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Iron, Oxidative Stress, and Δ9 Stearoyl-Coenzyme A Desaturase Index (C16:1/C16:0): An Analysis Applying the National Health and Nutrition Examination Survey 2003–04

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Abstract

Background: Stearoyl-coenzyme A desaturase (SCD) is a key enzyme in fatty acid metabolism, and elevated SCD activity is associated with multiple adverse health outcomes. Diet, hormone levels, and environmental exposures are potential factors affecting SCD activity. Less is known about the relationship between micronutrients, including iron, and SCD activity.

Objective: The aim of this study was to investigate the association between serum ferritin level, a biomarker of circulating iron levels, and the Δ9 desaturase index (C16:1/C16:0), a biomarker of estimated SCD activity, among women in the United States.

Methods: The association between serum ferritin and the Δ9 desaturase index was assessed in a cross-sectional study of 447 female participants, aged 20–49 y, from NHANES 2003–2004. The multivariate analyses were performed utilizing generalized linear modeling, adjusting for potential confounders. Mediation of the relationship between serum ferritin and Δ9 desaturase index by γ-glutamyltranspeptidase (GGT), a biomarker of oxidative stress, was also assessed.

Results: Increased ferritin was significantly associated with a higher Δ9 desaturase index. Adjusting for waist circumference, age, race, and cotinine levels, an interquartile range increase in serum ferritin corresponded to 3.92% (95% CI: 0.88%, 7.05%) higher Δ9 desaturase index. GGT, the biomarker used to measure oxidative stress level, did not appear to mediate the association between ferritin and Δ9 desaturase index. After stratifying by pregnancy status, these associations were limited to nonpregnant individuals.

Conclusions: Elevated SCD activity may be associated with increased iron storage inside the human body; the association did not appear to be mediated via oxidative stress, as estimated by GGT levels. *Curr Dev Nutr* 2018;2:nzx001.

Keywords: ferritin, iron, oxidative stress, stearoyl-coA desaturase, NHANES

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Supplemental Tables 1–4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/cdn/.

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Abbreviations used: CRP, C-reactive protein; GGT, γ-glutamyltranspeptidase; SCD, stearyol-CoA desaturase.

Introduction

Stearoyl-CoA desaturase (SCD) is an FA desaturase that catalyzes the introduction of double bonds into methylene-interrupted fatty acyl chains (1). The MUFA products generated by SCD are the major substrates for the synthesis of complex lipids, including diacylglycerols, phospholipids, triglycerides, wax esters, and cholesterol esters, which play essential roles in cell signaling and membrane fluidity (2). SCD also regulates lipogenesis events to provide essential lipid resources for rapid proliferation and cell structure in cancer cells. Upregulation of SCD expression and increased biosynthesis of MUFAs have been widely reported in several common metabolic disorders, such as diabetes, obesity, cardiovascular disease, and cancer (3).

Due to the well-documented associations between SCD expression and chronic diseases (4, 5), understanding the extrinsic risk factors that regulate SCD activity can have broad implications in disease treatment and prevention. The association between increased intake of

macronutrients and SCD activity as well as its biological implications have been extensively studied (2). For instance, SCD activity induced by diets high in carbohydrates and SFAs is associated with increased hepatic lipogenesis, hypertriglyceridemia, weight gain, insulin resistance, and inflammation status (2). Others have reported that higher consumption of vegetable oil–based margarine and high-fiber products was associated with low SCD activity, whereas higher consumption of simple carbohydrates was associated with high SCD activity (6). However, the impact of micronutrients, such as iron, on SCD activity or expression is not as well understood. Accumulating studies suggest a link between body iron excess and metabolic syndromes (7). Thus, studying the association between iron and SCD may contribute to an understanding of the micronutrient's role in energy metabolism and chronic disease development.

