ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Intervention Program Methods and Outcomes

Reduction in Ferritin Concentrations among Patients Consuming a Dark-Green Leafy Vegetable–Rich, Low Inflammatory Foods Everyday (LIFE) Diet

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ABSTRACT

Background: Ferritin is an iron-containing protein and acute-phase reactant, which may be elevated due to systemic iron overload or inflammation. Various diseases are associated with excess iron, but therapeutic iron chelation is suboptimal. Prior studies suggest that several plant phytochemicals possess iron-chelating properties, indicating that a plant-based diet may benefit patients with iron overload.

Objectives: The aim was to investigate whether patients who consume a nutrient-dense, dark-green leafy vegetable–rich diet, called the Low Inflammatory Foods Everyday (LIFE) diet, experience reductions in ferritin concentrations.

Methods: This was a retrospective study in which patients were intensively counseled to follow the LIFE diet. Compliance was assessed by patient interviews and serum B-carotene measurements. Primary outcomes included changes in ferritin, B-carotene, and C-reactive protein (CRP). Patients with elevated CRP concentrations at baseline were excluded in order to separate the impact of inflammation from iron overload on ferritin concentrations. Premenopausal women, who lose iron from menstruation, were also excluded.

Results: Thirty-two patients met the inclusion criteria. The median follow-up was 183 d. Following the dietary intervention, ferritin decreased (-81 μg/L, $P = 0.006$) and B-carotene increased (46 μg/L, $P < 0.0001$), whereas CRP remained unchanged (-0.02 mg/L, $P = 0.86$). Adherent patients had greater reductions in ferritin compared with nonadherent patients (-138 μg/L vs. 15 μg/L, $P = 0.001$). Among all patients, there was an inverse relation between B-carotene and ferritin (-2.02, $P = 0.03$).

Conclusions: The LIFE diet, or similar dark-green leafy vegetable–rich, whole-food plant-based diets, may benefit patients with disorders of iron overload and iron-induced oxidative stress. *Curr Dev Nutr* 2022;6:nzac095.

Keywords: iron chelation, beta-carotene, C-reactive protein, dark-green leafy vegetables, whole-food plant-based diet

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Abbreviations used: CKD, chronic kidney disease; CRP, C-reactive protein; CVD, cardiovascular disease; DFO, deferoxamine mesylate; DGLV, dark-green leafy vegetable; hsCRP, high-sensitivity C-reactive protein; LIFE, Low Inflammatory Foods Everyday.

Introduction

While iron is essential for life, it can also prove toxic by generating tissue-damaging free radicals and oxidative stress. Pathological iron accumulation catalyzes the generation of hydroxy radicals, and these reactive oxygen species may induce DNA damage, promoting cell death and tissue injury (1). Imbalances in iron homeostasis have been implicated in numerous diseases such as hemochromatosis (2), cardiovascular disease (CVD) (3), chronic kidney disease (CKD) (4), age-related macular degeneration (5), stroke (6), diabetes (7), and cancer (8). Ferritin is the body's intracellular iron storage protein. Some liver ferritin is secreted into the blood, making it a useful marker of liver iron stores. Ferritin is also an acute-phase reactant, upregulated in response to inflammatory hormones and cytokines (9). In order to distinguish elevations in ferritin due to excess iron versus inflammation, high-sensitivity C-reactive protein (hsCRP) (10), a widely accepted test of systemic inflammation, was utilized. High ferritin with normal CRP concentrations suggests iron overload; on the other hand, elevations in both CRP and ferritin may indicate inflammation.

The current treatment for disorders of iron overload includes phlebotomy for hemochromatosis or iron chelators for thalassemia. Unfortunately, iron chelators are associated with serious side effects, including renal impairment (11), retinal toxicity (12), gastrointestinal upset (13), and neutropenia (14). For this reason, it is worthwhile to investigate alternative methods of iron elimination. Prior evidence suggests that the polyphenols and flavonoid compounds in plants, such as

catechin, quercetin, and epigallocatechin gallate, are iron chelators (15, 16). Plant polyphenols and phytate also inhibit iron absorption (17), indicating that a whole-food plant-based diet may offer a safe and effective alternative to reduce iron stores, thereby treating diseases of iron overload as well as disorders related to oxidative stress such as cancer or CVD. Indeed, several prior studies showed that plant-based foods are associated with lower ferritin concentrations (18) and possibly reduced risk for anemia (19) or metabolic syndrome (20). However, these studies did not control for the potential confounding changes in inflammation, comment on the possibility of dietary iron chelators, or lay out specific plant-based diets that stipulate ingredients, frequency of consumption, or portion sizes.

