ORIGINAL ARTICLE

Iron deficiency and biomarkers of inflammation: a 3-year prospective analysis of the DO-HEALTH trial

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Abstract
Background The longitudinal association between iron deficiency and inflammatory biomarkers levels has not been fully explored among relatively healthy older adults.
Aims To assess whether iron deficiency at baseline and at any yearly follow-up time point, with or without anemia, was associated with changes from baseline in high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6) levels over 3 years.
Methods This is a post-hoc observational analysis of DO-HEALTH, a double-blind, randomized controlled trial including 2157 European community-dwelling adults age 70+. The outcomes were changes from baseline in hs-CRP and IL-6 levels, measured at 12, 24, and 36 months of follow-up. Iron deficiency was defined by soluble transferrin receptor levels > 28.1 nmol/L and baseline anemia by hemoglobin levels < 130 g/L for men and < 120 g/L for women.
Results In total, 2141 participants were included in the analyses (mean age: 74.9 years, 61.5% of women, 26.8% with iron deficiency). Baseline iron deficiency was associated with greater increase in IL-6 levels (mean difference in change: 0.52 ng/L, 95%CI 0.03–1.00, $P = .04$) over 3 years. Iron deficiency at any yearly time point was associated with higher increases in hs-CRP (mean difference in change: 1.62 mg/L, 95%CI 0.98–2.26, $P < .001$) and IL-6 levels (mean difference in change: 1.33 ng/L, 95%CI 0.87–1.79, $P < .001$) over 3 years. No significant interaction between iron deficiency and anemia was found, suggesting that the results are independent of the anemic status.
Conclusions These findings suggest that iron deficiency may play a role in low-grade chronic inflammation among relatively healthy older adults.

Keywords Iron deficiency · Anemia · Hs-CRP · IL-6 · Inflammaging · DO-HEALTH

Introduction

Iron deficiency (ID) is the most frequent nutritional disorder worldwide affecting populations of all ages, sexes, and ethnic descent. [1–3] Iron is an essential micronutrient and its homeostasis plays an important role in several physiological

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human processes, including cellular activities, erythropoiesis, and innate immune function. [4] Inflammatory stimuli induce macrophage production of interleukin-6 (IL-6), which stimulates hepatocytes to produce acute-phase proteins including C-reactive protein (CRP) [5, 6] and hepcidin. [7] This inflammation state may lead to extended hypoferremia and cause anemia. [8]

While there is good scientific evidence that inflammation may predict ID, evidence on the influence of ID on pre-existing low-grade inflammation remains limited. With aging, loss of homeostasis alters the production of cytokines, leading to a low-grade chronic systemic pro-inflammatory state characterized by permanently elevated levels of IL-6 and CRP. This state, also called "inflammaging" [9], is considered to be a promotor of accelerated aging [10], age-related syndromes such as frailty [11–14], and mortality [15–18] in older adults. Thus, capturing iron status among older adults may be relevant to the promotion of healthy aging and the prevention of age-related chronic diseases.

Recently, high prevalence of ID was reported in a population of community-dwelling older adults. [19] To our knowledge, so far, no longitudinal prospective study has investigated the association between ID and low-grade chronic inflammation in relatively healthy older individuals. In a recent cross-sectional study, iron-deficient women with mean age 58 years showed increased levels of CRP and IL-6 compared to healthy controls. [20] However, the use of a different ID definition prevents the accurate comparison of results between studies. [21, 22]

Therefore, the aim of the present study was to assess whether the presence of ID at baseline and at yearly follow-up visits, with or without anemia, was associated with changes from baseline in high-sensitivity CRP (hs-CRP) and IL-6 levels over 3 years in a large European cohort of relatively healthy community-dwelling older adults.

Methods

Study design and participants

The study is a post-hoc observational analysis of the DO-HEALTH clinical trial with a prospective, longitudinal approach. DO-HEALTH is a multi-center, double-blind, randomized controlled clinical trial designed to support healthy aging in European older adults (NCT01745263). The trial examined the individual and combined effects of omega-3 fatty acids, vitamin D, and a simple home exercise program over 3 years of follow-up. A total of 2157 community-dwelling healthy and pre-frail seniors aged 70 years and older were recruited from 7 centers in 5 European countries: Zurich, Basel, Geneva (Switzerland), Berlin (Germany),

Innsbruck (Austria), Toulouse (France), and Coimbra (Portugal). Inclusion criteria were absence of major health events in the 5 years prior to enrollment, sufficient mobility, and good cognitive status. Further details are provided elsewhere. [23]

Primary outcomes: biomarkers of inflammation

For the present study, the 3-year-follow-up data were used. Fasting blood samples, collected in the morning were taken at baseline, 12, 24, and 36 months of follow-up. Hs-CRP levels were measured with the C-Reactive Protein Gen. 3 test on a cobas e 701 analyser (Roche) using an Immunoturbidimetric assay. IL-6 levels were measured using the Elecsys IL-6 assay on cobas e 801 analyser (Roche) with ElektroChemiLumineszenz-ImmunoAssay "ECLIA" technology. Higher levels of hs-CRP and IL-6 indicate greater inflammation.