Based upon previous biological evidence, there are 3 potentially interrelated reasons to hypothesize an iron-induced SCD index elevation and lipid abnormalities. First, SCD is an iron-containing enzyme and its dimetal-centered structure has been identified using X-ray crystallography (8). In animal studies, reduced SCD activity has been observed among rats fed iron-deficient diets (1, 9). Second, there is extensive literature documenting circulating or hepatic iron-related abnormal lipid profiles, and lipid metabolism-related chronic disease risks (10–12). Thirdly, iron is also known to be associated with oxidative stress (13), an imbalance between the production of reactive oxygen species and antioxidant defenses (14). Elevated oxidative stress is a well-characterized indicator for an increasing number of chronic diseases (15, 16); in animal models, mice fed diets with elevated iron have shown an increased oxidative stress and inflammatory response, which leads to impaired liver and kidney function (17). Increased oxidative stress also attenuates lipid synthesis, increases mitochondrial FA oxidation, and induces abnormal lipogenesis (18, 19). A series of epidemiologic and clinical studies have consistently suggested that serum γ-glutamyltransferase (GGT) positively predicted F2-isoprostanes, an oxidative damage product of arachidonic acid, and fibrinogen and C-reactive protein (CRP), markers of inflammation, and stress-related issues (20). These findings suggest that serum GGT is an early and sensitive enzyme related to oxidative stress. With such potential clinical and health significance, however, the relationship between iron, oxidative stress, and SCD activity remains poorly understood in human studies.

The primary goal of this study was to test the hypothesis that total body iron storage status is associated with SCD activity. We examined associations between a well-characterized biomarker of iron storage status, serum ferritin, and an established biomarker of estimated SCD activity, the ratio of palmitoleic acid and palmitic acid from plasma cholesteryl esters (21). The secondary goal of this study was to test the hypothesis that the association between total body iron and SCD activity is mediated through oxidative stress, which in our dataset is estimated by the biochemical indicator GGT (22, 23). All data and related information were collected from NHANES 2003–2004 (24).

Methods

Study population

The NHANES is a nationally representative, cross-sectional survey of non-institutionalized civilian US residents, collected by the National Center for Health Statistics of the CDC. Written informed consent was obtained for all participants, and the survey protocol was approved by the Research Ethics Review Board at the National Center for Health Statistics. NHANES has applied survey weights to account for the complex survey design, survey nonresponse, and population stratification. To ensure that the results of this study are generalizable to the US civilian noninstitutionalized population, these survey weights were incorporated into all analyses processes utilizing the R "survey" package.

Laboratory measurements

Serum ferritin was measured in samples collected in 2003 using single-incubation 2-site immunoradiometric antibody reagent, and using animmuno-tubidimetry clinical analyzer in samples collected in 2004 (25). Long-term estimates of method precision of ferritin measurements from calendar years 1991 and 1992 for NHANES 1999+ show total CVs of ∼4–6% at 30–400 ng/mL and ∼9–10% at 5–10 ng/mL (26).

Plasma palmitoleic acid and palmitic acid were measured in fasting ($\geq$8 h) participants. Esterified FAs were hydrolyzed from cholesteryl ester using sequential treatment with acid then base. Following base hydrolysis, the samples were reacidified and total FAs were hexane extracted from the matrix along with internal standards. Extracts were then derivatized with pentaflurobenzyl bromide in the presence of triethylamine to form pentaflurobenzyl esters and then reconstituted in hexane. Pentaflurobenzyl FA derivatives were injected into a GC column, and palmitoleic acid and palmitic acid were detected using negative-ion MS (27). Bench quality-control pools were characterized to determine the quality-control limits by analyzing duplicate samples in 22 assays. Mean CV (SD) for all analytes in the 3-bench quality-control pool was 6% (2% SD) (28). The Δ9 desaturase index was calculated as the ratio of palmitoleic acid to palmitic acid, as previously described (29).

The activity of GGT was measured by the enzymatic rate method. GGT catalyzes the reaction with transferring a γ-glutamyl group from the colorless substrate, γ-glutamyl-*p*-nitroaniline, to the acceptor glycylglycine with production of the colored product, *p*-nitroaniline. The rate of change in absorbance was directly proportional to the activity of GGT and was measured by a Beckman Synchron LX20 at 410 nm (30).