In 2 prior studies, we showed that the highly specific Low Inflammatory Foods Everyday (LIFE) diet, which is rich in dark-green leafy vegetables (DGLVs), rapidly and significantly lowers systemic inflammation (21, 22). This was validated by reductions in mean CRP and elevations in mean B-carotene, an antioxidant that is abundant in DGLVs (23). The LIFE diet may have the added benefit of curbing iron concentrations via DGLVs' iron-chelating effects. We hypothesized that adherence to the LIFE diet will diminish ferritin concentrations in individuals with low levels of systemic inflammation at baseline (hsCRP <2mg/L), which may reduce their risk of chronic diseases associated with high ferritin and iron-induced oxidative stress. Herein, we report a longitudinal retrospective study examining changes in ferritin, hsCRP, and B-carotene concentrations among 32 patients in a community practice who were instructed to consume the LIFE diet.

Methods

Subject population

Patients were identified using the electronic medical records of author DMD's integrative medicine practice. The first criterion for eligibility was that subjects' ferritin and hsCRP concentrations had to be >100 μg/L and <2 mg/L, respectively, at baseline. The reason for selecting ferritin >100 μg/L is that several studies have shown an association between these levels and disease risk, providing a rationale to lower the ferritin below 100 μg/L (6, 24–26). When hsCRP concentrations are ≥2 mg/L, there is increased risk for cardiovascular events (10). Normal CRP concentrations fall within 0–3 mg/L, while optimal hsCRP is defined as <1 mg/L. Mandating a low level of inflammation was essential to preclude inflammation as a possible confounder of changes in ferritin concentrations throughout the study (10). B-Carotene concentrations had to be <91 μg/dL for patients using Labcorp, Quest, or Enzo or <200 μg/dL for subjects using Sunrise laboratories, which has a different scale. Subjects were required to use the same laboratory throughout the study. Women had to be postmenopausal to be included, as systemic iron concentrations are reduced by menstruation (27).

Subjects were excluded from the study if they experienced an infection, flare-up of seasonal allergies, or physical trauma, as these events can impact ferritin concentrations through inflammatory pathways (9, 28). Participants were also excluded if they received Epogen injections for CKD, donated blood, had a history of bleeding intestinal ulcers, or consumed red meat, pork, or iron supplements (including multivitamin with iron), all of which may affect systemic iron concentrations (29–32).

Study design

This study was approved by the University of Pennsylvania's Institutional Review Board (831,566; exempt category 4) under the protocol "Effect of Plant Based Diet on Blood Chemistries." The study design was a retrospective review of de-identified patient records. There was no control group who did not receive nutritional counseling. All patients in the practice of DMD were advised to eat the LIFE diet. Changes in ferritin concentrations over time were compared among patients who happened to be adherent with those who were not. Outcomes were ferritin, B-carotene, and CRP concentrations. All participants were required to follow the LIFE diet as designed by DMD, which was adapted from Dr. Joel Fuhrman's high-nutrient-density (HND) diet. All patients were informed of the inclusion of their de-identified data in the study and the study's results.

Components of the LIFE diet included the following:

1) Drinking one 32-ounce LIFE smoothie every day. The LIFE smoothie consists of 8 ounces (by weight) of DGLVs, 2.25 cups of blueberries (preferably frozen), 1 banana, 1 tablespoon of unsweetened cocoa powder, 1 tablespoon of ground flaxseed, 0.5 cup of soy milk (plain or vanilla) or unsweetened vanilla almond milk, and 0.5 cup of water blended together with a high-intensity blender (1200 peak watt power). DMD counseled each patient on how to properly measure and blend the ingredients.
2) Consumption of at least 5 ounces by weight of DGLVs in salad or cooked vegetables per day. Examples of DGLVs include spinach, kale, collard greens, bok choy, broccoli, cauliflower, cabbage, Brussels sprouts, arugula, Swiss chard, endive, asparagus, mustard greens, beet greens, mache, broccolini, broccoli rabe, radish, watercress, escarole, romaine, and green leaf lettuce. Other nonstarchy vegetables, such as onions, mushrooms, garlic, green and yellow zucchini, eggplants, peppers, or tomatoes are also recommended.
3) Consumption of fruit is unlimited. Berries are encouraged.
4) Daily consumption of at least 0.5 cup of beans or legumes or 3 ounces of bean pasta is required.
5) Consumption of whole grains and starchy vegetables (e.g., potatoes, winter squashes, peas, and corn) is limited to no more than 2 servings total per day. Also, patients could not eat more than 1 medium cooked or uncooked sweet potato or 4 cooked carrots per week during the study period because these foods contain B-carotene and could confound the use of serum B-carotene concentrations as an indicator of DGLV consumption.
6) No more than 1 serving or 4 ounces of refined grains can be consumed over 7 d.
7) No more than 24 g added sugar/d, which is in accordance with the American Heart Association and the WHO's guidelines, can be consumed.
8) No more than 2 ounces/d of raw seeds and nuts can be consumed.
9) No more than 6 ounces of fish, eggs, or dairy products can be consumed per day.
10) No more than 1 tablespoon of oil can be consumed per day.
11) Red meat, pork, cold cuts, bacon, butter, and cheese are not allowed.

