

Reduced-carbohydrate, weight loss diet is associated with greater bone mineral content in obese African-American girls

Lynae J. Hanks¹, Anna L. Newton¹, Ambika P. Ashraf², Orlando M. Gutierrez³ and Krista Casazza^{1*}

Correspondence: kristac@uab.edu

¹Department of Nutrition Sciences, 1720 University of Alabama at Birmingham, 2nd Avenue S, Webb 445, Birmingham, Alabama.

²Department of Pediatrics, CPP 230, University of Alabama at Birmingham, 1604 4th Ave S, Birmingham, Alabama.

³Division of Nephrology, Department of Medicine, University of Alabama at Birmingham, 2nd Avenue S, ZRB 614 Birmingham, Alabama.

Abstract

Background: Postprandial insulinemia the related physiologic response, associated with a high carbohydrate meal, may impact the bone-fat axis. In the context of dietary intervention such metabolic challenges, may impart significant influence. This may be particularly salient in African American females who have exaggerated acute insulin response and high propensity for adipose tissue accrual. The objective of this study was to evaluate the association of a standard- versus reduced-carbohydrate diet on bone mineral content in obese African American females.

Methods: This dietary intervention study included 26 African American girls (≥ 96 th BMI percentile), ages eight to 15 years. All of the meals were provided and tailored to meet the estimated energy requirements in a weight stable (eucaloric, five weeks), followed by a weight loss (1000 kcal deficit, 11 weeks) phase by random assignment to either a reduced- (42%, n=11) or a standard- (55%, n=15) carbohydrate diet for 16 weeks. DXA was used to evaluate body composition at baseline and after each diet phase. The contribution of diet to bone mineral content after each diet phase was evaluated using multiple linear regression analyses, controlling for baseline bone mineral content and fat mass index. The contribution of insulin and osteocalcin (a marker of bone deposition) to bone mineral content were also investigated by diet group at baseline and after the weight stable diet phase.

Results: Dietary carbohydrate proportion significantly contributed to bone mineral content, such that consumption of the reduced-carbohydrate diet was associated with greater bone mineral content after the weight loss phase. After the weight stable phase of the study, both insulin and osteocalcin significantly contributed to bone mineral content ($p < 0.05$) in those on the reduced-carbohydrate diet only.

Conclusions: A reduced relative to standard carbohydrate diet to promote weight loss was associated with increased bone mineral content in obese African American females. Optimization of skeletal maturation processes is an important consideration when designing weight loss interventions among this age group. Our support the need for careful evaluation of macronutrient profile.

Keywords: puberty, diet, weight-loss, insulin dynamics, obesity

Trial registration: NCT01410643

Background

Dietary carbohydrate content is commonly targeted to limit adipose tissue accrual and maintain insulin homeostasis [1]. A greater glycemic, and consequentially insulinemic, response to a high compared to a lower carbohydrate-containing meal enhances lipogenic pathways [1]. The metabolic consequences associated with chronically high carbohydrate load challenge the weight/adiposity homeostatic mechanisms, setting a more permissive stage for adipogenesis. This is likely of paramount importance in puberty when the foundation for body composition trajectory is established. While limiting excess fat mass accrual is important, doing so without disturbing

cumulative optimization of growth-related processes (e.g. skeletal development) remains a challenge. As weight loss in and of itself is associated with bone loss [2], direct impact of weight change during this critical period can be had on skeletal maturation, with potentially unintended consequences. Thus, understanding implications of dietary intervention strategies and influence exerted system-wide is of the essence.

Dietary carbohydrate content may influence individuals with inherently high insulin secretion. Transient insulin resistance, characteristic of the pubertal transition, differentially impacts racial groups, with African American (AA) females more adversely affected [3,4] than European American (EA) females.

