

Original Article

Iron status from diet and serum in relation to biological aging acceleration: A NHANES cross-sectional study

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Background and Objectives: Iron is essential but potentially toxic; both deficiency and overload may affect aging. Population-level evidence linking iron exposure to biological aging is limited, and aging-based optimal iron ranges are undefined. We examined associations of total, dietary, and supplemental iron intake and serum iron with three biological age (BA) measures and derived optimal ranges. **Methods and Study Design:** We analyzed 13,284 U.S. adults (≥ 20 years) from NHANES 2007–2010 and 2015–2018. BA deviations (PA, GoldBA, LightBA) were derived from clinical biomarkers. Weighted linear and restricted cubic spline (RCS) models assessed associations; optimal ranges were exposure intervals with significantly beneficial associations. Models adjusted for demographics, lifestyle, comorbidities, and diet quality. Subgroup analyses examined sex, age, menopause, and chronic disease. **Results:** Dietary iron was associated with younger BA (PA $\beta = -0.07$; GoldBA $\beta = -0.09$ per 2-fold increase), whereas supplemental iron accelerated aging above 18 mg/day (GoldBA $\beta = 0.35$ for >18 mg/day). Serum iron showed the strongest benefit (GoldBA $\beta = -0.25$ per 2-fold increase), with a U/J-shaped optimal range of 15.2–18.8 $\mu\text{mol/L}$. Optimal ranges were also identified for total iron (15.7–37.3 mg/day) and dietary iron (≥ 13.6 mg/day). The optimal serum iron range varied by subgroup (wider in postmenopausal women and those with chronic diseases; interaction $p < 0.05$). **Conclusions:** The iron-aging relationship is source-dependent: dietary iron is protective, supplemental iron >18 mg/day is detrimental, and serum iron 15.2–18.8 $\mu\text{mol/L}$ is optimal. Optimal ranges should be stratified by population.

Key Words: iron status, biological aging, age acceleration, NHANES, serum iron

INTRODUCTION

Iron, an essential trace element in the human body, plays an indispensable role in many biological processes, such as oxygen transport, mitochondrial function, DNA synthesis, and energy metabolism.¹ Iron functions as a double-edged sword: while iron deficiency restricts erythropoiesis and leads to anemia with consequent impairments in cognitive function and immunity, iron overload generates reactive oxygen species that inflict cellular and organ damage.^{1,2} Therefore, it is critical for the body to maintain systemic iron homeostasis by balancing its uptake, utilization, and storage to ensure an adequate iron supply while preventing its toxic accumulation.^{1,3}

Aging is accompanied by profound alterations in iron metabolism and systemic iron homeostasis.^{4,5} Advancing age is associated with progressive iron accumulation in multiple organs, including the liver, kidney, and brain, which has been linked to several age-related pathologies, such as type II diabetes, cardiovascular diseases, and neurodegenerative disorders.^{6–8} Meanwhile, inflammation-driven hepcidin upregulation and declining intestinal iron absorption and transport promote cellular iron sequestration and a decline in circulating iron, underlying the prevalent anemia in the elderly population.^{9,10} At the cellular level, disturbed iron metabolism is mechanistically linked to senescence and ferroptosis.^{4,11} Nevertheless,

despite these well-characterized age-related perturbations, our understanding of the relationship between iron nutritional status and human aging at the population level remains limited, and the optimal iron range for healthy aging has yet to be defined.

Accumulating evidence has linked iron status to biological aging; however, the direction and shape of the association vary markedly across different iron biomarkers and aging metrics. For dietary iron intake, the association varies depending on the intake level, as both low and high intake levels have been reported to be related to accelerated aging.^{12–15} Elevated serum ferritin, a biomarker for iron storage, has been consistently associated with greater epigenetic clock acceleration (GrimAge, PhenoAge, DunedinPACE) and shorter leukocyte telomeres.^{16–18} Transferrin saturation, a biomarker reflecting circulating iron

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Electronic supplementary information available. See apjcn.qdu.edu.cn/35_5_829_supp.pdf

Manuscript received 12 February 2026. Initial review completed 26 June 2026. Revision accepted 20 August 2026.

doi: 10.6133/apjcn.202610_35(5).0009

availability, showed mixed relations.¹⁷⁻¹⁹ Notably, serum iron, the compartment directly reflecting metabolically available iron, has yielded the least consistent picture: both positive and negative associations with epigenetic aging clocks have been reported,^{17, 18} and whether serum iron follows a linear or U/J-shaped pattern across multiple biological age indicators remains unresolved.^{20, 21} These discrepant findings likely reflect the fact that different biomarkers capture distinct iron compartments (storage versus transport).^{4, 22} More importantly, because biological age metrics differ substantially in their construction and exposure sensitivity, an association observed for one indicator may not generalize to others.²³ Current studies mostly rely on a single indicator, typically an epigenetic clock or leukocyte telomere length, and cross-metric consistency and validation are lacking.

In this study, we used data from the National Health and Nutrition Examination Survey (NHANES), a nationally representative survey of the US population, to (1) compare the consistency of iron-biological age associations across three measures of biological aging estimating mortality hazard: phenotypic age (PA),²⁴ Gompertz Law-Based Biological Age (GoldBA), and Light BioAge (LightBA),²⁵ and (2) delineate the optimal iron range that protects against biological age acceleration. We focused on iron intake from diet and supplements as the primary indicators of nutritional status. We also used serum iron concentration, a blood biomarker available for all participants in the NHANES cycles, as a secondary exposure to validate the associations

METHODS

Study population

Data were obtained from the NHANES, a nationally representative cross-sectional survey of non-institutionalized US residents. The NHANES was conducted biennially by the National Center for Health Statistics (NCHS) in the United States. The survey employed a complex, multi-stage stratified probability sampling design with stratification and primary sampling units to obtain a weighted and representative sample of the U.S. population. The study protocol was approved by the NCHS Ethics Review Board, and all participants provided written informed consent. NHANES data were made public by the NCHS for statistical reporting and analysis (<https://www.cdc.gov/nchs/policy/data-user-agreement.html>). In this study, we used data from four cycles of 2007–2010 and 2015–2018, in which all biomarkers for calculating biological age (BA) were available, and the usage of dietary supplements was collected as part of the 24-hour dietary recall interviews. Among 39,911 participants in these cycles, we excluded participants aged less than 20 years ($n = 16,470$) and those with any missing data in the metrics required for BA calculation ($n = 3,962$), with any missing data in dietary iron or total energy intake ($n = 3,705$), with abnormal daily energy intake (<500 kcal or >5000 kcal) ($n = 193$) or serum iron level (≥ 3 SD) ($n = 238$), and with missing dietary components for calculating the alternative health eating index (AHEI) ($n = 2,059$). The final sample size was 13,284, representing approximately 134,596,046 adults in the US aged ≥ 20 years (Figure 1).

Outcome variables

Three BA algorithms based on mortality risk estimation, namely PA, GoldBA, and LightBA, were constructed from a set of biomarkers in accordance with the published literature.^{24, 25} PA was computed using the BioAge R package (https://github.com/dayoonkwon/NHANES_BioAge),²⁶ trained on NHANES III reference data and applied to the analytical sample. PA combines chronological age with twelve clinical biomarkers (albumin, alkaline phosphatase, C-reactive protein, total cholesterol, creatinine, glycosylated hemoglobin, blood urea nitrogen, uric acid, lymphocyte percentage, mean cell volume, white blood cell count, and systolic blood pressure) to predict the 10-year mortality risk.²⁴ GoldBA was computed using a Gompertz law-based algorithm, which links chronological age (CA) and a set of nine biomarkers (mean cell volume, red cell distribution width, alkaline phosphatase, lymphocyte percentage, white blood cell count, gamma-glutamyl transferase, creatinine, glucose, and albumin) to mortality hazard.²⁵ LightBA, a simplified model of GoldBA, was derived from CA and three blood biomarkers (creatinine, glucose, and log-transformed C-reactive protein).²⁵ LightBA achieved a C-index of 0.810 for all-cause mortality prediction, and GoldBA demonstrated the highest mortality prediction accuracy (C-index = 0.826) among the three algorithms.²⁵ Three BA measures, as well as CA, were highly correlated (all $r > 0.97$ and $p < 0.001$) (Supplementary Figure 1).

The standardized residuals of the regressions of three BA measures on CA (PA deviation, GoldBA deviation, and LightBA deviation) were used to estimate the biological age deviations of an individual's actual BAs from the predicted BAs at their CA.^{26, 27} A positive value of PA, GoldBA, or LightBA deviation indicates accelerated biological aging and increased risk of disease, disability, and mortality, and a negative value of deviation indicates a delayed biological aging process and reduced risk of disease, disability, and mortality.²⁶ Deviations of PA, GoldBA, and LightBA were inter-correlated, and PA deviation and LightBA deviation had a stronger correlation ($r = 0.52$) than the correlations between PA and GoldBA deviation ($r = 0.38$) and between GoldBA and LightBA deviation ($r = 0.34$) (Supplementary Figure 2).