Serum cotinine, a biomarker of tobacco smoke exposure, was measured by an isotope dilution-HPLC/atmospheric pressure chemical ionization tandem MS (31).

Pregnancy status was based on urine pregnancy test or self-reporting. Persons who reported that they were pregnant at the time of test were assumed to be pregnant; if the urine test was negative, but the subject reported that they were pregnant, the status was still coded as "pregnant." If the urine pregnancy results were negative and the subject said that they were "not pregnant," the respondent was coded not pregnant. Subjects with only self-reporting information were coded as "could not be determined."

Statistical methods

Primary variables included in the analysis were ferritin, Δ9 desaturase index (the ratio of palmitoleic acid to palmitic acid), and GGT. Confounders included a priori in adjusted models were age, race, waist circumference, and cotinine. The primary analysis of this study was to

test the association between ferritin and Δ9 desaturase activity; the secondary analysis was to test the association between GGT and Δ9 desaturase activity; the tertiary analysis was to test for mediation of the relationship between ferritin and Δ9 desaturase activity by GGT. After exclusion of study subjects with missing data, there were 447 subjects with information on all covariates of interest. Further stratification analysis was based on pregnancy status.

Our data analysis comprised 3 stages. The first stage was a univariate analysis of all continuous and categorical variables included in the study. Summary statistics for each variable, including minimum, median, maximum, geometric mean, and number of individual in each category, were presented. Right-skewed continuous variables were log transformed for further analyses. The second stage was the test of bivariate associations among predictor variables, outcome variables, and confounder variables by Spearman correlation analysis. In the third stage, generalized linear modeling was utilized for multivariate analysis. β Coefficients and P values from unadjusted and covariate-adjusted models were summarized and reported. CRP, a biomarker of systemic inflammation, was considered as a potential covariate; however, here serum ferritin and CRP were not correlated. Thus, we did not include CRP as a covariate or confounder in generalized linear modeling analyses. P values < 0.05 were considered statistically significant.

To test for nonlinearity in the relationship between ferritin and Δ9 desaturase activity index, ferritin concentrations were categorized into 4 groups. Multivariate generalized linear models were calculated using the first quartile of ferritin as the reference group. We assessed the statistical power of the model including ferritin quartiles using the F test. Smoothing parameters within a generalized additive model with adjusted confounders were also plotted to observe possible nonlinearity in the relationships between predictors and outcomes. To test the hypothesis that GGT was a mediator of the association between ferritin and Δ9 desaturase activity, Kenny's 4-step method for testing mediation with regression analysis was applied (32, 33).

Results

Table 1 presents the anthropometric, biochemical biomarkers, and demographic characteristics of the entire study population, as well as information stratified by pregnancy status. All 447 members of the study population were women, aged 20–49 y (mean = 34.4 y). The average waist circumference was 91.8 cm (range = 66.3–151 cm). The majority of the participants were non-Hispanic whites (69.7%), and not pregnant (81.2%). The serum palmitic acid level ranged from 1250 to 8150 μmol/L with a mean of 2576.8 μmol/L; the serum palmitoleic acid ranged from 51.6 to 1050 μmol/L with a mean of 201.6 μmol/L. The ferritin levels ranged from 2 to 562 ng/mL (mean = 37.1), with an IQR of 46 ng/mL (quartile 1 = 23, quartile 3 = 69); 28 participants had a ferritin level >150 ng/mL, which is the medical cut-off point of diagnosed iron overload (34).