Exposures: ID and anemia

We used soluble transferrin receptor levels (sTfR) levels as a clinical marker of ID at baseline and over the follow-up, since this parameter is not influenced by inflammation. [24] The threshold of more than 28.1 nmol/L was used to define ID as it was validated in older adults. [25–27] sTfR levels were measured with Tina-quant Transferrin ver.2 Test on a cobas c 502 analyser (Roche) using an Immunoturbidimetric assay. Considering the lack of consensus in the definitions of ID [7], we performed sensitivity analyses using serum ferritin levels and the sTfR-ferritin index (sTfR / log Ferritin) as alternate definitions of ID. Ferritin concentrations were measured with Elecsys Ferritin Test on a cobas e 801 analyser (Roche) using ElektroChemiLumineszenz-ImmunoAssay "ECLIA" technology. ID was then defined using the established cutoffs of ferritin less than 45 µg/L [28, 29] and 30 µg/L for older adults [30, 31]; and of sTfR-ferritin index over 1.5 ng/mL. [26, 32]

Hemoglobin was measured in the whole blood at baseline. We used hemoglobin levels less than 130 g/L for men and less than 120 g/L for women to define anemia, according to the World Health Organization guidelines. [33]

Baseline covariates

Participants' characteristics such as age, sex, tobacco consumption, and body mass index (BMI) were collected at baseline. Alcohol consumption (g/day) was derived from the Food Frequency Questionnaire. [34] Comorbidities were assessed with the self-administered comorbidity questionnaire. [35] Frailty status was determined

according to Fried criteria. [36] Polypharmacy was defined as the use of 5 or more medications. Frequency of physical activity (0, 1–2, ≥ 3 times per week) was measured using the Nurses' Health Study questionnaire. [37]

Statistical analysis

Baseline demographic and clinical characteristics, including baseline hs-CRP and IL-6 levels, were compared between ID and non-ID participants using Chi-square and *t* tests. Linear regression models based on generalized estimating equations (GEE) for repeated measurements were used to compare changes from baseline in hs-CRP and IL-6 levels in participants who were ID versus non-ID at baseline. Each participant had up to 3 outcome measures (hs-CRP or IL-6 changes from baseline at 1, 2, and 3 years) and the primary predictors were baseline ID status, follow-up year, and the interactions between these two predictors. Models were adjusted for treatment allocation, study site and baseline covariates: age [38], sex [39], number of comorbidities [22], alcohol consumption [40], tobacco consumption [41], polypharmacy [22], frailty status [11, 13], frequency of physical activity [5], and the baseline level of the outcome. Time-varying BMI [5] and the incidence rate of infections [42] (number of infections per year/person-days per year) in the year preceding the measurement of the inflammatory biomarkers were also used as adjustment variables. These models allowed us to estimate, at each yearly follow-up, whether the change from baseline in hs-CRP or IL-6 level was different between participants who were ID compared to non-ID. Additionally, we estimated the effect of baseline ID status on hs-CRP and IL-6 changes across all follow-up times simultaneously. Finally, to explore the effect of the presence of ID at each follow-up (rather than at baseline), we included a time-varying variable for iron status in separate, analogous GEE models controlling for baseline iron status. Mean differences in changes (MD) with 95% confidence intervals (CI) are presented.

In exploratory analyses, for each outcome, interactions between ID and age (70–74 years, 75 years and older), sex, and anemic status were tested. A sensitivity analysis was performed to investigate the association between recurrent iron deficiency (defined as sTfR levels > 28.1 nmol/L at 2 or more consecutive time points vs 1 time point—including baseline) and biomarkers levels. Furthermore, since liver and kidneys perform a major role in iron homeostasis [43–46], we excluded participants who reported baseline liver disease (*n* = 37), kidney disease (*n* = 54), and participants with incident invasive cancer over the follow-up (*n* = 74) in a second sensitivity analysis.

Statistical analyses involved using SAS version 9.4 (SAS Institute, Cary, NC). Two-sided *p* values < 0.05 were considered statistically significant.