Exposure variables

Four iron exposure metrics (total, dietary, and supplemental iron intake, and serum iron concentration) were assessed.

For iron intake, two 24-hour dietary recalls separated by 3–10 days were conducted to collect participants' dietary and supplement intake data. Reported food quantities were converted into gram weights, and nutrient composition was calculated using the USDA Food and Nutrient Database for Dietary Studies (FNDDS) (<https://fndds.nal.usda.gov/>).²⁸ Daily intake values of iron from food sources (mg/day) and supplements (mg/day) were calculated as the average of both recall days, and the total iron intake was calculated as the sum of iron from food sources and supplements.

For serum iron concentration measurement, participants' blood samples were collected by venipuncture according to the laboratory procedure manual of the

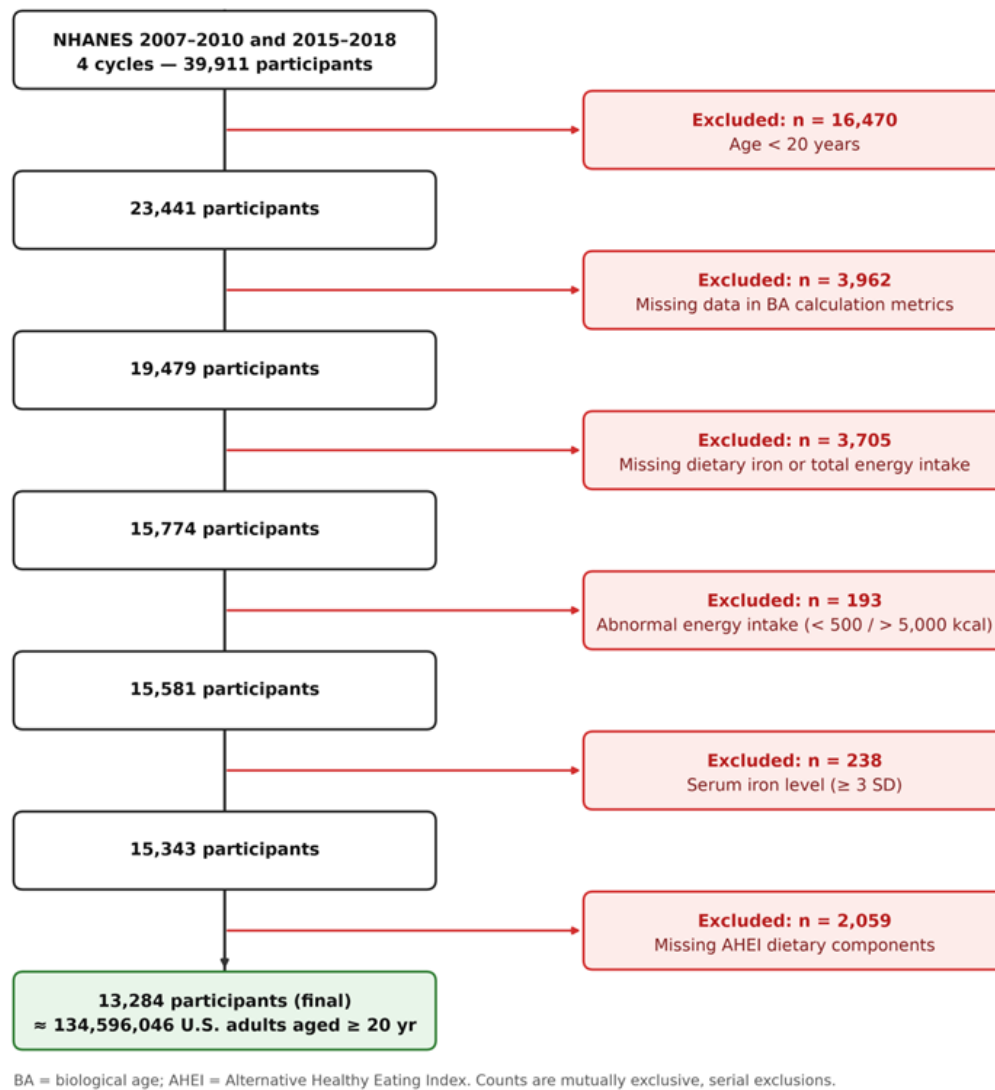


Figure 1. Flowchart for participant selection

survey.²⁸ Iron concentrations ($\mu\text{mol/L}$) in refrigerated or frozen serum samples were measured using a Roche Cobas 6000 analyzer with a three-step process using Ferrozine reagent. Briefly, ferric iron was liberated from transferrin by acid or detergent and reduced to ferrous iron by ascorbate, and the reduced iron was then reacted with the FerroZine reagent to form a colored complex, the intensity of which was detected by the absorbance at 560 nm.

Covariates

Covariates, including sex (men and women), race (non-Hispanic Whites, non-Hispanic Blacks, Mexican Americans, and others), educational level (less than high school graduate, high school graduate and some college, college graduate and higher), poverty income ratio (PIR), cigarette smoking (non-smoker, former smoker, and current smoker), alcohol consumption (non-drinker, moderate drinker, and heavy drinker), physical activity, AHEI, history of chronic diseases, and menopausal status for women, were obtained through questionnaires, examinations, and laboratory tests. The body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2). Participants who had smoked fewer than 100 cigarettes during their lifetime were defined as non-

smokers, participants who had smoked more than 100 cigarettes and were smoking at the time of the interview were considered current smokers, and those who had smoked more than 100 cigarettes but had quit smoking for more than 6 months were considered to be former smokers. Alcohol drinking status was categorized as non-drinker (less than 12 drinks in a lifetime or no drinking in the past 12 months), moderate drinker (less than 3 drinks per day for men and 2 drinks per day for women), or heavy drinker (3 drinks or more for men and 2 drinks or more for women). Daily physical activity levels were calculated based on the time and metabolic equivalent of the task (MET) for each activity and categorized as light, moderate, or vigorous according to the tertiles of the total MET. The AHEI was derived from dietary recall data according to the literature using the R package.²⁹ Participants who were diagnosed by a doctor as having high blood pressure, were currently taking antihypertensive medications, or had a systolic blood pressure ≥ 130 mmHg/diastolic blood pressure ≥ 80 mmHg were categorized as having hypertension. Participants who were diagnosed with diabetes by a doctor, were currently taking insulin or diabetes medication, or had an abnormal blood biomarker (HbA1c level $\geq 6.5\%$, fasting plasma glucose level ≥ 126 mg/dL, or oral glucose tolerance test level

≥ 200 mg/dL) were defined as having diabetes mellitus. Participants who were told by a doctor to have high blood lipid levels, were currently taking lipid-lowering medication, or had an abnormal lipid profile (HDL cholesterol < 40 mg/dL, LDL cholesterol ≥ 110 mg/dL, or triglyceride ≥ 150 mg/dL) were categorized as having dyslipidemia. Other medical conditions, including chronic respiratory disease (emphysema and chronic bronchitis), hepatopathy, cardiovascular disease (heart failure, coronary heart disease, angina pectoris, and heart attack), stroke, arthritis, and thyroid disease, were defined based on self-reports. Blood hemoglobin (g/dL) concentrations were obtained from the complete blood count (CBC) test, and the normal range was defined as 13.8–17.2 mg/dL for men and 12.1–15.1 mg/dL for women.³⁰

Statistical analysis

All data analyses were conducted according to the NHANES analysis guidelines, with survey sample weights calculated from four 2-year cycles and applied in all analyses to adjust for unequal selection probabilities and non-response bias.³¹ Serum iron analyses used the mobile examination center weight; all dietary and supplemental iron analyses used the dietary two-day weight. For the descriptive analysis, continuous variables are presented as weighted means (standard errors [SEs]) or weighted medians (interquartile range [IQR]), and categorical variables are presented as weighted proportions (SEs) in percentage.

Weighted linear regression models (Taylor-series linearization, R survey package) were used to estimate the linear association between iron exposure and biological age deviation. Iron exposure (serum iron, total iron intake, and dietary iron intake) was natural-log transformed so that the regression coefficient (β) could be expressed per two-fold increase in exposure ($\beta \times \ln 2$), together with its 95% confidence interval (CI) and two-sided p -value. Supplemental iron was modeled as a continuous (mg/day, restricted to supplement users) and three-level categorical variable (none, ≤ 18 mg/day, > 18 mg/day, in all sample). Four sequentially adjusted models were fitted: Model 1 adjusted for demographics (sex, ethnicity, education, PIR); Model 2 additionally adjusted for lifestyle factors (body mass index, smoking, alcohol intake, physical activity, total energy intake); Model 3 further included nine chronic diseases (hypertension, diabetes, dyslipidemia, arthritis, cardiovascular disease, stroke, respiratory disease, liver disease, thyroid disorders); and Model 4 (the fully adjusted model) additionally adjusted for diet quality (AHEI). For supplemental iron analyses, dietary iron was added as a covariate to account for shared exposure domains.

