, immune function, cognition and physical performance[1].

Fe overload or haemochromatosis (HC) is positive Fe balance characterised by the accumulation of excess Fe in the body. It has been reported that Fe overload is associated with an elevated risk for CVD and mortality risk in some studies[2–5] but not in others[6–7]. Further, cirrhosis, heart failure, diabetes mellitus and inflammatory disease are prevalent clinical manifestations of Fe overload because excess Fe accumulates in varied organs including the liver, heart and pancreas[8–10]. The central biological mechanism underlying the detrimental effects of Fe overload is that labile Fe is involved in the production of reactive oxygen species (ROS), which have the potential to damage DNA[11].

Telomeres, which cap the ends of a chromosome, consist of non-coding repeating sequences of hexameric DNA (TTAGGG in humans). The attrition of these telomeric sequences occurs naturally during DNA replication, but is accelerated under conditions of oxidative stress[12]. Currently, telomere length is considered an indicator of the replicative ageing of cells or cumulative oxidative stress[12]. Given the hypothesis that Fe causes oxidative stress, it is expected that Fe overload is associated with short telomere length. In fact, there is an epidemiological study that evaluated the association between Fe overload and leucocyte telomere length (LTL)[13], although other data are not available. In this sole study, the majority of participants were of European descent, and regardless of genetic predisposition, individuals with the phenotype of Fe overload, which was defined as elevated concentrations of serum ferritin (>300 ng/ml for men and >200 ng/ml for women) or transferrin saturation (TSAT) (≥50 % for men and ≥45 % for women), showed an approximately 2-fold higher OR of having shorter LTL than those without the Fe-overload phenotype[13]. However, whether normal concentrations of serum ferritin

Abbreviations: ALT, aspartate transaminase; AST, alanine transaminase; BP, blood pressure; hs-CRP, high-sensitivity C-reactive protein; LTL, leucocyte telomere length; MET, metabolic equivalent; ROS, reactive oxygen species; TIBC, total Fe-binding capacity; TSAT, transferrin saturation.

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(20–300 ng/ml for men and 20–200 ng/ml for women) and TSAT (20–50 % for men and 20–45 % for women) are associated with shorter LTL is unknown, although a hypothesis on the detrimental effects of even normal Fe status has been raised[14].

In the present study, we evaluated which Fe-status biomarkers and their concentrations were associated with LTL in a general population. In particular, we tested the hypothesis that even normal concentrations of serum ferritin or TSAT are associated with shorter LTL. We also evaluated whether lifestyle factors such as smoking, alcohol consumption and physical activity modify the association between an Fe-status biomarker and LTL.

Methods

Study design and population

We conducted a cross-sectional study in a population-based cohort of the Korean Genome Epidemiology Study, which is an ongoing longitudinal investigation. Detailed information regarding enrolment of cohort members and study procedures is available elsewhere[15,16]. In brief, 5015 cohort members aged 40–69 years were enrolled between 25 June 2001 and 29 January 2003. Since the baseline, they have been followed up biennially and have undergone a comprehensive health examination and an on-site interview at the Korea University Ansan Hospital. During the health examination, cohort members provided blood samples for biochemical assays and participated in anthropometric and clinical evaluations. They also participated in questionnaire-based interviews administered by trained personnel to collect information on sociodemographics, medical history, health conditions and lifestyle factors. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Human Subjects Review Committee at the Korea University Ansan Hospital (ED0624). At each visit, written informed consent was obtained from all subjects.

Among cohort members, 1243 individuals provided blood samples for LTL assays and biochemical assessments for Fe-status biomarkers and others between February 2011 and November 2012. During this period, individuals who reported a diagnosis of cancer, CVD or hepatitis (n 2) within the past 2 years or those with potential infection (high-sensitivity C-reactive protein (hs-CRP) concentrations >10 mg/l) or Fe deficiency (ferritin concentrations <20 ng/ml) (n 67) were excluded to minimise pathophysiologic alterations in Fe-status biomarkers. After this exclusion, 1174 participants were included in present study. We used G*Power program (version 3.1.9.2; HHU, Düsseldorf Universität) to confirm the appropriateness of the sample size. After applying an α of 0·05, a power of 0·80 and 15 predictors in the calculation, we obtained minimum sample sizes as 139 for a medium effect size ($f^2 = 0{\cdot}15$) and 954 for a small effect size ($f^2 = 0{\cdot}02$). When we compared baseline characteristics between the study participants and remaining cohort members who did not participate in this study, non-smokers and those with a higher education level were more likely to participate in this study, whereas BMI, alcohol consumption and physical activity level were similar between the two groups.