Results

Baseline characteristics of the study population

Of the 2157 trial participants, 16 (0.7%) had missing baseline sTfR levels. In total, 2141 participants were included in the analyses. At baseline, 573 (26.8%) of them suffered from ID, and over the 3-year follow-up, a total of 262 new cases of ID were identified among non-ID participants at baseline. Baseline characteristics of subjects are presented in Table 1. Overall, the mean age was 74.9 (4.5) years, including 1317 (61.5%) women. The mean levels of hs-CRP and IL-6 were 2.9 mg/L (5.6) and 3.8 ng/L (6.9), respectively. At baseline, ID participants were more likely to be older (*P* < 0.001), less physically active (*P* = 0.009), at least pre-frail (*P* = 0.007), subject to polypharmacy (*P* < 0.001), and anemic (*P* < 0.001). In addition, they were more likely to have fewer years of education (*P* < 0.001), a higher BMI (*P* < 0.001), lower alcohol consumption (*P* = 0.008), and more comorbidities (*P* < 0.001), compared to the non-ID participants.

Association between baseline ID and inflammatory biomarkers

Overall, a significant difference in baseline hs-CRP levels was observed according to the iron status, with higher levels in ID subjects (unadjusted MD: 1.49 mg/L, 95% CI: 0.78–2.21, *P* < 0.001) (Table 2). However, there was no statistically significant difference in hs-CRP levels over time between participants with ID and non-ID at baseline (Fig. 1a).

Significantly higher baseline IL-6 levels were observed in ID participants compared to non-ID participants (unadjusted MD: 1.26 ng/L, 95% CI: 0.45–2.07, *P* = 0.003). Over time, we found a significant increase in IL-6 levels among both non-ID (0.52 ng/L) and ID participants (1.04 ng/L), with a significantly greater increase in the latter (MD: 0.52 ng/L, 95% CI: 0.03–1.00, *P* = 0.04) (Fig. 1b).

The interaction between ID and baseline anemic status was not statistically significant in hs-CRP (*P* = 0.23) and IL-6 models (*P* = 0.42), suggesting that results are independent of anemic status. A significant interaction between sex and baseline ID was found (*P* = 0.03). We observed a significant decrease in CRP levels between

Table. 1 Baseline characteristics of the study population

	Baseline iron deficiency sTfR > 28.1 nmol/L	No baseline iron deficiency sTfR ≤ 28.1 nmol/L	p value [a]	Overall
	$n = 573$ (26.8)	$n = 1568$ (73.2)		$n = 2141$
Sex, N (%)			0.29	
Women	363 (63.4)	954 (60.8)		1317 (61.5)
Men	210 (36.7)	614 (39.2)		824 (38.5)
Age, mean (SD), years	75.6 (4.7)	74.7 (4.3)	< 0.001	74.9 (4.5)
Education, years	12.1 (4.4)	12.9 (4.2)	< 0.001	12.7 (4.3)
BMI, mean (SD), kg/m^2	27.1 (4.5)	26.0 (4.2)	< 0.001	26.3 (4.3)
Alcohol, mean (SD), g/day	7.7 (11.1)	9.3 (11.9)	0.008	8.9 (11.7)
Current smokers, N (%)	14 (2.4)	111 (7.1)	< 0.001	125 (5.8)
Live alone, N (%)	242 (42.2)	655 (41.8)	0.85	897 (41.9)
Physical activity, N (%)			0.009	
None	123 (21.5)	249 (15.9)		372 (17.4)
1–2 times per week	170 (29.7)	474 (30.3)		644 (30.1)
≥ 3 times per week	280 (48.9)	843 (53.8)		1123 (52.5)
Frailty status [b], N (%)			0.007	
Robust	272 (48.4)	852 (55.1)		1124 (53.3)
At least pre-frail	290 (51.6)	695 (44.9)		985 (46.7)
Polypharmacy [c], N (%)	191 (33.3)	384 (24.5)	< 0.001	575 (26.9)
Iron supplementation, N (%)	37 (6.5)	83 (5.3)	0.30	120 (5.6)
Number of comorbidities [d], mean (SD)	2 (1.5)	1.6 (1.4)	< 0.001	1.7 (1.4)
Anemia [e], N (%)	63 (11.0)	77 (4.9)	< 0.001	140 (6.5)
hs-CRP, mean (SD), mg/L	4.0 (8.4)	2.5 (4.2)	< 0.001	2.9 (5.6)
IL-6, mean (SD), ng/L	4.7 (9.3)	3.5 (5.9)	0.003	3.8 (6.9)
Countries, N (%)			0.02	
Austria	55 (9.6)	143 (9.1)		198 (9.3)
France	73 (12.7)	226 (14.1)		299 (14.0)
Germany	92 (16.1)	254 (16.2)		346 (16.2)
Portugal	101 (17.6)	192 (12.2)		293 (13.7)
Switzerland	252 (44.0)	753 (48.0)		1005 (46.9)

BMI Body Mass Index, *sTfR* soluble Transferrin Receptor, *hs-CRP* high-sensitivity C-Reactive Protein, *IL-6* Interleukin-6

[a] Differences between iron-deficient and non-iron-deficient participants at baseline were assessed by an independent *t* test for continuous variables and Chi-square test for categorical variables.

