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Dual-Energy X-ray Absorptiometry With Serum Ferritin Predicts Liver Iron Concentration and Changes in Concentration Better Than Ferritin Alone

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Abstract

Accurate assessment of liver iron concentration (LIC) is critical for optimal monitoring of iron toxicity in multi-transfused patients. Serum ferritin is the most widely used although its association to LIC is only modest. We studied if a liver-specific measure using dual-energy X-ray absorptiometry (DXA) systems could improve LIC estimates over ferritin alone in Thalassemia (Thal) patients. Thirty-seven patients with Thal (19.2 ± 9.0 yr, 20 male) were studied and 10 had multiple visits. Height, weight, ferritin, whole-body DXA, and hepatic superconducting quantum interference device (SQUID) were measured within 5 wk. DXA hepatic density was measured using right rib, whole liver, and multiple subliver regions. The best agreement to SQUID LIC was found using a combination of ferritin, weight, DXA subliver region 3 bone mineral content (BMC), and right rib BMC. DXA with ferritin improved the ferritin alone correlation from $R^2=0.35$ to $R^2=0.62$. Serial LIC changes using DXA were associated with serial SQUID changes ($r=0.73$, $p=0.02$). Changes in ferritin alone were not significant ($p=0.06$). We conclude that the addition of whole-body DXA measures and body weight substantially increased the accuracy of LIC and change in LIC estimates over the use of ferritin alone and could be useful when magnetic resonance imaging or SQUID is not available.

Keywords

Body composition; LIC; SQUID; thalassemia; transfusion; whole-body DXA

Introduction

Accurate assessment of iron is critical for optimal monitoring of iron-related organ toxicity in multitransfused patients, such as those with β -thalassemia. Thalassemia (Thal) is a hemolytic disorder, where inherited mutations affect specific genes of the alpha or beta-

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globin chains of hemoglobin. Chronic transfusion therapy, initiated soon after birth, has been used for decades to alleviate the severe anemia and associated complications in Thal (1). Although beneficial, transfusion therapy results in iron overload in patients without strict adherence to chelation therapy. Excess iron, primarily stored in the reticuloendothelial system organs such as the liver, also accumulates in the heart, pancreas, and endocrine organs leading to cardiomyopathy, diabetes, hypogonadism, hypothyroidism, as well as low bone mass and increased fracture risk (2–5).

Currently, there are several clinically available noninvasive methods to measure liver iron concentration (LIC); these include serum ferritin, magnetic resonance imaging (MRI), and liver biosusceptometry more commonly known as the superconducting quantum interference device (SQUID). Under steady-state conditions, serum ferritin correlates reasonably with total-body iron stores (6). Serum ferritin is the most convenient and widely used laboratory test for iron assessment because of the cost and availability. However, the ability of serum ferritin to accurately reflect total-body iron stores in patients with hemoglobinopathies was called into question as far back as 1993 (7). Single measures of ferritin are unreliable in predicting LIC because of broad confidence intervals, particularly in nontransfused patients (8–10). To date, the most accurate noninvasive measures of LIC are made using either X-ray computed tomography (CT) (11), MRI (12), or liver biosusceptometry (SQUID) (8,13). Unfortunately, these methods are of limited utility in developing nations with a high prevalence of Thal because of either radiation exposure to the patient (CT), instrument cost (MRI and SQUID), and availability (SQUID, MRI) as well as insufficient technical training (CT, MRI, and SQUID).

Previous reports of measurements using dual-energy X-ray absorptiometry (DXA) have found associations with serum ferritin levels, when special liver regions of interest (ROIs) were analyzed (14). Variables obtained by DXA include bone mineral content (BMC) in grams and areal bone mineral density (BMD) in grams per square centimeter. Liver tissue with excess iron appears as mineral content to the DXA scanner. Chatterton et al (14) compared the DXA BMD measure in the liver region to serum ferritin and found a high correlation, $r=0.90$. However, as outlined above, serum ferritin can be highly variable, and thus the findings have limited clinical utility. Our hypothesis is that the combination of a subregional DXA body composition measure placed on the liver with serum ferritin could be used as an indicator of the need for transfusion to reduce LIC when the alternative is ferritin alone. The purpose of this study is to measure the DXA liver density and ferritin levels to create an estimate of LIC and compare with LIC gold standard techniques, SQUID. If successful, this methodology could provide a simple, inexpensive, and accurate assessment of LIC for patients in rural or less-developed parts of the world.

