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RESEARCH ARTICLE

Ferritin and Percent Transferrin Saturation Levels Predict Type 2 Diabetes Risk and Cardiovascular Disease Outcomes

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Abstract: ***Introduction*:** Type 2 diabetes (T2D) and cardiovascular disease (CVD) risk associate with ferritin and percent transferrin saturation (%TS) levels. However, increased risk has been observed at levels considered within the "normal range" for these markers.

***Objective*:** To define normative ferritin and %TS levels associated with T2D and CVD risk.

***Methods*:** Six-monthly ferritin, %TS and hemoglobin levels from 1,277 iron reduction clinical trial participants with CVD (peripheral arterial disease, 37% diabetic) permitted pair-wise analysis using Loess Locally Weighted Smoothing plots. Curves showed continuous quantitative ferritin, hemoglobin (reflecting physiologic iron requirements), and %TS (reflecting iron transport and sequestration) levels over a wide range of values. Inflection points in the curves were compared to ferritin and %TS levels indicating increased T2D and CVD risk in epidemiologic and intervention studies.

***Results*:** Increasing ferritin up to about 80 ng/mL and %TS up to about 25% TS corresponded to increasing hemoglobin levels, and minimal T2D and CVD risk. Displaced Loess trajectories reflected lower hemoglobin levels in diabetics compared to non-diabetics. Ferritin levels up to about 100 ng/mL paralleled proportionately increasing %TS levels up to about 55%TS corresponding to further limitation of T2D and CVD risk. Ferritin levels over 100 ng/mL did not associate with hemoglobin levels and coincided with increased T2D and CVD risk.

***Conclusions*:** Recognition of modified normal ranges for ferritin from about 15 ng/mL up to about 80-100 ng/mL and %TS from about 15% up to about 25-55% may improve the value of iron biomarkers to assess and possibly lower T2D and CVD risk.

Keywords: Iron, ferritin, transferrin, diabetes, normal values.

INTRODUCTION

Diabetes is associated with bidirectional abnormalities of glucose and iron metabolism [1-5]. Meta-analyses of observational studies have shown that ferritin levels in the general population predict type 2 diabetes (T2D) and gestational diabetes mellitus (GDM) risk [6-11]. A longitudinal epidemiologic study showed dose-over-time relationships between elevated ferritin levels and increased mortality from diabetes and other age-related diseases [12]. Podmore and colleagues reported the predictive value of measures of iron status for subsequent diabetes risk [13]. These investigators documented continuous linear dose-related associations between ferritin levels and increased T2D risk in men and women with highly significant differences across ferritin quintiles. Continuously increasing hazard ratios began at ferritin levels above about 100 ng/mL, a level generally considered to be within the normal range. Quintile boundaries placed rising risk in the second quintile for males having low and high cut points of 68 ng/mL and 117 ng/mL respectively, and in the fourth risk quintile for women having cut points of 73 ng/mL and 121 ng/mL respectively. In contrast, reduced T2D risk with higher percent transferrin saturation (%TS) levels was observed in women at a cutoff approximating 45%TS. This association diminished above 50%TS and was lost at a cutoff above 55%TS. These findings were consistent with progressively reduced capacity for transferrin binding of excess iron or redox-active non-transferrin bound iron (NTBI) at %TS levels above about 50-55% [13, 14]. A

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similar trend in men did not reach statistical significance possibly due to overall higher ferritin levels in men [14-16].

We observed that higher ferritin levels correlated with mortality during a prospective randomized clinical trial of iron reduction in primarily males with cardiovascular disease (CVD, peripheral arterial disease, VACSP 410) [17] along with increased levels of inflammatory markers: TNF alpha (receptor 1), IL6 and hsCRP [18, 19]. Significantly improved disease outcomes related to lower ferritin levels [17, 20, 21] and with increasing %TS levels in the range of 25-50%TS [21]. Mean ferritin levels in participants in CSP 410 at entry were about 122 ng/mL and iron reduction targeted ferritin levels of 60 ng/mL [17]. Ferritin levels in participants having no mortality, or non-fatal myocardial infarction or stroke, over 6-years of follow-up averaged 76 ng/mL [17]. In a participating institution sub-study, the mean ferritin level corresponding to mortality was 132.5 ng/mL while the mean ferritin in those with no mortality was 82.6 ng/mL [18, 19]. These steep dose-effect levels approximated ferritin levels associated with reduced morbidity and increased longevity observed in other population studies [20, 22-25], as well as threshold levels of ferritin and protective %TS levels observed by for T2D risk by Podmore and associates [13].