[b] Frailty status was defined using the Fried Physical Frailty Phenotype which evaluates 5 criteria: fatigue (self-reported), unintentional weight loss (self-reported loss more than 5% of total body weight), reduced physical activity (self-reported), slowness (impaired walking speed) and weakness (low grip strength). Participants are classified as at least pre-frail when one or more of the criteria are presented, and otherwise classified as robust.

[c] Polypharmacy was defined as the concomitant use of 5 or more medications.

[d] Self-reported number of comorbidities was assessed by the Sangha questionnaire, range 0–13.

[e] Anemia was defined as hemoglobin < 130 g/L for men and < 120 g/L for women.

baseline and year 3 in male ID subjects, while there was no significant change in hs-CRP levels over time in both female subgroups (Online Resource 1). A significant interaction was also found between baseline ID and age groups ($P = 0.01$). In younger participants, IL-6 levels increased regardless the iron status, without any significant difference across all time points. In older subjects, ID was significantly associated with a greater increase in IL-6 levels over time (Online Resource 2).

Table. 2 Changes from baseline in hs-CRP and IL-6 levels by baseline iron status

	Baseline iron deficiency sTfR levels > 28.1 nmol/L n = 573	No baseline iron deficiency sTfR levels ≤ 28.1 nmol/L n = 1568	Mean difference in change from baseline (95% CI)	P value for mean difference in change from baseline between groups [a]
hs-CRP (mg/L)				
Unadjusted at baseline, mean (SD)	3.99 (0.35)	2.49 (0.10)	**1.49 (0.78 to 2.21)**	**< 0.001**
Adjusted change at Year 1 (95% CI)	0.07 (− 0.37 to 0.50)	**− 0.29 (− 0.54 to − 0.05)**	0.36 (0.15 to 0.87)	0.16
Adjusted change at Year 2 (95% CI)	0.19 (− 0.29 to 0.68)	0.00 (-0.35 to 0.35)	0.19 (− 0.40 to 0.79)	0.52
Adjusted change at Year 3 (95% CI)	− 0.26 (− 0.57 to 0.05)	− 0.26 (− 0.56 to 0.04)	− 0.01 (− 0.44 to 0.43)	0.98
Adjusted change across all time points (95% CI)	− 0.00 (− 0.28 to 0.28)	− 0.18 (− 0.38 to 0.01)	0.18 (− 0.16 to 0.52)	0.29
IL-6 (ng/L)				
Unadjusted at baseline, mean (SD)	4.72 (0.39)	3.46 (0.15)	**1.26 (0.45 to 2.07)**	**0.003**
Adjusted change at Year 1 (95% CI)	**0.93 (0.43 to 1.43)**	**0.43 (0.15 to 0.70)**	0.51 (− 0.09 to 1.10)	0.09
Adjusted change at Year 2 (95% CI)	**0.98 (0.50 to 1.47)**	**0.70 (0.41 to 0.98)**	0.29 (− 0.31 to 0.89)	0.35
Adjusted change at Year 3 (95% CI)	**1.20 (0.55 to 1.85)**	**0.44 (0.20 to 0.69)**	**0.75 (0.03 to 1.48)**	0.04
Adjusted change across all time points (95% CI)	**1.04 (0.65 to 1.43)**	**0.52 (0.31 to 0.73)**	**0.52 (0.03 to 1.00)**	**0.04**

[a] *p* values correspond to the mean differences in biomarkers levels or changes in biomarker levels between iron-deficient and non-iron-deficient groups. Baseline levels are compared using a *t* test. Yearly changes from baseline are compared by repeated measures linear regression with interaction terms between iron deficiency status and time. Overall differences across all time points are compared by repeated measures linear regression with a main effect for iron deficiency

Models are adjusted for treatment allocation, age, sex, center, body mass index over the follow-up, alcohol consumption, tobacco consumption, polypharmacy, number of comorbidities, frailty status (pre-frailty), frequency of physical activity, yearly incidence rate of infections, and baseline level of the outcome

sTfR soluble Transferrin Receptor

Values in bold indicate significant P values

Association between yearly-assessed ID and inflammatory biomarkers

Participants with ID at any yearly follow-up time point had 1.59 mg/L higher concentration of hs-CRP at baseline (unadjusted, 95% CI 0.89–2.29, *P* < 0.001) (Table 3). Across all yearly follow-ups, there was a significant increase in hs-CRP levels among subjects with ID at a particular follow-up time point (1.07 mg/L), while there was a significant decrease in hs-CRP levels in participants who did not have ID at these time points (− 0.55 mg/L). The presence of ID at a yearly follow-up time point was associated with a statistically significant greater increase in hs-CRP levels (MD: 1.62 mg/L, 95% CI 0.98–2.26, *P* < 0.001) (Fig. 1c).