Design and Methods

The approach used in the study was to model liver iron content as an increase in apparent BMC in the region of the liver as measured from a DXA whole-body scan. Our gold standard for LIC was a SQUID measure. Special ROIs in the vicinity of the liver were used to separate the apparent liver BMC from the whole-body BMC. In addition, we investigated other covariates such as serum ferritin levels at the time of the DXA scan to increase the

predictability of the model. Lastly, we investigated other BMC regions from the whole-body scan in an attempt to control for overall bone mass.

Subjects

Thirty-seven patients with Thal (18.1 ± 6.4 yr, 20 male) had SQUID, DXA, and serum ferritin assessments within a 5-wk interval; 10 of whom were measured on more than one visit. Patients with transfusion-dependent ($n=26$) and non-transfusion-dependent Thal ($n=11$) were referred from the outpatient comprehensive thalassemia program at the Children's Hospital & Research Center Oakland (CHRCO) for routine noninvasive assessment of LIC by SQUID. Transfusion-dependent patients were transfused every 2 to 4 wk and received either deferoxamine (30–60 mg/kg) or deferasirox (20–30 mg/kg/d) as chelation therapy to minimize their iron load.

At each time point, the following measurements were performed: height was measured using a wall-mounted Holtain stadiometer and weight by an electronic scale, and a 5-mL blood sample was drawn for assessment of serum ferritin. Serum ferritin was measured at the CHRCO clinical laboratory using a one-step immunoassay (FERR-FlexR; Dada Behring, Newark, NJ) with an assay range of 1 to 1000 $\mu\text{g/L}$ (without dilution) and an interassay variation of 20%.

A healthy non-Thal control group ($n=32$) was also assessed for comparison to the DXA measures. These controls (7–30 yr, 15 male) were similar to the Thal group with respect to age, body mass index (BMI), and ethnicity, and they were recruited from hospital staff, their children and summer research interns at the hospital. This protocol for secondary analysis of the blinded data was given expedited approval by the institutional review board at CHRCO and did not require additional consenting.

DXA Scan

The whole-body DXA scans were acquired on a Hologic Discovery/A (Hologic Inc., Bedford, MA) using standard positioning as recommended by the manufacturer and software Discovery A, software version 11.3 or 11.6. The DXA images were centrally analyzed using Hologic's current software version Apex 2.3 by one licensed (California) technologist with 2-yr DXA experience. The whole-body scans were first analyzed using the standard whole-body analysis, then specific bone mineral density (BMD) and BMC measures were made in the region of the liver using a variety of special subregions using Hologic's subregional analysis tool. The standard whole-body analysis is automated, and the subregional analysis is more custom and manual in the placement of the ROIs. In all, we investigated four custom ROIs as shown in Fig. 1. R1 and R2 were small (21×3 cm) square regions on either side of the spine between the ribs and the iliac crest. The intention of these regions was to place a small ROI within the liver (R1) and a contralateral region (R2) to subtract off effects of rib BMC. R3 was a region that was recreated from the Chatterton paper, a rectangle (20×5 cm [pediatric] 24×5 cm [adult]) over the liver to the right of the midline. Region R4 is an outline of the entire liver. Lastly, the following standard whole-body subregions were also used in our analysis. The right rib ROI was used because it would include any BMC identified in the liver. The left rib ROI, whole body, and spine regions

were used as potential normalizing measures of skeletal BMC. The DXA system was monitored for stability over time using quality control procedures outlined by the manufacturer. The calibration of the device was stable within $\pm 0.5\%$ during the course of this study.

