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ORIGINAL ARTICLE

Effects of habitual diet on ethnic differences in serum total ghrelin

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Abstract Ghrelin, an orexigenic hormone, may be involved in the etiology of obesity. African Americans (AA) experience higher obesity rates than European Americans (EA), but it is unclear whether ghrelin differs with ethnicity. This study was designed to compare ghrelin concentrations between overweight AA and EA adults in a post absorptive state, in response to a standard meal, and after 8-week habituation to diets of differing macronutrient profiles. Sixty-one overweight men and women (31 EA and 30 AA) were assigned to either a higher-carbohydrate/lower-fat diet (55 % CHO, 18 % PRO, 27 % FAT) or a lower-carbohydrate/higher-fat diet (43 % CHO, 18 % PRO, 39 % FAT) for 8 weeks. At baseline and week 8, participants ingested a standard liquid mixed meal. Blood was sampled before the meal and serially after ingestion to measure total ghrelin and insulin. Hunger was assessed with a visual analog scale. Composite scores for ghrelin, insulin, and hunger were calculated as area under the curve (AUC), and ghrelin suppression was calculated as the change from fasting concentration. Fasting ghrelin and ghrelin AUC were higher among EA at baseline and week 8 ($p < 0.001$), and these differences were not affected by diet habituation. Despite greater postprandial ghrelin suppression, EA displayed greater hunger immediately following the test meal ($p < 0.05$). Overweight EA displayed higher circulating ghrelin and greater ghrelin suppression compared to AA. Further study is warranted to explore the physiological basis for these ethnic differences and to determine whether they may relate to higher obesity rates among AA.

Keywords Ghrelin · Hunger · Ethnicity · African American

Clinical trials registration This study is registered on www.clinicaltrials.gov (ClinicalTrials.gov ID: NCT00726908).

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Introduction

Ghrelin is a peptide hormone known to stimulate hunger and food intake [1]. Rising obesity rates nationwide [2] have prompted the pharmaceutical industry to pursue development of anti-obesity drugs that would target ghrelin action [3, 4]. African Americans (AA) are more prone to obesity than European Americans (EA) [2], suggesting that the underpinnings of obesity may differ between these two groups. Thus, it is of interest to determine whether the physiology of ghrelin may also differ with ethnicity.

Circulating ghrelin is thought to be regulated in part by circulating insulin, as inverse associations between ghrelin and insulin have been reported, and insulin infusions have been shown to decrease ghrelin concentrations [5–7]. Previous studies have identified ethnic differences in post-challenge insulin, with AA displaying higher peak insulin levels compared to EA [8–10]. However, it is unclear whether a higher acute insulin response among AA may be associated with ethnic differences in postprandial ghrelin concentrations.

Ghrelin is also acutely influenced by diet composition. Studies examining effects of one-time test meals have reported that carbohydrate induces the most potent ghrelin suppression, whereas lipids have the weakest effect on suppression [11–14]. However, whether ghrelin response to

habitual macronutrient intake differs between EA and AA has not been investigated.

The primary aims of this study were to determine whether fasting ghrelin and ghrelin response to a standard liquid mixed meal would differ between EA and AA and whether these responses would change after 8-week habituation to diets differing in macronutrient composition. Secondary aims were to determine whether ethnic differences in ghrelin would correspond to circulating insulin and/or subjective hunger.

Methods

Participants

Participants were 61 sedentary men and women, ages 21–49, recruited by newspaper and electronic media advertisements in Birmingham, Alabama. Forty-nine percent of subjects were AA and 51 % were EA. Ethnicity was assessed by self report during a telephone screen and a medical history questionnaire. All participants were overweight but otherwise healthy, with body mass index (BMI) 25.0–45.5 kg/m^2 and reported weight change no greater than ±2.3 kg within the previous 6 months. Exclusion criteria included any disorders of lipid or glucose metabolism, any medication known to affect body composition (including oral contraceptives, cholesterol medications, or blood pressure medications), exercise >2 h per week, tobacco use, and irregular menstrual cycles. All participants provided written consent, and the protocol was approved by the Institutional Review Board for Human Use at the University of Alabama at Birmingham (UAB).

Protocol

After a 3-day run-in phase on a standard diet (55 % CHO, 18 % PRO, 27 % FAT), subjects were provided with all food for either a higher-carbohydrate/lower-fat (High-CHO/Low-FAT; 55 % CHO, 18 % PRO, 27 % FAT) diet or a lower-carbohydrate/higher-fat diet (Low-CHO/High-FAT; 43 % CHO, 18 % PRO, 39 % FAT) for 8 weeks (Fig. 1). Participants were assigned in blocks to one of the two diet groups as previously described [15], and they were blinded to their diet assignment for the duration of the study. Both diets were comprised of foods known to be frequently consumed within our geographic location [16], and sample menus of specific food items have been previously published [15]. Details about carbohydrate, glycemic loads, and fatty acids provided by the respective diets are described in Table 1. Registered dietitians at the General Clinical Research Center (GCRC) calculated energy needs for weight maintenance using the Harris Benedict