Such changes in insulin dynamics provide a mechanism for facilitating rapid somatic growth [5]. However, the anabolic properties of insulin essential to support maturation may be impaired in the setting of chronic hyperinsulinemia, where there is exaggerated fat mass accrual, leading to impaired bone integrity [6]. AA females have been consistently observed to have higher insulin concentration and greater adiposity relative to EA counterparts, although AA have historically been identified as “protected” from poor bone health parameters. Contradicting this axiom, a recent report indicates that despite having higher bone mineral content (BMC), AA have exponentially increased fractures rate across multiple anatomical sites [7]. Similarly, type 2 diabetic individuals have greater fracture prevalence as well as delayed healing time relative to non-diabetics [8,9] suggesting perturbations in insulin homeostasis may adversely affect bone integrity. A dietary strategy to improve insulin dynamics via carbohydrate reduction and consequent decreased insulin response may be beneficial to the unique metabolic and growth characteristics of AA females.

The extent to which the metabolic response to manipulations in macronutrient dietary intake influences the bone-fat axis during growth has not been explored. As both dietary composition and adipogenic pathways can impact skeletal maturation, understanding the relationship during puberty is essential, particularly among AA females. Therefore, the objective of this study was to evaluate the association of a standard- versus reduced-carbohydrate diet on BMC, with consideration of the influence of insulin and bone deposition in obese AA females.

Methods

Participants included were 26 obese AA girls $\geq 96^{\text{th}}$ BMI percentile, ages eight to 15 years, and pubertal stages Tanner II to V [10,11]. Exclusion criteria included medical diagnoses of diabetes, cardiac diseases, and/or taking medications known to affect body composition, metabolism, cardiac function, etc. Girls were recruited to participate in a weight loss study through newspaper advertisement, distribution of flyers (posted at various community partnerships), and word of mouth. The nature, purpose, and possible risks and benefits of the study were carefully explained to the girls and parent(s), and informed assent and consent, respectively, were obtained. The protocol was approved by the Institutional Review Board (IRB) for human subjects at the University of Alabama at Birmingham (UAB). All measurements were performed at the Clinical Research Unit (CRU) and the Department of Nutrition Sciences (DNS) at UAB between 2008 and 2009.

Protocol

Participants were block randomized to one of two diets which they remained on for the duration of the study: reduced- or standard-carbohydrate diet as previously described [12]. Briefly, the intervention diet comprised 42% and 40%, and

the intervention diet comprised 55% and 27% of energy from carbohydrate and fat, respectively. Each group received ~18% of energy from protein, and <10% of fat was saturated. The 16-week intervention included two phases: a five-week weight stable, and an 11-week weight loss (1,000 kcal deficit) phase. All food was provided for both phases of the study, with daily caloric needs determined via indirect calorimetry, multiplying assessed resting energy requirements by an activity factor of 1.2.

At baseline, participants attended two visits. The first included a physical examination including pubertal staging by the study pediatrician, physical activity questionnaire distribution, and whole-body dual-energy x-ray absorptiometry (DXA) scanning. For the second visit, participants arrived to the CRU in the fasted state in the morning for metabolic testing. After resting for 30 minutes, participants underwent indirect calorimetry. Participants were also provided meals according to the randomized diet groups, marking the beginning of the weight stable phase. Participants were weighed twice per week throughout to ensure weight stability, with changes exceeding two kg from baseline resulting in caloric modification to maintain weight. At the end of the weight stable phase, participants repeated the DXA scan and indirect calorimetry. During the 11-week weight loss phase, participants received a 1,000 calorie/day deficit. Participants continued biweekly weight evaluation to ensure compliance, with changes of >two kg requiring dietary modification (although this was not necessary in any case). Upon completion of the weight loss phase, an additional DXA scan was performed. Girls were encouraged to maintain baseline level of physical activity for the duration of the study.

Anthropometric assessment

The same registered dietitian obtained anthropometric measurements on all of the participants, obtaining weight to the nearest 0.1 kg (Scale-tronix 6702W; Scale-tronix, Carol Stream, IL) and height using a digital stadiometer (Heightronic 235; Measurement Concepts, Snoqualmie, WA) in minimal clothing without shoes. BMI percentile was calculated using Centers for Disease Control and Prevention growth charts based on these measurements (<http://apps.nccd.cdc.gov/dnpabmi>).