SQUID Measurements

Liver biosusceptometry is the quantification of LIC by magnetizing a small section of the liver and measuring the tissue magnetic susceptibility. The technical details and the measurement procedure of the biosusceptometer at CHRCO (Ferritometer[®]; Model 5700, Tristan Technologies, San Diego, CA) are described elsewhere (15,16). Biosusceptometry was performed according to the Hamburg-Torino-Oakland protocol (16,17), and results of this in vivo method are reported in units of microgram iron per gram of wet liver tissue (microgram Fe/g wet weight). In previous studies, SQUID estimates of LIC have shown to be quantitatively equivalent to biochemical determinations with correlation coefficients of $r=0.98-0.99$ (18,19). The long-term reproducibility was determined in one stable Thal patient, measured 12 times over 1 yr. The coefficient of variation (CV) of LIC by SQUID was 12%, whereas the CV for serum ferritin over the similar time interval was 32% (15).

Analysis

Ferritin levels of our population were compared with those reported by Chatterton et al using histogram analysis. Because of the skewed distribution of serum ferritin and LIC, nonparametric statistics were used (median, 95% confidence intervals, and others), Spearman rank correlation was used to determine the relationship between each of the clinical measures. Multivariate regression was used to determine the relationship between the most promising combinations of measures and LIC by SQUID. To meet the normality assumption in the multivariate analysis, ferritin, SQUID as well as the DXA BMD and BMC measures were transformed to natural log (ln) values. The 95% prediction intervals for hepatic iron concentration were calculated for either ferritin only or ferritin with other covariates. The model R^2 value was used as proportion of total variations from the known factors associate with SQUID LIC using stepwise multivariate regression procedures.

Results

Table 1 is a description of our Thal and reference populations at baseline with respect to the measures studied including age, weight, height, BMI, and the LIC-associated measures (SQUID, ferritin, right rib BMC, and R3 BMC). Of note is that our Thal population had a hepatic iron load that ranged from 275 to 5524 $\mu\text{g Fe/g wet wt}$ by SQUID. The normal range for hepatic iron is 90–340 mg Fe/g wet wt. Our population of Thal subjects had considerably lower serum ferritin levels than that reported by Chatterton et al. The mean $\pm$ standard deviation (SD) ferritin levels were $2130 \pm 2115 \mu\text{g/L}$ and $3884 \pm 3622 \mu\text{g/L}$ (extracted from (4)), respectively for our sample compared with Chatterton. In general, R3 showed the largest and most significant separation between the Thal subjects and controls. No difference was seen in the whole-body BMD values between the Thal and control groups ($p=0.88$). Table 2 lists the same variables from Table 1 for the participants with follow-up visits. There were no statistically significant differences noted between the baseline and follow-up

visits for any of the variables assessed. Table 3 reports the Spearman rank correlation coefficients (r_s) between each of the study variables. The best univariate correlation to SQUID was R3 BMC and ferritin with $r_s=0.77$ and 0.61, respectively. Table 4 shows the multivariate modeling for select combinations of variables to predict LIC by SQUID. Equation (1) describes the relation of ferritin alone to SQUID,

$$\ln SQUID = \ln FERRITIN * 0.42 + 4.39 \quad (1)$$

Equation (3) was found to be the model with the highest correlation to LIC by SQUID:

$$\ln SQUID = \ln R3BMC * 0.17 + \ln FERRITIN * 0.22 + WT * 0.03 + (\text{right rib BMC}) * 0.02 + 5.73 \quad (3)$$

The R^2 for these models improved from 35% (Eq. 1), to 56% (Eq. 2), to 66% (Eq. 3), which indicated 21%, 1%, and 9% improvement from the addition of R3 BMC, weight and right rib BMC, respectively. Adding either the left rib or R2 BMC to control for the right rib BMC did not improve the model. The differences between the predicted SQUID and the actual SQUID are shown for Eq. 3 in Fig. 2. The plots are shown for the measures themselves instead of their natural log for clarity. The 95% confidence interval is plotted as twice the SD of the difference. The confidence interval around each estimate is much smaller for Eq. 3 and Eq. 1 (4396 vs 3116 $\mu\text{g Fe/g wet wt}$, respectively).