These epidemiologic and clinical trial associations suggested revision of normative values for ferritin and %TS levels, common measures of iron metabolism, considered indicative of the transition from low to increased disease risk. Similarities in iron biomarker levels associated with T2D risk [13, 15] and adverse vascular disease outcomes [17, 21] suggested that examination of graded biomarker levels during iron reduction would provide further information on quantitative relationships between these biomarkers of iron metabolism and disease risk.

This report demonstrates quantitative relationships between a wide range of levels of ferritin, %TS and hemoglobin (representing physiologic iron) obtained by direct clinical measurement using Loess plots [26] to display a transition from physiologic to pathologic markers of iron metabolism predictive of adverse clinical outcomes. We reported previously the utility of Loess methodology to characterize racial variation in red cell and iron homeostasis [27]. Our present findings clarify normative ferritin and %TS levels as measures of iron metabolism predictive of lower disease risk.

MATERIALS AND METHODS

The Iron and Atherosclerosis Study, FeAST, VA Cooperative Study Program # 410 (CSP 410); a prospective, randomized, controlled single-blinded clinical trial of iron reduction; used graded phlebotomy based on ferritin levels (Trial Registration: www.clinicaltrials.gov, Identifier NCT00032357) [27-29] to test the hypothesis that improved clinical outcomes might be achieved in subjects with established CVD by reducing iron stores to levels typical of children and pre-menopausal women (about 25 to 60 ng/mL). The Consort Diagram [28] and methodological details of study protocols; informed consent, randomization and intervention procedures; outcome assessment and study administration have been reported [28-30]. Institutional Review Boards (IRBs) at each of the 24 participating hospitals and a national IRB approved the protocol. Consenting (98.8% male) patients over age 21 (average age 67) with stable CVD (peripheral arterial disease) were computer randomized by diabetes and smoking status, age, ferritin level, HDL/LDL cholesterol ratio, and medical center [28-30]. Thirty-seven percent of the 1,277 subjects had a clinical diagnosis of diabetes at entry. This study was designed to optimize opportunity to detect an effect of follow-up ferritin levels on the primary outcome, all-cause mortality, and the secondary outcome, death plus non-fatal myocardial infarction and stroke. Patients were required to have stable CVD for the past 6 months, no disorder of iron metabolism including iron deficiency or hemochromatosis, no malignancy within the past 5 years, hematocrit of >35%, ferritin level of <400 ng/mL, and creatinine <3.0 mg/dL [28-30]. Serum ferritin levels up to 400 ng/mL, encountered commonly in practice [12], were similar for diabetics and non-diabetics (see results section). Because diabetes was a randomization variable, equivalent numbers of diabetics and non-diabetics were randomized to iron reduction versus control groups. The use of calibrated phlebotomy based on ferritin levels (ml blood to be removed = (ferritin level-25) X10) for iron reduction provided a wide range of ferritin, %TS and hemoglobin values observed in laboratory test panels obtained at 6-monthly intervals during six years of observation. Ferritin and %TS levels reported here represent the 6-monthly (maximum) levels measured during follow-up used to calculate the need for further phlebotomy. For purposes of this report, quantitative data at entry were examined for normality using the Shapiro-Wilk test [31] and found to be not normally distributed. Thus, quantitative data are presented below as medians with 25th and 75th percentiles. Statistical significance between groups was tested using the Wilcoxon test [32].