At baseline, IL-6 levels were significantly higher in participants who had ID at any yearly follow-up time point (unadjusted MD: 1.19 ng/L, 95% CI 0.52–1.86, *P* < 0.001).

Over time, we observed a significant increase in IL-6 levels among both participants with ID (1.65 ng/L) and without ID (0.32 ng/L). The presence of ID at any yearly time point over the follow-up was associated with a greater increase in IL-6 levels (MD: 1.33 mg/L, 95% CI 0.87–1.79, *P* < 0.001) (Fig. 1d).

We did not find any significant interaction between ID at any yearly follow-up time point and anemic status (*P* = 0.24 and *P* = 0.46 in hs-CRP and IL-6 models, respectively), suggesting that the results are independent of the presence of anemia. Results were similar for the interaction between ID at any yearly time point and sex (*P* = 0.25 in hs-CRP model and *P* = 0.72 in IL-6 model) or age groups (*P* = 0.59 in hs-CRP model and *P* = 0.32 in IL-6 model).
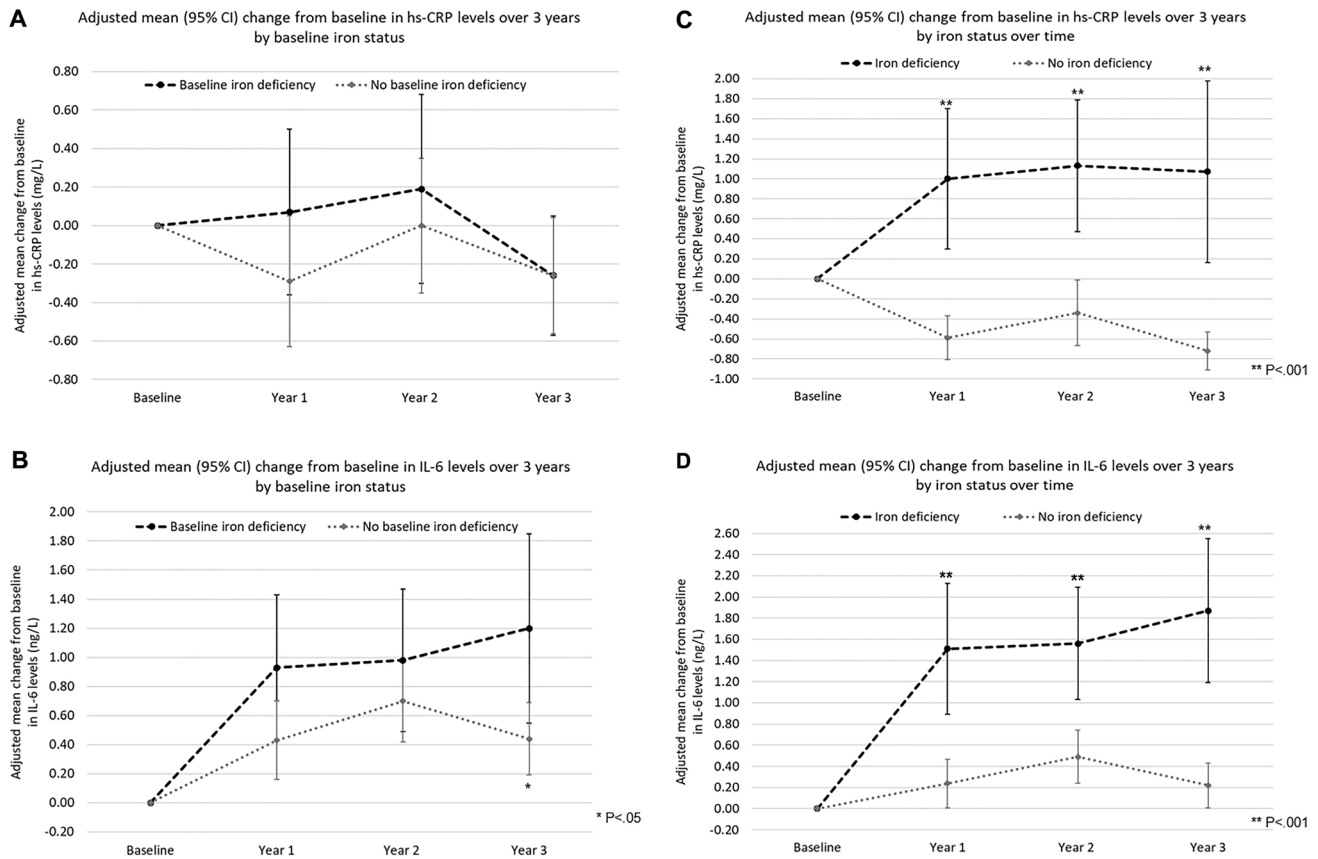
Fig 1 Changes in inflammatory biomarkers levels over time. The adjusted mean change from baseline over three years is shown for (**a**) hs-CRP levels by baseline iron status, **b** IL-6 levels by baseline iron status, **c** hs-CRP levels by iron status at each yearly time point over the follow-up, and **d** IL-6 levels by iron status at each yearly time point over the follow-up