Pubertal Status

The Tanner staging method according to the criteria of Marshall and Tanner [10,11] was used to characterize pubertal status, which has been demonstrated to be a reliable indicator of pubertal development [13]. The staging is based upon direct observation by a pediatrician, and differentiates among the five stages of maturity according to both breast and pubic hair development, with one composite number assigned representing the higher of the two values.

Body Composition

Whole-body composition was measured by DXA using a GE

Lunar Prodigy densitometer (GE LUNAR Radiation, Madison, WI). Participants were scanned in light clothing while lying flat on their backs with their arms at their sides. Girls not fitting within the scanning box were right-sided hemi-scanned with the left side estimated as per instrument protocol.

Resting energy expenditure

REE was determined in the fasted state upon arrival in the morning of the outpatient visit (Deltatrac, Sensormedics Corp, Yorba Linda, CA) in the PCIR. The instrument was calibrated before each test against standard gases. Before beginning testing, girls rested in attempt to return to "basal" state. During testing, all of the subjects were instructed to lie still while remaining awake. A canopy hood was used to collect expired air for 20 minutes after a 10-minute equilibration period, and oxygen consumption and carbon dioxide production were measured continuously during this time. After completion, REE was calculated using the equation of Weir [14].

Assay of metabolites

Fasting insulin and osteocalcin were evaluated and included as covariates due to a mitogenic role in adipogenesis and osteogenesis. Insulin is integral in various growth-related processes, including osteoblast transcription [15]. Osteocalcin, a protein secreted exclusively by osteoblasts and coordinator of bone with energy homeostasis [16], served as a proxy of bone deposition. Fasting insulin and osteocalcin values were derived from sera obtained during the baseline visit during metabolic testing. Insulin was analyzed using a Tosoh A1A 1800 Automated Immunoassay Analyzer (Tosoh Bioscience, South San Francisco, CA). Assay sensitivity is 15.42 pmol/L, mean intraassay coefficient of variability is 4.69%, and interassay coefficient of variability is 6.0%. Osteocalcin was analyzed by radioimmunoassay with the intra-assay and inter-assay coefficients of variation of 5.36 and 5.76%, respectively.

Statistical analysis

Differences in descriptive statistics and independent variables by diet group at baseline and after each phase were examined by t-test. The associations of diet group and BMC at week five (post weight stable phase) and at week 16 (post weight loss phase) were evaluated using multiple linear regression analyses. All models were adjusted for baseline BMC z-score, fat mass index (percent fat divided by height), and pubertal stage, as well as dietary calcium intake. Fasting insulin and osteocalcin levels were available at baseline and immediately following the weight-stable phase. Accordingly, the association of osteocalcin and insulin to BMC was evaluated across diet groups at baseline and post-weight stable phase of the study. To conform to the assumptions of linear regression, all of the statistical models were evaluated for residual normality and logarithmic transformations were performed when appropriate. Log transformation of BMC was used to account for the curvilinear relationship observed between bone mineral and

Table 1. Baseline demographics, clinical and diet characteristics (mean, standard error).

	Total Sample (n=26)	Standard-Carbohydrate (n=15)	Reduced-Carbohydrate (n=11)
Age (yrs)	11.9 ± 0.3	12.1 ± 0.4	11.6 ± 0.5
Pubertal stage	4.1 ± 0.2	4.0 ± 0.3	4.3 ± 0.3
Height (in)	62.3 ± 0.7	61.6 ± 0.8	63.1 ± 1.1
Weight (lbs)	192.5 ± 9.7	189.4 ± 14.6	196.3 ± 13.1
BMI percentile	98.2 ± 0.2	97.8 ± 0.3	98.6 ± 0.2 ^a
BMC z-score	1.5 ± 0.2	-0.1 ± 0.2	0.2 ± 0.3
BMC (mg)	2,290 ± 99.9	2,225.8 ± 120.2	2,378.9 ± 173.2
% Fat	43.9 ± 1.0	44.5 ± 1.1	43.2 ± 1.8
Osteocalcin (ng/mL)	6.6 ± 0.7	7.0 ± 0.9	5.9 ± 1.2
Insulin (microunit/mL)	14.9 ± 2.2	14.6 ± 3.6	15.3 ± 1.1
Energy Intake (kcal/d)	1,753 ± 74	1,779 ± 109	1,717 ± 98
Calcium Intake (mg/d)	706 ± 40	715 ± 59	694 ± 53

BMC=bone mineral content; ^aP < 0.05 for difference between diet groups.

body size [17,18]. Statistical significance was determined at $\alpha=0.05$. All of the data were analyzed using SAS 9.2 software (SAS Institute, Cary, NC).