Loess Locally Weighted Scatter Smoothing Plot analysis [26] was used to detect interrelationships between these variables. This method permits construction of a smooth curve in order to visualize relationships between two variables over a continuum not otherwise recognizable within large populations [33-35]. Inflection point relationships between two variables of interest allow identification of subpopulations within a larger clinical trial population with unique characteristics associated with graded risk of adverse clinical outcomes [36, 37]. Measures of interest included relationships between ferritin, %TS reflecting iron transport and sequestration, and hemoglobin levels reflecting physiologic iron status. Loess methodology has been used to show relationships between individual laboratory variables and clinical outcomes in studies of diabetes [36-39] and CVD [27, 40, 41]. The present study plotted relationships between ferritin and hemoglobin levels (8388 paired samples, Fig. **1**), between %TS and hemoglobin levels (8371 paired samples, Fig. **2**) and between ferritin and %TS levels (8371 paired samples, Fig. **3**). Loess curves were examined for inflections below or above which adverse clinical events were less or more likely to occur. Relevance of observed inflection points to clinical outcomes was based on outcomes from clinical trial findings [17, 21, 28] as well as epidemiologic studies assessing iron biomarker levels and risk of T2D and CVD [12, 13, 16].

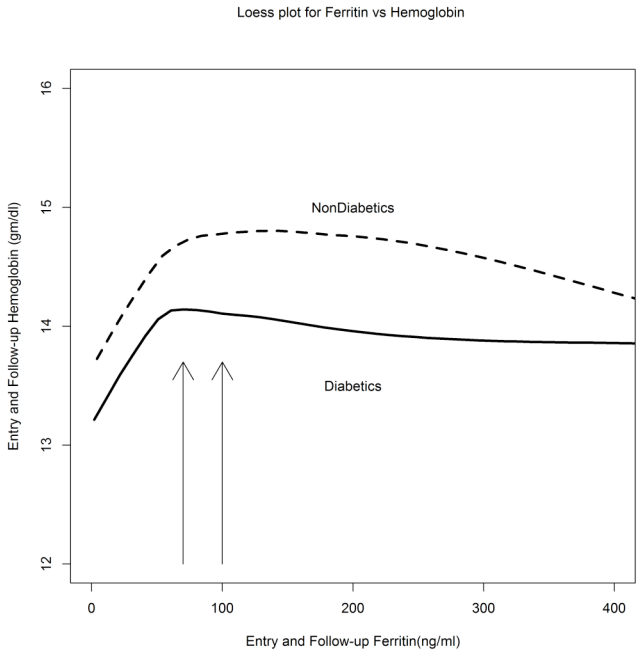

Fig. (1). Loess smoothing plots of 8388 paired values of hemoglobin and ferritin in diabetic and non-diabetic participants during the iron reduction trial, CSP 410. The range of values is depicted in the axes of the figure. Vertical arrows approximate inflection points associated with the limits of hemoglobin iron requirement, and altered T2D and CVD risk.

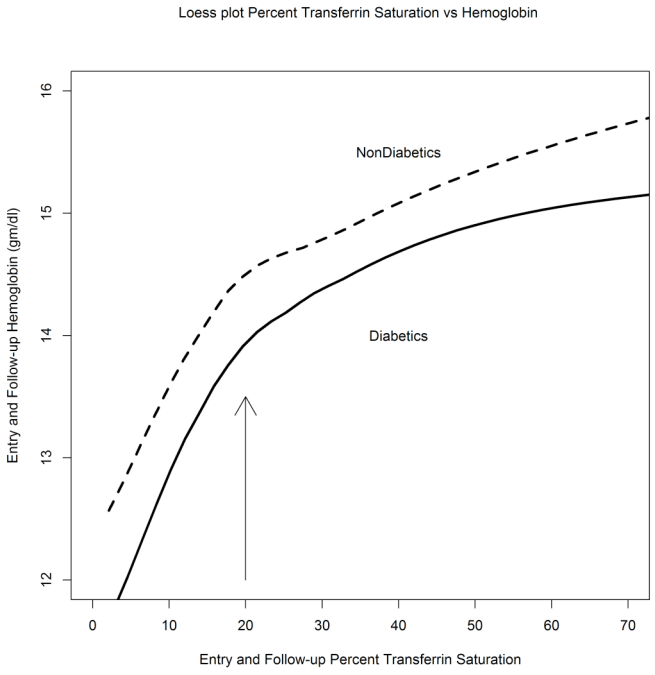

Fig. (2). Loess plots of 8371 paired values of hemoglobin and %TS in diabetic and non-diabetic participants during the iron reduction trial, CSP 410. The range of values is depicted in the axes of the figure. The vertical arrow shown here at about 20%TS approximates the inflection point (of 20-25%TS) representing transport iron for erythropoiesis.

