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Alterations in hemoglobin and serum 25-hydroxyvitamin D are related before and after weight loss independent of African admixture

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Abstract

African American (AA) and European American (EA) women often exhibit differences in hemoglobin (Hb) and 25-hydroxyvitamin D [25(OH)D], both of which can be altered by calorie restriction leading to weight loss. Given these known differences it is of clinical interest to examine the potential for race-specific, adverse responses to weight loss. Sixty-four overweight (BMI 27–29.9 kg/m²), premenopausal women consumed a standardized, very-low calorie diet to reduce BMI < 25 kg/m². Ancestry informative markers provided estimates of African admixture, an objective mean of expressing race. Blood sampling and anthropometric measures were performed at baseline and upon meeting target BMI. At baseline, in the overweight state, Hb (g/dL) (AA, 11.7 ± 0.9 vs. EA, 12.5 ± 0.8; $p < 0.01$) and 25(OH)D (nmol/L) (AA, 35.7 ± 12.9 vs. EA, 57.0 ± 20.0; $p < 0.01$) were lower in AAs. After weight loss, Hb decreased (AA, -0.5 ± 0.7 vs. EA, -0.4 ± 0.6; $p = 0.48$) to a similar extent among races. Conversely, 25(OH)D increased (AA, 43.4 ± 14.0 vs. EA, 68.2 ± 24.3; $p < 0.01$) though the magnitude of change () was not different (AA, +7.8 ± 13.5 vs. EA, +11.2 ± 16.7; $p = 0.37$) between races. Multiple linear regression revealed a positive association between Hb and 25(OH)D ($r = 0.386$; $p < 0.01$) adjusted for African admixture, testosterone, and body fat%. Path analyses revealed a significant indirect effect of body fat% on Hb through 25(OH)D, $\beta = -0.023$, CI [-0.06, -0.004]. Following 15% weight loss, participants with the largest increase in serum 25(OH)D exhibited the smallest decrease in Hb. Future research should clarify the optimal degree of calorie restriction to stimulate weight loss while mitigating the potential risk of anemia associated with dieting efforts.

Keywords

Adiposity; Dieting; Hormones; Race Disparity

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Introduction

Complications arising from adiposity-induced, low-grade inflammation are thought to contribute to iron dysregulation (Aeberli et al., 2009) and the consequent risk of developing anemia. Though weight loss can alleviate micronutrient absorption difficulties through a decrease in pro-inflammatory cytokines and hepcidin (Ausk & Ioannou, 2008; Tussing-Humphreys et al., 2009), severe calorie restriction can inadvertently decrease hemoglobin (Hb) (Gunga et al., 1996; Reljic et al., 2013). Testosterone (Leenen et al., 1994) and 25-hydroxyvitamin D [25(OH)D] (Rock et al., 2012), both of which are involved in erythropoiesis, can be readily influenced by weight loss. Complementing the known erythrogenic role of testosterone (Gordon et al., 1970), *in vitro* work has shown that 1,25-hydroxyvitamin D increases erythropoietin-receptor expression, and along with erythropoietin, up-regulates hematopoietic stem cell proliferation (Alon et al., 2002). Given that *in vivo* human studies have demonstrated an association between suboptimal 25(OH)D and prevalence of anemia (Yoo & Cho, 2015), it is of interest to evaluate how calorie restriction-induced changes in testosterone and 25(OH)D can influence Hb.

Despite having higher testosterone (Stolzenberg-Solomon et al., 2012) African American (AA) women frequently have lower Hb (Williams, 1981) and lower 25(OH)D (Smith et al., 2015) compared to European American (EA) women. Even when controlling for age, adiposity, and various parameters of iron status, AA women often exhibit mean Hb values ≈ 1.0 g/dL less than EA women (Williams, 1981) and thus may be at greater risk for anemia. This is especially important given that low Hb is an independent predictor of cardiovascular disease (Sarnak et al., 2002) and linked with reduced exercise tolerance and fatigue.

