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

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Footnotes to the Title Disclosing:
i: Supplemental Table 1, Supplemental Table 2, Supplemental Table 3, Supplemental Table 4
ii: NHANES: National Health and Nutrition Examination Survey
SCD: Stearyol-CoA Desaturase
GGT: Gamma-Glutamyl Transpeptidase
PFB: Pentafluorobenzyl
GLM: Generalized Linear Modeling
CRP: C-Reactive Protein
WBC: White Blood Cell
IMCL: Intramyocellular Lipid
PC: Protein Carbonyls
MDA: Malondialdehyde
8-OHdG: 8-Hydroxydeoxyguanosine (8-OHdG)

CV: Coefficient of Variation

iii: Support for this work was provided by the Ravitz Foundation

iv: Yue Wu, Ana Baylin, Justin A. Colacino: no conflict of interest
ABSTRACT

Background: Stearoyl-coa 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: 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 females 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 from 20 to 49 years old 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 gamma-glutamyl transpeptidase (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 non-pregnant 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.
**Keywords**: ferritin, iron, oxidative stress, stearoyl-coA desaturase, NHANES

**INTRODUCTION**

Stearoyl-CoA Desaturase (SCD) is a fatty acid desaturase that catalyzes the introduction of double bonds into methylene-interrupted fatty acyl chains (1). The monounsaturated fatty acid (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 MUFA has 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, high carbohydrate and high saturated fatty acids diets induced SCD activity 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 while 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 three potentially interrelated reasons to hypothesize an iron-induced SCD index elevation and lipid abnormalities. Firstly, SCD is an iron-containing enzyme and its di-metal 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). Secondly, there is an extensive literature documenting circulating or hepatic iron-related abnormal lipid profiles, and lipid metabolism-related chronic disease risks (10, 11, 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 fatty acid oxidation and induces abnormal lipogenesis (18, 19). A series of epidemiological and clinical studies have consistently suggested that serum gamma-glutamyl transferase (GGT) positively predicted F2-isoprostanes, an oxidative damage product of arachidonic acid, and fibrinogen and C-reactive protein, 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 is to test the hypothesis that total body iron storage status is associated with SCD activity. We examine 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 is 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 gamma-glutamyl transpeptidase (GGT) (22, 23). All data and related information were collected from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 (24).

METHODS

3.1 Study population

The NHANES is a nationally representative, cross-sectional survey of non-institutionalized, civilian U.S. residents, collected by National Center for Health Statistics of the Centers for Disease Control and Prevention. 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 non-response, and population stratification. To ensure that the results of this study are generalizable to the U.S civilian non-institutionalized population, these survey weights were incorporated into all analyses processes utilizing the R “Survey” package.

3.2 Laboratory Measurements

Serum ferritin was measured in samples collected in 2003 using single-incubation two-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 coefficients of variations (CVs) of about 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 (≥ 8 hours) participants. Esterified fatty acids were hydrolyzed from cholesteryl ester using sequential treatment with acid then base. Following base hydrolysis, the samples were re-acidified and total fatty acids were hexane-extracted from the matrix along with internal standards. Extracts were then derivatized with pentafluorobenzyl bromide in the presence of triethylamine to form pentafluorobenzyl (PFB) esters and then reconstituted in hexane. PFB-fatty acid derivatives were injected into a gas chromatographic column, and palmitoleic acid and palmitic acid were detected using negative-ion mass spectrometry (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 three 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 gamma-glutamyl transpeptidase (GGT) was measured by the enzymatic rate method. GGT catalyzes the reaction with transferring a gamma-glutamyl group from the colorless substrate, gamma-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 it was measured by Beckman Synchron LX20 at 410 nm (30).

Serum cotinine, a biomarker of tobacco smoke exposure, was measured by an isotope dilution-high performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry (31).
Pregnancy status was based on urine pregnancy test or self-reporting. Persons who reported they were pregnant at the time of test were assumed to be pregnant; if the urine test was negative, but the subject reported they were pregnant, the status was still coded as “pregnant”. If the urine pregnancy results were negative and the subject said they were “not pregnant”, the respondent was coded not pregnant. Subjects with only self-reporting information were coded as “could not be determined”.

3.3 Statistical methods:

Primary variables included in the analysis were ferritin, Δ9 desaturase index (the ratio of palmitoleic acid to palmitic acid), and gamma-glutamyl transpeptidase (GGT). Confounders included \textit{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 was comprised of three 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 (GLM) was utilized for multivariate analysis. Beta
coefficients, p values from unadjusted and covariates adjusted models were summarized and reported. C-Reactive Protein (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/confounder in GLM analyses. P-values less than 0.05 were considered statistically significant.