Blood collection and assessments

All participants fasted for at least 8 h before blood collection. Whole blood and serum samples were collected, some of which were delivered to the Seoul Clinical Laboratories for assays of leucocyte counts and glucose, total cholesterol, HDL-cholesterol, TAG, hs-CRP, aspartate transaminase (ALT) and alanine transaminase (AST) concentrations as well as for concentrations of Fe-status biomarkers including Hb and serum Fe, ferritin and unsaturated Fe-binding capacity (UIBC), which were used to calculate total Fe-binding capacity (TIBC) and TSAT; TIBC was obtained by adding serum Fe and UIBC concentrations, and TSAT was calculated by multiplying the ratio of serum Fe:TIBC by 100. Regarding assay methods, flow cytometry for Hb and leucocyte counts, colorimetry for glucose, Fe and UIBC, turbidimetric immunoassay for hs-CRP and two-step chemiluminescent sandwich immunoassay for ferritin were used. Serum total cholesterol, HDL-cholesterol, TAG, ALT and AST were measured enzymatically using the Siemens Advia 1800 Analyzer (Siemens Healthcare Diagnostics Inc.). Applicable assay kits for these biomarkers, except hs-CRP, were purchased from Siemens, and those for hs-CRP from Nittobo. All assays were carried out based on the standardised protocol of quality control in the commercial laboratory. The laboratory reported CV from routine assays; 1·7 % for leucocyte counts, 0·8 % for glucose, 0·9 % for total cholesterol, 1·7 % for HDL-cholesterol, 1·8 % for TAG, 1·9 % for hs-CRP, 4·8 % for ALT, 3·3 % for AST, 0·9 % for Hb, 1·6 % for Fe, 5·8 % for ferritin and 2·1 % for UIBC.

Leucocyte telomere length measurement

Whole blood samples were immediately frozen using dry ice before storage at −80°C. Within 2 months of storage, leucocyte genomic DNA was extracted from the thawed samples using a QIAamp DNA Blood Mini Kit (QIAGEN). Purified DNA samples were diluted and quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). Relative LTL was measured using quantitative real-time PCR[17]. The ratio of the telomere-repeat copy number:the single-copy gene (*36B4* encoding acidic ribosomal phosphoprotein) copy number was determined to assess the relative LTL using the iQ Multi-Color Real-Time PCR Detection System (Bio-Rad). The final concentrations of the PCR reagents were 1× SYBR Green SuperMix (Bio-Rad), 50 ng of DNA, 0·2 μM telomere primers (forward, 5'-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3'; reverse, 5'-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3') and 0·3 μM *36B4* primers (forward, 5'-CAGCAAGTGGGAAGGTGTAATCC-3'; reverse, 5'-CCCATTCTATCATCAACGGGTACAA-3'). The reactions were performed using telomere and *36B4* primers in the same ninety-six-well plate, and each plate included a reference DNA sample. A four-point standard curve was established to transform the cycle threshold into nanograms of DNA. A validity test showed that the Pearson intra- and inter-assay correlation coefficients were 0·78 and 0·69, respectively, when twenty-five samples were run in triplicate.

Potential confounding variables

Information on potential confounding variables including age, sex, income, BMI, smoking status, alcohol consumption,

physical activity level, the presence of diabetes mellitus, hypertension or dyslipidaemia, leucocyte counts and some biochemical measures were obtained in the same follow-up period when blood samples for assays of LTL and other biomarkers were collected. These variables were selected on the basis of earlier epidemiological studies[3–5] and the results of the association analysis for our study population. Because dietary information was not measured concurrently in the present study, nutritional variables including Fe intake were not taken into account. Demographic data, information on lifestyle factors such as smoking status, alcohol consumption and physical activity as well as medication history were collected through questionnaire-based interviews, and anthropometric data were collected from a health examination. According to a standardised protocol for anthropometric measurements, height (cm) and body weight (kg) were measured to the nearest 0·1 cm or 0·1 kg, respectively, while the subjects were not wearing footwear, and BMI (kg/m^2) was calculated. Information on smoking status (never/current/ex-smoker) and the number of cigarettes smoked was obtained. Information on the average frequency of alcohol-drinking occasions, the amount of alcohol consumed on a typical occasion and the volume of one standard drink for each alcoholic beverage was obtained, and the daily amount of alcohol consumed (g/d) was calculated. Information on physical activity level was obtained using five categories for activity intensity with open-ended questions about the hours spent in a typical day per level of intensity. A total metabolic equivalent score (MET-h/d) was calculated by multiplying the hours spent at a particular activity intensity according to MET values (1·0 for sleep or sedentary, 1·5 for very light activity, 2·4 for light activity, 5·0 for moderate activity and 7·5 for vigorous activity), which were determined based on examples of activities given for each category. The presence of diabetes mellitus, hypertension or dyslipidaemia was confirmed if the following criteria were met: fasting plasma glucose level of ≥120 mg/dl (6·66 mmol/l) or postprandial glucose level of ≥200 mg/dl (11·10 mmol/l) for diabetes mellitus; use of antihypertensive medications or systolic blood pressure (BP) of ≥140 mmHg or diastolic BP of ≥90 mmHg for hypertension; use of hypolipidaemic medications or serum total cholesterol concentrations of ≥240 mg/dl (6·22 mmol/l), serum HDL-cholesterol concentrations of <50 mg/dl (1·30 mmol/l) (women) or <40 mg/dl (1·04 mmol/l) (men); or serum TAG concentrations of ≥150 mg/dl (1·69 mmol/l) for dyslipidaemia. BP was measured in a sitting position using a mercury sphygmomanometer after a rest period of at least 5 min. Repeated BP measurements were performed at approximately 30 s intervals and recorded to the nearest 2 mmHg. The average of two measurements each in the left and right arms was calculated for systolic and diastolic BP.