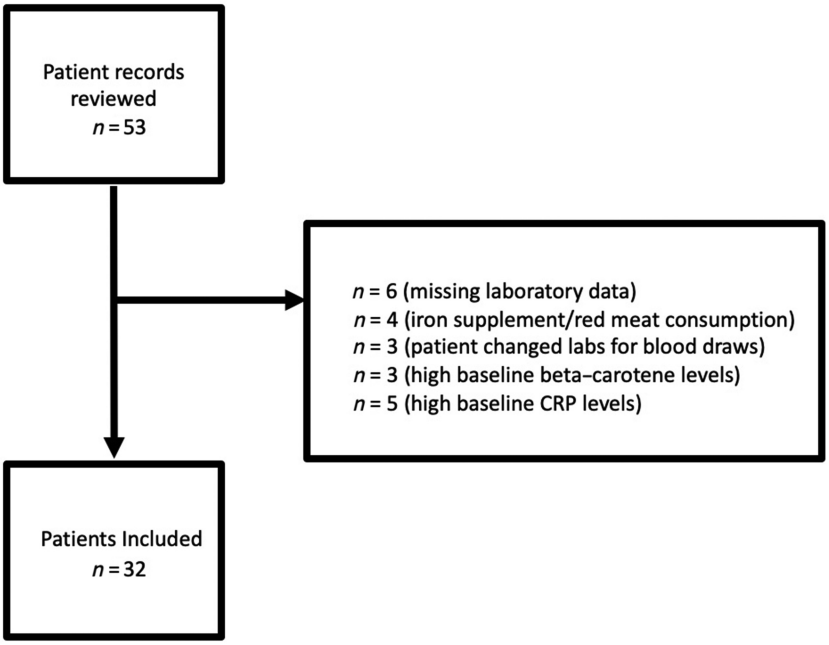

FIGURE 1 Flow diagram of patient selection process.

12) No more than 2 medium-sized dates can be consumed per day as they have a high sugar to micronutrient ratio.

13) Patients with low vitamin B-12 (<550 pg/mL according to European standards) or high methylmalonic acid concentrations are instructed to take 500 to 1000 μg vitamin B-12 daily.

No patients in the study utilized special diets such as vegetarian, vegan, paleo, or keto diet. Compliance was assessed subjectively by DMD during patient interviews by asking 24-h detailed recall data including specific foods and number of servings with each patient at every visit. Compliance was assessed objectively by measuring plasma B-carotene concentrations. DGLVs are rich in carotenoids such as B-carotene; therefore, an increase in plasma concentrations suggest at least some adherence to the diet.

Laboratory measurements

Plasma hsCRP, ferritin, and B-carotene were measured at any one of the following laboratories: Quest, LabCorp, or Sunrise. Blood samples were collected in fasting patients between 07:00 and 09:00 h. Patients were required to undergo blood testing before they started the diet and then again at each follow-up visit.

Statistical analyses

Descriptive analyses were performed using mean (SD, median, range) for continuous measures and percentage for categorical measures. Comparisons of means (e.g., CRP, or B-carotene, ferritin) between baseline and the last visit were performed using paired t test, and the comparisons of mean change from baseline between adherent and nonadherent groups were made using 2-sample t test. The association between change in B-carotene and change in ferritin among all subjects was evaluated using a regression model that was adjusted by age, gender, race, and CRP change. In the statistical comparisons of B-carotene, CRP, and ferritin between adherent and nonadherent patients, to account for the different lengths of follow-up, we also calculated the daily change as the linear slope from the linear regression model by using measures from all visits. All of the statistical analyses were made in SAS version 9.4 (SAS Institute, Inc.), and 2-sided P values <0.05 were considered statistically significant.

Results

Characteristics of study participants

In total, 53 charts over an 8-y period (2013–2021) were reviewed for eligibility by DMD. Thirty-two patients met the inclusion criteria and 21 were excluded. Patients were excluded due to missing laboratory measurements ($n = 6$), consumption of iron supplements or red meat/pork ($n = 4$), switching labs ($n = 3$), and high B-carotene ($n = 3$) or high CRP ($n = 5$) concentrations at baseline (**Figure 1**). A comprehensive summary of the baseline characteristics of participants is shown in **Table 1**. Eleven patients were female (34.4%). The mean age was 62 y, ranging from 43 to 78 y. Racial demographics included 26 (81.3%) White patients, 3 (9.4%) Black patients, 2 (6.3%) Asian patients, and 1 (3.1%) Hispanic patient. Six (18.8%) patients had diabetes, 20 (62.5%) had high cholesterol, 13 (40.6%) had hypertension, 6 (18.8%) had CVD, 3 (9.4%) had CKD, 7 (21.9%) had osteoarthritis, 4 (12.5%) had gastroesophageal reflux disease, and 5 (15.6%) had autoimmune diseases. Baseline CRP ranged from 0.02 mg/L to 1.9 mg/L, with a mean of 0.82 mg/L. Baseline B-carotene ranged from 4 μg/dL to 184 μg/dL, with a mean of 53.0 μg/dL. Baseline ferritin ranged from 111 μg/L to 1176 μg/L, with a mean of 277 μg/L (**Figure 2**).