Table 2 presents the association between the Δ9 desaturase index and serum ferritin from both unadjusted and fully adjusted regression models. Before further adjustment for age, waist circumference, race/ethnicity, and serum cotinine level, an IQR increase in serum ferritin corresponded to 6.23% (95% CI: 2.22%, 10.39%) higher Δ9 desaturase index ($P = 0.009$). After adjusting for potential confounders, an IQR increase in serum ferritin corresponded to 3.92% (95% CI: 0.88%, 7.05%) higher Δ9 desaturase index among this study population ($P = 0.041$). Additionally, we observed that waist circumference and cotinine were positively associated with higher Δ9 desaturase index ($P < 0.0001$; $P = 0.025$). These results highlight that body composition and smoking are both independently associated with estimated SCD activity. African American study participants, on average, had a significantly lower Δ9 desaturase index compared to non-Hispanic whites (the reference category, $P < 0.0001$). Moreover, given that pregnancy status usually leads to higher iron requirements and decreased ferritin storage, the association between ferritin and Δ9 desaturase index may be modified at different life periods (35). We observed that the

TABLE 1 Demographic characteristics of the study population: overall and stratified by pregnancy status[1]

Variables	Entire population (n = 447)	Pregnant (n = 78)	Nonpregnant (n = 363)	P value
Gender				
Female, n (%)	447 (100)	78 (17.4)	363 (81.2)	
Race/ethnicity, n (%)				0.67
Mexican American	88 (8.8)	20 (25.6)	68 (18.7)	
Other Hispanic	13 (3.0)	2 (2.6)	11 (3.0)	
Non-Hispanic white	232 (69.7)	39 (50.0)	189 (52.1)	
Non-Hispanic black	92 (12.5)	11 (14.1)	79 (21.8)	
Other	22 (6.0)	6 (7.7)	16 (4.4)	
Age, y	35.5, 34.4 (20, 49)	27.5, 27.1 (20, 39)	36.0, 34.9 (20, 49)	**0.004**
Waist circumference, cm	93.2, 91.8 (66.3, 151)	93.5, 92.2 (67, 145)	93.3, 91.9 (66.3, 151)	0.43
Palmitic acid, mmol/L	2696.4, 2576.8 (1250, 8150)	3484, 3312.6 (1710, 8150)	2664, 2550.9 (1250, 7180)	**0.003**
Palmitoleic acid, mmol/L	232.7, 201.6 (51.6, 1050)	290.3, 241.1 (67.2, 1050)	230.6, 200.3 (51.6, 851)	0.15
Δ9 Desaturase index	0.08, 0.08 (0.03, 0.21)	0.08, 0.07 (0.03, 0.15)	0.08, 0.08 (0.03, 0.21)	0.57
Ferritin, ng/mL	55.4, 37.1 (2, 562)	35.3, 27.8 (4, 180)	56.6, 37.6 (2, 562)	0.06
GGT, U/L	17.7, 14.8 (5, 310)	11.8, 10.7 (5, 57)	17.9, 14.9 (5, 310)	0.06
Cotinine, ng/mL	54.67, 0.44 (0.01, 631)	8.7, 0.1 (0.01, 186)	56.0, 0.5 (0.01, 631)	**0.006**

[1] Values are mean, geometric mean (minimum, maximum) unless otherwise indicated. Bold type indicates P value is < 0.05, which means this specific variable is significantly different between groups (Pregnant vs. Nonpregnant). GGT, γ-glutamyltranspeptidase.

TABLE 2 Associations between log-transformed ferritin and log-transformed Δ9 desaturase index, in unadjusted and adjusted generalized linear models ($n = 447$)[1]

	Unadjusted		Adjusted[2]	
	β coefficient (95% CI)	*P* value[3]	*β* coefficient (95% CI)	*P* value
Log(ferritin)	0.055 (0.020, 0.090)	**0.009**	0.035 (0.008, 0.062)	**0.041**
Waist circumference, cm			0.005 (0.004, 0.006)	**<0.0001**
Age, y			–0.002 (–0.007, 0.003)	0.48
Race/ethnicity[4]				
Mexican American			0.001 (–0.106, 0.108)	0.99
Other Hispanic			–0.146 (–0.275, –0.016)	0.06
Non-Hispanic black			–0.307 (–0.378, –0.236)	**<0.0001**
Other			0.146 (0.035, 0.257)	**0.0371**
Log(cotinine)			0.017 (0.006, 0.028)	**0.025**

[1]Bold type indicates *P* value is < 0.05, which means this specific variable is significantly different between groups (Pregnant vs. Non-pregnant).
[2]Values adjusted for waist circumference, age, race/ethnicity, and cotinine level.
[3]$P = 0.05$ was the cutoff point in order to determine significance.
[4]Non-Hispanic white was the reference group.

positive association between serum ferritin and Δ9 desaturase activity was restricted to nonpregnant study subjects, who have, on average, higher serum ferritin concentrations ($P = 0.041$, **Supplemental Table 1**).