Statistical analysis

According to the quartiles of LTL, descriptive statistics for demographic and clinical characteristics, information on lifestyle factors and blood assay results of study participants were determined. The Cochran–Armitage test for trend and the ANOVA test with trend analysis were used, as appropriate. To evaluate the associations between Fe-status biomarkers and LTL, LTL was transformed using the natural-logarithm function to minimise the effect of outliers and was fitted as a dependent variable for linear regression analysis. Quartiles of each Fe-status biomarker were fitted as independent variables. In the multiple linear regression models, covariates including age (continuous), sex, income (average monthly wage of $<1{\cdot}5\times10^6$ Won or $\geq1{\cdot}5\times10^6$ Won, which approximately corresponds to the government-set minimum wage for a family of three persons), BMI (continuous), smoking status (non-smoking or smoking of 1–10, 11–20 or ≥21 cigarettes/d), alcohol consumption (non-drinking or alcohol consumption of 1–15, 16–30 or >30 g/d), physical activity level (quartiles of MET-h/d), the presence of diabetes mellitus, hypertension or dyslipidaemia, leucocyte counts (continuous) as well as some biochemical measures (continuous) such as hs-CRP, ALT and AST concentrations were included. In addition, interaction terms between Fe-status biomarkers and sex and lifestyle factors such as smoking status (non-smoking and smoking), alcohol consumption (non-drinking and drinking), physical activity level (less than the median value and greater than or equal to the median value) and the BMI groups classified using the cut-off point of 25 kg/m^2 to define obesity in Asians (non-obese and obese)[18] were entered and tested in multiple models because sex and these factors are known to be associated with LTL. Multiple linear regression analyses stratified by sex were also performed. For the comparison of LTL across a categorical variable, Scheffé's *post hoc* multiple-comparison tests were performed. All statistical analyses were performed using SAS version 9.1.3 software (SAS Institute).

Results

This cross-sectional study included 623 men (53 %) and 551 women (47 %). Elevated concentrations of Fe-status biomarkers were observed in some men; Hb >170 g/l in 1·8 %, serum Fe >150 μg/dl (26·85 μmol/l) in 24·2 %, ferritin >300 ng/ml in 5·8 %, TIBC >400 μg/dl (71·60 μmol/l) in 2·3 % and TSAT >50 % in 21·0 % of men. Similarly, Hb >150 g/l in 1·5 %, serum Fe >145 μg/dl (25·96 μmol/l) in 13·4 %, ferritin >200 ng/ml in 3·1 %, TIBC >400 μg/dl (71·60 μmol/l) in 3·1 % and TSAT >45 % in 14·9 % of women were observed.

Table 1 shows the descriptive statistics for Fe-status biomarkers and potential confounding variables according to the quartiles of LTL. Participants with longer LTL were more likely to be younger and have higher concentrations of TIBC and lower concentrations of TSAT ($P<0{\cdot}001$).

Table 2 shows the coefficient estimates and standard errors obtained from linear regression analyses for the association of Fe-status biomarkers and LTL. After adjustment of potential confounding variables including leucocyte counts and serum hs-CRP, ALT and AST concentrations, LTL was positively associated with TIBC ($P_{\text{for trend}}<0{\cdot}001$) and was inversely associated with TSAT ($P_{\text{for trend}}<0{\cdot}01$) (Table 2). The highest quartile of TIBC (>335 μg/dl (59·96 μmol/l)) was strongly associated with longer LTL ($P<0{\cdot}001$), whereas the second and third quartiles of TSAT, indicating normal concentrations (30·3–44·7 %), as well as the highest quartile (>44·7 %) were significantly associated with shorter LTL ($P<0{\cdot}05$ and $P<0{\cdot}01$, respectively) (Table 2).