TABLE 1 Baseline characteristics of the study subjects[1]

Baseline characteristics	Values
Age, y	
Mean (SD)	62.0 (9.3)
Median (Q1, Q3)	61 (55.5, 69.5)
Min, Max	43, 78
Gender, *n* (%)	
Male	21 (65.6%)
Female	11 (34.4%)
Race/ethnicity, *n* (%)	
White	26 (81.3%)
Black	3 (9.4%)
Asian	2(6.3%)
Latino	1 (3.1%)
Systemic disease, *n* (%)	
High cholesterol	20 (62.5%)
Hypertension	13 (40.6%)
Diabetes	6 (18.8%)
CVD	6 (18.8%)
CKD	3 (9.4%)
Osteoarthritis	7 (21.9%)
GERD	4 (12.5%)
Autoimmune disease	5 (15.6%)
Baseline C-creative protein, mg/L	
Mean (SD)	0.82 (0.47)
Median (Q1, Q3)	0.75 (0.45, 1.20)
Min, max	0.02, 1.90
Baseline B-carotene, μg/dL	
Mean (SD)	53.0 (37.9)
Median (Q1, Q3)	45.5 (26.0, 71.5)
Min, max	4, 184
Baseline ferritin, μg/L	
Mean	277 (247)
Median (Q1, Q3)	181 (132, 302)
Min, max	111, 1176

[1] $n = 32$. CKD, chronic kidney disease; CRP, C-reactive protein; CVD, cardiovascular disease; GERD, gastroesophageal reflux disease; Min, max, minimum, maximum; Q, quartile.

The number of visits a patient completed throughout the study ranged from 2 to 7. The median length of follow-up with complete laboratory results was 183 d, ranging from 32 to 868 d. Twenty (62.5%) patients were classified as adherent to the LIFE diet based on patient interviews, which was objectively corroborated by increases in B-carotene over the course of the study. Twelve patients (37.5%) were classified as nonadherent.

Alterations in CRP, B-carotene, and ferritin among all patients, adherent patients, and nonadherent patients

Over a mean of 214 d of follow-up, the mean changes in B-carotene, CRP, and ferritin among all patients were 46 μg/dL ($P < 0.0001$), −0.02 mg/L ($P = 0.86$), and −81 μg/L ($P = 0.006$), respectively (**Table 2**, **Figure 3**).

Patients were separated into 2 groups: the "adherent" ($n = 20$) and "nonadherent" ($n = 12$) groups based on their compliance with the LIFE diet components described previously. Examples of the 24-h dietary recalls of 4 randomly selected adherent ($n = 2$) and nonadherent ($n = 2$) patients are shown (**Supplemental Results**). Mean B-carotene concentrations from the initial visit to the final visit increased from 64.6 μg/dL to 137 μg/dL (177% increase) among adherent patients (**Table 3**, **Figure 4**). Among the nonadherent patients, B-carotene concentrations remained relatively stable, increasing from 33.7 μg/dL to 36.1 μg/dL (6.7%) from the initial visit to the final visit. The mean change in B-carotene concentrations between baseline and the final visit was significantly different between adherent (72.5 μg/dL) and nonadherent patients (2.4 μg/dL; $P < 0.0001$).

As expected in this patient population selected for their low CRP on the initial visit, CRP concentrations remained stable from baseline to the final visit in both the adherent (−0.06 mg/L) and nonadherent (+0.06 mg/L) groups. Final concentrations of CRP also did not vary significantly between groups ($P = 0.5$).

Mean ferritin concentrations from the initial to the final visit decreased among adherent patients from 273 μg/L to 135 μg/L (mean % decrease: 45.4%), whereas mean ferritin concentrations increased from 284 μg/L to 299 μg/L (mean % increase: 2.2%) among nonadherent patients (**Figure 5**). The mean change in ferritin between baseline and the final visit was significantly different between adherent patients (−138 μg/L) compared with nonadherent patients (14.5 μg/L; $P = 0.001$). The daily change in ferritin was also significantly different in the adherent versus nonadherent group (−0.72 μg/L vs. 0.07; $P = 0.002$) (Table 3).