In testing for nonlinearity in the relationship between ferritin and Δ9 desaturase index, we also ran models with ferritin categorized into quartiles and found that individuals in the 3rd and 4th quartiles of serum ferritin had statistically higher Δ9 desaturase index than individuals in the 1st quartile ($P = 0.047$, $P = 0.014$, respectively. **Figure 1**). A similar nonlinear relationship was observed when plotting the association between ferritin and Δ9 desaturase index utilizing smoothing parameters within a generalized additive model (**Figure 2**).

To test the hypothesis that GGT was a mediator of the association between serum ferritin and Δ9 desaturase activity, we analyzed the association of GGT with serum ferritin (**Supplemental Table 2**) as well as the association of Δ9 desaturase index with GGT (**Supplemental Table 3**) in both unadjusted and fully adjusted regression models.

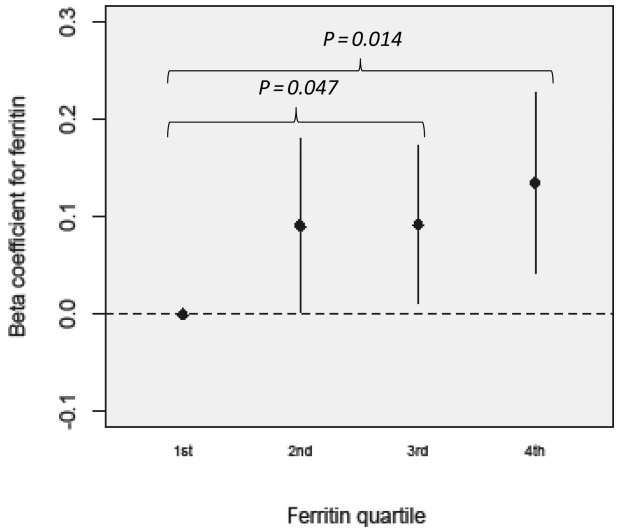

FIGURE 1 Association between the Δ9 desaturase index and quartiles of serum ferritin. Quartile 1 of serum ferritin is the reference quartile.

GGT was positively associated with the level of ferritin in adjusted models ($P = 0.042$), but it was not associated with Δ9 desaturase index ($P = 0.08$). According to the results from the mediation analysis presented in **Table 3**, GGT did not appear to mediate the association between ferritin and Δ9 desaturase activity. In a sensitivity analysis, we compared the Δ9 desaturase index between iron-overloaded and non-iron-overloaded individuals and identified that iron overloading was associated with 16.2% higher Δ9 desaturase index ($P = 0.12$, **Supplemental Table 4**).

Discussion

This study is the first to explore the impact of ferritin on SCD activity in a sample representative of the US population, testing oxidative stress as the mediator of this relationship. Increased iron storage among 447 female study subjects was associated with elevated SCD activity after adjusting for age, race/ethnicity, waist circumference, and cotinine level; this association became stronger in the highest iron storage population. Based on mediation analysis, however, the association between iron and elevated SCD expression did not appear to be mediated via oxidative stress, as estimated by GGT levels. Moreover, this is the first study identifying differences in baseline SCD activity between non-Hispanic black and other race/ethnicity individuals. By stratifying study participants into pregnant and nonpregnant groups, we also detected lower SCD activity among women at gestation stage with higher iron requirements.