In the 10 Thal subjects with multiple measures, change in iron load was significantly correlated to the change in the estimated iron content from Eq. 3 ($R^2=0.53$, $p=0.02$). Changes in ferritin alone were not significantly related to changes in iron load ($R^2=0.37$, $p=0.06$).

Discussion

We found that the inclusion of a simple DXA measure of the density of the liver with serum ferritin significantly increased the ability to accurately assess whole-body iron stores and change in iron concentration with time over ferritin measures alone. More than 25% of the variance between ferritin and SQUID measures was accounted for by including the DXA regional measures. Our R3 region showed the most significant differences between the Thal and control subjects. The whole-liver region, R4, showed less striking differences than the midregion R3. DXA is a projection technique, and the overlapping soft tissue above (anterior) and below (posterior) to the liver contribute to the regional measures. R3 was placed at the thickest part of the liver, and this may account for its stronger association than R4 because extending the regional area all the way to the liver edges would most likely proportionally bring in more nonliver soft tissue. These DXA findings are significant, given that in many parts of the world, MRI and SQUID measures are not available for serial measures because of availability, appropriate training, and cost. In addition, DXA can be a valuable tool for assessment of bone health in patients with Thal. Low bone mass and bone loss are prevalent in children with Thal and continues in adulthood with increased bone resorption (20,21). Bone disease in Thal is multifactorial. Undertransfused patients with Thal have marked bone marrow hyperplasia leading to expansion of the medullary spaces

and the typical mongoloid features. In chronically transfused patients, iron overload slows bone formation and reduces mineral deposition because of deposition of iron in the osteoblasts (21). Dysplastic changes of the vertebrae (platyspondyly) occur secondary to iron chelation therapy, especially with high doses of desferioxamine (22). Decreased lumbar BMD has been associated with age of chelation therapy initiation and length of time on chelation therapy (23). Additionally, osteoporosis in male and female patients with Thal is associated with iron-induced hypogonadism and hypothyroidism (24,25).

Di Stefano et al (22) demonstrated that with proper treatment, BMD improves in Thal patients, due primarily to a reduction in endocrinopathies observed. Therefore, the addition of whole-body DXA to acquire the BMC measurement of the rib region could provide a more comprehensive assessment of a patient's iron overload status, while also providing valuable clinical information regarding bone health.

Our study showed that serum ferritin was a poor predictor of LIC. This has been reported previously by many others (7,8,26). Pakbaz et al (8) showed that serum ferritin levels underestimate LIC as measured by SQUID, particularly in nontransfused patients. Earlier, Nielsen et al (26) concluded that the use of serum ferritin for iron quantitation was marginal in patients with iron overload disease (8,26). However, ferritin is still the only practical cost-effective clinical reference. The Italian Society of Hematology gives guidelines to start chelation after 10 units of blood or when ferritin levels exceed 1000 µg/L (27). The Children's Hospital & Research Center Oakland stated in their most recent publication of the "Standards of Care Guidelines for Thalassemia" that chelation therapy should be started after about 1 yr of chronic transfusions that correlates with a serum ferritin of approximately 1000 µg/L (28). Brittenham et al reported that the predicted LIC 95% CI for this example was 0 to 6948 µg Fe/g wet wt. From Table 4, this study showed that for a predicted value of 3474 µg Fe/g wet wt (midrange of the Brittenham et al CI), the confidence interval was from 1276 to 5672 µg Fe/g wet wt. Our confidence range is smaller, most likely because we focused primarily on subjects with Thal, whereas Brittenham et al looked at a mixed population of Thal and sickle cell subjects. The inclusion of DXA with ferritin in Eq. 3 for the same predicted LIC resulted in a smaller similar 95% confidence of 1916 to 5032 µg Fe/g wet wt.

SQUID and MRI measures of hepatic iron storage provide very high accuracy when compared with liver biopsy measures. Brittenham et al (18) reported correlations of $r=0.98$ for SQUID compared with liver biopsy. Anderson et al (29) reported LIC correlations of $r=0.68$ (fibrotic liver) to $r=0.93$ (nonfibrotic liver) for a T2-star MRI protocol compared with biopsy. MRI has the added advantage of being able to estimate myocardial iron depositions. However, these modalities are still difficult to perform in many parts of the world where Thal is prevalent. Our estimated iron content using DXA and ferritin was not as predictive as these reported findings using MRI and SQUID; therefore, these modalities should continue to be used when possible to monitor iron load. When these modalities are not practical, DXA in combination with ferritin is superior to ferritin alone.