Association between change in B-carotene, CRP, and ferritin among all patients

Among all subjects, the mean increase in B-carotene was significantly associated with a mean reduction in ferritin (regression coefficient = −1.25, $P = 0.007$). This relation remained significant when adjusted by age, gender, and race (regression coefficient = −1.41, $P = 0.005$) as well

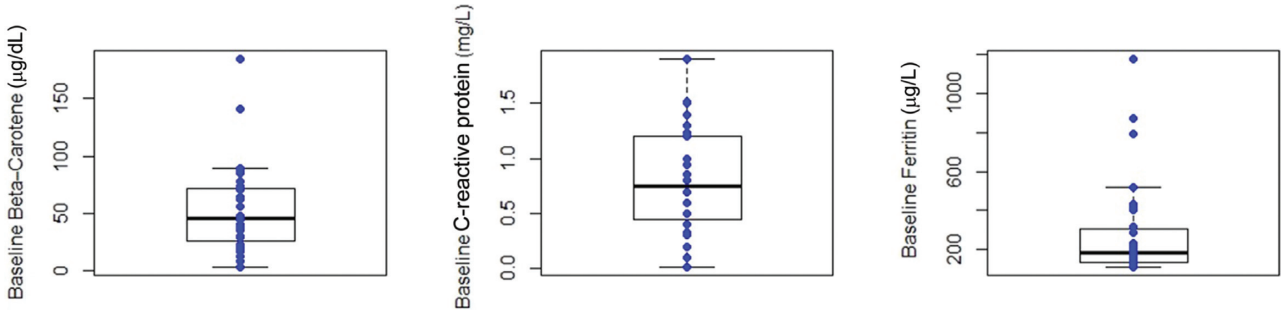

FIGURE 2 Boxplots of baseline B-carotene, CRP, and ferritin concentrations among all patients. CRP, C-reactive protein.

TABLE 2 B-Carotene, CRP, and ferritin at baseline and at last follow-up visit[1]

	Baseline	Last visit	Change from baseline	P[2]
B-Carotene (μg/dL)				
n	32	32	32	
Mean (SD)	53 (37.9)	99 (65.8)	46 (41.6)	<0.0001
Median (Q1, Q3)	45.5 (26.0, 71.5)	92 (39.5, 138)	46.5 (5.5, 76.0)	
CRP (mg/L)				
n	32	26	26	
Mean (SD)	0.82 (0.47)	0.81 (0.57)	−0.02 (0.43)	0.86
Median (Q1, Q3)	0.75 (0.45, 1.20)	0.65 (0.32, 1.10)	0 (−0.3, 0.1)	
Ferritin (μg/L)				
n	32	32	32	
Mean (SD)	277 (247)	196 (190)	−81 (157)	0.006
Median (Q1, Q3)	181 (132,302)	128 (81, 225)	−46 (−81, −7)	

[1]CRP, C-reactive protein; Q, quartile.
[2]For testing whether change from baseline is statistically significant using paired *t* test.

as further adjusted by change in CRP (regression coefficient = −2.02, $P = 0.03$). In contrast, the association between the change in CRP and change in ferritin over the course of the study was not statistically significant (−4.15, $P = 0.91$; **Table 4**).

Discussion

In this retrospective longitudinal study, we confirmed our hypothesis that the LIFE diet would significantly decrease ferritin concentrations and increase B-carotene concentrations. Subjectively adherent patients benefitted from further reductions in ferritin and greater increases in B-carotene compared with nonadherent patients. Importantly, the inverse relation between ferritin and B-carotene remained among all patients, even when adjusted for changes in systemic inflammation as measured by hsCRP. This result suggests that the LIFE diet decreased the body's total iron concentrations rather than its inflammatory load, which was optimal in this group at baseline (mean hsCRP <1).

This study adds important information to the 2 prior LIFE diet studies. In the first, we found that the LIFE diet resulted in substantial decreases in hsCRP and increased B-carotene over a mean follow-up of 6 mo (33). In the second, patients who consumed a standard American diet supplemented with 1 component of the LIFE diet, a daily 32-ounce LIFE smoothie, experienced significant reductions in CRP in only 7 d (21). In both of these analyses, patients had higher than optimal mean CRP concentrations at baseline: 6.67 mg/L and 2.86 mg/L, respectively. Herein, we present a patient population with already optimized mean CRP concentrations of 0.82 mg/L. While the adherent patients had a further 25% decrease in their CRP, this decrease was not significantly different from the change in CRP among nonadherent patients and was not associated with change in ferritin concentrations. This was critical in order to isolate the effects of the LIFE diet on iron metabolism rather than inflammation. To our knowledge, this is the first study to investigate the relation between ferritin and a plant-based diet while controlling for CRP. By reducing the possibility that inflammation was a confounder, our study supports the