Sensitivity analysis

Association between baseline ID defined with ferritin levels and transferrin–ferritin index and inflammatory biomarkers

Over 3 years, no statistically significant difference over time in hs-CRP levels was found between ID and non-ID groups, when ID was defined by both < 45 µg/L and < 30 µg/L ferritin levels (Online Resource 3). IL-6 levels significantly increased in all subjects, without any significant difference between the 2 groups over time, regardless of the ferritin cut-off used.

When defining ID with transferrin–ferritin index, ID was associated with a greater increase in hs-CRP and IL-6 concentrations, across all time points (Online Resource 3).

Association between ID defined with ferritin levels and transferrin–ferritin index over the follow-up and inflammatory biomarkers

Regardless of the cut-off applied for ferritin levels, the presence of ID at any yearly time point was associated with a decrease in hs-CRP levels across all time points (Online Resource 4). There was no significant difference in IL-6 levels between participants with ID at a particular time over the follow-up and those without. When defined by transferrin–ferritin index > 1.5, the presence of ID at any yearly time point over the follow-up was associated with a greater increase in IL-6 across all time points (Online Resource 4).

Association between recurrent ID and inflammatory biomarkers

Across all time points, the presence of recurrent iron deficiency was associated with statistically significant greater increases in hs-CRP and IL-6 levels (Online Resource 5).

Exclusion of participants with baseline liver, kidney disease, and incident invasive cancers

When excluding participants with baseline liver disease, kidney disease, and incident invasive cancers, results were consistent with those observed in the main analysis (Online Resource 6).

Table 3 Changes from baseline in hs-CRP and IL-6 levels by yearly-assessed iron status

	Iron deficiency at any yearly follow-up time point sTfR levels > 28.1 nmol/L	No iron deficiency at any yearly follow-up time point sTfR levels ≤ 28.1 nmol/L	Mean difference in change from baseline (95% CI)	P value for mean difference in change from baseline between groups [a]
hs-CRP (mg/L)				
Unadjusted at baseline, mean (SD)	4.05 (0.34)	2.47 (0.11)	**1.59 (0.89 to 2.29)**	**< 0.001**
Adjusted change at Year 1 (95% CI)	**1.00 (0.30 to 1.70)** [n = 481]	**− 0.59 (-0.81 to -0.37)** [n = 1419]	**1.59 (0.82 to 2.37)**	**< 0.001**
Adjusted change at Year 2 (95% CI)	**1.13 (0.47 to 1.79)** [n = 485]	**− 0.34 (− 0.67 to − 0.01)** [n = 1346]	**1.47 (0.70 to 2.24)**	**< 0.001**
Adjusted change at Year 3 (95% CI)	**1.07 (0.17 to 1.98)** [n = 462]	**− 0.72 (− 0.90 to − 0.53)** [n = 1367]	**1.80 (0.83 to 2.77)**	**< 0.001**
Adjusted change across all time points (95% CI)	**1.07 (0.50 to 1.63)**	**− 0.55 (− 0.72 to − 0.38)**	**1.62 (0.98 to 2.26)**	**< 0.001**
IL-6 (ng/L)				
Unadjusted at baseline, mean (SD)	4.66 (0.33)	3.48 (0.15)	**1.19 (0.52 to 1.86)**	**< 0.001**
Adjusted change at Year 1 (95% CI)	**1.51 (0.88 to 2.13)** [n = 481]	**0.24 (0.01–0.47)** [n = 1419]	**1.27 (0.60 to 1.94)**	**< 0.001**
Adjusted change at Year 2 (95% CI)	**1.56 (1.04 to 2.09)** [n = 485]	**0.49 (0.24–0.74)** [n = 1346]	**1.07 (0.48 to 1.67)**	**< 0.001**
Adjusted change at Year 3 (95% CI)	**1.87 (1.18 to 2.55)** [n = 462]	**0.22 (0.01–0.43)** [n = 1367]	**1.65 (0.92 to 2.37)**	**< 0.001**
Adjusted change across all time points (95% CI)	**1.65 (1.23 to 2.06)**	**0.32 (0.14–0.49)**	**1.33 (0.87 to 1.79)**	**< 0.001**