To this end, the current study sought to examine the potential for race-specific, adverse effects (i.e., reduced Hb) to a standardized, very-low calorie diet leading to weight loss. To appropriately investigate phenotypic differences among races, genetic admixture techniques were used to quantitatively evaluate participant ancestry. As such, the interrelationships of Hb, serum 25(OH)D (metabolite reflective of overall vitamin D status), total testosterone, and C-reactive protein (CRP; marker of inflammation) were evaluated before and after weight loss among premenopausal AA and EA women. The following hypotheses were made: 1) AA women would have lower Hb compared to EA women; 2) calorie restriction would decrease Hb; and 3) the magnitude of observed changes in Hb would be greater among AA women compared to EA women.

Methods

Overweight (BMI 27–29.9 kg/m²) AA ($n = 33$) and EA ($n = 31$) women aged 20–45 years reporting normal menstrual cycles and not taking oral contraceptives or any medications known to influence iron, glucose or lipid metabolism volunteered for this study. Further inclusion criteria were: i) nonsmoker; ii) normoglycemic as evaluated by 75-g oral glucose tolerance test; and iii) sedentary as defined by participating in exercise-related activities less than one time per week. All participants provided written informed consent prior to

inclusion. Study procedures were approved by the local Institutional Review Board at University of Alabama at Birmingham.

Procedures

Participants included in this secondary analysis were part of a much larger study designed to evaluate the influence of exercise training on visceral fat regain 1 year after weight loss (Hunter et al., 2010). After initial screening, weight was stabilized ($< 1\%$ fluctuation) over a 4 week period via dietary control, during which body weight was measured 3–5×/week. At baseline, body composition analyses and blood sampling were performed after a 24-hour fast during the follicular phase of the menstrual cycle. Participants were randomly assigned to one of three weight loss interventions: i) diet only; ii) diet +aerobic exercise; or iii) diet +resistance exercise. A very-low, 800 kcal/day diet was prepared by the local Clinical Research Center (CRC) consisting of 20–22% energy as fat, 18–22% as protein, and 58–62% as carbohydrate. Posthoc dietary analyses of average iron intake was ≈ 10 mg/day and ≈ 24 mg/day during the weight loss and weight maintenance phases, respectively. Participants visited the CRC 2×/week for food pick-up and had their body weight measured. Upon reaching a BMI of < 25 kg/m², participants were held in energy balance via dietary control to stabilize weight for an additional 4 weeks. Similar to baseline, body composition and blood sampling were performed in the same manner as previously described following an overnight fast during the follicular phase.

Body composition

Body composition was determined by dual-energy X-ray absorptiometry (DXA) (Prodigy; Lunar Radiation, WI). Scans were analyzed with ADULT software, LUNAR DPX-L version 1.33 (GE Medical Systems Lunar).

Serum assays

Whole blood was collected by venipuncture following an overnight fast. Serum was centrifuged and stored at -80°C until analyses. Hb was measured by coulter method using the Beckman-Coulter hematology analyzer (Beckman Coulter, Inc., GA). Serum 25(OH)D was measured in duplicate by IDS ELISA assay (Immuno Diagnostic Systems, AZ). Mean intra- and inter-assay coefficient of variations (CV) were 6.2% and 4.9%, respectively. Total testosterone was measured via radioimmunoassay in duplicate by Coat-a-Count from Siemens (previously Diagnostic Products Corp, CA). Mean intra- and inter-assay CVs were 5.0% and 4.1%, respectively. CRP was measured in duplicate by high-sensitivity ELISA assay (ALPCO, NH). Mean intra- and inter-assay CVs were 5.6% and 7.8%, respectively.

Genetic admixture

Estimates of genetic admixture were used to account for the ancestral genetic components underlying racial classification, as previously described (Hunter et al., 2010). In short, a panel of 85 ancestry informative markers (AIMs) were genotyped and translated into estimates of African and European American ancestry using the maximum likelihood estimation techniques described by Hanis (Hanis et al., 1986) then implemented into the

ADMIXMAP software (McKeigue, 2000). Admixture estimate ranged from 1–100 for each participant.