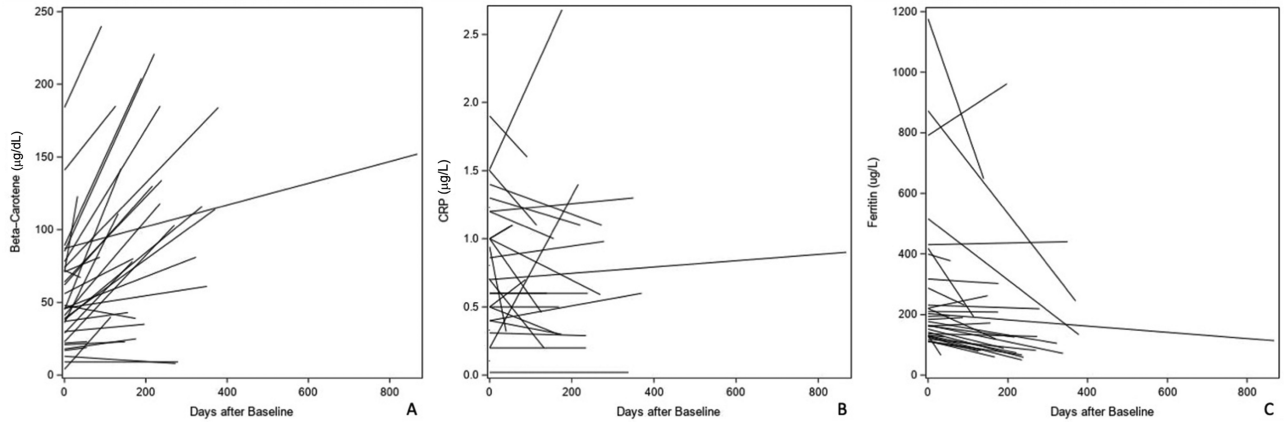

FIGURE 3 Change in B-carotene, CRP, and ferritin concentrations from baseline. These line plots show B-carotene (A), CRP (B), and ferritin (C) concentrations at baseline and the last visit with a line connecting these 2 time points for each subject, respectively. The daily change rate was calculated as the linear slope from the linear regression model with laboratory measures as the outcome and follow-up time as the predictor. CRP, C-reactive protein.

TABLE 3 Comparison of B-carotene, CRP, and ferritin and their changes between adherent patients and nonadherent patients[1]

Measure	Adherent patients ($n = 20$)	Nonadherent patients ($n = 12$)	P[2]
Length of follow-up (days)			
Mean (SD)	243 (174)	166 (98)	0.18
Median (Q1, Q3)	219 (136,297)	166 (72,235)	
B-Carotene (μg/dL)			
Baseline	64.6 (41.1)	33.7 (21.5)	0.009
Last visit	137 (52.3)	36.1 (23.2)	<0.001
Change from baseline at last visit	72.5 (29.4)	2.4 (6.8)	<0.0001
Percent change from baseline	177 (187)	6.7 (21.1)	0.0006
Daily change[3]	0.42 (0.35)	0.01 (0.06)	<0.0001
CRP (mg/L)			
Baseline	0.75 (0.52)	0.92 (0.38)	0.34
Last visit	0.65 (0.45)	1.06 (0.66)	0.08
Change from baseline at last visit	− 0.06 (0.41)	0.06 (0.46)	0.50
Percent change from baseline at last visit	25.5 (156)	3.1 (39.0)	0.59
Daily change[3]	− 0.0014 (0.0034)	− 0.0005 (0.0052)	0.56
Ferritin (μg/L)			
Baseline	273 (282)	284 (187)	0.90
Last visit	135 (133)	299 (229)	0.04
Change from baseline at last visit	− 138 (171)	14.5 (51.6)	0.001
Percent change from baseline	− 45.4 (14.6)	2.2 (9.3)	<0.0001
Daily change[3]	− 0.72 (0.93)	0.07 (0.32)	0.002

[1]CRP, C-reactive protein; Q, quartile.
[2]From 2-sample *t* test.
[3]Daily change was calculated from the linear regression model by using measures from all visits.

hypothesis that some of the phytochemicals in plants may be effective iron chelators.

The most commonly used medicinal chelators include siderophores and synthetic chelators. Siderophores such as deferoxamine mesylate (DFO) are Fe^{3+}-specific chelators derived from iron-dependent microorganisms (11). DFO was once the standard of treatment for iron chelation, which increased survival in patients with thalassemia major, sickle cell disorders, and myelodysplasia (11, 34). Prior studies showed that DFO may have strong therapeutic value for those with atherosclerosis or diabetes (35) as well. However, DFO has poor oral