To characterize potential non-linear dose-response relationships, restricted cubic splines (RCS) with five knots placed at the 5th, 27.5th, 50th, 72.5th, and 95th percentiles of the exposure distribution (Hmisc::rcspline.eval, inclx = TRUE) were fitted within the fully adjusted Model 4 using survey-weighted generalized linear models (svyglm, Gaussian family). The β coefficient was centered at the overall population median exposure, such that the y -axis represented the difference in biological age deviation relative to the median. The prediction grid

spanned the 5th to 95th percentile of exposure. The overall association (p -overall) was assessed using a Wald test of all spline terms jointly and deviation from linearity (p -nonlinearity) using a Wald test of the spline terms excluding the linear component (survey::regTermTest). The optimal iron range was defined for each exposure as the widest contiguous interval over which all outcomes with significant p -overall simultaneously exhibited $\beta < 0$, indicating a beneficial (younger biological age) direction; regions below this interval were classified as deficiency and regions above as excess.

The same Model 4 RCS framework was applied within four pre-specified subgroup dimensions: age (< 65 vs. ≥ 65 years), sex (men vs. women), menopausal status (pre- vs. postmenopausal, restricted to women), and chronic disease status (non-communicable disease [NCD] absent vs. present). For the NCD subgroup analysis, nine disease covariates were removed from the model to avoid over-adjustment. To ensure cross-subgroup comparability, the knot positions, reference median, and prediction grid were fixed to the values derived from the overall population rather than re-estimated within each subgroup. Subgroup heterogeneity was formally tested by introducing a multiplicative interaction term between $\ln(\text{exposure})$ and the subgroup indicator in the survey-weighted linear model and assessing its significance using a Wald test (regTermTest).

All analyses were performed in R (version 4.6.1) with the survey and Hmisc packages; a two-sided p -value < 0.05 was considered statistically significant.

RESULTS

Characters of study participants

The demographic characteristics, dietary and serum iron levels, and biological ages of the 13,242 participants are summarized in Table 1. The participants' weighted mean age was 47.8 years, the weighted proportion of men was 48.6%, and most participants were non-Hispanic White (69.3%). The median total iron intake was 14.95 mg/day (IQR: 10.54–22.44), with 2,181 participants (17.6%) reporting iron supplement use. The median dietary iron intake was 13.44 mg/day (IQR: 10.01, 18.18). The weighted mean serum iron level was 15.73 $\mu\text{mol/L}$ (Table 1). Both total (Pearson's $r = 0.038$, $p < 0.001$) and dietary iron intake (Pearson's $r = 0.090$, $p < 0.001$) were weakly related to serum iron levels (Supplementary Figure 3). Men had higher total and dietary iron intakes (16.30 mg/day and 12.95 mg/day, respectively) than premenopausal (13.48 mg/day and 11.81 mg/day, respectively) and postmenopausal women (13.29 mg/day and 11.07 mg/day, respectively). Similarly, men had higher serum iron concentrations (17.09 $\mu\text{mol/L}$) than premenopausal (14.24 $\mu\text{mol/L}$) and postmenopausal women (14.69 $\mu\text{mol/L}$). Women (22.3% of premenopausal women and 24.9% of postmenopausal women) had a higher proportion of iron supplement intake than men (11.5%). Postmenopausal women had the highest prevalence of hypertension (46.7%), diabetes (10.8%), and thyroid problems (26.3%) (Table 1).

Table 1. Characteristics of participants, total and stratified by sex and menopausal status[†]

Variable	Total sample (n = 13,284)	Men (n = 6,503)	Premenopausal women (n = 3,668)	Postmenopausal women (n = 3,113)
Weighted n, millions	134	65.4	39.7	29.5
Total iron intake, mg/day	16.0 (10.5, 22.4)	16.3 (11.9, 22.6)	13.4 (9.78, 22.6)	13.3 (9.39, 20.8)
Dietary iron intake, mg/day	13.4 (10.0, 18.2)	13.0 (9.63, 17.7)	11.8 (8.92, 15.8)	11.1 (8.30, 14.7)
Iron supplement user, %	17.6 (0.5)	11.5 (0.6)	22.3 (1.0)	24.9 (1.0)
Supplemental iron intake, mg/day	4.02 (0.16)	2.22 (0.14)	5.94 (0.33)	5.49 (0.39)
Serum iron, mmol/L	15.7 (0.09)	17.1 (0.11)	14.2 (0.16)	14.7 (0.17)
Age, years	47.8 (0.29)	47.3 (0.32)	36.3 (0.23)	64.2 (0.23)
Age group, %				
< 65 yrs	81.0 (0.6)	82.4 (0.7)	100 (0.0)	52.2 (1.0)
≥ 65 yrs	19.0 (0.6)	17.6 (0.7)	-	47.8 (1.0)
Sex, %				
Men	48.6 (0.5)	100 (0.0)	-	-
Women	51.4 (0.5)	-	100 (0.0)	100 (0.0)
Race, %				
Non-Hispanic white	69.3 (1.7)	69.9 (1.7)	61.9 (2.2)	78.0 (1.7)
Non-Hispanic black	10.3 (0.9)	9.5 (0.8)	12.5 (1.1)	9.1 (0.9)
Mexican American	8.1 (0.9)	8.4 (1.0)	10.4 (1.2)	4.4 (0.6)
Other	12.3 (0.8)	12.2 (0.9)	15.1 (1.1)	8.5 (0.9)
Education level, %				
Below high school	14.5 (0.7)	14.9 (0.8)	13.0 (0.8)	15.5 (0.9)
High school	24.6 (0.7)	25.7 (1.1)	20.5 (0.9)	28.0 (1.0)
Beyond high school	60.9 (1.2)	59.4 (1.5)	66.5 (1.3)	56.6 (1.4)
Marital status, %				
Married	57.1 (0.9)	60.3 (1.1)	52.2 (1.3)	56.6 (1.2)
Divorced or separated	9.6 (0.4)	7.9 (0.5)	8.3 (0.4)	15.2 (1.0)
Widowed	7.8 (0.3)	4.1 (0.3)	4.4 (0.4)	20.3 (0.7)
Never married	17.4 (0.7)	18.8 (0.8)	24.3 (1.1)	4.8 (0.6)
Liver with partner	8.2 (0.4)	8.9 (0.5)	10.8 (0.7)	3.0 (0.5)
Poverty-to-income ratio	3.04 (0.04)	3.13 (0.04)	2.82 (0.05)	3.13 (0.06)
Cigarette smoking, %				
Non-smoker	55.4 (0.9)	47.8 (1.0)	65.8 (1.3)	58.1 (1.2)
Former smoker	25.0 (0.6)	30.5 (0.8)	13.6 (0.9)	28.3 (1.1)
Current smoker	19.6 (0.6)	21.7 (0.8)	20.6 (1.0)	13.6 (0.9)
Alcohol drinking, %				
Non-drinker	28.0 (0.7)	23.5 (0.8)	27.8 (0.9)	38.1 (1.4)
Moderate drinker	36.3 (0.9)	42.4 (1.3)	25.6 (1.1)	37.3 (1.4)
Heavy drinker	35.7 (0.7)	34.1 (1.0)	46.6 (1.1)	24.6 (1.3)
Physical activity levels, %				
Light	27.8 (0.7)	21.4 (0.6)	28.7 (1.1)	40.8 (1.3)
Moderate	35.8 (0.7)	32.9 (0.9)	39.4 (1.1)	37.2 (1.2)
Vigorous	36.4 (0.6)	45.7 (0.9)	31.9 (1.0)	21.9 (1.2)
BMI, kg/m ²	28.8 (0.11)	28.8 (0.13)	28.6 (0.19)	28.9 (0.16)
Hemoglobin, g/dL	14.3 (0.03)	15.2 (0.03)	13.3 (0.04)	13.6 (0.05)
Energy intake, kcal/day	2,116 (10.9)	2,431 (14.9)	1,873 (12.4)	1,725 (18.7)
AHEI score	38.6 (0.31)	37.6 (0.35)	38.1 (0.36)	41.7 (0.46)
Hypertension, %	28.8 (0.7)	29.6 (1.0)	14.1 (0.7)	46.7 (1.2)
Diabetes, %	7.0 (0.4)	7.8 (0.5)	2.7 (0.3)	10.8 (0.9)
Dyslipidemia, %	30.6 (0.7)	31.3 (1.0)	16.4 (0.8)	48.5 (1.2)
Arthritis, %	25.5 (0.7)	22.2 (0.8)	13.4 (0.9)	48.9 (1.3)
Cardiovascular disease, %	5.5 (0.3)	7.3 (0.5)	1.0 (0.2)	7.7 (0.6)
Stroke, %	2.6 (0.2)	2.4 (0.2)	0.9 (0.2)	5.2 (0.4)
Respiratory disease, %	6.4 (0.4)	4.9 (0.4)	5.6 (0.5)	11.0 (0.9)
Liver disease, %	3.5 (0.2)	4.0 (0.3)	2.2 (0.3)	3.9 (0.5)
Thyroid disorders, %	10.7 (0.3)	3.9 (0.3)	10.3 (0.5)	26.3 (1.0)
PA, years	45.8 (0.30)	45.9 (0.33)	33.7 (0.24)	61.8 (0.29)
GoldBA, years	56.3 (0.32)	55.5 (0.35)	44.7 (0.27)	73.9 (0.29)
LightBA, years	47.3 (0.30)	47.6 (0.33)	34.6 (0.24)	63.6 (0.26)
PA deviation, years	-0.13 (0.02)	0.02 (0.02)	-0.27 (0.03)	-0.27 (0.03)
GoldBA deviation, years	-0.04 (0.02)	-0.13 (0.02)	0.13 (0.03)	-0.09 (0.03)
LightBA deviation, years	-0.08 (0.01)	0.22 (0.02)	-0.32 (0.02)	-0.44 (0.02)

PA, phenotypic age; GoldBA, the Gompertz Law-Based Biological Age; LightBA, the Light BioAge. PA, GoldBA, and LightBA deviations were standardized residuals of the regressions of PA, GoldBA, and LightBA on chronological age, respectively.