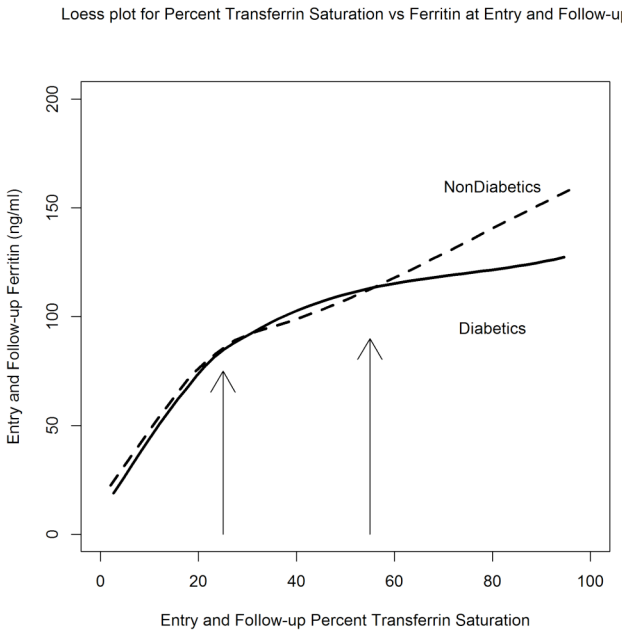

Fig. (3). Loess plots of 8371 paired ferritin and %TS values in diabetic and non-diabetic participants during the iron reduction trial, CSP 410. The range of values is depicted in the axes of the figure. Rising curves overlap with an inflection point marked by the left-hand vertical arrow at about 25%TS and about 80 ng/mL ferritin (similar to values shown in figures 1 and 2). The right-hand vertical arrow marks a second inflection point at about 55%TS and 100 ng/mL of ferritin.

RESULTS

The FeAST study included 1,277 entrants, 473 of whom were diabetic and 804 non-diabetic at entry. Levels of ferritin, %TS and hemoglobin found at entry are expressed here as medians with 25th and 75th percentiles in parentheses. Median levels of ferritin of 103 ng/mL (61 ng/mL, 170 ng/mL) and 103 (58, 168) ng/mL, (p=0.681); and median levels of %TS were 23.4% (18.7%, 30.1%) and 24.7% (18.8%, 30.8%), (p=0.141) were similar at entry for diabetics and non-diabetics respectively. Median entry levels of hemoglobin were slightly but statistically significantly lower in diabetics compared to non-diabetics: 14.5 g/dL (13.5 g/dL, 15.3 g/dL) versus 14.8 g/dL (13.9 g/dL, 15.7 g/dL), (p<0.001). Loess plot trajectories for diabetic and non-diabetic participants were similar but displaced for diabetics as shown in figures 1 and 2 owing to lower hemoglobin levels in diabetics.

Fig. (**1**) shows Loess smoothing plots for hemoglobin and ferritin paired values. Levels of both increase in parallel up to an inflection point at ferritin levels approximating 80-100 ng/mL reflecting a possible mean upper limit for the physiologic "normal range" (represented by hemoglobin levels) for both measures. Loess curves for diabetics and non-diabetics were roughly parallel but displaced downward in diabetics as predicted by the significantly lower hemoglobin levels in diabetic participants. The inflection point for the hemoglobin level may be interpreted as occurring at a somewhat lower ferritin level in diabetics compared to non-diabetics. However, ferritin levels below about 80-100 ng/mL followed a similar trajectory in diabetics and non-

diabetics. Ferritin levels above the transition at about 80-100 ng/mL lacked physiologic correspondence to increasing hemoglobin levels, an effect that was exaggerated in diabetics and possibly contributory to "anemia" in certain diabetics [42].