To test for non-linearity in the relationship between ferritin and Δ9 desaturase activity index, ferritin concentrations were categorized into four groups. Multivariate GLMs were calculated using 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 non-linearity 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; David Kenny’s 4-step method in 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 female from 20 to 49 years old (mean = 34.4). The average waist circumference was 91.8 cm (range =66.3, 151cm). 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 micromol/L with the mean of 2576.8 micromol/L; the serum palmitoleic acid ranged from 51.6 to 1050 micromol/L with the mean of 201.6 micromol/L. The ferritin levels ranged from 2 to 562 ng/ml (mean = 37.1) and the interquartile
range is 46 ng/ml (Q1=23, Q3=69); 28 participants had ferritin level higher than 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 interquartile range 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 interquartile range 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 with non-Hispanic White (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 positive association between serum ferritin and Δ9 desaturase activity was restricted to non-pregnant study subjects, who have, on average, higher serum ferritin concentrations (P=0.041, Supplemental Table 1).

In testing for non-linearity in the relationship between ferritin and Δ9 desaturase index, we also ran models with ferritin categorized into quartiles and found that individuals in 3rd and 4th quartiles of serum ferritin had statistically higher Δ9 desaturase index than individuals in the first quartile (P=0.047, P=0.014, respectively. Figure 1). A similar non-linear 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. 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 United States 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 non-pregnant
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 (ROS) (37, 38). Elevated levels of ROS compromise intracellular biochemical reactions, triggers apoptotic cell death, and leads 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 diagnosed iron-overloads. Given we have found elevated SCD activity among the population with highest daily iron requirements (women within reproduction age range), in future studies it 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 fatty acid 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 epidemiological studies, elevated SCD activity is significantly correlated with 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 years, 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 high public health significance. Our findings suggest that maintaining moderate ferritin storage associated with normal SCD activity (49), which has applicable potential in the prevention and treatment of a range of chronic diseases.

The correlation between iron and SCD expression have been previously studied \textit{in vivo}, and these findings support the primary result here. Pigeon et al. observed SCD mRNA overexpression in iron overloaded male mice liver cells (50). In another study, Novikoff found that hepatoma microsomes with a complete absence of cytochrome b5 (the iron-containing component and terminal desaturase) showed a lack of SCD expression (9). 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 fatty acid 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 years (76.9% males), researchers observed a higher but insignificant change of the $\Delta 9$ desaturase index with respect to elevated plasma ferritin levels among males. No significant correlations were observed among females (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 epidemiological study, our data is a snapshot of a certain time-frame, which can lead to prevalence-incidence bias and difficulty in making causal inferences. More importantly, the uniformity of subjects is a limitation. Due the limitation of NHANES data
availability, the participants were all female from 20 to 49 years old (51.9% Caucasians) with child-bearing ability, and women in this age range have higher iron requirements than elder women. Of the women 78 out of 447 were pregnant with even higher daily iron requirements, which suggests 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 non-pregnant woman, an interquartile range increase in serum ferritin corresponded to 3.85% (95% CI: 0.78%, 7.02%) higher $\Delta 9$ desaturase index, which indicates that people with lower iron requirements have 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 hypothesis 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 was that, on average, non-Hispanic Black women have lower SCD activity than non-Hispanic White women. Since elevated SCD activity is associated with lipotoxicity and it is a key contributing factor of various chronic diseases, we would expect higher SCD activity among non-Hispanic Black women (52). Previous researchers found African Americans population have a genetic background that may attenuate lipotoxicity, given that, as compared with Caucasian women, they demonstrated lower expression of PPAR-responsive genes, lower plasma adiponectin, and decreased intramyocellular lipid (IMCL) 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 health care 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 fatty acid concentrations compared to erythrocyte membrane
fatty acid measurements that would reflect long-term fatty acid 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 three 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 (8-OHdG), protein
carbonyls (PC), and malondialdehyde (MDA) 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. Secondly, regulation of lipid metabolism is a complicated
system; inflammation status and hormone levels are additional known factors contributing 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 triglycerides 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 the 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 significant impact on 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 adverse health outcomes possibility caused by excess iron storage. With the emphasis on high iron intake and supplements in various literatures (57, 58), this research provides some evidence for the importance of moderate iron intake and storage to health outcomes.
ACKNOWLEDGEMENTS:

Statement of Authors’ Contribution to Manuscript:

W.Y., B.A., and C.A.J. designed the research; W.Y., and C.A.J. conducted research; W.Y. analyzed the data; W.Y., B.A., and C.A.J. wrote the paper; C.A.J has primary responsibility for final content. All authors read and approved the final manuscript.
References:


Table 1: Demographic characteristics of the study population: overall and stratified by pregnancy status.