[†]Data are given as weighted proportion (SE) in percentage for categorical and weighted mean (SE) or median (IQR) for continuous variables.

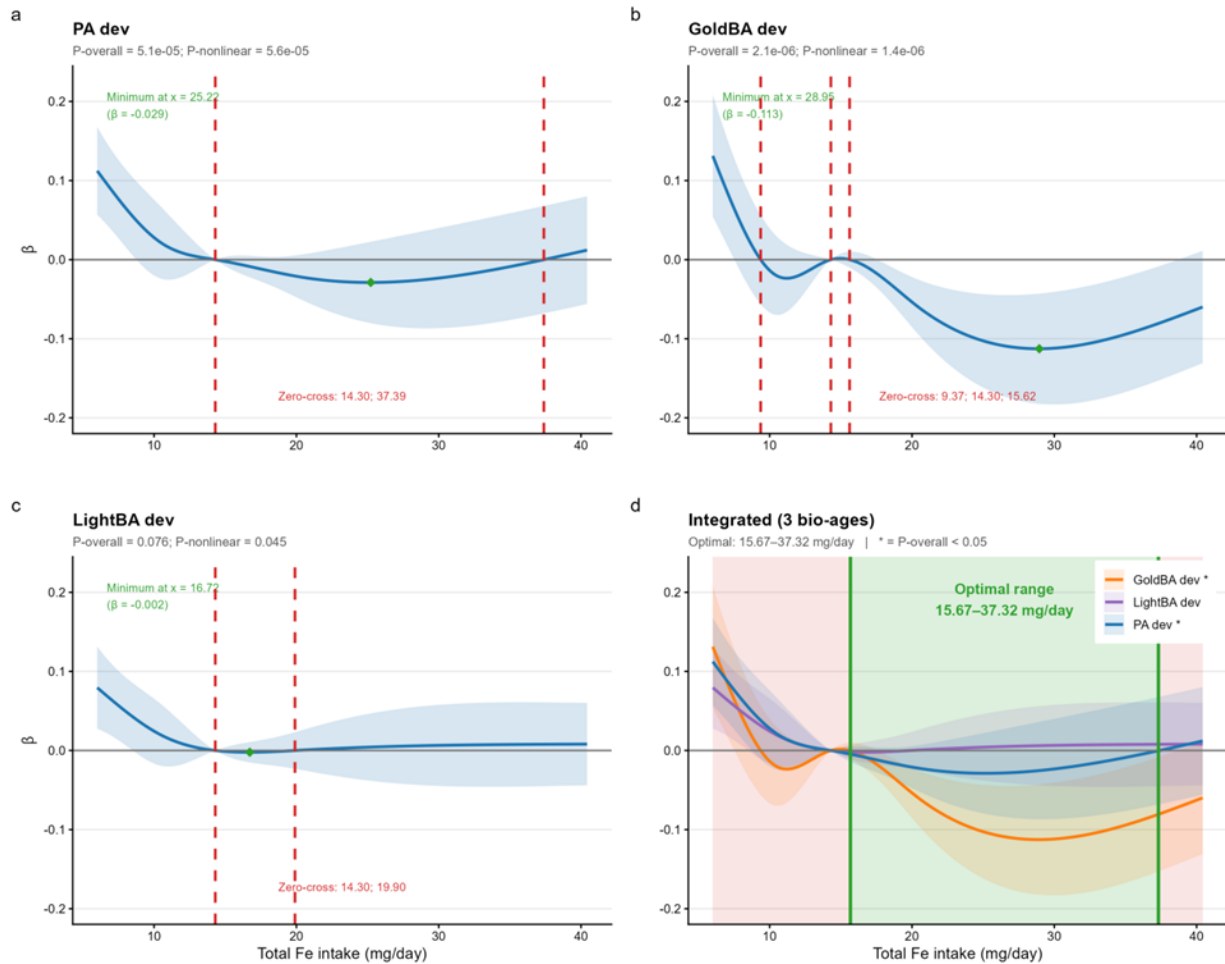


Figure 2. The RCS regression and optimal range of total iron intake over deviations of PA, GoldBA, and LightBA in the NHANES sample ($n = 13,284$). Models adjusted for sex, ethnicity, education levels, poverty income ratio, BMI, cigarette smoking, alcohol consumption, physical activity levels, the presence of chronic diseases, energy intake, and AHEI.

Associations between iron intake and biological aging

Weighted linear regression analyses showed inverse associations between total iron intake and all three BA deviations in the demographic-adjusted models (Model 1), which were attenuated after further adjustment. In the fully adjusted model (Model 4), higher total iron intake (per 2-fold increase) was not significantly associated with any biological age deviation (Table 2). However, RCS analysis revealed that deviations of PA and GoldBA (p -overall and p -nonlinearity < 0.001 for both) exhibited highly significant nonlinear associations with total iron intake, with a U/L-shaped trajectory (minimum $\beta = -0.03$ at 25.22 mg/day and zero-crossings at 14.3 and 37.4 mg/day for PA deviation; minimum $\beta = -0.11$ at 28.95 mg/day and zero-crossings at 14.3 and 15.62 mg/day for GoldBA deviation) (Table 3 and Figure 2). LightBA deviation did not exhibit significant association with total iron intake (p -overall = 0.08 and p -nonlinearity = 0.05) (Figure 2). The optimal intake range for total iron was 15.7–37.3 mg/day, defined by the intersection of the two significant outcomes (PA and GoldBA deviations) (Figure 2).

Among the 11,103 individuals without supplemental iron intake, dietary iron intake was inversely associated with deviations of all three BA measures in models adjusted for demographic factors, lifestyle, and presence of chronic diseases (Models 1, 2, and 3). After further ad-

justment for AHEI (Model 4 and fully adjusted model), the associations remained significant for PA and GoldBA deviations, and per-2-fold increases in dietary iron intake were associated with approximately 0.8 months ($\beta = -0.07$, 95% CI: -0.11, -0.03; $p = 0.001$) and 1.1 months ($\beta = -0.09$, 95% CI: -0.14, -0.05; $p < 0.001$) less advanced PA and GoldBA, respectively (Table 2). Non-linearity was present for GoldBA deviation (p -nonlinear < 0.01) but absent for PA deviation (p -nonlinear = 0.07), indicating an essentially linear dose-response for the latter (Table 3 and Figure 3). The optimal dietary iron intake was ≥ 13.6 mg/day, with a deficiency region < 13.6 mg/day and no excess region within the observed intake range (Figure 3). Similar associations between dietary iron intake and deviations of the three biological ages were found when individuals taking iron supplements were included in the analysis (Supplementary Table 1 and Supplementary Figure 4).

Iron supplementation had an opposite effect. Among 2,181 supplement users, per-2-fold increases were positively associated with 0.7 months ($\beta = 0.06$, 95% CI: 0.03, 0.09; $p < 0.001$) advanced PA, 0.8 months ($\beta = 0.07$, 95% CI: 0.04, 0.10; $p < 0.001$) advanced GoldBA, and 0.4 months ($\beta = 0.03$, 95% CI: 0.01, 0.06; $p = 0.02$) advanced LightBA in the fully adjusted model (Model 4) (Table 2). The RCS curves were non-linear for PA deviation (p -overall < 0.01 , p -nonlinear = 0.02) and rose

Table 2. Weighted linear association (β , 95%CI; p) between serum iron or iron intake and biological aging in the NHANES sample[†]