Table 1. Characteristics of 1174 study participants according to the quartiles of leucocyte telomere length (LTL) (Mean values and standard deviations; percentages)

	Quartiles of LTL (median, range)								
	1st quartile (0·72, 0·09–0·82)		2nd quartile (0·91, 0·83–0·99)		3rd quartile (1·12, 1·00–1·29)		4th quartile (1·67, 1·30–10·23)		
Characteristics	Mean	SD	Mean	SD	Mean	SD	Mean	SD	$P_{for\ trend}$*
Age (years)	60·5	7·4	60·0	7·3	59·1	7·1	58·7	6·9	<0·001
Men (%)	53·9		58·8		50·0		49·5		0·09
Low income (%)	22·5		20·4		17·7		20·5		0·40
BMI (kg/m^2)	24·9	2·9	24·6	3·0	24·8	3·1	24·7	3·0	0·54
Current smokers (%)	12·0		14·6		10·2		12·6		0·78
Current alcohol drinkers (%)	49·5		50·3		51·4		44·4		0·27
Physical activity (MET-h/d)	40·8	6·0	40·8	7·5	41·2	7·1	41·4	5·6	0·19
Presence of diabetes mellitus (%)	21·2		23·5		19·7		19·8		0·46
Presence of hypertension (%)	37·9		36·4		34·4		39·9		0·75
Presence of dyslipidaemia (%)	56·0		59·5		51·0		50·9		0·07
Leucocyte counts	5·2	1·5	5·0	1·4	5·1	1·4	5·4	1·5	0·07
Biochemical measures									
Hb (g/dl)	14·1	1·3	14·2	1·2	14·1	1·2	14·2	1·3	0·72
Fe (μg/dl)	118·7	39·0	116·3	39·6	120·0	43·4	116·8	44·9	0·85
Ferritin (ng/ml)	109·2	75·1	126·3	86·7	112·2	106·1	106·5	87·5	0·35
Total Fe-binding capacity (μg/dl)	298·4	44·8	292·8	41·7	307·9	45·5	323·1	48·4	<0·001
Transferrin saturation (%)	39·7	11·9	39·6	12·0	39·1	13·2	36·3	12·6	<0·001
hs-CRP (mg/l)	1·17	1·44	1·34	1·66	0·95	0·98	1·26	1·64	0·72
Alanine transaminase (U/l)	25·3	14·0	24·6	13·1	24·2	14·9	27·2	21·8	0·21
Aspartate transaminase (U/l)	27·5	9·9	27·2	9·5	26·9	8·5	28·9	16·2	0·19

MET, metabolic equivalent; hs-CRP, high-sensitivity C-reactive protein.
* Obtained using the Cochran–Armitage test or the trend test of ANOVA.

In additional multiple linear regression analysis for TSAT, which was classified into six groups, coefficient estimates were −0·04 (SE 0·04) (P=0·25) for the group of 30·1–35·0 % (n 197), −0·09 (SE 0·03) (P<0·01) for that of 35·1–40·0 % (n 222), −0·13 (SE 0·04) (P<0·001) for that of 40·1–45·0 % (n 179), −0·09 (SE 0·04) (P<0·05) for that of 45·1–50·0 % (n 115) and −0·10 (SE 0·04) (P<0·01) for that of >50·0 % (n 172) compared with the reference (≤30·0 %) (n 289). On the basis of these results, the threshold value of TSAT associated with shorter LTL appeared to be 35 %.

When we compared LTL across the four groups of TSAT (≤30·0, 30·1–35·0, 35·1–45·0 and >45 %), participants with high-normal concentrations (35·1–45·0 %) and abnormally high concentrations (>45 %) of TSAT had shorter LTL compared with those who had TSAT concentrations within the reference range (≤30·0 %) after adjustment for potential confounding variables (Fig. 1).

Table 3 presents results stratified by sex for the associations between Fe-status biomarkers and LTL. The positive association between TIBC and LTL was stronger among men (P<0·001) than among women (P<0·05), and thus an interaction term between TIBC and sex was significant (P<0·05). An inverse association between TSAT and LTL was observed in both men and women (P<0·05). In particular, the third quartiles of TSAT, which indicate normal concentrations for men (<50 %) and women (<45 %), were associated with shorter LTL (P=0·06 for men and P<0·01 for women).

Next, we evaluated interactions between TSAT and lifestyle factors such as smoking, alcohol consumption, physical activity and obesity associated with LTL. As shown in Table 4, the interaction terms between TSAT and lifestyle factors were not significant. In analyses stratified by smoking status, higher concentrations of TSAT were significantly associated with shorter LTL only among non-smokers (P<0·01). In addition, alcohol drinkers in the fourth quartile of TSAT had significantly shorter LTL compared with non-drinkers in the lowest quartile (P<0·05). Less-active persons with MET-h/d of <40 (a median value) had significantly shorter LTL (P<0·01) compared with the reference, but active persons with MET-h/d of ≥40 did not, when they were in the highest TSAT quartile. Obese persons in the third and fourth quartiles had a strong association with LTL compared with the non-obese in the lowest quartile (P<0·05) (Table 4).