[a] p values correspond to the mean differences in biomarkers levels or changes in biomarker levels between iron-deficient and non-iron-deficient groups. Baseline levels are compared using a t test. Yearly changes from baseline are compared by repeated measures linear regression with interaction terms between iron deficiency status and time. Overall differences across all time points are compared by repeated measures linear regression with a main effect for iron deficiency

Models are adjusted for treatment allocation, age, sex, center, body mass index over the follow-up, alcohol consumption, tobacco consumption, polypharmacy, number of comorbidities, frailty status (pre-frailty), frequency of physical activity, yearly incidence rate of infections, baseline iron status and baseline level of the outcome

Numbers between squared brackets indicate the number of iron-deficient and non-iron-deficient participants at each yearly time point

sTfR soluble Transferrin Receptor

Values in bold indicate significant P values

Discussion

To the best of our knowledge, the present study is the first to investigate the longitudinal association between ID and low-grade chronic inflammation in a large cohort of relatively healthy European community-dwelling older adults.

At baseline, ID defined by sTfR levels was cross-sectionally associated with higher levels of hs-CRP and IL-6. Across all time points, the change from baseline in hs-CRP levels did not differ according to the iron status, while a significantly greater increase in IL-6 levels was observed in participants with ID at baseline. Participants with ID at any yearly time point over the follow-up had higher levels of inflammatory biomarkers at baseline. Similarly, we observed significant increases in hs-CRP levels and in IL-6 levels at

follow-up time points when participants had ID at that time point.

Our results are partly consistent with those of a recent cross-sectional study. [20] Askar et al. found that CRP and IL-6 levels significantly increased in 20 middle-aged women with ID anemia (defined by ferritin < 15 μg/L and hemoglobin level of < 11.5 g/dL), when compared to their disease-free counterparts. Nevertheless, in our large study population of older adults, we did not find any statistically significant interaction between ID and baseline anemia, suggesting the results herein are independent of anemic status.

While the presence of ID at any yearly time point over the follow-up was associated with high levels of inflammatory biomarkers, we found opposite trends in non-ID participants. We observed a significant decrease in hs-CRP levels while IL-6 significantly increased over time. These different trends

in inflammatory biomarkers levels were also observed in another large 3-year observational study including 4979 older adults, regardless of the iron status. [47] Since IL-6 is known to induce the production of CRP, this lack of timely overlap between IL-6 and hs-CRP levels could be explained by the intervention of hepcidin [48] and other cytokines not measured in the present study (e.g., IL-1, IL-17). [49] More recently, a review described that elevated IL-6 levels may also indicate protection, preservation, and/or repair of somatic tissue in an aging organism. [5].

Additionally, we found that baseline ID was associated with a significantly greater increase in IL-6 levels across all time points. These results may suggest ID as a promotor of low-grade chronic inflammation measured with IL-6. Given limited current scientific evidence on ID predicting inflammation and the observational nature of our study, our results are hypothesis generating. Thus, this work constitutes a first step to further investigate the role of ID and other regulators of iron on inflammation homeostasis such as hepcidin and their implications in healthy aging.

Furthermore, there is currently no consensus to define ID in older adults. We conducted sensitivity analyses to consider the most used definitions in clinical practice. Compared to our main analysis, contradictory results were obtained when using ferritin levels to define ID. Since ferritin is influenced by inflammatory conditions due to its additional function as acute-phase protein [50], our results confirmed that it might be less suitable to investigate ID in older adults. [51] Another point that has to be mentioned is the absence of evidence-based clinical thresholds to draw conclusions about the clinical relevance of increases or decreases in IL-6 and hs-CRP levels in our study population. For hs-CRP levels, a previous longitudinal large study used a cut-off of more than 3 mg/L to define the presence of systemic inflammation, since mildly elevated hs-CRP levels are particularly common in older adults. [52] When using this cut-off, it confirmed that our participants with ID at baseline or at any yearly follow-up time point presented a systemic inflammation since their hs-CRP levels were continually over 3 mg/L, while not in non-iron-deficient participants. Concerning IL-6 levels, current literature stated that IL-6 serum levels higher than 2 ng/L can be considered to define chronic inflammation in an older adult population. [53, 54] In the DO-HEALTH participants, IL-6 levels were higher than 2 ng/L at baseline and over time, regardless of the iron status. Nevertheless, the presence of ID over the follow-up was associated with higher increases of inflammatory biomarkers over time, compounding pre-existing chronic inflammation. Describing and interpreting the variations in inflammatory biomarkers over time could be of importance, especially when considering the concepts of aging and inflammaging. As evidenced by current literature, permanently elevated levels of IL-6 and CRP have been suggested as promoters of accelerated aging [10],