Fig. (**2**) shows the Loess plots of paired hemoglobin and %TS levels. As in Fig. (**1**), these plots also were roughly parallel but displaced downward in diabetics. The inflection point for hemoglobin levels depicted in Fig. (**2**) occurred at about 20%TS in both diabetics and non-diabetics. Levels plateaued above about 25%TS and followed roughly similar trajectories in relation to hemoglobin levels in diabetics and non-diabetics throughout the range of values in this data set.

Fig. (**3**) shows Loess plots of paired ferritin and %TS levels which increase synchronously with overlapping curves in diabetics and non-diabetics up to levels of about 55%TS. The plateau above about 25%TS, the presumed cutoff for physiologic levels of transport iron for erythropoiesis represented by the %TS level, is similar to that in Fig. (**2**). Curves flatten but continue to overlap as levels of both rise in proportion to each other for ferritin levels up to about 100 ng/mL and %TS levels up to about 55%TS. However, curves for diabetics versus non-diabetics diverge at ferritin levels above about 100 ng/mL and %TS levels above about 55%. Above these levels, dissociation of these measures appeared in which a further increase in ferritin levels is accompanied by a proportionate rise in %TS in non-diabetics while a less proportionate ferritin/%TS response occurred in diabetics.

DISCUSSION

Loess plots for diabetic and non-diabetic cohorts differed because of slightly lower hemoglobin values in diabetics related presumably to "anemia of chronic disease" occurring in some diabetics [42]. However, inflection points in relation to hemoglobin levels occurred in both diabetic and non-diabetic cohorts for ferritin levels at about 80-100 ng/mL, and for %TS levels at about 25% TS (Figs. **1** and **2**, respectively). Increasing ferritin levels up to these inflection points suggest increasing physiologic incorporation of iron into hemoglobin.

Data presented in Fig. (**1**) suggest that ferritin levels approximating 80-100 ng/mL represent an upper normal limit of physiologic levels of body iron as related to corresponding hemoglobin levels. Ferritin levels over the 80-100 ng/mL range appear to lack physiologic correspondence with hemoglobin levels. Thus, normative values for the serum ferritin may range from the commonly accepted lower limit of less than about 15 ng/mL to an upper limit of about 80-100 ng/mL as observed in this study. This threshold ferritin level coincides with levels associated with increased T2D risk observed epidemiologically [13] as well as adverse outcomes observed in clinical trial data [17, 21].

Fig. (**2**) shows proportionately increasing hemoglobin and %TS levels up to about 20-25%TS. Rising %TS levels gradually plateau above an inflection point of about 25% in relation to corresponding hemoglobin levels. Transferrin saturation levels below about 20-25% may be consistent with transport of iron as holotransferrin for erythropoiesis [14]. Levels above about 25%TS imply a possible switch to incorporation of iron in physiologic excess (or possibly redox-active NTBI) onto transferrin [14].

The proportionate increase in ferritin and %TS values shown in Figure 3 up to ferritin levels of about 80 ng/mL and %TS levels of about 25% suggests strict physiologic correspondence or compensation of these functionally different proteins representing balance between ferritin and transferrin as they interact to maintain optimal (compensated) iron homeostasis [14] associated with minimal disease risk [13, 17, 21]. The curves flatten above ferritin levels of about 80 ng/mL and %TS levels above about 25% but then increase proportionately up to about 100 ng/mL of ferritin of and about 50-55%TS. These relationships suggest sustained compensation by an increase in %TS as related to increasing ferritin levels. Podmore and colleagues [13] also observed apparent clinical compensation for increasing ferritin levels with increasing %TS levels up to about 50-55%TS which appeared to protect against T2D risk. The transition from physiologic to apparently de-compensated (disproportionately increasing) levels above about 50-55%TS in women was associated with increased T2D risk in that study [13]. We also noted a similar inflection point in our male participants with CVD at about 25%TS (Figs. **2** and **3**). The present data suggest a transition from presumably protective to potentially toxic %TS levels above a range of about 25-55%TS in males (Figs. **2** and **3**). Progressive iron loading may exceed the capacity for physiologic compensation by transferrin that may be most efficient when ferritin levels remain below about 80-100 ng/mL [17, 21]. Iron biomarker relationships shown in Figures 2 and 3 corresponding closely to the epidemiologic findings of Podmore and colleagues [13] for their observed hazard ratios for ferritin and %TS for T2D risk. The apparent functional dissociation of ferritin and %TS above these levels suggests a testable hypothesis that transferrin may become occupied increasingly with potentially toxic NTBI at ferritin levels considered previously to be normal [14, 43-45].