Outcomes and exposure [‡]	n	Model 1	Model 2	Model 3	Model 4
PA deviation					
Serum iron concentration (mmol/L)	13242	-0.16 (-0.20, -0.12); <0.001	-0.05 (-0.09, -0.01); 0.01	-0.05 (-0.08, -0.01); 0.02	-0.04 (-0.07, 0.00); 0.05
Total iron intake (mg/day)	13284	-0.04 (-0.07, -0.01); 0.02	-0.02 (-0.05, 0.01); 0.30	-0.03 (-0.06, 0.00); 0.10	-0.01 (-0.04, 0.02); 0.73
Dietary iron intake (mg/day) [§]	11103	-0.09 (-0.12, -0.05); <0.001	-0.1 (-0.14, -0.06); <0.001	-0.11 (-0.15, -0.07); <0.001	-0.07 (-0.11, -0.03); 0.001
Supplemental iron intake (mg/day) [¶]	2181	0.08 (0.04, 0.11); <0.001	0.07 (0.04, 0.10); <0.001	0.06 (0.03, 0.09); <0.001	0.06 (0.03, 0.09); <0.001
GlodBA deviation					
Serum iron concentration (mmol/L)	13242	-0.31 (-0.35, -0.26); <0.001	-0.26 (-0.31, -0.22); <0.001	-0.25 (-0.30, -0.21); <0.001	-0.25 (-0.29, -0.20); <0.001
Total iron intake (mg/day)	13284	-0.04 (-0.07, -0.01); 0.01	-0.02 (-0.05, 0.01); 0.25	-0.02 (-0.06, 0.01); 0.16	-0.02 (-0.05, 0.02); 0.35
Dietary iron intake (mg/day)	11103	-0.08 (-0.12, -0.04); <0.001	-0.10 (-0.15, -0.05); <0.001	-0.11 (-0.15, -0.06); <0.001	-0.09 (-0.14, -0.05); <0.001
Supplemental iron intake (mg/day)	2181	0.07 (0.04, 0.11); <0.001	0.07 (0.04, 0.10); <0.001	0.07 (0.04, 0.10); <0.001	0.07 (0.04, 0.10); <0.001
LightBA deviation					
Serum iron concentration (mmol/L)	13242	-0.28 (-0.32, -0.24); <0.001	-0.20 (-0.24, -0.16); <0.001	-0.19 (-0.22, -0.15); <0.001	-0.18 (-0.22, -0.14); <0.001
Total iron intake (mg/day)	13284	-0.05 (-0.07, -0.03); <0.001	-0.02 (-0.04, 0.00); 0.04	-0.03 (-0.05, -0.01); <0.01	-0.02 (-0.04, 0.00); 0.12
Dietary iron intake (mg/day)	11103	-0.07 (-0.11, -0.04); <0.001	-0.05 (-0.08, -0.01); 0.02	-0.06 (-0.09, -0.02); <0.01	-0.03 (-0.06, 0.01); 0.16
Supplemental iron intake (mg/day)	2181	0.04 (0.02, 0.07); 0.001	0.04 (0.02, 0.07); <0.01	0.03 (0.01, 0.06); 0.01	0.03 (0.01, 0.06); 0.02

[†]Model 1 adjusted for sex (men/women), ethnicity (non-Hispanic Whites, non-Hispanic Blacks, Mexican Americans, and others), education levels (less than high school graduate, high school graduate to some college, college graduate or higher), and poverty income ratio; model 2 further adjusted for BMI (kg/m²), cigarette smoking (non-smoker, former smoker, current smoker), alcohol consumption (non-drinker, moderate drinker, and heavy drinker), physical activity levels (low, moderate, and vigorous), and energy intake (kcal/day); model 3 further adjusted for the presence of chronic diseases (hypertension, diabetes, dyslipidemia, chronic respiratory disease, hepatopathy, cardiovascular disease, stroke, arthritis, and thyroid diseases) (yes and not); and model 4 further adjusted for AHEI.

[‡]The exposure levels were log₂-transformed, and the coefficient was rescaled to the effect per 2-fold increase in exposure ($\beta \times \ln 2$), with 95% CIs.

[§]The analysis was limited to participants without iron supplements.

[¶]The analysis was limited to iron supplement users.

Table 3. Weighted non-linear association between serum iron concentrations or iron intake and biological aging in the NHANES sample[†]

Exposure and outcome	n	<i>p</i> _overall	<i>p</i> _nonlinear	Median exposure	Knots	Lowest point		Highest point		Zero crossing x
						x	<i>β</i>	x	<i>β</i>	
Serum iron										
PA deviation	13242	<0.001	<0.001	14.50	6.40; 11.50; 14.50; 18.30; 26.10	16.13	-0.02	9.96	0.14	14.50; 18.85
GoldBA deviation	13242	<0.001	<0.001	14.50	6.40; 11.50; 14.50; 18.30; 26.10	21.99	-0.06	6.40	0.52	11.59; 14.50; 15.20
LightBA deviation	13242	<0.001	<0.001	14.50	6.40; 11.50; 14.50; 18.30; 26.10	22.94	-0.08	6.40	0.29	14.50
Total iron intake										
PA deviation	13284	<0.001	<0.001	14.30	6.01; 10.49; 14.30; 20.37; 40.42	25.22	-0.03	6.01	0.11	14.30; 37.39
GoldBA deviation	13284	<0.001	<0.001	14.30	6.01; 10.49; 14.30; 20.37; 40.42	28.95	-0.11	6.01	0.13	9.37; 14.30; 15.62
LightBA deviation	13284	0.080	0.050	14.30	6.01; 10.49; 14.30; 20.37; 40.42	16.65	0.00	6.01	0.08	14.30; 19.90
Dietary iron intake [‡]										
PA deviation	11103	<0.001	0.070	12.87	5.77; 9.85; 12.87; 16.94; 27.30	27.30	-0.07	5.77	0.11	12.86
GoldBA deviation	11103	<0.001	<0.01	12.87	5.77; 9.85; 12.87; 16.94; 27.30	27.30	-0.08	5.77	0.16	10.47; 12.87; 13.54
LightBA deviation	11103	0.200	0.190	12.87	5.77; 9.85; 12.87; 16.94; 27.30	13.99	0.00	5.77	0.08	12.87; 15.28
Supplemental iron intake [§]										
PA deviation	2181	<0.01	0.020	18.00	1.00; 18.00; 65.00	1.00	-0.19	65.00	0.20	18.00
GoldBA deviation	2181	<0.001	0.110	18.00	1.00; 18.00; 65.00	1.00	-0.22	65.00	0.33	18.00
LightBA deviation	2181	0.160	0.110	18.00	1.00; 18.00; 65.00	1.00	-0.11	53.18	0.07	18.00

[†]Models adjusted for sex (men/women), ethnicity (non-Hispanic Whites, non-Hispanic Blacks, Mexican Americans, and others), education levels (less than high school graduate, high school graduate to some college, college graduate or higher), poverty income ratio, BMI (kg/m²), cigarette smoking (non-smoker, former smoker, current smoker), alcohol consumption (non-drinker, moderate drinker, and heavy drinker), physical activity levels (low, moderate, and vigorous), energy intake (kcal/day), the presence of chronic diseases (hypertension, diabetes, dyslipidemia, chronic respiratory disease, hepatopathy, cardiovascular disease, stroke, arthritis, and thyroid disease) (yes and not) and AHEI.
[‡]The analysis was limited to participants without iron supplements.
[§]The analysis was limited to iron supplement users.

Table 4. Linear association (β, 95% CI; *p*) between supplemental iron intake levels and biological aging in the NHANES sample (n = 13,284)[†]

	n	PA deviation	GoldBA deviation	LightBA deviation
No iron supplement	11103	Ref.	Ref.	Ref.
≤18 mg/day	1614	-0.06 (-0.11, -0.01); 0.03	-0.15 (-0.22, -0.08); < 0.001	-0.06 (-0.11, -0.01); 0.02
>18 mg/day	567	0.20 (0.07, 0.33); < 0.01	0.35 (0.25, 0.45); < 0.001	-0.01 (-0.07, 0.06); 0.88

[†]Linear regression models adjusted for sex (men/women), ethnicity (non-Hispanic Whites, non-Hispanic Blacks, Mexican Americans, and others), education levels (less than high school graduate, high school graduate to some college, college graduate or higher), poverty income ratio, BMI (kg/m²), cigarette smoking (non-smoker, former smoker, current smoker), alcohol consumption (non-drinker, moderate drinker, and heavy drinker), physical activity levels (low, moderate, and vigorous), the present of chronic diseases (hypertension, diabetes, dyslipidemia, chronic respiratory disease, hepatopathy, cardiovascular disease, stroke, arthritis, and thyroid disease) (yes and not), energy intake (kcal/day), AHEI, and dietary iron intake (mg/day).