Table 1: Original to this manuscript.

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<th>Variables</th>
<th>Entire Population (N=447)</th>
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<th>Non-Pregnant (N=363)</th>
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<td>N (%)</td>
<td>N (%)</td>
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<td>Gender</td>
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<td>447 (100%)</td>
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<td>363 (81.2%)</td>
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<td>2 (2.6%)</td>
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<td>Non-Hispanic White</td>
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<td>39 (50.0%)</td>
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<td>Non-Hispanic Black</td>
<td>92 (12.5%)</td>
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<td>22 (6.0%)</td>
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<td>(Min, Max)</td>
<td>(Min, Max)</td>
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<td>Age (year)</td>
<td>35.5, 34.4 (20, 49)</td>
<td>27.5, 27.1 (20, 39)</td>
<td>36.0, 34.9 (20, 49)</td>
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<td>Waist Circumference (cm)</td>
<td>93.2, 91.8 (66.3, 151)</td>
<td>93.5, 92.2 (67, 145)</td>
<td>93.3, 91.9 (66.3, 151)</td>
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<td>Palmitic Acid (mmol/L)</td>
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<td>(1710, 8150)</td>
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<td>Palmitoleic Acid (mmol/L)</td>
<td>232.7, 201.6 (51.6, 1050)</td>
<td>290.3, 241.1 (67.2, 1050)</td>
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<td>Δ9 desaturase index</td>
<td>0.08, 0.08 (0.03, 0.21)</td>
<td>0.08, 0.07 (0.03, 0.15)</td>
<td>0.08, 0.08 (0.03, 0.21)</td>
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<td>Ferritin (ng/ml)</td>
<td>55.4, 37.1 (2, 562)</td>
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<td>GGT (U/L)</td>
<td>17.7, 14.8 (5, 310)</td>
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<td>17.9, 14.9 (5, 310)</td>
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<td>Cotinine (ng/ml)</td>
<td>54.67, 0.44 (0.01, 631)</td>
<td>8.7, 0.1 (0.01, 186)</td>
<td>56.0, 0.5 (0.01, 631)</td>
<td>0.006</td>
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*6 out of 447 study subjects have “could not be determined” pregnancy status.

P-value from Chi-squared (categorical variable) or t-test (continuous variable)

GGT: Gamma-Glutamyl Transpeptidase.
**Table 2:** Associations between log-transformed ferritin and log-transformed Δ9 desaturase index, in unadjusted and adjusted generalized linear models (N=447).

**Table 2:** Original to this manuscript.

<table>
<thead>
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</tr>
<tr>
<td>Log(Ferritin)</td>
<td>0.055 (0.020, 0.090)</td>
<td><strong>0.009</strong></td>
<td>0.035 (0.008, 0.062)</td>
<td><strong>0.041</strong></td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>0.005 (0.004, 0.006)</td>
<td>&lt; <strong>0.0001</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>-0.002 (-0.007, 0.003)</td>
<td>0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race³</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican American</td>
<td>0.001 (-0.106, 0.108)</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Hispanic</td>
<td>-0.146 (-0.275, -0.016)</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>-0.307 (-0.378, -0.236)</td>
<td>&lt; <strong>0.0001</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.146 (0.035, 0.257)</td>
<td><strong>0.0371</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log(Cotinine)</td>
<td>0.017 (0.006, 0.028)</td>
<td><strong>0.025</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹: Adjusted for waist circumference, age, race/ethnicity, cotinine level.

²: P=0.05 was the cutoff point in order to determine significance.

³: Use non-Hispanic White as reference group.
Table 3: Testing the role of gamma-glutamyl transpeptidase (GGT) in mediating the relationship between Δ9 desaturase index and serum ferritin concentrations (N=447).

Table 3: Original to this manuscript.