Our study had the following limitations. First, our model included a limited number of subjects with substantially higher iron overload concentrations. The highest LIC level in our

cohort was 5524 $\mu\text{g Fe/g wet wt}$ (SQUID), which calculates to an LIC by biopsy of 33.1 mg/g dry weight. Hepatic iron concentrations can be as high as 9000 $\mu\text{g Fe/g wet wt}$ in some disorders such as Blackfan Diamond syndrome or subjects with Thal who are receiving inadequate chelation therapy. Additional subjects with higher hepatic iron loads would need to be studied to insure the relationship holds for very high LIC concentrations. However, most well-chelated patients on chronic transfusion therapy exhibit serum ferritin levels in the range of 1000 to 2000 $\mu\text{g/L}$. Secondly, the equations we derived are only valid for whole-body DXA scans acquired on a Hologic fan beam densitometer with the Apex whole-body software. There is currently no standardization in DXA whole-body BMD and BMC results. Differences as high as 20% in the BMD values are known to exist between Hologic and GE systems (30). Lastly, our cohort was small, and these results should be verified in a larger population and in subjects with disorders other than Thal to insure the generalizability and validity of the relationship. We conclude that when more advanced methods of LIC measurements, such as MRI or SQUID, are not available, DXA can be used to substantially increase the power to predict LIC above what is possible by measuring serum ferritin alone.

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References

1. Zurlo MG, De Stefano P, Borgna-Pignatti C, et al. Survival and causes of death in thalassaemia major. *Lancet*. 1989; 2(8653):27–30. [PubMed: 2567801]
2. Borgna-Pignatti C, Cappellini MD, De Stefano P, et al. Survival and complications in thalassemia. *Ann N Y Acad Sci*. 2005; 1054:40–47. [PubMed: 16339650]
3. Fung EB, Harmatz PR, Milet M, et al. Disparity in the management of iron overload between patients with sickle cell disease and thalassemia who received transfusions. *Transfusion*. 2008; 48(9):1971–1980. [PubMed: 18513257]
4. Vogiatzi MG, Macklin EA, Fung EB, et al. Prevalence of fractures among the Thalassemia syndromes in North America. *Bone*. 2006; 38(4):571–575. [PubMed: 16298178]
5. Bishop N, Braillon P, Burnham J, et al. Dual-energy X-ray absorptiometry assessment in children and adolescents with diseases that may affect the skeleton: the 2007 ISCD Pediatric Official Positions. *J Clin Densitom*. 2008; 11(1):29–42. [PubMed: 18442751]
6. Borgna-Pignatti C, Castriota-Scanderbeg A. Methods for evaluating iron stores and efficacy of chelation in transfusional hemosiderosis. *Haematologica*. 1991; 76(5):409–413. [PubMed: 1806447]
7. Brittenham GM, Cohen AR, McLaren CE, et al. Hepatic iron stores and plasma ferritin concentration in patients with sickle cell anemia and thalassemia major. *Am J Hematol*. 1993; 42(1):81–85. [PubMed: 8416302]
8. Pakbaz Z, Fischer R, Fung E, et al. Serum ferritin underestimates liver iron concentration in transfusion independent thalassemia patients as compared to regularly transfused thalassemia and sickle cell patients. *Pediatr Blood Cancer*. 2007; 49(3):329–332. [PubMed: 17554789]
9. Harmatz P, Butensky E, Quirolo K, et al. Severity of iron overload in patients with sickle cell disease receiving chronic red blood cell transfusion therapy. *Blood*. 2000; 96(1):76–79. [PubMed: 10891433]
10. Telfer PT, Prestcott E, Holden S, et al. Hepatic iron concentration combined with long-term monitoring of serum ferritin to predict complications of iron overload in thalassaemia major. *Br J Haematol*. 2000; 110(4):971–977. [PubMed: 11054091]