Table 5. Optimal ranges of iron exposures in the NHANES sample, all population and stratified by subgroups[†]

Subgroup	Total iron intake			Dietary iron intake			Supplemental iron intake			Serum iron concentration		
	n	Optimal range (mg/day)	Significant outcomes [‡]	n	Optimal range (mg/day)	Significant outcomes [‡]	n	Optimal range (mg/day)	Significant outcomes [‡]	n	Optimal range (mmol/L)	Significant outcomes [‡]
All population	13284	15.7 - 37.3	1, 2	11103	≥13.0	1, 2	2181	≤17.9	1, 2	13242	15.2 - 18.8	1, 2, 3
Sex												
Men	6503	≥15.4	1, 2	5759	≥12.9	1, 2	744	≤17.9	1, 2, 3	6472	14.7 - 17.9	1, 2, 3
Women	6781	16.2 - 27.0	1, 2	5344	≥13.4	2	1437	≤17.9	1, 2	6770	14.9 - 19.4	1, 2, 3
Age group												
< 65 yrs	9987	14.4 - 33.9	1, 2	8468	≥12.9	2	1519	≤17.9	1, 2	9952	15.4 - 17.2	1, 2, 3
≥ 65 yrs	3297	≥18.9	1, 2	2635	≥16.4	1, 2	662	≤17.9	1, 2	3290	≥14.7	1, 2, 3
Menopausal status												
Premenopause	3668	16.2 - 21.2	1, 2	2912	≥13.9	2	756	NA	2, 3	3658	15.0 - 16.1	1, 2, 3
Postmenopause	3113	≥18.3	1, 2	2432	19.7 - 26.4	2, 3	681	≤17.9	2, 3	3112	≥14.8	2, 3
Presence of chronic diseases												
No	3835	14.4 - 32.1	1, 2	3126	≥13.0	2	709	≤17.9	1, 2, 3	3824	16.5 - 17.7	1, 2, 3
Yes	9449	≥17.1	1, 2	7977	≥15.5	2	1472	≤17.9	1, 2	9418	≥14.6	1, 2, 3

[†]Models adjusted for sex (men/women), ethnicity (non-Hispanic Whites, non-Hispanic Blacks, Mexican Americans, and others), education levels (less than high school graduate, high school graduate to some college, college graduate or higher), poverty income ratio, BMI (kg/m²), cigarette smoking (non-smoker, former smoker, current smoker), alcohol consumption (non-drinker, moderate drinker, and heavy drinker), physical activity levels (low, moderate, and vigorous), energy intake (kcal/day), the presence of chronic diseases (hypertension, diabetes, dyslipidemia, chronic respiratory disease, hepatopathy, cardiovascular disease, stroke, arthritis, and thyroid disease) (yes and not) and alternative healthy eating index (AHEI).

[‡]1, PA deviation; 2, GoldBA deviation; 3, LightBA deviation.

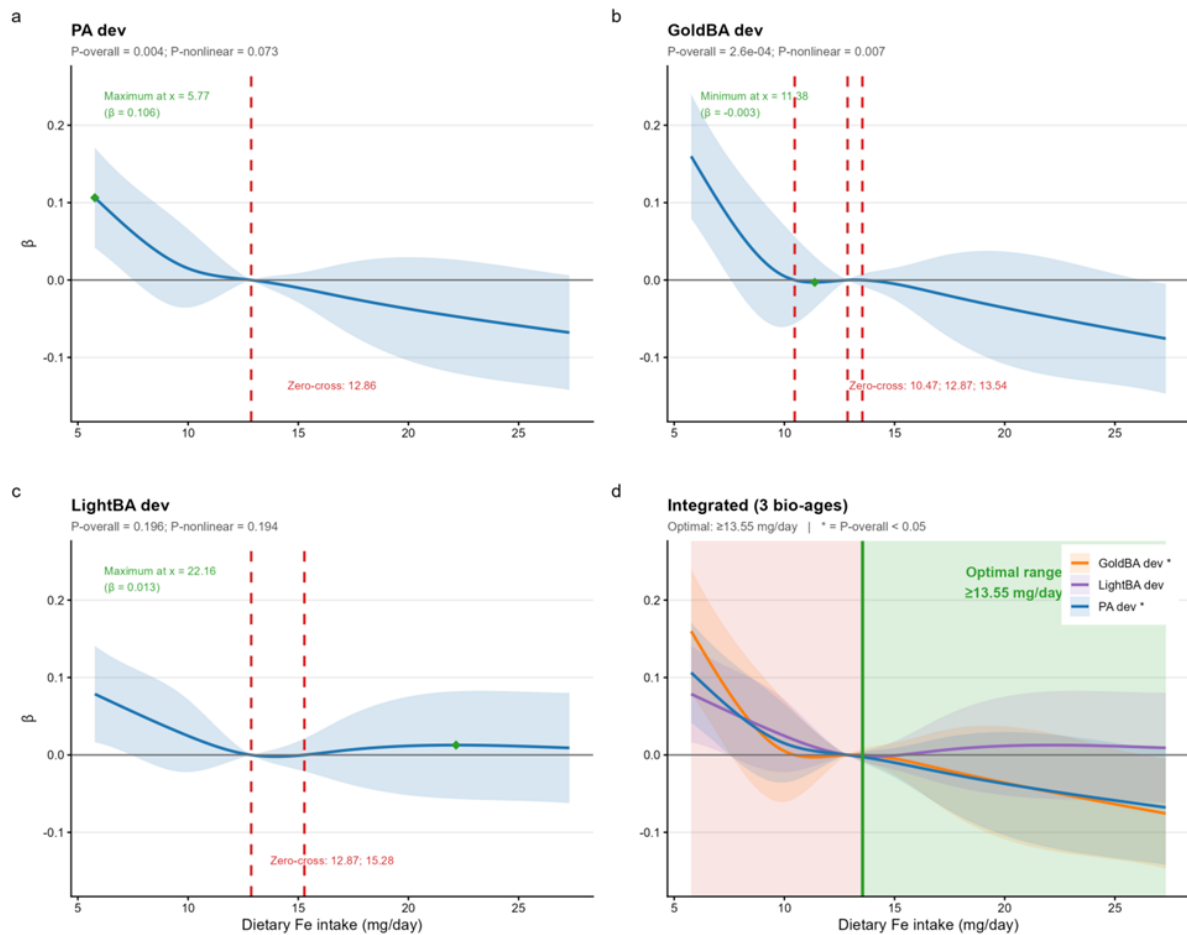


Figure 3. The RCS regression and optimal range of dietary iron intake over deviations of PA, GlodBA, and LightBA in the NHANES participants without taking iron supplements ($n = 11,103$). Models adjusted for sex, ethnicity, education levels, poverty income ratio, BMI, cigarette smoking, alcohol consumption, physical activity levels, the presence of chronic diseases, energy intake, and AHEI

monotonically above the reference, with β turning positive at 18 mg/day (Table 3 and Supplementary Figure 5). A categorical analysis confirmed a dose threshold: compared with non-users, a dose of ≤ 18 mg/day was associated with 0.7 months less advanced PA ($\beta = -0.06$, 95% CI: $-0.11, -0.01$; $p = 0.03$) and LightBA ($\beta = -0.06$, 95% CI: $-0.11, -0.01$; $p = 0.02$), and 1.8 months less advanced GoldBA ($\beta = -0.15$, 95% CI: $-0.22, -0.08$; $p < 0.001$), whereas a dose of > 18 mg/day was associated with 2.4 months and 4.1 months more advanced PA ($\beta = 0.20$, 95% CI: $0.07, 0.33$; $p < 0.01$) and GoldBA ($\beta = 0.35$, 95% CI: $0.25, 0.45$; $p < 0.001$), respectively (Table 4). The optimal supplemental range was ≤ 17.9 mg/day (Supplementary Figure 5).

Associations between serum iron concentration and biological aging

Weighted linear regression analyses consistently found inverse associations between serum iron concentrations and the three BA deviations in sequentially adjusted models (Table 2). In the fully adjusted models, per-2-fold increases were associated with approximately 0.5 months ($\beta = -0.04$, 95% CI: $-0.07, 0.00$; $p = 0.05$), 3 months ($\beta = -0.25$, 95% CI: $-0.29, -0.20$; $p < 0.001$), and 1.5 months ($\beta = -0.12$, 95% CI: $-0.22, -0.14$; $p < 0.001$) less advanced PA, GoldBA, and LightBA, respectively (Table 2). RCS analyses further indicated L-shaped associations of serum iron with GoldBA and LightBA deviations and a U-

shaped relationship with PA deviation (all p -overall < 0.001 and p -nonlinear < 0.001) (Table 3; Figure 4). The lowest point was found to be near 16.0 and 23.0 mmol/L and beta crossing zero at 14.5 mmol/L and 18.9 mmol/L for PA deviation, and at 14.5 mmol/L for LightBA deviation. The optimal serum iron range was 15.2–18.8 mmol/L, which was significant for all three biological age deviations (Table 3; Figure 4).

Subgroup analysis

Subgroup analysis found that heterogeneity was most pronounced for serum iron, where the interaction was significant for the three biological age measures across sex, age group, menopausal status, and the presence of chronic disease. Total iron levels showed heterogeneity only by sex. Dietary iron showed scattered significant interactions (e.g., sex for PA, age for PA and LightBA, and chronic disease for PA). Supplemental iron heterogeneity was limited to sex (Supplementary Table 2–5).