<table>
<thead>
<tr>
<th>Model</th>
<th>ß-coefficient (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1 Log(Δ9 desaturase index) = β₀ + β₁*Log(Ferritin) + E</td>
<td>β₁: 0.035 (0.041)</td>
</tr>
<tr>
<td>Step 2 Log(GGT) = β₀ + β₁*Log(Ferritin) + E</td>
<td>β₁: 0.109 (0.042)</td>
</tr>
<tr>
<td>Step 3 Log(Δ9 desaturase index) = β₀ + β₁*Log(GGT) + E</td>
<td>β₁: 0.071 (0.08)</td>
</tr>
<tr>
<td>Step 4 Log(Δ9 desaturase index) = β₀ + β₁<em>Log(Ferritin) + β₂</em>Log(GGT) + E</td>
<td>β₁: 0.028 (0.15); β₂: 0.060 (0.16)</td>
</tr>
</tbody>
</table>

¹: If one or more ß-coefficients among step 1-3 are nonsignificant, the mediation is not possible or likely. Assuming there are significant relationships from step 1-3, full mediation is supported if β₂ in step 4 is not significant; otherwise, partial mediation is supported (45).
Figure Legends

Figure 1: Association between the delta-9 desaturase index and quartiles of serum ferritin. Quartile 1 of serum ferritin is the reference quartile.

Figure 1: Original to this manuscript.

Figure 2: Top-Left (A): Non-linear modeling of the change of delta-9 desaturase index with increased ferritin. Top-Right (B): change of GGT with increased ferritin. Bottom-Left(C): change of delta-9 desaturase index with increased GGT (C). 95% confidence intervals are denoted with dashed lines.

Figure 2: Original to this manuscript.
A: $P=0.041$
B: $P=0.042$
C: $P=0.079$
**Supplemental Table 1** (Stratification Analysis): Associations between log-transformed ferritin and log-transformed Δ9 desaturase index stratified with pregnancy status, in unadjusted and adjusted generalized linear models (Pregnant: N=78; Non-pregnant: N=363)\(^1\).

Supplemental Table 1: Original to this manuscript.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pregnant (N=78)</th>
<th>Non-Pregnant (N=363)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Adjusted</td>
</tr>
<tr>
<td></td>
<td>β-coefficient (95% CI)</td>
<td>P-value(^4)</td>
</tr>
<tr>
<td>Log(Ferritin)</td>
<td>0.070 (0.016, 0.124)</td>
<td>0.22</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>0.008 (0.004, 0.012)</td>
<td>0.011</td>
</tr>
<tr>
<td>Age (year)</td>
<td>-0.008 (-0.017, 0.001)</td>
<td>0.56</td>
</tr>
<tr>
<td>Race(^3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexico American</td>
<td>-0.180 (-0.465, 0.105)</td>
<td>0.27</td>
</tr>
<tr>
<td>Other Hispanic</td>
<td>-0.037 (-0.600, 0.526)</td>
<td>0.90</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>-0.480 (-0.674, -0.286)</td>
<td>0.005</td>
</tr>
<tr>
<td>Other</td>
<td>0.019 (-0.137, 0.175)</td>
<td>0.83</td>
</tr>
<tr>
<td>Log(Cotinine)</td>
<td>0.033 (0.011, 0.055)</td>
<td>0.033</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables</th>
<th>Non-Pregnant (N=363)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
</tr>
<tr>
<td>Log(Ferritin)</td>
<td>0.053 (0.034, 0.072)</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>0.005 (0.004, 0.006)</td>
</tr>
<tr>
<td>Age (year)</td>
<td>-0.002 (-0.007, 0.003)</td>
</tr>
<tr>
<td>Race(^3)</td>
<td></td>
</tr>
<tr>
<td>Mexico American</td>
<td>0.013 (-0.094, 0.120)</td>
</tr>
<tr>
<td>Other Hispanic</td>
<td>-0.148 (-0.273, -0.023)</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>-0.304 (-0.381, -0.227)</td>
</tr>
<tr>
<td>Other</td>
<td>0.155 (0.034, 0.276)</td>
</tr>
<tr>
<td>Log(Cotinine)</td>
<td>0.017 (0.005, 0.029)</td>
</tr>
</tbody>
</table>

\(^1\): N=6 subjects had “could not be determined” pregnancy status.

\(^2\): Adjusted for waist circumference, age, race/ethnicity, cotinine level.

\(^3\): P=0.05 was the cutoff point in order to determine significance.

\(^4\): Use non-Hispanic White as reference group.
**Supplemental Table 2**: Associations between log-transformed ferritin and log-transformed gamma-glutamyl transpeptidase (GGT), in unadjusted and adjusted generalized linear models (N=447).