Aerobic exercise

Continuous walking/jogging was performed on a treadmill 3×/week, beginning with a 3–5 minute period for warm-up/stretching at each session. Through week 1, continuous exercise was maintained for 20 minutes at ≈67% of maximum heart rate (MHR). Gradually over an 8-week period, exercise intensity and duration increased so that by the final week, exercise was consistently maintained for 40 minutes at ≈80% MHR. Participants cooled down for 3–5 minutes, following cessation of exercise.

Resistance exercise

A brief, 3–5 minutes warm-up was performed on either a treadmill or cycle ergometer followed by light stretching before completing a whole-body resistance training circuit of upper-body and lower-body exercises 3×/week. During the initial 4 weeks of training, one set of 10 repetitions were performed for each exercise separated by a 2 minute rest period. Every 3 weeks strength was re-evaluated to ensure a progressive training load based on ≈80% of one-repetition max.

Statistical analyses

Means and standard deviations were calculated for each weight loss group. Exploratory analyses revealed the days needed to reach target BMI, total weight reduction, and total decrease in body fat% were not different among groups, therefore, group data was pooled then dichotomized by race. A 2×2 analysis of variance with repeated measures (pre- and post-) was used to compare differences for primary variables of interest at baseline and following weight loss. Comparisons of change (deltas; Δ) were made by determining the differences between weight loss and baseline values. Bivariate correlation analyses were used to examine the relationship among variables at baseline, following weight loss, and deltas (occurring with weight loss). Based on simple correlations, a multiple linear regression was used to evaluate the independent effects of 25(OH)D (from baseline to weight loss) on Hb, independent of African admixture, testosterone, and body fat%. Collinearity diagnostics for all variables were within acceptable limits and variable inflation factors for all models were < 1.14. Additionally, the direct and indirect effects of body fat % on Hb through 25(OH)D were assessed using a path-analytic approach. The PROCESS macro for SPSS described by Hayes (Hayes, 2013) was selected as it utilizes a bootstrapping approach which is less influenced by sample size. In short, the bootstrapping procedure is accomplished by taking a large number of samples from the original dataset via random sampling with replacement. The result is an empirically derived sampling distribution of the indirect effect in which the upper and lower bounds of the 95% confidence interval (CI) match the 2.5% and 97.5% points of the sampling distribution. When the CIs do not contain zero, mediation is indicated. Data were analyzed with SPSS (v22; IBM Corporation, NY). Statistical significance was assumed if p -values were ≤ 0.05.

Results

Overview

By design, all participants lost body weight ($p < 0.001$) and body fat% ($p < 0.001$) (Table 1). No significant time by race interactions were observed nor were there differences in the number of days required to reach target BMI ($< 25 \text{ kg/m}^2$) among the weight loss groups: [1] diet only, 154 ± 43 days; 2) diet +aerobic exercise, 150 ± 71 days; 3) diet +resistance exercise, 149 ± 58 days; $p = 0.97$].

Overweight state

Prior to weight loss, AA women had lower 25(OH)D and lower Hb compared to EA women. Total testosterone and CRP were not different between races prior to weight loss. Negative associations were found between African admixture and 25(OH)D ($r = -0.459$; $p < 0.01$), as well as, African admixture and Hb ($r = -0.389$; $p < 0.01$). Positive associations were found between 25(OH)D and Hb ($r = 0.376$; $p < 0.01$) and 25(OH)D and body fat% ($r = 0.254$; $p < 0.05$).

Post-weight loss

Following weight loss, 25(OH)D increased while Hb decreased among both races. Mean values for total testosterone tended to decrease but the change was not significant. A significant time effect ($p < 0.01$) was observed for CRP following weight loss. Negative associations were found between African admixture and 25(OH)D ($r = -0.456$; $p < 0.01$) and Hb ($r = -0.396$; $p < 0.01$). A positive association was found between 25(OH)D and Hb ($r = 0.319$; $p < 0.01$).