Discussion

We observed significant associations of serum TIBC and TSAT concentrations with LTL in middle-aged and older adults from a population-based study. Those with higher TIBC concentrations showed longer LTL, whereas those with higher TSAT concentrations had shorter LTL; in particular, an inverse association between TSAT and LTL was observed among participants with normal TSAT concentrations between 35 and 45 % as well as among those with abnormally high TSAT concentrations above 45 %. In addition, less-active or obese persons who had high TSAT concentrations were likely to have shorter LTL.

TSAT has been used as a more sensitive biomarker of Fe status than just serum Fe or TIBC. TSAT indicates the proportion of Fe-binding sites of transferrin loaded with Fe contained in plasma or serum, whereas TIBC is defined as the maximum amount of Fe that can bind with transferrin. TSAT level is determined by sex, race, Fe consumption and presence of

Table 2. Associations between iron-status biomarkers and leucocyte telomere length (LTL) (Coefficient estimates with their standard errors)

Fe-status biomarkers (quartiles (range))	LTL*								
	Model 1†			Model 2†			Model 3†		
	Coefficient estimate	SE	*P*	Coefficient estimate	SE	*P*	Coefficient estimate	SE	*P*
Hb (g/dl)									
1st quartile (10·5–13·2)	Ref.			Ref.			Ref.		
2nd quartile (13·3–14·1)	0·05	0·03	0·13	0·05	0·03	0·17	0·04	0·03	0·21
3rd quartile (14·2–15·0)	−0·01	0·04	0·82	−0·01	0·04	0·87	−0·02	0·04	0·70
4th quartile (>15·0)	0·05	0·04	0·28	0·05	0·04	0·22	0·04	0·05	0·33
$P_{for\ trend}$	0·41			0·32			0·45		
Fe (μg/dl)									
1st quartile (28–89)	Ref.			Ref.			Ref.		
2nd quartile (90–113)	0·01	0·03	0·88	0·003	0·03	0·94	0·002	0·03	0·94
3rd quartile (114–138)	−0·05	0·03	0·11	−0·04	0·03	0·17	−0·05	0·03	0·16
4th quartile (>138)	−0·04	0·03	0·21	−0·04	0·03	0·27	−0·04	0·03	0·28
$P_{for\ trend}$	0·10			0·16			0·16		
Ferritin (ng/ml)									
1st quartile (20·1–59·4)	Ref.			Ref.			Ref.		
2nd quartile (59·5–92·2)	0·03	0·03	0·29	0·03	0·03	0·30	0·03	0·03	0·32
3rd quartile (92·3–139·9)	0·02	0·03	0·56	0·03	0·03	0·42	0·03	0·03	0·45
4th quartile (>139·9)	−0·002	0·03	0·95	0·02	0·04	0·63	0·01	0·04	0·78
$P_{for\ trend}$	0·68			0·83			0·99		
Total Fe-binding capacity (μg/dl)									
1st quartile (148–272)	Ref.			Ref.			Ref.		
2nd quartile (273–302)	0·04	0·03	0·24	0·04	0·03	0·20	0·04	0·03	0·20
3rd quartile (303–335)	0·06	0·03	0·07	0·06	0·03	0·08	0·06	0·03	0·09
4th quartile (>335)	0·17	0·03	<0·001	0·17	0·03	<0·001	0·17	0·03	<0·001
$P_{for\ trend}$	<0·001			<0·001			<0·001		
Transferrin saturation (%)									
1st quartile (9·0–30·2)	Ref.			Ref.			Ref.		
2nd quartile (30·3–37·1)	−0·07	0·03	0·02	−0·07	0·03	0·02	−0·07	0·03	0·02
3rd quartile (37·2–44·7)	−0·10	0·03	0·002	−0·09	0·03	0·004	−0·09	0·03	0·005
4th quartile (>44·7)	−0·10	0·03	0·002	−0·09	0·03	0·005	−0·09	0·03	0·006
$P_{for\ trend}$	0·003			0·006			0·008		

Ref., referent group.
* Log-transformed value.
† In model 1, data were adjusted for age and sex. In model 2, data were adjusted for age, sex, income status, BMI, smoking status, alcohol consumption, physical activity and presence of diabetes mellitus, hypertension or dyslipidaemia. In model 3, data were adjusted for leucocyte counts as well as for serum high-sensitivity C-reactive protein, alanine transaminase and aspartate transaminase concentrations with the covariates in model 2.