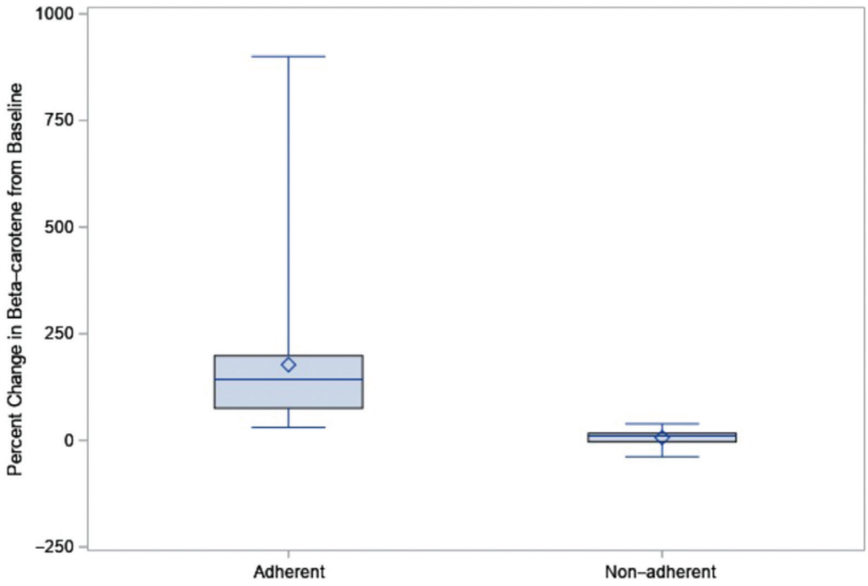

FIGURE 4 Boxplot for the percentage change from baseline in B-carotene at last visit in adherent and nonadherence patients calculated by 2-sample *t* test ($P = 0.0006$).

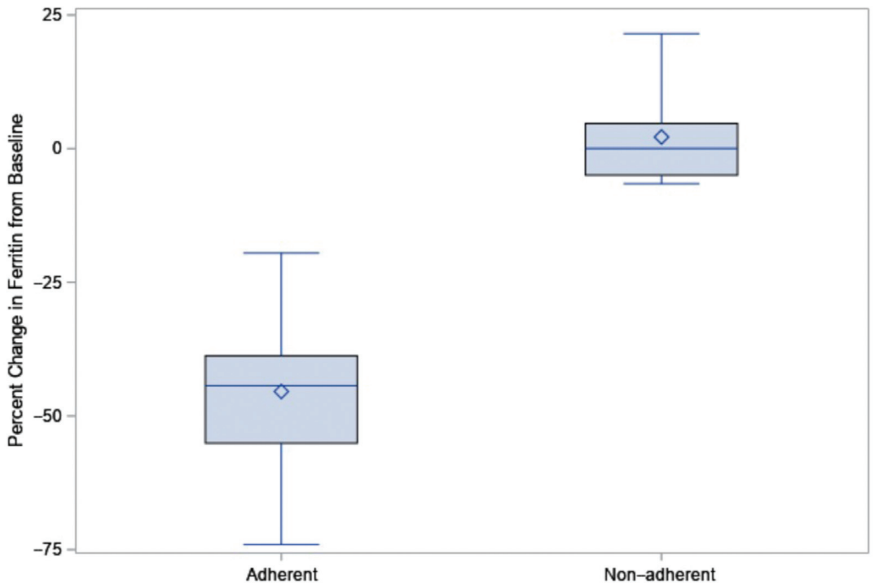

FIGURE 5 Boxplot for the percentage change from baseline in ferritin at the last visit in adherent and nonadherent patients calculated by 2-sample *t* test ($P < 0.0001$).

bioavailability, mandating less-convenient parenteral administration up to 7 d/wk, which lends itself to frequent patient noncompliance. It also has significant retinal and ototoxicity.

Synthetic oral chelators are alternatives to DFO or used in combination with it. One of the most commonly used is the bidentate ligand deferiprone (Ferriprox®, Chiesi Inc.), which demonstrates efficacy in treating iron-related cardiac dysfunction (34). Deferiprone, however, carries the serious side effect of reversible agranulocytosis in 1% of patients (36). Deferasirox (Exjade®, Chiesi Inc.), another synthetic oral chelator, is a tridentate ligand with FDA approval for iron overload secondary to chronic transfusions (37). A major drawback to this treatment incudes the requirement for monitoring creatinine due to instances of renal failure. Cases of cytopenias, rash, and gastrointestinal upset have been reported as well (11). Nutritional interventions such as the LIFE diet may be safer alternatives or adjunctive approaches to treating iron overload. While prospective trials are needed to confirm this, our results indicate that such studies may be warranted.

Over the last 2 decades, evidence supporting the potential role of dietary plant phenols in iron homeostasis has emerged. The flavonoids found in leafy vegetables and fruits, for example, possess iron-binding motifs similar to those in microbial siderophores (15). In an in vitro study under physiologically relevant conditions, the flavonol quercetin, which is found in high concentrations in cranberries, completely inhibited Fenton reaction–induced oxidative damage by chelating iron (38). In another in vitro experiment, the polyphenol epigallocatechin gallate exhibited ferrous iron–chelating activity that was similar in potency to DFO based on the 2 compounds' ability to compete with ferrozine for ferrous ions (39). Although the only phytochemical we measured in this study was B-carotene, the LIFE diet contains many additional phytonutrients including carotenoids, bioflavonoids, polyphenols, and glucosinolates (38). B-Carotene, therefore, simply serves as a biomarker for all of the phytonutrients consumed in the LIFE diet, several of which likely chelated iron and resulted in significant reductions in ferritin concentrations.