11. Houang MT, Arozana X, Skalicka A, et al. Correlation between computed tomographic values and liver iron content in thalassaemia major with iron overload. *Lancet*. 1979; 1(8130):1322–1323. [PubMed: 87781]
12. St Pierre TG, Clark PR, Chua-anusorn W, et al. Noninvasive measurement and imaging of liver iron concentrations using proton magnetic resonance. *Blood*. 2005; 105(2):855–861. [PubMed: 15256427]
13. Brittenham GM, Badman DG. Noninvasive measurement of iron: report of an NIDDK workshop. *Blood*. 2003; 101(1):15–19. [PubMed: 12393526]
14. Chatterton BE, Thomas CM, Schultz CG. Liver density measured by DEXA correlates with serum ferritin in patients with beta-Thalassemia Major. *J Clin Densitom*. 2003; 6(3):283–288. [PubMed: 14514999]
15. Fung EB, Fischer R, Pakbaz Z, et al. The new SQUID biosusceptometer at Oakland: first year of experience. *Neurol Clin Neurophysiol*. 2004; 2004:5. [PubMed: 16012600]
16. Fischer R, Tiemann CD, Engelhardt R, Nielsen P, Dürken M, Gabbe EE, Janka GE. Assessment of iron stores in children with transfusion siderosis by biomagnetic liver susceptometry. *Am J Heme*. 1999; 60:289–299.
17. Fischer, R. Liver iron susceptometry.. In: Andrae, W.; Nowak, H., editors. *Magnetism in Medicine —A Handbook*. Wiley-VCH; Berlin, Germany: 1998. p. 286-301.
18. Brittenham GM, Farrell DE, Harris JW, et al. Magnetic-susceptibility measurement of human iron stores. *N Engl J Med*. 1982; 307(27):1671–1675. [PubMed: 7144866]
19. Fischer, R.; Engelhardt, R.; Nielsen, P., et al. Liver iron quantification in the diagnosis and therapy control of iron overload patients.. In: Hoke, M.; Erne, S.; Okada, Y., et al., editors. *Advances in Biomagnetism: Clinical Aspects*. Elsevier; Amsterdam, The Netherlands: 1992. p. 585-588.
20. Dresner Pollack R, Rachmilewitz E, Blumenfeld A, et al. Bone mineral metabolism in adults with beta-thalassaemia major and intermedia. *Br J Haematol*. 2000; 111(3):902–907. [PubMed: 11122154]
21. Vogiatzi MG, Autio KA, Schneider R, Giardina PJ. Low bone mass in prepubertal children with thalassemia major: insights into the pathogenesis of low bone mass in thalassemia. *J Pediatr Endocrinol Metab*. 2004; 17(10):1415–1421. [PubMed: 15526720]
22. Di Stefano M, Chiabotto P, Roggia C, et al. Bone mass and metabolism in thalassemic children and adolescents treated with different iron-chelating drugs. *J Bone Miner Metab*. 2004; 22(1):53–57. [PubMed: 14691688]
23. Origa R, Galanello R, Ganz T, et al. Liver iron concentrations and urinary hepcidin in beta-thalassemia. *Haematologica*. 2007; 92(5):583–588. [PubMed: 17488680]
24. Origa R, Fiumana E, Gamberini MR, et al. Osteoporosis in beta-thalassemia: clinical and genetic aspects. *Ann N Y Acad Sci*. 2005; 1054:451–456. [PubMed: 16339696]
25. Vogiatzi MG, Macklin EA, Fung EB, et al. Bone disease in thalassemia: a frequent and still unresolved problem. *J Bone Miner Res*. 2009; 24(3):543–557. [PubMed: 18505376]
26. Nielsen P, Gunther U, Durken M, et al. Serum ferritin iron in iron overload and liver damage: correlation to body iron stores and diagnostic relevance. *J Lab Clin Med*. 2000; 135(5):413–418. [PubMed: 10811057]
27. Angelucci E, Barosi G, Camaschella C, et al. Italian Society of Hematology practice guidelines for the management of iron overload in thalassemia major and related disorders. *Haematologica*. 2008; 93(5):741–752. [PubMed: 18413891]
28. Vichinsky, E.; Levine, L.; Bhatia, S., et al. Standards of care guidelines for thalassemia.. In: Levine, M., editor. *Children's Hospital & Research Center Oakland*. Oakland, CA: 2008. http://www.thalassemia.com/SOC_guidelines.html. [June 20, 2010]
29. Anderson LJ, Holden S, Davis B, et al. Cardiovascular T2-star (T2*) magnetic resonance for the early diagnosis of myocardial iron overload. *Eur Heart J*. 2001; 22(23):2171–2179. [PubMed: 11913479]
30. Genant HK, Grampp S, Gluer CC, et al. Universal standardization for dual x-ray absorptiometry: patient and phantom cross-calibration results. *J Bone Miner Res*. 1994; 9(10):1503–1514. [PubMed: 7817795]