infection or metabolic diseases as well as by genetic factors, and its normal values are approximately 20–50 %[8,19]. Women with >45 % or men with >50 % are likely to have manifestations of Fe overload (or HC), which are mostly due to increased Fe absorption caused genetically or by large amounts of Fe consumption[8,19–21]. The HC gene (*HFE*) has been reported as a major genetic determinant of hereditary HC among populations of European descent, whereas the role of this gene in Fe overload seems to be trivial among Asians[10]. A large dose of Fe supplementation or high consumption of Fe-fortified foods or haem-Fe-rich foods such as red meats and organ meats may be a risk factor for Fe overload[21,22]. In particular, Fe overload with high TSAT concentrations of over 60 % leads to the accumulation of non-transferrin-bound Fe or labile Fe, which is involved in the production of ROS via the Fenton reaction or the Haber–Weiss reaction[11,23]. Because ROS have the potency to damage cells and DNA, increased Fe accumulation has been hypothesised to accelerate telomere attrition[24]. Although data on the association between Fe status and telomere length are very limited, one study demonstrated that high concentrations of serum ferritin and TSAT reflecting Fe overload are associated with short LTL regardless of the presence of *HFE* mutation[13]. In our study, the prevalence of Fe overload, defined as TSAT of >45 % or ferritin of >200 ng/ml for women and TSAT of >50 % or ferritin of >300 ng/ml for men, was 21·3 %. Among those without Fe overload, 41·6 % were observed to have high-normal TSAT concentrations between 35 and 45 %. On the basis of the findings regarding the association between TSAT and LTL, these individuals were likely to have shorter LTL than those with lower TSAT concentrations.

LTL has been measured in epidemiological studies regarding ageing-associated diseases and mortality risk[25–27]. Although telomere length in leucocytes is shorter than that in somatic cells, because the rates of telomere shortening are found to be similar in leucocytes and somatic cells[28] and because leucocyte samples are easily obtainable, LTL may be a useful biomarker of cellular ageing as well as of cumulative oxidative damage[29]. Given the hypothesis that Fe is involved in the production of ROS, our findings on the association between TSAT and LTL suggest that individuals with high-normal concentrations and abnormally high concentrations of TSAT are

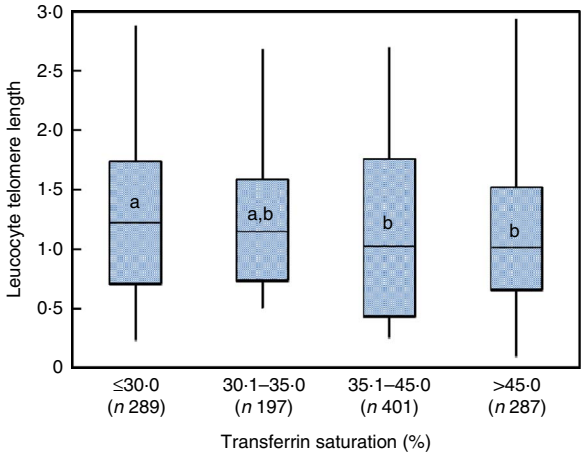

Fig. 1. Box plots of leucocyte telomere length for the groups of serum transferrin saturation levels. In the box plot, the range of a box and its internal horizontal line indicate the values of (mean + standard deviation) or (mean − standard deviation) and the mean, respectively, and the range of whiskers for each box indicate the minimum and maximum values that are not outliers. Multiple-comparison tests between groups were conducted after adjustment for age, sex, income status, BMI, smoking status, alcohol consumption, physical activity, presence of diabetes mellitus, hypertension or dyslipidaemia, leucocyte counts as well as for serum high-sensitivity C-reactive protein, alanine transaminase and aspartate transaminase concentrations. [a,b] Different letters indicate *post hoc* (Scheffé's test) significance ($P < 0·05$).

exposed to Fe-induced oxidative damage and further expected to be at risk for ageing-associated diseases. A previous study, which investigated the association between TSAT concentrations and the risk for all-cause mortality during the 9-year follow-up period of the Third National Health and Nutrition Examination Survey, suggested TSAT concentrations between 23 and 40 % as an optimal value range to demonstrate the lowest mortality risk[5]. Danish population-based follow-up studies also observed similar findings[3].

In this study, we also observed that TIBC was strongly associated with LTL, whereas ferritin was not. TIBC, which indicates the maximum amount of Fe that can be bound to transferrin, is known to reflect the antioxidant properties of transferrin in the blood[30]. Serum ferritin concentrations indicate the amount of Fe stored in tissues and has been used to identify Fe overload with TSAT[8]. However, serum ferritin may not be a proper indicator of oxidative stress partly because ferritin appears to play both pro- and antioxidative roles in the regulation of the labile Fe pool[31]. Indeed, the risk for myocardial infarction was inversely associated with TIBC but not with ferritin concentrations[32].