Among the changes (), from pre- and post-weight loss, a positive association between 25(OH)D and Hb ($r = 0.302$; $p < 0.05$) was observed while 25(OH)D was negatively associated with body fat% ($r = -0.316$; $p < 0.01$) (Table 2). A multiple linear regression was used to test the independent effects of 25(OH)D on Hb with weight loss. A positive association persisted ($r = 0.386$; $p = 0.007$) after adjusting for African admixture, testosterone, and body fat% (Table 3). Of note, adjusting for weight loss group did not affect the regression model suggesting the changes in Hb were not a product of group allocation/treatment. The PROCESS macro for SPSS (Hayes, 2013), tested the direct and indirect effects of body fat% on Hb through 25(OH)D (Figure 2). Delta body fat% was negatively associated with 25(OH)D, $\beta = -0.71$, 95% CI $[-1.24, -0.17]$. Delta 25(OH)D was positively associated with Hb, $\beta = 0.03$, 95% CI $[0.004, 0.06]$ such that a greater increase in 25(OH)D corresponded with an attenuated decrease in Hb, independent of body fat%. Finally, a negative indirect effect of body fat% on Hb through 25(OH)D was observed, $\beta = -0.023$, 95% CI $[-0.06, -0.004]$. Since the confidence interval did not include zero, mediation was confirmed ($p < 0.05$).

Discussion

The current study revealed that calorie restriction (i.e., 800 kcal/day) leading to significant weight loss decreases Hb in both AA and EA women, and these changes () were

independently associated with 25(OH)D. Simply put, participants with the largest increase in serum 25(OH)D, presumably from reduced adiposity, also exhibited the smallest decrease in Hb. Path analyses revealed a significant indirect effect of body fat% on Hb through 25(OH)D. Since low Hb is an independent predictor of cardiovascular disease (Sarnak et al., 2002) and impairs aerobic capacity, further research should clarify optimal calorie restriction to stimulate weight loss while also mitigating the potential risk of anemia associated with dieting efforts.

Associations between obesity and low 25(OH)D have previously been reported (Aasheim et al., 2008; Lagunova et al., 2009; Snijder et al., 2005). While some debate exists about the exact nature of this association, it is generally believed that 25(OH)D is sequestered in adipose tissue which lowers bioavailability (Wortsman et al., 2000). However, weight loss with a concomitant loss in fat mass, has been shown to increase serum 25(OH)D in a dose-response manner (Rock et al., 2012). From that study, participants who lost >10% of their initial body weight expressed the largest increase in serum 25(OH)D (+12.5 nmol/L). Given the relatively homogenous cohort in the current study, all participants achieved a similar ~15% reduction from their initial body weight which corresponded to a ~23% reduction in body fat. Despite an average increase of 25(OH)D, a notable proportion of AA women from the present study had 25(OH)D levels below the recommended threshold (50 nmol/L) proposed by the Institute of Medicine (Dietary Reference Intakes for Vitamin D and Calcium, 2011). However, bioavailable, rather than total 25(OH)D is the clinically meaningful fraction (Ashraf et al., 2014), and as such, it is not clear whether our participants were actually vitamin D deficient or insufficient. Despite efforts to correct low 25(OH)D (Vieth et al., 2007), a degree of ambiguity exists regarding the appropriate 25(OH)D range (Sorkin et al., 2014). Nevertheless, participants from the current study exhibiting the largest increase in 25(OH)D also had the smallest decrease in Hb, suggesting a shared (albeit limited) role of 25(OH)D concerning erythrocyte regulation. In light of previous work showing 1,25(OH)₂D administration stimulates erythropoietin receptor expression (Alon et al., 2002), the weight loss-induced increase in 25(OH)D, may have expanded bioavailability and thus worked synergistically with erythropoietin to preserve Hb during dieting.

Subclinical inflammation associated with excess adiposity is thought to upset iron availability through an upregulation of hepcidin (Yanoff et al., 2007). Hence, circulating Hb could be affected through a disruption in iron homeostasis. However, we did not find an association before or after weight loss between CRP and body fat%. Recently, Cheng and colleagues (Cheng et al., 2013) proposed a notable degree of comorbidities (e.g., obesity, age) may be obligatory before iron kinetics are adversely affected. Apart from being overweight upon enrollment, our participant sample were relatively young, free of comorbidities, with very few participants exceeding (>3 mg/L) the clinically relevant CRP threshold for cardiovascular disease. Thus, it is unlikely that perturbations in Hb following weight loss were due to elevated CRP.