Results

Descriptive statistics at baseline are presented in **Table 1**. There were no significant differences between diet groups. **Table 2** represents post-intervention values of independent variables. The only difference between groups was that of calcium intake, which was significantly less among the standardized diet group in both the weight stable and weight loss phase ($p<0.0001$).

Multiple regression analyses identified an independent effect of diet group on BMC, such that BMC was greater among those on the reduced-carbohydrate diet after 16 weeks ($\beta=0.09$; $p=0.02$), despite no significant effect after only five weeks of the weight stable phase ($\beta=0.04$, $p=0.70$). At baseline, insulin and osteocalcin were not associated with BMC in either diet group. However, after the weight stable phase of the study, both insulin and osteocalcin significantly contributed to BMC ($p=0.04$; $p=0.03$, respectively) in those on the reduced-carbohydrate diet; whereas neither were significant in the standard diet group.

Discussion

Recent investigations suggest the protective effects of bone strength among AA display evidence of compromise, even though a higher BMC in AA has remained consistent [7]. A potential mechanism explaining contemporary reports of compromised bone health in AA could be the added insult of puberty-related perturbations in insulin dynamics, which is more severe in AA females [7]. As the main dietary component regulating insulin homeostasis, a reduction in carbohydrate content of the diet could optimize body composition changes as well as bone mineralization. Our main finding in this study is that BMC was greater among those on a reduced-carbohydrate,

Table 2. Post-intervention independent variables.

Intervention	Standard-Carbohydrate (n=15)		Reduced-Carbohydrate (n=11)	
	Weight stable	Weight loss	Weight stable	Weight loss
Phase				
Height (in)	61.7 ± 0.8	62.4 ± 0.7	63.4 ± 1.1	63.6 ± 1.1
Weight (lbs)	180.5 ± 15.1	175.4 ± 13.7	190.7 ± 13.2	186.0 ± 14.0
BMC z-score	-0.1 ± 0.2	-0.2 ± 0.2	0.2 ± 0.3	0.3 ± 0.3
BMC (g)	2,260.4 ± 121.9	2,290.7 ± 111.6	2,434.6 ± 176.5	2,549.4 ± 146.8
% Fat	43.9 ± 1.2	41.4 ± 1.4	42.8 ± 5.4	41.0 ± 4.8
Osteocalcin (ng/mL)	7.6 ± 1.1	N/A	6.8 ± 1.7	N/A
Insulin (microunit/mL)	16.5 ± 2.9	N/A	21.4 ± 2.4	N/A
Energy Intake (kcal/d)	2,020 ± 74	1,047 ± 66	2,055 ± 67	1,055 ± 67
Calcium Intake (mg/d)	733 ± 30 ^a	377 ± 23 ^a	1,168 ± 71	636 ± 69

Weight stable phase, five weeks; weight loss phase, 11 weeks; BMC=bone mineral content; aP < 0.01 for difference between diet groups; N/A=measures not available.

weight loss diet after 16 weeks, despite no significant change in BMC after five weeks of the weight stable phase. During periods of growth, there is high remodeling rate with the balance between resorption and formation being anabolic, so that there is net bone formation. This intricate ongoing bone remodeling process during growth and development is critical for optimizing peak bone mass and strength; thus any metabolic impairments can affect bone integrity through multiple, interconnected pathways leading to greater bone fragility. Our results suggest that a reduced-carbohydrate diet during the peri-pubertal period may be beneficial to long-term bone health.