Sex and lifestyle factors such as smoking, alcohol consumption, physical activity and obesity were reported to be associated with LTL[33–36]. Although this study did not obtain

Table 3. Associations between iron-status biomarkers and leucocyte telomere length (LTL) by sex*
(Coefficient estimates with their standard errors)

		LTL†										
		2nd quartile			3rd quartile			4th quartile				
Fe-status biomarkers by sex	1st quartile	Coefficient estimate	SE	*P*	Coefficient estimate	SE	*P*	Coefficient estimate	SE	*P*	$P_{for\ trend}$	$P_{for\ interaction}$‡
Hb (g/dl)												0·11
Men (range)	11·2–14·2	14·3–14·9			15·0–15·6			15·7–18·5				
	Ref.	−0·01	0·04	0·76	0·01	0·04	0·78	0·07	0·05	0·12	0·08	
Women (range)	10·5–12·7	12·8–13·3			13·4–13·7			13·8–16·0				
	Ref.	−0·01	0·05	0·77	0·09	0·05	0·06	−0·03	0·05	0·58	0·99	
Fe (μg/dl)												0·30
Men (range)	28–95	96–121			122–149			150–400				
	Ref.	0·06	0·04	0·16	−0·01	0·04	0·83	0·02	0·04	0·65	0·98	
Women (range)	36–84	85–104			105–127			128–226				
	Ref.	−0·06	0·05	0·23	−0·05	0·05	0·28	−0·08	0·05	0·11	0·14	
Ferritin (ng/ml)												0·91
Men (range)	20·2–78·9	79·0–120·9			121·0–174·0			174·1–1156·7				
	Ref.	0·04	0·04	0·33	0·02	0·04	0·64	−0·01	0·04	0·79	0·54	
Women (range)	20·1–47·4	47·5–69·9			70·0–101·7			101·8–431·9				
	Ref.	0·05	0·05	0·34	0·11	0·05	0·02	0·02	0·05	0·66	0·70	
Total Fe-binding capacity (μg/dl)												0·03
Men (range)	156–270	271–298			299–329			330–501				
	Ref.	0·11	0·04	0·01	0·08	0·04	0·04	0·23	0·04	<0·001	<0·001	
Women (range)	148–274	275–308			309–340			341–479				
	Ref.	−0·05	0·05	0·33	0·02	0·05	0·71	0·09	0·05	0·09	0·04	
Transferrin saturation (%)												0·69
Men (range)	9·0–32·8	32·9–40·2			40·3–48·2			48·3–98·1				
	Ref.	−0·02	0·04	0·73	−0·08	0·04	0·06	−0·08	0·04	0·08	0·04	
Women (range)	10·8–28·3	28·4–34·4			34·5–40·6			40·7–96·9				
	Ref.	0·01	0·05	0·93	−0·13	0·05	0·009	−0·08	0·05	0·10	0·03	

Ref., referent group.
* Data were adjusted for age, income status, BMI, smoking status, alcohol consumption, physical activity, presence of diabetes mellitus, hypertension or dyslipidaemia, leucocyte counts as well as for serum high-sensitivity C-reactive protein, alanine transaminase and aspartate transaminase concentrations.
† Log-transformed value.
‡ Interaction terms between sex and Fe-status biomarkers were tested using multiple linear regression models.

Table 4. Associations between transferrin saturation (TSAT) and leucocyte telomere length (LTL) stratified by lifestyle factors* (Coefficient estimates with their standard errors)

Lifestyle factors and quartiles of TSAT (range)...	LTL†												
	1st quartile (<30·3%)			2nd quartile (30·3–37·1 %)			3rd quartile (37·2–44·7 %)			4th quartile (>44·7 %)			
	Coefficient estimate	SE	*P*	Coefficient estimate	SE	*P*	Coefficient estimate	SE	*P*	Coefficient estimate	SE	*P*	$P_{for\ interaction}$
Smoking status													
Non-smoker	Ref.			−0·08	0·03	0·02	−0·11	0·03	0·002	−0·10	0·04	0·004	0·27
Smoker	−0·11	0·08	0·19	−0·10	0·08	0·17	−0·08	0·07	0·25	−0·10	0·06	0·10	
Alcohol-drinking status													
Non-drinker	Ref.			−0·07	0·04	0·12	−0·09	0·04	0·05	−0·05	0·05	0·25	0·07
Drinker	0·03	0·05	0·58	−0·06	0·05	0·18	−0·08	0·05	0·08	−0·11	0·04	0·02	
Physical activity (MET-h/d)													
<Median value (<40)	Ref.			−0·07	0·05	0·13	−0·08	0·05	0·09	−0·15	0·05	0·002	0·72
≥Median value (≥40)	0·04	0·05	0·35	−0·03	0·05	0·50	−0·07	0·05	0·14	−0·01	0·05	0·88	
BMI (kg/m^2)													
<25	Ref.			−0·09	0·04	0·04	−0·07	0·04	0·12	−0·06	0·04	0·18	0·06
≥25	−0·01	0·05	0·78	−0·04	0·05	0·33	−0·12	0·05	0·02	−0·12	0·05	0·01	