Fig. 1. Whole-body dual-energy X-ray absorptiometry images showing the regions investigated. (A) Standard Hologic whole-body regions of analysis. The right rib region is shown in bold. (B) Special regions of interest, R1, R2, R3, and R4, defined for this study using Hologic's subregion software.

Fig. 2.
Plot of predicted superconducting quantum interference device (SQUID) (ferritin and DXA) vs actual SQUID (A) using Eq. 1 and (B) Eq. 3.

Table 1

Demographic Data of Participants at Baseline

Variable	Control N	Control Mean (SD) [range]	Thal N	Thal Mean (SD) [range]	<i>p</i> value (2-sided)
Age (yr)	32	18.1 (6.4) [7.5–30.0]	37	19.2 (9.0) [7.6–43.9]	NS
Weight (kg)	32	57.1 (25.5) [22.9–166.3]	37	46.1 (15.5) [19.4–72.4]	0.04
Height (cm)	32	157.4 (24.6) [53.3–179.4]	37	151.1 (16.2) [114.8–180.5]	NS
BMI (kg/m ²)	32	20.1 (3.2) [14.3–26.1]	37	19.5 (3.8) [12.9–29.0]	NS
Whole-body BMD (g/cm ²)	32	0.98 (0.15) [0.68–1.26]	37	0.84 (0.15) [0.59–1.21]	NS
SQUID (µg Fe/g wet wt)	ND		37	2120.0 (1333.7) [275.0–5524.0]	
Ferritin (µg/L)	ND		37	2129.9 (2115.4) [180.0–8821.0]	
Right rib BMC (g)	32	70.6 (24.5) [27.3–111.4]	37	58.8 (22.5) [21.4–107.3]	0.04
R3 BMC (g)	32	0.7 (0.8) [0.0–3.6]	37	6.1 (5.9) [0.2–28.7]	<0.01

Note: There were no differences in age and height of the control and Thal populations. However, the weight, BMI, right rib BMC and R3 BMC were significantly different.

Abbr: SD, standard deviation; NS, not significant; BMI, body mass index; BMD, bone mineral density; SQUID, superconducting quantum interference device; BMC, bone mineral content, ND, not done.

Table 2

Demographic Data of Participants With a Follow-up Visit

Variable	N	Baseline Mean (SD) [range]	Follow up Mean (SD) [range]
Follow-up time (mo)	10	0	17.3 (8.8) [6–36]
Weight (kg)	10	46.1 (13.6) [28.2–68.7]	50.7 (13.2) [30.2–71.7]
Height (cm)	10	153.2 (13.2) [133.8–180.5]	158.1 (13.5) [138.8–179.0]
BMI (kg/m ²)	10	19.3 (4.1) [14.9–29.0]	20.1 (4.1) [15.7–29.5]
SQUID (µg Fe/g wet wt)	10	2082.6 (1047.0) [860.0–4396.0]	1831.8 (944.5) [769.0–3229.0]
Ferritin (µg/L)	10	2778.7 (2110.0) [421.0–7551.0]	3064.1 (2564.4) [269.0–7280.0]
Right rib BMC (g)	10	61.8 (22.0) [40.8–104.6]	68.2 (22.0) [42.5–108.4]
R3 BMC (g)	10	5.6 (4.6) [0.2–14.7]	5.1 (5.6) [0.2–19.1]

Note: There were no statistically significant differences between baseline and follow-up visits for any of the variables (*p* values all greater than 0.4).