Differing functions and regulatory control of ferritin as compared to %TS levels may also explain the positive association of ferritin levels with the inflammatory markers, TNF alpha, IL6 and hsCRP, [18, 19, 43] as well as the negative association of these inflammatory markers with %TS levels [42]. In accord with these findings, Podmore and colleagues also recorded that lower ferritin levels and higher %TS associate with lower hsCRP levels [13]. Side-by-side comparison of quantitative %TS data reported here coincides with clinical outcome data reported in epidemiologic [13] and clinical trial outcome [21] data. Protective effects appear to exist for %TS levels up to about 50-55% both diabetes risk [13] and CVD outcomes [21].

REVISED NORMAL RANGES FOR IRON STATUS AND LONGEVITY

The revised normal ranges for these biomarkers of iron metabolism suggested here bear striking resemblance to other estimates of disease risk in relation to increasing ferritin and %TS levels. Population mean ferritin levels vary considerably by age, race and sex while %TS levels appear to be relatively stable [20, 21, 27]. Mean ferritin levels up to approximately 60-70 ng/mL in the late teens in males and

females associate with low disease risk [17, 23-25]. Population levels in males increase at a rate of about 4 to 5 ng/mL per year and plateau at a mean of about 140-150 ng/mL associated with increasing disease risk by middle-age [20]. Cross-sectional measures of ferritin decline gradually with increasing age to range at about 80-100 ng/mL suggesting that such lower levels select for greater longevity [20, 24, 25] as illustrated in CSP 410 outcomes data [17]. Low risk mean ferritin levels below about 50 ng/mL in pre-menopausal women rise after menopause to plateau at approximately 80-100 ng/mL about 30 years earlier in women compared to men [20]. The increase in ferritin levels above this threshold associate with increased post-menopausal disease risk in women [46]. Occurrence of ferritin levels in this range at an earlier age in women correspond to their increased risk for T2D above this threshold [13]. Higher ferritin levels relate to increasingly impaired beta cell function, insulin resistance and metabolic abnormalities characterizing diabetes [5, 47, 48].

Ferritin levels below about 80-100 ng/mL relate to minimal disease and maximum longevity observed in other studies [17, 20, 22-25]. Individuals in the elderly Framingham Heart Study cohort having a mean age of 75 (age range 68-93) but lacking a clinical disease diagnosis had mean ferritin levels of 86 ng/mL whereas members of the same elderly cohort having a clinical disease diagnosis had a slightly higher mean ferritin level of 94 ng/mL [22]. Percent TS levels in the Framingham Heart Study cohort were lower in individuals without a clinical diagnosis, 25%TS, compared to individuals having a clinical disease diagnosis, 35%TS. Based on data shown in Figure 3, 25%TS would be considered within the low risk range and 35%TS, while higher, would remain in the compensated range. Data from a cohort of males from the Mediterranean region of Europe over age 80 having relatively low morbidity and mortality had mean ferritin levels of 68 ng/mL [23]. These levels were significantly lower than levels found in a male population of similar age from The Netherlands having significantly greater morbidity associated with mean ferritin levels of 137 ng/mL. Adjusted mean %TS levels were 20% in the healthier Mediterranean population versus 34% in the sicker Northern European population. Based on our findings, the healthier Mediterranean population having mean %TS levels of 20% would be within the low risk range while the higher risk Northern Europeans of the same age having mean %TS levels of 35%, while slightly higher, would remain in the compensated range. Residents of the Mediterranean region are known to have a lower risk of T2D [49], vascular disease and malignancy [23]. These estimates resemble NHANES III data showing a similar pattern for ferritin levels in free-living older age individuals [20].