In general, insulin sensitivity characteristically increases prior to the pubertal transition, progressively decreases from the onset and throughout the pubertal period, and rebounds toward the end of the maturation process [4]. This normal physiological response is thought to promote growth and resource partitioning via effects on energy metabolism. In addition, peak bone mass, a major determinant of adult bone health is largely achieved by the end of reproductive maturity. Approximately 28% of adult BMC is accrued during the two-year period surrounding peak BMC velocity [19]. The transient puberty-related hyperinsulinemia stimulates not only osteoblastic cell differentiation, but also (through insulin receptor substrate activation) osteoblastogenesis and thus plays a pivotal role in bone metabolism [5,9]. It is speculated that chronic hyperinsulinemia and consequential impairment in insulin homeostasis uncouples the dynamic process giving rise to impaired bone mass phenotype [20]. Germane to the present study, there has been consistent demonstration of impaired bone phenotype and subsequent increased fracture/osteoporosis risk in individuals with T1DM and T2DM [21]. However, it is not currently understood whether the effects of impaired glucose homeostasis on fracture risk are due to changes in bone structure (*i.e.* activation of inflammatory

processes favoring resorption), inherent properties of bone tissue (e.g. via mechanisms associated with impaired insulin cascade) or both [6,9,15,17]. Nonetheless, macronutrient composition of diet via influence of insulin homeostasis during the pubertal period may have particular clinical significance to bone (re)modeling.

In addition to the contribution of insulin, the osteoblast-derived protein osteocalcin may in part govern mitogenic effects on bone development. Osteocalcin is a regulator of energy metabolism exerting effects on the pancreas (insulin secretion) and adipose tissue (insulin sensitivity) [16,22]. Although a negligible presence exists in the literature concerning the interrelation of osteocalcin and insulin in children, it is plausible that interplay of these factors may dictate the influence of excess adiposity on bone maturation during the pubertal years. Only among those on the reduced carbohydrate diet, insulin and osteocalcin positively associated with BMC after only five weeks of dietary intervention. Our findings are in concordance with that of Pollock *et al.*, involving the association between insulin sensitivity and circulating osteocalcin [23], showing higher osteocalcin levels associates positively with insulin sensitivity. Although absolute concentration between diet groups did not differ, the physiologic response to macronutrients elicits differential impact upon bone phenotypes.

Robust measurement for assessment of metabolism and physiology as well as provision of all food for the duration of the study served to strengthen this study. Although 100% compliance cannot be assured, bi-weekly weigh-ins helped to increase adherence to the diet. Nevertheless, as a pilot study, a small sample size and inclusion of only one racial/ethnic group limited our findings. Another recognized limitation of the study is the difference in dietary calcium of the two diets. Although all analyses controlled for calcium intake, the biological significance of the differing quantities cannot

wholly be accounted for statistically. Both the diet groups provided lower than required amounts set forth for calcium; however, those on standard- had significantly lower levels than those on reduced- carbohydrate diet, which likely played a role in the lowered BMC among that group. Thus, future investigations targeting dietary carbohydrate effect on bone should ensure fulfilling individual micronutrient requirements, particularly those integral to bone development. In addition, the findings from this study may not be generalizable to non-obese subjects. Inclusion of multiple races/ethnicities and individuals with a wider range of body habitus would add valuable insight into the reported findings.

Conclusions

A reduced- relative to standard-carbohydrate diet to promote weight loss was associated with differences in metabolic bone parameters, with improved BMC in obese AA females. Indeed, early onset obesity has profound implications for long-term health. However, efforts to improve metabolic and obesity-related phenotypes, require consideration of the involvement of different, yet partially overlapping sub-systems which converge during puberty. These findings warrant future studies regarding assessment of changes within bone in response to dietary macronutrient profile during growth and development.

Competing Interests

The authors declare that they have no competing interests.

Authors contributions

LH conceived the analysis, participated in statistical analysis and drafted the manuscript. KC is the Principle Investigator of the study, participated in the statistical analysis, contributed to the construction of the manuscript draft and provided critical review. AN participated in construction of the manuscript draft and provided critical review. AA provided critical review of the draft. OG reviewed the statistical analyses and gave critical review of the draft. All authors read and approved the final manuscript.

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