Even individuals with ferritin concentrations in the normal range experienced substantial reductions in ferritin concentrations in this study, which is significant because ferritin concentrations that technically fall within the high physiologic or "normal" range may still result in adverse health outcomes due to oxidative stress (40). While our results imply that some of the phytochemicals within the LIFE diet chelated iron effectively, they did not do so to the point that any patients became anemic or iron deficient, which is associated with

TABLE 4 Association between change of CRP and change in ferritin among all subjects[1]

	Univariate analysis		Multivariable analysis (adjusted by age, gender, and race)	
	Regression coefficient (SE)[2]	*P*	Regression coefficient (SE)[2]	*P*
All subjects ($n = 32$)	− 4.15 (36.5)	0.91	− 58.0 (64.2)	0.37

[1]CRP, C-reactive protein.
[2]Determination of regression coefficient was used to represent how much change in ferritin corresponded to per unit increase in CRP.

TABLE 5 Iron content in sample LIFE diet[1]

	Values
Smoothie	
Raw kale (8 ounces)	5 mg
Blueberries (2.25 cups)	1 mg
Banana	0.2 mg
Ground flaxseed (1 tablespoon)	1 mg
Soymilk (0.5 cup)	0.4 mg
Whole foods	
Cooked spinach (5 ounces)	4 mg
Lentil pasta (1 cup)	6 mg
Chicken breast (6 ounces)	2 mg
Quinoa (4 ounces)	2 mg
Almonds (1 ounce)	1 mg
Blueberries (0.5 cup)	0.5 mg
Total	23.1 mg

[1]LIFE, Low Inflammatory Foods Everyday.

increased morbidity and mortality (12). Similarly, Armah et al. (41) demonstrated that young women with suboptimal ferritin concentrations at baseline do not become anemic after consuming a high-phytate (an inhibitor of iron absorption) diet for 8 wk. Together, these studies suggest that the longitudinal impact of phytonutrients and phytate on systemic iron concentrations is influenced by baseline iron concentrations. Furthermore, the iron content of the LIFE diet lies well above the minimum 8-mg dietary requirement in men and nonmenstruating women (42) (see sample LIFE diet; **Table 5**). It is also important to consider that patients consuming the LIFE diet were not forbidden from consuming heme-iron–containing animal protein; rather, they were limited to 6 ounces/d (not including red meat). In 11 y of full-time practice, one of the authors (DMD) has not encountered a single patient who developed iron deficiency anemia attributed to the LIFE diet, regardless of the patient's animal protein consumption.

There are several additional strengths of this study. This was a longitudinal study, allowing us to observe the relation between the change in B-carotene and the change in ferritin over time. The study population was also diverse; multiple genders, races, chronic disease statuses, and levels of adherence to the LIFE diet were represented. Finally, the LIFE diet is an easily quantifiable dietary intervention. Patients were intensively counseled on how often to consume components of the LIFE diet and the precise way to measure ingredients, which was done to ensure that assessments of the diet's efficacy were based on measurable doses and frequencies, similar to medications. In this way, we hoped to demonstrate that larger portion sizes and more frequent consumption could result in proportional increases in the LIFE diet's systemic effects (described previously as health benefit = frequency × dose) (21). As we highlighted in the second LIFE study, the "dose" of certain ingredients may be affected by the physical state of the food as well (21). There is some evidence to suggest that the B-carotene in liquefied DGLVs is better absorbed compared with whole vegetables (43). Significant increases in B-carotene after 1 wk of consuming a daily LIFE smoothie objectively demonstrated that B-carotene was very well absorbed when DGLVs were liquefied. In the context of the LIFE diet, therefore, the LIFE smoothie may produce potent effects on both inflammation and iron metabolism quickly.

There were several limitations to this study. Most notably, its small sample size and retrospective data collection. While excluding menstruating women and patients with high levels of systemic inflammation was important to remove potential confounders, this also limited the generalizability of our results. In addition, while we excluded patients with systemic inflammation, serum ferritin concentrations are still only an indirect measure of total body iron stores.

Overall, this study shows that the LIFE diet is associated with decreased total body iron as demonstrated by significant reductions in ferritin while controlling for the potential confounding variable inflammation. Importantly, the decreases in ferritin due to the LIFE diet were not associated with the development of anemia. Elevations in ferritin concentrations increase morbidity and mortality from CVD and are associated with diabetes, cancer, metabolic syndrome, and even severe disease from coronavirus disease 2019 (COVID-19) (44). The LIFE diet, therefore, may offer a fast, effective, and safe way to chelate bodily iron, thereby treating iron overload and reducing the risk for these chronic diseases. The positive results of this retrospective study justify a prospective clinical trial.

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The authors' responsibilities were as follows—DMD and JLD: designed the research; DMD: conducted the research and provided essential reagents or provided essential materials; G-SY: analyzed data or performed statistical analysis); BMP: wrote the manuscript and had primary responsibility for final content; and all authors: read and approved the final manuscript.

Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request.

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