REVISED NORMAL RANGES FOR IRON MEASURES AND DIABETES RISK

The validity of the revised normal values for measures of iron status reported here is supported by several studies of T2D risk. A prospective randomized interventional trial in T2D examined effects of rigorous dietary iron restriction (total n = 191, evaluable n = 170) on development of end stage renal disease requiring transplantation or all-cause mortality over a mean follow-up of about 4 years [50]. The mean ferritin level for the total cohort at entry was 301 ng/mL. Levels remained unchanged during follow-up in control subjects but declined to a mean of 36 ng/mL ($p<0.001$) in subjects randomized to dietary iron restriction. Iron reduction was associated with a significant reduction in the primary study endpoint, $p<0.01$, and significantly reduced risk of doubling of the serum creatinine, $p<0.02$.

Ogawa, et al [25] reported clinical outcome data from a cohort of patients on chronic hemodialysis, 21% of whom had diabetic nephropathy. Ferritin levels between about 30 ng/mL and 80 ng/mL were associated with significantly improved long-term survival ($p=0.013$) compared to survival with higher or lower ferritin levels and hemoglobin status.

Strong continuous statistical associations exist between ferritin levels and insulin resistance (IR) measured typically by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) assay [51-54]. Correlations between IR and ferritin levels above about 99 ng/mL in men and above about 86 ng/mL in women have been reported [50]. Kim et al [54] showed significant positive statistical associations between levels of insulin, fasting glucose and HOMA-IR with ferritin levels above 98 ng/mL in post-menopausal women ($p<0.001$ for each comparison). Relationships between iron measures and HOMA-IR were tracked longitudinally in a cohort of otherwise healthy non-diabetic women followed from pre-menopause to one-year post-menopause [55]. During menopausal transition, fasting glucose ($p=0.05$) ferritin ($p<0.01$) and HOMA-IR ($p=0.022$) increased significantly. Mean pre-menopausal ferritin levels were 69.5 ng/mL and mean post-menopausal ferritin levels 128.8 ng/mL, resembling low versus increased risk levels observed in the present and other studies [20,22,23]. Improved IR with removal of a single unit of blood (500 mL, estimated to reduce ferritin levels by about 50 ng/mL in a healthy individual) reinforces the concept that IR risk may be a continuum related to the ferritin level [56].

Relationships between iron measures and T2D risk [57,58] are relevant to vulnerable populations predisposed metabolically to diabetes associated with overall increased ferritin levels [21,27,58-60]. Living at high altitude correlates with both reduced diabetes risk [61] along with lower ferritin levels [62]. Treatment of diabetes with the oral hypoglycemic agent, metformin, significantly reduces risk of diabetic co-morbidities [63-70]. The anti-diabetic properties of metformin may be linked to the metal binding properties of this drug [71]. A 3-month course of metformin reduced ferritin levels by about 50% ($p<0.001$) and improved insulin resistance in a cohort of women with polycystic ovary syndrome [72].

LIMITATIONS OF THIS STUDY

CSP 410 focused on clarification of the role of iron excess in the pathogenesis of CVD that may be potentially linked to multiple factors that were not investigated in this clinical trial. Free-living CVD patients with and without diabetes likely have various co-morbidities or more advanced disease associated with higher ferritin levels than are represented in this cohort [73]. Elevated ferritin levels may relate to increase in iron stores, inflammatory response, or a combination of both. The plots obtained from the present partici-

pants may not be characteristic of the general population. Loess curves reported here were derived from a population consisting almost entirely of males. Curves derived from a population of females may also differ from those obtained in CSP 410. Measures of hepcidin, NTBI [74-76], glycated hemoglobin, insulin resistance, inflammation and possibly other variables were not available in this study. T2D risk may also be attributable to socio-economic factors, environmental contaminants [77-79] or Western Lifestyles [80]. These require further investigation of their effects on iron biomarkers and associated iron metabolism.