Subgroup analyses revealed no marked upper bound for optimal dietary iron intake across subpopulations, except for postmenopausal women (26.4 mg/day); however, the lower bound shifted upward in older individuals (16.4 mg/day for ≥ 65 years vs. 12.9 mg/day for < 65 years), postmenopausal women (19.7 mg/day vs. 13.9 mg/day), and participants with chronic diseases (15.5 mg/day vs. 13.0 mg/day), indicating a higher deficiency threshold in these groups. For total iron intake, the upper bound was

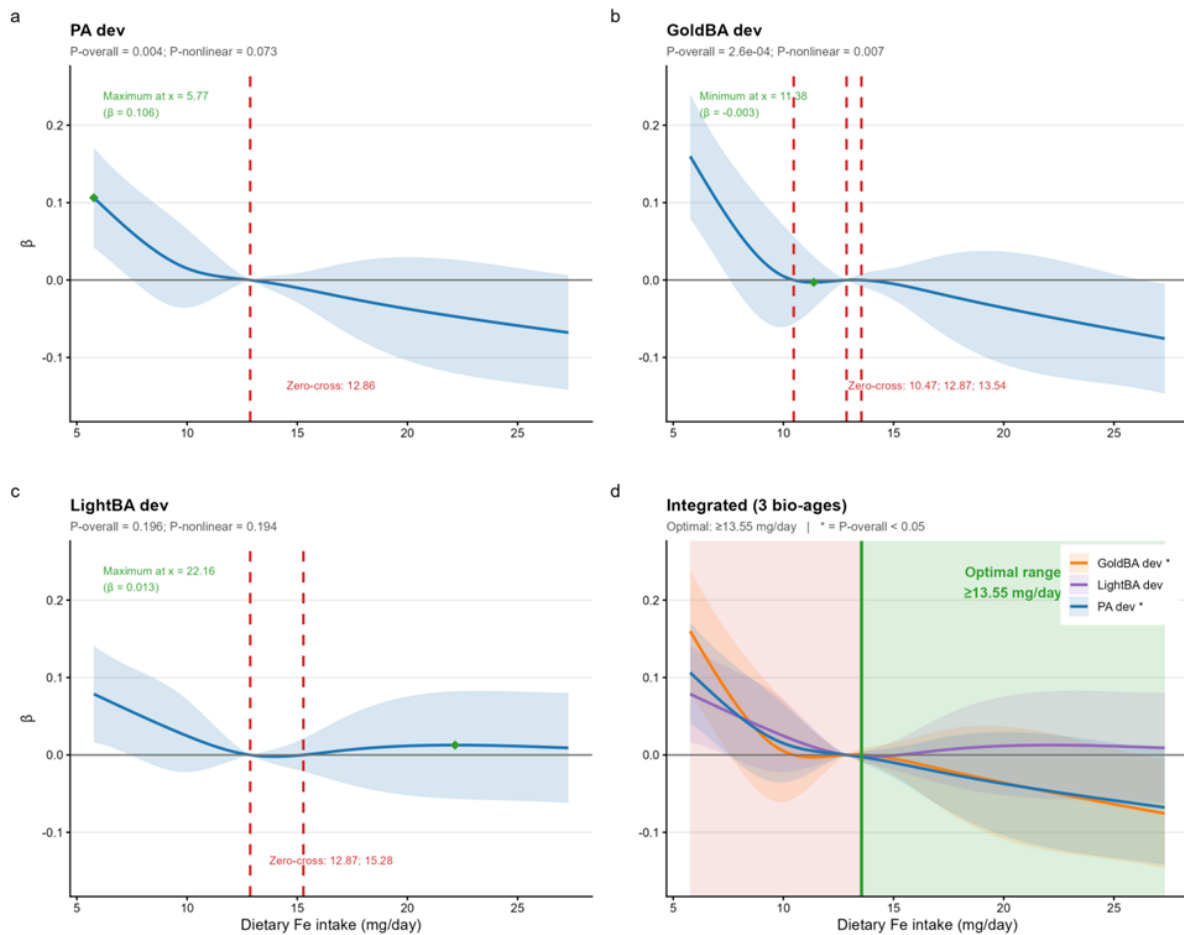


Figure 4. The RCS regression and optimal range of serum iron concentrations over deviations of PA, GlodBA, and LightBA in the NHANES participants ($n = 13,242$). Models adjusted for sex, ethnicity, education levels, poverty income ratio, BMI, cigarette smoking, alcohol consumption, physical activity levels, the presence of chronic diseases, energy intake, and AHEI.

wider in men (≥ 15.4 mg/day) than in women (16.2–27.0 mg/day), and older people (≥ 8.9 mg/day) and people with chronic diseases (≥ 17.1 mg/day) had both higher lower bounds and wider ranges than younger people (14.44–33.93 mg/day) and those without chronic diseases (14.44–32.13 mg/day). For supplemental iron, the optimal range (≤ 17.9 mg/day) was essentially invariant across sex, age, and the presence of chronic diseases. For serum iron, the optimal range was wider in individuals aged ≥ 65 years (≥ 14.7 mmol/L) than in younger individuals (15.42–17.24 mmol/L) and in participants with chronic diseases (≥ 14.55 mmol/L) than in those without chronic medical conditions (16.45–17.71 mmol/L) (Table 5 and Supplementary Figure 6–9).

DISCUSSION

In this cross-sectional study involving a representative sample of US adults, we explored the relationships between four iron exposures (total, dietary, and supplemental iron intake and serum iron) and three biological ages derived from clinical biomarkers. We also identified the optimal iron range for aging. Our study yielded three main results. First, the total iron intake exhibited significant U/J-shaped relationships with the three biological age metrics. Dietary iron intake was consistently linked to a less advanced biological age, whereas supplemental iron intake was associated with a more advanced biological age, with negative effects starting at approximately 18

mg/day. The adverse impact of high total iron intake on biological aging is primarily due to supplemental iron. Second, serum iron demonstrated the strongest and most consistent nonlinear associations, with an optimal range of 15.2–18.8 $\mu\text{mol/L}$. Both low intake levels and low serum concentrations are associated with accelerated biological aging. Finally, the optimal iron range varied significantly based on age, sex, menopausal status, and chronic disease status, with the greatest variation observed in the serum iron levels. These findings indicate that the relationship between iron and aging depends on both the iron source and the biological age metric, suggesting that personalized iron recommendations may be necessary for optimal aging outcomes.

Numerous BA assessments based on clinical biomarkers have been developed.³² Due to the differences in biomarkers and algorithm models used, as well as the different targets of evaluation (e.g., mortality, physiological function, or intervention effect), these BA indicators may differ significantly, making the comparison across different indicators critically important for result interpretation.^{24, 32} GoldBA is derived from the Gompertz mortality law,²⁵ and LightBA is a simplified indicator of GoldBA, using fewer biomarkers, and is less expensive and easier to apply in clinical settings.²⁵ Although these indicators were strongly intercorrelated, the aging processes measured by these BAs (the differences between the three BAs and CA) were less consistent, with a mod-

erate correlation coefficient. We found concordant directions of the association but different effect sizes among the three BA measurements. Serum iron was significant for all three (GoldBA $\beta = -0.25$, LightBA $\beta = -0.18$, PA $\beta = -0.04$ per two-fold increase), indicating the strongest and most consistent signal. Dietary iron was significantly protective against PA and GoldBA but not against LightBA, whereas total iron intake was non-linearly associated only with PA and GoldBA. In contrast, supplemental iron was significantly associated with all three metrics but in a harmful direction. The weaker sensitivity of LightBA to total and dietary iron likely reflects its fewer input indicators, which reduce exposure-driven variation. PA, which incorporates metabolic and inflammatory biomarkers directly influenced by iron metabolism,¹⁵ and GoldBA, which is anchored to mortality, appear more responsive to iron status.²⁵ The convergence of PA and GoldBA, two mechanistically distinct constructs, for serum and dietary iron strengthens the inference that these associations reflect genuine biological aging processes rather than artifacts of a single algorithm.

Our finding that dietary iron intake is associated with a less advanced biological age (linear and protective; optimal ≥ 13.6 mg/day) is in consistence with the findings of a Chinese cohort study showing a U-shaped relationship between dietary iron intake and biological age measurements¹⁴ and a sex-specific inverse association between iron intake and cellular aging markers mediated by TNF- α .¹² The non-linear (U/J-shaped) relationship of total iron intake with PA and GoldBA deviations - beneficial within an optimal window (15.7–37.3 mg/day) but detrimental at both low and high intakes - extends the "optimal range" paradigm from cardiometabolic outcomes¹⁵ to multi-metric biological aging. The most striking contrast was observed between the effects of dietary and supplemental iron. Whereas food-sourced iron was protective, supplemental iron was associated with a more advanced biological age, with a dose threshold near 18 mg/day, above which the association became clearly detrimental (GoldBA $\beta = 0.35$ for >18 mg/day vs. -0.15 for ≤ 18 mg/day). This directly mirrors evidence that supplement-driven iron overload accelerates phenotypic aging through inflammatory responses.¹⁵ This can be explained by the fact that dietary non-heme and heme iron absorption is tightly regulated by hepcidin and intestinal transporters,¹ so excess intake is largely withheld, whereas high-dose supplements can bypass this regulation, expanding the labile iron pool and catalyzing Fenton/Haber–Weiss reactive oxygen species generation and ferroptosis.² Our data add population-level evidence that the source of iron, not merely the amount, determines its aging-related effects, and foods should be the primary source for adequate intake.