The findings regarding the adverse effects of iron are consistent with previous studies. Iron is essential to the cell and plays a catalytic role in multiple redox reactions to support basic metabolic functions (36). Excesses of iron are known to favor the Fenton reaction, which leads to an overproduction of reactive oxygen species (37, 38). Elevated levels of reactive oxygen species compromise intracellular biochemical reactions, trigger apoptotic cell death, and lead to abnormal lipid metabolism (39). Experimental animal studies have indicated that elevated iron is an independent determinant of chronic disease risk and overall survival length (40, 41). Similarly, excess iron storage is associated with higher risks of chronic diseases among humans (42). This study found that an elevated Δ9 desaturase index was correlated with higher serum ferritin levels. However, for our study, only 6% of subjects had clinically

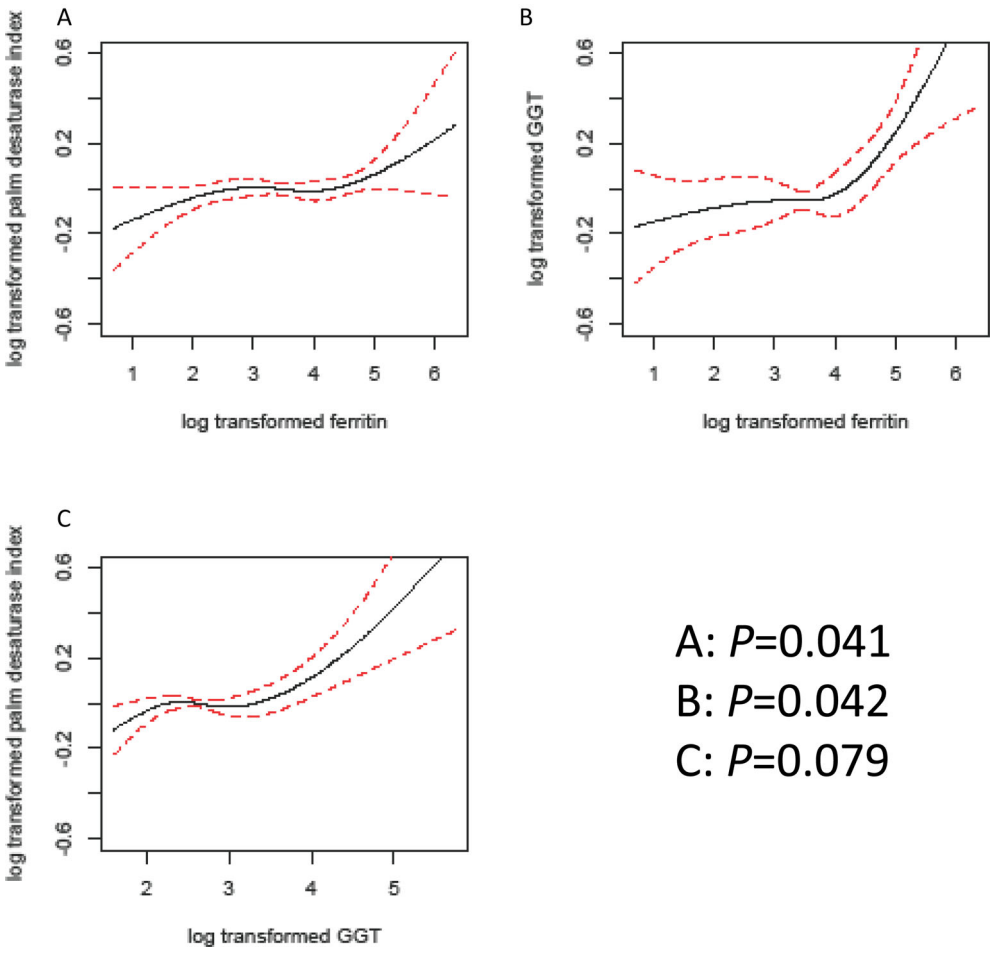

FIGURE 2 Nonlinear modeling of the change of Δ9 desaturase index with increased ferritin (A); change of GGT with increased ferritin (B); and change of Δ9 desaturase index with increased GGT (C). Dashed lines indicate 95% CIs. GGT, γ-glutamyltranspeptidase.

diagnosed iron overloads. Given that we have found elevated SCD activity among the population with highest daily iron requirements (women within reproduction age range), in future studies it would be helpful to recruit a greater range of subjects with larger differences in iron requirements.