Given the known erythrogenic properties of testosterone and previous work indicating that weight loss reduces testosterone in overweight women (Leenen et al., 1994), we initially expected that Hb would be affected through this mechanism. To our surprise, we did not find an association between testosterone and Hb before or after weight loss. Recently,

testosterone administration has been shown to upregulate splenic iron release via hepcidin inhibition which increases iron availability for Hb synthesis (Guo et al., 2013). However, only modest changes in testosterone were observed in the present study and it is unlikely that altered testosterone profiles contributed to the observed changes in Hb.

Though the NIDDK has indicated very-low calorie dieting can permit weight loss up to 3–5 lbs./week, average time to reach target BMI ($< 25 \text{ kg/m}^2$) in the present work was ≈ 150 days. Subsequently, participants were held in energy balance for a further 4 weeks before post-weight loss blood samples were taken. It is possible that erythropoiesis may have affected, due in-part to length of calorie restriction, as previous work has shown calorie restriction combined with intense exercise directly suppresses erythropoietin (Gunga et al., 1996). Additionally, Caro et al. (Caro et al., 1981) have previously established that calorie restriction can readily effect erythropoietin production through a decrease in triiodothyronine (T_3). Although erythropoietin and T_3 were not measured in the present study, we have previously shown that energy restriction to 800 kcal/day decreases T_3 (Weinsier et al., 2000). More recently, moderate weight loss ($\approx 6.5\%$) over a 12-month period has been shown to lower T_3 (Agnihotri et al., 2014). Due to the severity of caloric restriction in the present study, body mass ($\approx 15\%$) and body fat% ($\approx 23\%$) were reduced to a greater extent and at a faster rate, thus it seems plausible that Hb could have been affected due to changes in T_3 .

Several limitations arose during this investigation. The noted associations were based on single blood draws before and after weight loss. Causal relationships cannot be made nor can we account for the potential seasonal variations (i.e., sunlight exposure) in the observed outcomes. Given the notable time-frame needed to reach target BMI, we can only speculate that some cheat foods were being consumed during the dieting phase of the investigation. As such, potential variation in nutrient intake over time slightly restricts the extent our findings can be extrapolated to other very-low calorie dieting paradigms. Indeed low dietary iron intake could have contributed to the observed changes in Hb, however, recent work has indicated that depleted muscle glycogen can upregulate hepcidin thus interfering with iron absorption (Badenhorst et al., 2015). Since all participants consumed between ≈ 116 – 124 grams of carbohydrates per day, it is possible Hb could have been altered through this mechanism. However, during initial exploratory analyses there were no detectable differences found between exercising and non-exercising weight loss groups suggesting that changes in Hb were not a product of group allocation/treatment. Given the significant decrease Hb, we cannot definitively rule out the possibility of a ‘new-baseline’ following weight loss. Yet, it should be noted that a large proportion of women, particularly AA, were well-below the widely accepted threshold ($< 12 \text{ g/dL}$) for anemia in non-pregnant women (WHO, 2011). Finally, only total 25(OH)D values were available, future work should examine the relationship between Hb and the bioavailable fraction of 25(OH)D following weight loss.

Novelty Statement

Similar to others, we found that reduced adiposity is related to increased serum 25(OH)D. Importantly though, participants who expressed the largest increase in 25(OH)D after weight

loss also had the smallest decrease in Hb. As shown in Figure 1, 15% of the total variance in Hb was accounted for by 25(OH)D adjusted for African admixture, testosterone, and body fat%. Finally, path analyses revealed a significant indirect effect of body fat% on Hb through 25(OH)D.

Practical Application

Given the observed link between Hb and 25(OH)D before and after weight loss, additional research should determine if vitamin D supplementation can preserve Hb during dieting efforts. Our results indicate very-low calorie dieting may, in some, inadvertently lower Hb though it is unknown if such changes interfere with exercise tolerance and/or engagement in free-living physical activity.

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Figure 1.

Figure 2.