Abbr: SD, standard deviation; BMI, body mass index; SQUID, superconducting quantum interference device; BMC, bone mineral content.

Table 3

Spearman Rank Correlation Coefficients Showing the Association Between the DXA, Ferritin, and SQUID Measures for the Baseline Visit^a

Spearman Correlation Coefficients (<i>p</i> Value)										
	R1 BMD	R2 BMD	R3 BMD	R3 BMC	R4 BMD	R4 BMC	Right rib BMC	Right rib BMD	LnSQUID	LnFERRITIN
R1 BMD	1	0.5 (<0.01)	0.39 (0.02)	0.37 (0.03)	0.22 (0.19)	0.35 (0.04)	0.27 (0.11)	0.23 (0.17)	0.16 (0.36)	0.14 (0.41)
R2 BMD		1	0.08 (0.64)	−0.08 (0.65)	0.03 (0.88)	0 (0.99)	0.21 (0.20)	0.13 (0.43)	−0.14 (0.40)	−0.09 (0.60)
R3 BMD			1	0.60 (<0.01)	0.68 (<0.01)	0.66 (<0.01)	0.72 (<0.01)	0.74 (<0.01)	0.40 (0.01)	0.38 (0.02)
R3 BMC				1	0.24 (0.16)	0.68 (<0.01)	0.45 (0.01)	0.26 (0.13)	0.77 (<0.01)	0.57 (<0.01)
R4 BMD					1	0.67 (<0.01)	0.64 (<0.01)	0.82 (<0.01)	0.15 (0.37)	0.16 (0.33)
R4 BMC						1	0.57 (<0.01)	0.48 (<0.01)	0.51 (<0.01)	0.38 (0.02)
Right rib BMC							1	0.85 (<0.01)	0.25 (0–13)	0.14 (0.42)
Right rib BMD								1	0.17 (0.30)	0.14 (0.40)
LnSQUID									1	0.61 (<0.01)

Note: The *p* values are shown in parentheses.

Abbr: DXA, dual-energy X-ray absorptiometry; SQUID, superconducting quantum interference device; BMD, bone mineral density; BMC, bone mineral content.

^aR1 and R2 were small square regions on either side of the spine between the ribs and the iliac. R3 was a rectangular region over the liver to the right of the midline. Region R4 is an outline of the entire liver. The right rib region of interest is taken from the standard whole-body DXA analysis.

Table 4

Regression Analysis Table for ln(SQUID) Using the Baseline Results^a

Equation	Variable	Variables in Model	Value	R ² Change	Model R ²	Model R ² _{adjusted}	RMSE	Cp	F Value	p Value
(1)	lnFERRITIN	1	0.42	0.35	0.35	0.35	0.58	2.00	18.47	<0.01
	Constant		4.39							
(2)	lnR3BMC	1	0.29	0.48	0.48			6.95	32.56	<0.01
	lnFERRITIN	2	0.23	0.08	0.56	0.53	0.48	3.00	5.95	0.020
(3)	Constant		5.4							
	lnR3BMC	1	0.17	0.48	0.48			16.01	32.56	<0.01
	lnFERRITIN	2	0.22	0.08	0.56			10.71	5.95	0.02
	Weight	3	0.03	0.01	0.57			11.70	0.81	0.37
	Right rib BMC	4	0.02	0.09	0.66	0.62	0.43	5.00	8.70	0.01
	Constant		5.73							

Note: By adding the dual-energy X-ray absorptiometry results of ROI3 BMC or right rib BMC plus weight increased the ability to predict the liver iron load dramatically from using the ferritin alone.

Abbr: RMSE, root mean square error; Cp, Mallows' Cp statistic; BMC, bone mineral content.

^a R² change denotes how each variable improved the correlation coefficient.