The role of SCD in chronic disease risk is well documented (43–48). As an iron-containing, eukaryotic FA desaturase enzyme, SCD plays an important role in the modulation of cell proliferation (43). We recently reported that SCD is an important regulator of normal breast stem cells (44). As observed in a wide range of epidemiologic studies, elevated SCD activity is significantly correlated with an increased risk of chronic diseases. In a case-cohort study of 27,548 adult subjects, elevated SCD activity was linked with higher type 2 diabetes risk (45). In another cross-sectional study of Finnish children aged 6–8 y, elevated SCD enzyme activity was associated with cardiometabolic risk factors (46). Similar results have been identified in cancer epidemiology fields (47, 48). Therefore, given the growing chronic disease burden worldwide, identification of factors that influence SCD activity is of great public health significance. Our findings suggest that maintaining moderate ferritin storage is associated with normal SCD activity (49), which has applicable potential in the prevention and treatment of a range of chronic diseases.

TABLE 3 Testing the role of GGT in mediating the relationship between Δ9 desaturase index and serum ferritin concentrations ($n = 447$)[1]

	Model	β coefficient (*P* value)
Step 1	Log(Δ9 desaturase index) = $\beta_0 + \beta_1 \times$ log(ferritin) + E	β_1: 0.035 (**0.041**)
Step 2	Log(GGT) = $\beta_0 + \beta_1 \times$ log(ferritin) + E	β_1: 0.109 (**0.042**)
Step 3	Log(Δ9 desaturase index) = $\beta_0 + \beta_1 \times$ log(GGT) + E	β_1: 0.071 (0.08)
Step 4	Log(Δ9 desaturase index) = $\beta_0 + \beta_1 \times$ log(ferritin) + $\beta_2 \times$ log(GGT) + E	β_1: 0.028 (0.15); β_2: 0.060 (0.16)

[1] If ≥1 β coefficients among steps 1–3 are nonsignificant, the mediation is not possible or likely. Assuming there are significant relationships from steps 1 to 3, full mediation is supported if β_2 in step 4 is not significant; otherwise, partial mediation is supported (45). Bold type indicates *P* value is <0.05, which means this specific variable is significantly different between groups (Pregnant vs. Nonpregnant). GGT, γ-glutamyltranspeptidase.

The correlation between iron and SCD expression has been previously studied in vivo, and these findings support the primary result here. Pigeon et al. (50) observed SCD mRNA overexpression in iron-overloaded male mice liver cells. In another study, researchers (9) found that Novikoff hepatoma microsomes with a complete absence of cytochrome b5 (the iron-containing component and terminal desaturase) showed a lack of SCD expression. However, there has been very limited research quantifying the association between iron and SCD activity research in human studies (51). To test the "iron-heart hypothesis," the relation of erythrocyte membrane FA composition to overloaded plasma ferritin was analyzed in Chinese study participants with angiographic coronary artery diseases. Among study subjects with a mean age of 63.1 y (76.9% men), researchers observed a higher but insignificant change of the Δ9 desaturase index with respect to elevated plasma ferritin levels among men. No significant correlations were observed among women (51). These inconclusive SCD activity results among different genders and population, as well as the unclear mechanism involved in this association, indicate an important research gap from animal to human studies, which was the initial motivation for our research.