Table 1

Descriptive characteristics and corresponding changes with weight loss.

Variables	African American (n = 33)	European American (n = 31)	Time	P-values	T × R
African admixture (%)	62 ± 15	16 ± 4		< 0.001	
Age (yr)	34 ± 7	34 ± 6		0.58	
Height (cm)	166 ± 6	164 ± 7		0.25	
Body weight (kg)					
Overweight	78.3 ± 4.7	76.5 ± 7.5			
Post-weight loss	66.6 ± 4.6	64.6 ± 7.7	< 0.001	0.24	0.61
Body mass index (kg/m ²)					
Overweight	28.3 ± 1.2	28.2 ± 1.2			
Post-weight loss	24.0 ± 0.9	23.8 ± 1.2	< 0.001	0.48	0.56
Body fat (%)					
Overweight	44.3 ± 4.3	45.6 ± 3.1			
Post-weight loss	33.9 ± 5.0	34.9 ± 4.2	< 0.001	0.25	0.80
Hemoglobin (g/dL)					
Overweight	11.7 ± 0.9	12.5 ± 0.8			
Post-weight loss	11.2 ± 1.1	12.1 ± 0.9	< 0.001	< 0.001	0.48
25(OH)D (nmol/L)					
Overweight	35.7 ± 12.9	57.0 ± 20.0			
Post-weight loss	43.4 ± 14.0	68.2 ± 24.3	< 0.001	< 0.001	0.37
Testosterone (ng/dL)					
Overweight	40.2 ± 29.9	38.2 ± 16.1			
Post-weight loss	37.0 ± 24.3	34.9 ± 15.5	0.17	0.70	0.97
C-reactive protein (mg/L)					
Overweight	3.3 ± 4.3	2.1 ± 1.8			
Post-weight loss	1.8 ± 2.2	1.4 ± 2.2	< 0.001	0.22	0.24

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Based on reference ranges for non-pregnant, premenopausal women according to the World Health Organization: 15 (12 AA) of 64 (23%) participants had moderate-anemia (8.0–10.9 g/dL), 19 (12 AA) of 64 (30%) participants had iron-deficiency anemia (11.0–11.9 g/dL) while 30 (9 AA) of 64 (47%) participants had normal hemoglobin (>12.0 g/dL) after weight loss. Reference values follow: body mass index, 18.5–24.9 kg/m² (normal); 25.0–29.9 kg/m² (overweight); 25(OH)D >50 nmol/L (normal); total testosterone 15–60 ng/dL.

All values are presented as means $\pm$ SD; T $\times$ R, time by race interaction; AA, African Americans.

Table 2

Correlation matrix among the variable changes () from baseline with weight loss ($n = 64$).

Variables	African admixture	Hemoglobin	25(OH)D	Testosterone	CRP	Percent Fat
African admixture	--	--	--	--	--	--
Hemoglobin	-0.117	--	--	--	--	--
25(OH)D	-0.093	0.302 [†]	--	--	--	--
Testosterone	0.113	0.185	0.023	--	--	--
CRP	-0.062	-0.002	0.102	-0.118	--	--
Percent Fat	0.038	-0.123	-0.316 [*]	-0.020	-0.065	--

^{*} Significance at $p < 0.01$;

[†] Significance at $p < 0.05$. , changes from baseline with weight loss; hemoglobin (g/dL); 25(OH)D, 25-hydroxyvitamin D (nmol/L); testosterone (ng/dL); CRP, C-reactive protein (mg/L).

Table 3

Model estimation for changes () in hemoglobin with weight loss.

	Model R	R ²	Slope	Standardized β	Partial r	p -value
Intercept	0.43	0.18	-0.223			
African admixture			-0.221	-0.086	-0.093	0.535
25(OH)D (nmol/L)			0.017	0.403	0.386*	0.007
Testosterone (ng/dL)			0.006	0.156	0.169	0.257
Body Fat (%)			0.030	0.120	0.124	0.407

To independently evaluate plausible factors influencing Hb the following variables were included for analyses: African admixture, 25(OH)D, testosterone, and body fat%
* Significant at $p < 0.01$.