Ref., referent group; MET, metabolic equivalent.
* Data were adjusted for age, sex, income status, BMI, smoking status, alcohol consumption, physical activity, presence of diabetes mellitus, hypertension or dyslipidaemia, leucocyte counts as well as for serum high-sensitivity C-reactive protein, alanine transaminase and aspartate transaminase concentrations.
† Log-transformed value.

significant findings from the interaction tests, our findings that less-active or obese persons with high TSAT concentrations had shorter LTL than others suggest that obesity and low physical activity may contribute to biological ageing along with high Fe levels. Associations of obesity and physical activity with LTL have been observed in other studies[33,34]. Persons with Fe overload need to undergo therapeutic phlebotomy and Fe chelation to delay organ damage or complications. In addition, they are generally recommended to avoid high Fe consumption[20]. On the basis of our findings, physical activity and maintaining normal body weight can be recommended for those with Fe overload as well as for those with high-normal TSAT concentrations to reduce the potential risk for Fe-induced oxidative damage. In a cross-sectional study, decreased superoxide dismutase (SOD) activity and increased lipid peroxidation were observed in obese healthy participants compared with those with normal BMI[37]. On the contrary, an intervention study showed that SOD activity increases significantly without elevated lipid peroxidation after exercise among postmenopausal women[38]. So, such pro- and antioxidative conditions may affect Fe-induced oxidative damage.

The strengths of our study include the measurement of varied Fe-status biomarkers, observation on the association between normal values of Fe-status biomarkers and LTL as well as the consideration of a broad range of confounding factors. However, some study limitations should be taken into account for the interpretation of our findings. Because we were unable to have opportunities to conduct assays of Fe-status biomarkers and LTL in previous visits, the study design was cross-sectional. Thus, a causal relationship between Fe-status biomarkers and LTL remained undetermined and should be investigated in longitudinal studies. Similar to other studies, our study used leucocytes instead of somatic cells to assess telomere length. The study participants might not be representative of the general population as well as of the primary study's cohort members. Because they were unaware of the hypothesis of present study, however, the association between Fe-status biomarkers and LTL might be biased towards the null. We did not collect information on dietary Fe intake during the period when Fe-status biomarkers and LTL were analysed. However, earlier studies showed no association between dietary Fe intake and LTL[39,40]. This observation may be due to the fact that circulating Fe levels are more influenced by non-dietary factors including age, body weight, menopausal status, alcohol consumption and genetic predisposition that determine the ability of intestinal Fe absorption, rather than due to dietary Fe intake[21]. Thus, Fe biomarkers in blood may be more useful measures reflecting Fe status in the body than dietary Fe intake. The findings of our study might be generalisable to middle-aged and older Asians, but not to other age groups or other ethnicities, in terms of biological relevance. Future studies need to evaluate the association between high-normal TSAT concentrations and LTL in populations with broader ranges of age and other ethnicities, especially in those with genetic predisposition to Fe overload as well as in those with potential factors to elevate TSAT, such as alcohol consumption or high consumption of Fe supplements.

In this study, we observed that higher serum concentrations of TSAT and lower concentrations of TIBC were associated with shorter LTL in middle-aged and older participants. In particular, participants with high-normal TSAT concentrations between 35 and 45 % as well as those with abnormally high TSAT concentrations reflecting Fe overload were likely to have shorter LTL. These findings were more evident among less-active or obese participants than among the active and lean. On the basis of the findings, we suggest that circulating TSAT concentrations may need to be controlled normally below 35 % to delay biological ageing and further prevent ageing-associated diseases,

and that middle-aged and older adults with such high TSAT concentrations may need to increase physical activity and maintain normal body weight through lifestyle modification. In addition, less-active or obese persons may need to pay more attention to potential detrimental effects of Fe overload caused by a large excess of Fe consumption, such as the use of Fe supplements.

Acknowledgements

This study was supported by a National Research Foundation of Korea Grant funded by the Korean Government (I. B., NRF-2017R1A2A2A05001380) and a fund (C. S., 2011-E71004-00, 2012-E71005-00, 2013-E71005-00) for research from Korea Centers for Disease Control and Prevention. The funders had no role in the design, analysis or writing of this article.

I. B. provided study inference, conducted statistical analysis and wrote the manuscript; C. S. conducted the study. Both contributed to interpreting the results and finalising the manuscript.

Neither of the authors has any conflicts of interest to declare.

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