frailty, decline in cognitive functions, age-related chronic disease, and a higher risk of mortality. [38] Hence, screening older adults for ID may be relevant for healthy aging, and preventing chronic diseases, and loss of autonomy in an increasingly, aging population. Once ID has been identified, education on dietary sources of iron as well as substances inhibiting iron absorption should be provided. [30] Alternatively, iron supplementation might be discussed. In octogenarians, low-dose supplementation of 15 mg of oral iron per day was reported to be safe and effective [55].

Strengths and limitations

Our study took advantage of data collected in the DO-HEALTH trial, the largest European study on aging that included and followed 2157 community-dwelling older adults over 3 years. We assessed long-term chronic inflammation with annually repeated measurements of two reliable inflammatory biomarkers, broadly used in clinical practice. Besides, we extensively investigated the influence of ID on inflammatory biomarkers levels using four different definitions. We also strengthened the validity of our results in adjusting on a wide range of potential confounders and in performing several sensitivity analyses. However, a few limitations need consideration. Due to the observational nature of our study and despite adjustment for several relevant sociodemographic and clinical factors, we cannot exclude the possibility that the observed associations between ID and high levels of inflammatory biomarkers may be explained in part by residual confounding.

Conclusion

Our results suggest that ID may play a relevant role in chronic low-grade inflammation measured by hs-CRP and IL-6 levels among relatively healthy older adults.

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Data availability In a first step, no data will be made available to researchers external to DO-HEALTH Research Group to allow primary researchers to fully exploit the dataset. The data will be shared in a second step according to a controlled access system.

Declarations

Conflict of interest LA is currently an employee of MSD, Switzerland. **DRS** reports personal fees from Danube University of Krems, US Department of Defense, European Society of Anesthesiology, Korean Society for Patient Blood Management, Korean Society of Anesthesiologists, Network for the Advancement of Patient Blood Management, Haemostasis and Thrombosis, Bayer AG, Zürich, B. Braun Melsungen AG, Boehringer Ingelheim GmbH, Bristol-Myers-Squibb, CSL Behring GmbH, Celgene International II Sàrl, Daiichi Sankyo AG, Haemonetics, Instrumentation Laboratory (Werfen), LFB Biomédicaments, Merck Sharp & Dohme, PAION Deutschland GmbH, Pharmacosmos A/S, Pfizer AG, Pierre Fabre Pharma, Portola Schweiz GmbH, Roche Diagnostics International Ltd, Sarstedt AG & Co., Shire Switzerland GmbH, Tem International GmbH, Vifor Pharma, Vifor (International) AG, Zuellig Pharma Holdings. DRS's academic department is receiving grant support from the Swiss National Science Foundation, the Swiss Society of Anesthesiology and Reanimation (SGAR), the Swiss Foundation for Anesthesia Research, Vifor SA and Vifor (International) AG. DRS is co-chair of the ABC-Trauma Faculty, sponsored by unrestricted educational grants from Novo Nordisk Health Care AG, CSL Behring GmbH, LFB Biomédicaments, and Octapharma AG. **EJO** reports grants from Zurich University, during the conduct of the study. As part of the DO-HEALTH independent and investigator-initiated clinical trial, **HABF** reports as the PI of the DO-HEALTH trial, grants from European Commission, from University of Zurich, from NESTEC, from PFIZER Consumer Healthcare, from Streuli Pharma, plus nonfinancial support from DSM Nutritional Products and nonfinancial support from Roche Diagnostics. Further, Prof. Bischoff-Ferrari reports speaker fees from Wild, Pfizer, Vifor, Mylan, Roche Diagnostics, and independent and investigator-initiated grants from Pfizer and from Vifor. All other authors declare no competing interests.

Ethics approval statement The Cantonal Ethical Committee of the Canton of Zurich approved this ancillary analysis (BASEC N° 2018–01755).

Informed consent Informed consent was obtained from all individual participants included in the study.

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