Our finding that lower serum iron levels were associated with a more advanced biological age measured by all three metrics in both linear and non-linear regression models suggests a detrimental effect of iron insufficiency on health in the elderly and highlights the importance of maintaining an optimal iron status for healthy aging. Iron deficiency or inadequacy is a common nutritional problem in the elderly population.³ Indeed, iron deficiency anemia has been related to various age-related diseases,

such as cardiovascular diseases,^{9, 33} neurodegenerative disorders,³⁴ cancer,³⁵ and total mortality.³⁶ Our results are supported by a previous study showing that higher levels of serum iron and transferrin saturation, another indicator of circulating iron, were related to less advanced aging, as measured by DNA methylation, in a group of non-Hispanic women.¹⁸

However, our findings contradict those of a Mendelian randomization study that showed a positive association between genetically predicted serum iron levels and advanced epigenetic biological age.¹⁷ Furthermore, studies using other biomarkers of iron homeostasis have also shown positive correlations with biological aging. For example, higher serum ferritin, a biomarker for body iron storage, has been consistently associated with accelerated aging, as measured by leukocyte telomere length^{16, 37} or DNA methylation-based clocks,^{17, 18} and higher serum transferrin saturation has also been related to accelerated aging in most studies.^{17, 19, 37} These studies emphasized the potential detrimental effects of iron overload, which involves excess iron-induced generation of reactive oxygen species that cause oxidative damage to molecules, induce cellular senescence, and lead to various health issues such as Alzheimer's disease, type 2 diabetes, liver cirrhosis, and cancer.^{3, 5, 38} However, in our study, the detrimental association beyond the optimal range (15.2–18.8 $\mu\text{mol/L}$) was only observed for PA with a relatively weak effect size. One explanation for this discrepancy may be related to the methodological differences across BA measures, which involve the choice of biomarkers and the application of mathematical modelling techniques.³⁹ For example, one study showed that the highest quartile of serum ferritin was significantly associated with DNA methylation-based DunedinPACE acceleration but not PhenoAgeAccel acceleration, even in the same group of women.¹⁸ Another important reason may be the variation in the clinical significance of iron homeostasis biomarkers. Serum iron levels reflect the total amount of iron bound to transferrin in circulation and are more sensitive to deficiency, whereas serum ferritin levels indicate intracellular iron storage, and higher serum ferritin levels might suggest a higher possibility of overload.^{1, 22} Meanwhile, low serum iron concentrations and high ferritin levels can occur concurrently in the status of systemic inflammation.¹⁰ These findings highlight the complexity of iron metabolism and the importance of comprehensively interpreting the associations between different iron homeostasis biomarkers and aging.

A key novelty of this study is the derivation of aging-based optimal iron ranges. Our optimal dietary iron range (≥ 13.0 mg/day) exceeds the US Recommended Dietary Allowance (RDA) for most adults (8 mg/day for men and postmenopausal women; 18 mg/day for premenopausal women), and the optimal supplemental range (≤ 17.9 mg/day) is well below the Tolerable Upper Intake Level (45 mg/day).⁴⁰ Importantly, dietary reference intakes are established to meet physiological function and prevent deficiency (with a safety margin for toxicity), rather than optimize biological aging. Therefore, our ranges complement dietary reference intakes by introducing an aging-centered reference, as intakes that meet the RDA may still fall below the aging-optimal window, and intakes

below the upper limit (e.g., high-dose supplements) may be detrimental to aging.

The optimal range was strongly modified by the subgroups. For serum iron, the upper bound widened markedly in postmenopausal women (≥ 14.8 $\mu\text{mol/L}$), adults aged ≥ 65 years (≥ 14.7 $\mu\text{mol/L}$), and individuals with chronic disease (≥ 14.5 $\mu\text{mol/L}$), but narrowed in premenopausal women (15.0–16.1 $\mu\text{mol/L}$), younger adults (15.4–17.2 $\mu\text{mol/L}$), and those without chronic disease (16.5–17.7 $\mu\text{mol/L}$); the interaction p-values were significant across sex, menopause, age, and chronic disease. Total iron showed a similar but weaker pattern, with wider ranges in men, postmenopausal women, older adults, and participants with chronic diseases. The lower bound of dietary iron shifted upward in the older adult and chronic disease subgroups, indicating a higher deficiency threshold. These patterns are biologically interpretable. Menstrual iron loss in premenopausal women tightens the homeostatic requirement, producing a narrow optimal window. Loss of menses after menopause relaxes this constraint and alters iron handling, widening the range of iron levels.³ In older adults and participants with chronic diseases, inflammaging-driven hepcidin upregulation causes cellular iron sequestration and functional hypoferrremia,^{3, 9, 10} potentially shifting the apparent optimal range; however, because chronic disease covariates were removed from that model, residual confounding by inflammation and reverse causation cannot be excluded. The sex-specific iron-cellular aging interaction reported previously¹² supports the biological plausibility of our sex and menopause heterogeneity. Collectively, these findings suggest that iron guidance for healthy aging should be stratified rather than uniform.

Our study's findings have both theoretical and practical implications. Theoretically, by jointly comparing multiple biological age metrics across dietary, supplemental, and circulating iron exposures in a nationally representative sample, we demonstrated that the iron-aging association is metric-, source-, and population-dependent. The results extend the "ferro-senescence" concept^{4, 5} to population epidemiology and provide aging-based optimal ranges that complement deficiency-prevention-based dietary reference intake. Practically, the data favor iron obtained from diet over high-dose supplementation for aging health, caution against routine high-dose iron supplementation (>18 mg/day) in replete individuals, and support subgroup-stratified iron guidance, for example, a narrower serum iron target in premenopausal women and closer monitoring in older adults and chronic disease-bearing individuals.

The study is strengthened by the parallel use of three biological age metrics enabling cross-validation,²³⁻²⁶ the use of restricted cubic spline modelling that captured non-linear U/J-shaped relations invisible to linear models, the separate modelling of supplement and non-supplement users with mutual covariate adjustment, and a rigorous optimal-range definition as the intersection of significant, beneficial ($\beta < 0$) outcomes. However, this study had several limitations. First, the cross-sectional study design limited causal inference. Dysregulation of iron homeostasis can be a driving factor for aging, but it can also be a consequence of accelerated aging.^{1, 41} A prospective study

is needed to further clarify the causal effects. Second, owing to the unavailability of other biomarkers (ferritin, TSAT, or hepcidin) in the NHANES data, we relied on a single serum iron measurement. Although serum iron levels are sensitive to iron deficiency, they may not be reliable indicators of iron storage.^{1, 22} Moreover, serum iron levels can fluctuate due to factors such as dietary intake, circadian rhythm, and recent illnesses or medical conditions.⁴¹ Further studies are needed to comprehensively assess multiple biomarkers reflecting iron homeostasis. Third, in this study, we only focused on three mortality-based and blood biochemistry-derived BA indicators. The relationship between iron and the aging process across multiple systems and at the cellular level warrants further investigation using a broader range of BA metrics. Fourth, although we used the AHEI score to adjust for the confounding effects of overall dietary quality, it remained challenging to disentangle the specific impact of dietary iron from other diet-related factors influencing iron metabolism (e.g., vitamin C and polyphenols), given the inherent complexity of dietary patterns.⁴¹ Fifth, the optimal range is data-driven and sensitive to knot placement and significance thresholds, and formal multiple-comparison corrections across many exposure-outcome-subgroup tests should be further conducted. Sixth, dietary intake relied on 24-h recall, and residual confounding by unmeasured lifestyle factors remains possible. Furthermore, disease status (hypertension and diabetes) was defined using a single fasting plasma glucose measurement and limited blood pressure readings obtained under the NHANES protocol, which may not satisfy the repeated-measurement criteria required for a clinical diagnosis and could lead to some misclassification of chronic disease status. Finally, although a Chinese cohort reported a similar U-shaped correlation between dietary iron and biological age,¹⁴ our ranges derived from a US population may not be generalizable to other populations.

Conclusion

In this nationally representative study, higher dietary iron intake was associated with a less advanced biological age, whereas iron supplementation was associated with accelerated aging and was beneficial only at or below 18 mg/day. Higher serum iron concentrations were related to delayed biological aging with a narrow optimal window (approximately 15–19 $\mu\text{mol/L}$), which widened in postmenopausal women, older adults, and individuals with chronic diseases. The iron-aging association was consistent across biological age metrics for serum and dietary iron and exhibited the strongest subgroup heterogeneity for serum iron levels. These findings support the distinction between dietary and supplemental iron sources and the stratification of iron guidance by population characteristics, suggesting that an aging-based optimal iron range may complement existing dietary reference intakes. Longitudinal and interventional studies are needed to confirm causality and refine population-specific optimal ranges.

DISCLOSURE ON THE USE OF AI AND AI-ASSISTED TECHNOLOGIES

The authors acknowledge the use of Paperpal (accessed July 2026) for language editing and clarity improvement. All AI-assisted revisions were reviewed and approved by the authors.

CONFLICT OF INTEREST AND FUNDING DISCLOSURES

The authors declare no conflict of interest.

This research did not receive any specific grants from funding agencies in the public, commercial, or not-for-profit sectors.

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