**Supplemental Table 2: Original to this manuscript.**

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Adjusted¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-coefficient (95% CI)</td>
<td>P-value²</td>
</tr>
<tr>
<td>Log(Ferritin)</td>
<td>0.129 (0.037, 0.221)</td>
<td><strong>0.020</strong></td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>0.009 (0.005, 0.013)</td>
<td>&lt; <strong>0.001</strong></td>
</tr>
<tr>
<td>Age (year)</td>
<td>0.007 (0.003, 0.012)</td>
<td><strong>0.016</strong></td>
</tr>
<tr>
<td>Race³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican American</td>
<td>0.198 (0.015, 0.381)</td>
<td>0.07</td>
</tr>
<tr>
<td>Other Hispanic</td>
<td>0.030 (-0.199, 0.259)</td>
<td>0.80</td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>0.194 (0.091, 0.297)</td>
<td><strong>0.008</strong></td>
</tr>
<tr>
<td>Other</td>
<td>0.090 (-0.091, 0.271)</td>
<td>0.37</td>
</tr>
<tr>
<td>Log(Cotinine)</td>
<td>0.021 (0.006, 0.034)</td>
<td><strong>0.023</strong></td>
</tr>
</tbody>
</table>

¹: Adjusted for waist circumference, age, race/ethnicity, cotinine level.

²: P=0.05 was the cutoff point in order to determine significance.

³: Use non-Hispanic White as reference group.
**Supplemental Table 3:** Associations between log-transformed gamma-glutamyl transpeptidase (GGT) and log-transformed Δ9 desaturase index, in unadjusted and adjusted generalized linear models (N=447).

**Supplemental Table 3:** Original to this manuscript.

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Adjusted¹</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-coefficient (95% CI)</td>
<td>P-value²</td>
<td>β-coefficient (95% CI)</td>
<td>P-value²</td>
</tr>
<tr>
<td>Log(GGT)</td>
<td>0.100 (0.020, 0.180)</td>
<td><strong>0.030</strong></td>
<td>0.071 (0.002, 0.140)</td>
<td>0.08</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>0.004 (0.002, 0.006)</td>
<td>&lt; <strong>0.001</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>-0.002 (-0.006, 0.002)</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race²</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican American</td>
<td>-0.017 (-0.115, 0.081)</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Hispanic</td>
<td>-0.156 (-0.306, -0.006)</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>-0.326 (-0.392, -0.260)</td>
<td>&lt; <strong>0.0001</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.155 (0.044, 0.266)</td>
<td><strong>0.029</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log(Cotinine)</td>
<td>0.015 (0.003, 0.027)</td>
<td><strong>0.027</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹: Adjusted for waist circumference, age, race/ethnicity, cotinine level.

²: P=0.05 was the cutoff point in order to determine significance.

³: Use non-Hispanic White as reference group.
Supplemental Table 4 (Generalized Linear Regression): To test whether iron overloading and non-iron overloading were equally likely to correlate with similar change on log-transformed Δ9 desaturase index, in unadjusted and adjusted generalized linear models (Iron overloaded: N=28; Non-iron overloaded: N=419).

Supplemental Table 4: Original to this manuscript.

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th></th>
<th>Adjusted</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-coefficient (95% CI)</td>
<td>P-value</td>
<td>β-coefficient (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Iron Overloaded²</td>
<td>0.181 (0.118, 1.534)</td>
<td>0.15</td>
<td>0.162 (-0.014, 0.338)</td>
<td>0.12</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>0.005 (0.004, 0.006)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>-0.002 (-0.007, 0.003)</td>
<td>0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race⁴</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexican American</td>
<td>-0.004 (-0.111, 0.103)</td>
<td>0.95</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Hispanic</td>
<td>-0.148 (-0.282, -0.013)</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic Black</td>
<td>-0.321 (-0.386, -0.256)</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0.150 (0.041, 0.259)</td>
<td>0.031</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log(Cotinine)</td>
<td>0.017 (0.005, 0.029)</td>
<td>0.026</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1: Adjusted for waist circumference, age, race/ethnicity, cotinine level.

2: Ferritin=150 ng/ml were used as cutoff point to categorize iron overloading. Non-iron overloading subjects (ferritin < 150 ng/ml) was reference group.

3: P=0.05 was the cutoff point in order to determine significance.

4: Use non-Hispanic White as reference group.