As a cross-sectional epidemiologic study, our data are a snapshot of a certain timeframe, which can lead to prevalence-incidence bias and difficulty in making causal inferences. More importantly, the uniformity of subjects is a limitation. Due to the limitation of NHANES data availability, the participants were all women aged 20–49 y (51.9% Caucasians) with childbearing ability, and women in this age range have higher iron requirements than do elder women. Of the women, 78 out of 447 were pregnant, and hence had even higher daily iron requirements, which suggests that they are less likely to have elevated iron storage. In a sensitivity analysis, we divided the subjects by pregnancy status to compare the differences in the association between serum ferritin and SCD activity. Among nonpregnant woman, an IQR increase in serum ferritin corresponded to 3.85% (95% CI: 0.78%, 7.02%) higher Δ9 desaturase index, which indicates that people with lower iron requirements have a higher risk of elevated SCD activity. In future studies, to further understand the association between iron and SCD activity, it would be necessary to recruit a range of subjects with larger differences in iron requirements, such as menopausal women, male study subjects, and hemochromatosis patients. We would hypothesize that those individuals with lower biological requirements for iron are more susceptible to the observed association between ferritin and SCD activity.

An unexpected finding of this study is that, on average, non-Hispanic black women have lower SCD activity than do non-Hispanic white women. Since elevated SCD activity is associated with lipotoxicity and is a key contributing factor of various chronic diseases, we would expect higher SCD activity among non-Hispanic black women (52). Previous researchers found the African American population to have a genetic background that may attenuate lipotoxicity, given that, compared with Caucasian women, they demonstrated lower expression of PPAR-responsive genes, lower plasma adiponectin, and decreased intramyocellular lipid levels in adipose tissue. However, the biological findings are not coherent with the high prevalence of lipotoxicity-induced chronic conditions, including heart disease, cancer, diabetes, high blood pressure, and stroke, among African American individuals (52, 53). Resolving this paradox and understanding the integrated impacts of social determinants, environment exposures, community conditions, personal health behaviors, and healthcare access involved is a promising area for further inquiry.

There are several other limitations of this study, and the foremost one is due to the dataset availability of iron-level indicators. Ferritin is known as the storage form of the iron pool, a sensitive indicator of deficient, normal, and excessive iron levels. In terms of iron regulation, transferrin, transferrin receptor, ferroportin, and hormone hepcidin also play essential roles. In this study, transferrin receptor concentrations were also quantified. A Pearson correlation analysis of transferrin receptor and Δ9 desaturase index, however, did not identify a significant correlation ($r = -0.06$). Future studies should include other regulators to provide more comprehensive information about the impact of iron regulation on SCD expression. Additionally, we utilized plasma measurements of palmitoleic and palmitic acid, available in NHANES, which provide a shorter-term readout of FA concentrations compared to erythrocyte membrane FA measurements that would reflect long-term FA exposure.

Finally, according to the mediation analysis, the association between iron and elevated SCD expression appeared not to be mediated via oxidative stress, as estimated by GGT levels. There are 3 potential explanations for this negative result. First, GGT is one of many common biomarkers to characterize the oxidative status of human subjects; just like other biochemical indicators, it has limitations (54). Isoprostane, 8-hydroxydeoxyguanosine, protein carbonyls, and malondialdehyde are other common chemical indicators of oxidative stress; however, only GGT was available in this dataset. In future research, these other biomarkers can be used as a substitute for, or in addition to, GGT, which may provide information that is more robust. Second, regulation of lipid metabolism is a complicated system; inflammation status and hormone levels are additional factors known to contribute to this process. For example, bacterial infection and macrophage overproduction can decrease the expression of lipid-dependent regulators, which could modulate the expression of genes involved in lipid metabolism (55). Hormones produced by adipose tissue, such as leptin, adiponectin, and acylation-stimulating protein, also play a critical role in triglyceride synthesis and fat oxidation (56). Thus, the regulation of lipid synthesis and metabolism is likely a complex process where iron-induced oxidative stress may only play a modest role. Last but not least, iron itself is a pro-oxidant. Thus, collinearity between ferritin and GGT may influence the association analysis.

To summarize, the results presented here suggest that iron storage is an extrinsic factor that may have a significant impact on the regulation of Δ9 stearoyl-coA desaturase index (C16:1/C16:0), which may link to the synthesis of complex lipids and potential risks of chronic diseases. The positive association indicates the possibility of adverse health outcomes caused by excess iron storage. Given the emphasis on high iron intake and supplements noted in the literature (57, 58), this research provides some evidence for the importance of moderate iron intake and storage to health outcomes.

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