STRENGTHS OF THIS STUDY

Ferritin levels at entry to CSP 410 were a randomization variable considered a "signal" of iron metabolism conveying prognostic information. Ferritin levels at entry and intent-to-treat ferritin levels during follow-up were interpreted at face value to determine the amount of blood to be withdrawn by phlebotomy without attempting to differentiate between acute phase and non-acute phase levels. Strengths that render data on iron biomarkers suitable for the present analysis include study's prospective, randomized single-blinded design, and longitudinal analysis of a wide range of levels of the variables of interest. Iron reduction was based on levels of the primary variable of interest (the serum ferritin level) as modified with randomization to calibrated phlebotomy [17, 21]. Ferritin levels in this study are observed commonly in the general population and were subject to experimental variation [17] as well as natural variation in free-living populations [17, 20]. Diabetes status was also a randomization variable; similar numbers of diabetics and non-diabetics were randomized to iron reduction as compared to control [27-30]. Ferritin and %TS levels reported here were similar between diabetic and non-diabetic entrants while hemoglobin levels were significantly lower in diabetics. Loess methodology identified break points in a continuum of ferritin and %TS levels that corresponded to clinical risk [13, 17, 21]. Cut points in Loess curves may reflect an apparent transition from physiologic to pathologic iron biomarker levels that appeared to be similar between participants with or without T2D. Thus, the demonstrated quantitative relationships for ferritin and %TS levels that distinguish physiologic from non-physiologic ferritin levels of these biomarkers or iron metabolism may facilitate clinical correlation of observations on iron-mediated pathways and risk of T2D [81-83], its complications [84-86] and its co-morbidities [87-90]. Iron biomarker values reported here might be extrapolated to co-morbidities of diabetes besides CVD. For example, malignancy may have a similar hypothetical pathophysiologic narrative, i.e., damage to cells and tissues caused by iron-catalyzed oxygen free radical generation [24, 91]. CSP 410 data reported previously showed reduced risk of new visceral malignancy ($p=0.036$), and all-cause and cancer-specific mortality ($p=0.009$) with randomization to iron reduction [92]. Mean follow-up ferritin levels in entrants not developing cancer as compared to those developing cancer were 76.4 ng/mL versus 127.1 ng/mL respectively ($p=0.017$). These ferritin levels also correspond to lower versus higher risk values as suggested by the present report. Additionally, ferritin levels below about 100 ng/mL are recommended for optimal management of diseases of iron excess by the American Association for the Study of Liver Disease [92].

CONCLUSION

The wide range of putatively "normal" serum ferritin levels, 30-300 ng/mL in men and 15-200 ng/mL in women, a 10-fold variation, represent extreme variations that do not resemble the range of normal values for other laboratory metabolic measures [2, 24]. Revised normal values as reflected by relationships between ferritin, %TS and hemoglobin observed in the present study also associate with epidemiologic and clinical trial data predicting favorable clinical outcomes. Optimal (minimum risk) serum ferritin levels appear to range from a low of about 15 ng/ml to about 80-100 ng at an upper limit. Data analyzed here for %TS levels resemble the range of normal as accepted commonly having a lower limit below about 15% and an upper limit of about 55%. We suggest that %TS levels up to about 25% may reflect iron transport for erythropoiesis. Ferritin in relation to %TS levels ranging between about 25% and 55% may reflect compensation by transferrin scavenging of iron or NTBI up to ferritin levels of about 100 ng/mL. Ferritin and %TS levels within these ranges appear to associate with lower T2D [13] and CVD risk [21].

LIST OF ABBREVIATIONS

CSP 410	=	VA Cooperative Studies Program #410
CVD	=	cardiovascular disease
FeAST	=	The Iron and Atherosclerosis Study, CSP 410
GDM	=	gestational diabetes mellitus
HOMA-IR	=	Homeostasis Model Assessment of Insulin Resistance
HDL	=	High density lipoprotein cholesterol
hsCRP	=	high sensitivity C-reactive protein
IRB	=	Institutional review board
LDL	=	low density lipoprotein cholesterol
IL6	=	Interleukin 6
Loess	=	Locally weighted scatter plot smoothing
NHANES	=	National Health And Nutrition Examination Survey
NTBI	=	Non-transferrin bound iron
TNF	=	Tumor necrosis factor
TS	=	transferrin saturation
T2D	=	Type 2 diabetes
VA	=	Veterans Administration

AUTHOR CONTRIBUTIONS

L.R.Z. and R.G.D drafted the manuscript, G.S. and B.K. C. performed the statistical analyses and prepared the figures. L.R.Z., G.S., B.K.C. and R.G.D. contributed to writing and editing the manuscript, and approved the final manuscript for submission.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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