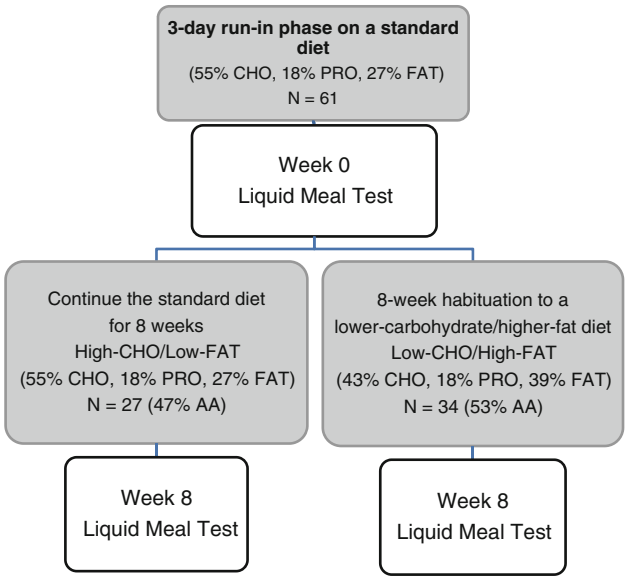

Fig. 1 Outline of the diet intervention. *CHO* carbohydrate, *PRO* protein, *AA* African American

formula [17] with an activity factor of 1.35 for women and 1.50 for men. All food was provided to participants by the GCRC Bionutrition Kitchen.

At baseline and week 8, each participant completed a liquid meal test following a 12-h overnight fast. Concentrations of total serum ghrelin and insulin from blood samples collected at −15 and −5 min before each meal test were averaged to yield fasting values. Participants ingested a standard liquid meal (Carnation Instant Breakfast and whole milk; 7 kcal/kg, 58.6 % CHO, 17.4 % PRO, 24 % FAT) within a 5-min period, and blood samples were collected at 15, 60, 90, 120, 180, and 240 min. At each of these time points, participants marked a 100 mm visual analog scale to rate their hunger [18].

Each weekday morning, body weight was recorded to the nearest 0.1 kg at the GCRC. Total fat mass, lean mass, and percent body (%Fat) were determined at baseline and 8 weeks by dual-energy X-ray absorptiometry (Lunar iDXA; GE Healthcare, Madison, WI; software version 12.3).

All analyses were conducted in the Core Laboratories of UAB's Nutrition Obesity Research Center, Diabetes Research and Training Center, and GCRC. Serum total ghrelin was measured in duplicate 20 µl aliquots by enzyme-linked immunoabsorbent assay (ELISA; Millipore Corporation; Billerica, MA). Mean intra-assay coefficient of variation (CV) was 7.10 %, and mean inter-assay CV was 5.98 % for this assay. Serum samples were pretreated with Pefabloc protease inhibitor (Roche Diagnostics; Mannheim, Germany) and DDP-IV protease inhibitor (Millipore Corporation; Billerica, MA). Fasting glucose was measured in 3 µl sera by a glucose oxidase method

Table 1 Diet composition for a sample energy level of 1800 kcal/d

	Total CHO (g)	Fiber (g)	Glycemic load[a] (points/1,000 kcal)	SFA (%)	MUFA (%)	PUFA (%)	ω-3FA (%)
High-CHO/Low-FAT	250	17	≥75	8.6	9.7	6.6	0.8
Low-CHO/High-FAT	187	22	≤45	11.9	14.2	11.1	1.5

Kcal kilocalories, *CHO* carbohydrate, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *ω-3FA* omega-3 fatty acids

[a] Glycemic load relative to glucose was calculated per 1,000 kcal to insure proportionate glycemic loads within diet groups

(Stanbio Sirrus analyzer; Stanbio Laboratory, Boerne, TX). The mean intra-assay CV for this assay was 1.21 %, and a mean interassay CV was 3.065 %. Insulin was measured in 50 µl aliquots by immunofluorescence (TOSOH AIA-II analyzer; TOSOH Corporation; South San Francisco, CA). Mean intra-assay CV and inter-assay CV for this analysis were 1.49 and 4.42 %, respectively.

Statistical analysis

Variables with non-normal distributions were log transformed for analyses (insulin, glucose, and lean mass). Chi-square tests were used to evaluate group differences in categorical variables, Mann–Whitney *U* tests were used to identify differences in age and fasting hunger as these variables were non-normally distributed after log transformation, and independent t-tests were used for group comparisons of all other variables. Area under the curve (AUC) was calculated by the trapezoidal method [19] for ghrelin, insulin, glucose, and hunger. Comparisons of AUC composites and fasting concentrations were evaluated by repeated measures ANOVA (RM-ANOVA) with diet and ethnicity as between-subjects factors. Paired *t* tests were also used to examine changes within diet and ethnic groups from baseline to 8 weeks for ghrelin, insulin, glucose and each AUC composite. ANCOVA was used to identify differences in ghrelin between groups with adjustment for diet, sex, insulin, %Fat. Ghrelin suppression was calculated as the value at each time point subtracted from the fasting concentration. Pre- and post-intervention comparisons of ghrelin suppression within group were assessed by non-parametric Wilcoxon signed ranks tests, while between-group comparisons were made by Mann–Whitney *U* tests. All analyses were two-sided with a Type I error rate of 0.05 and were performed using SPSS (version 19.0; Chicago, IL) and GraphPad Prism (version 5.0; La Jolla, CA) software.

Results

Participant characteristics are displayed as mean ± SD in Table 2 by ethnicity. The two groups were similar with the exception of higher fasting ghrelin among EA ($p < 0.001$). Despite provision of kilocalories for weight maintenance, study participants on average experienced slight weight change over the 8-week study period (−1.04 ± 1.51 kg; not shown). Weight change did not differ between diet or ethnic groups, nor was weight change correlated with any variable of interest.

Figure 2 displays differences in ghrelin between EA and AA. Both before and after the diet intervention, fasting ghrelin was higher among EA ($p < 0.001$), as was post-prandial ghrelin for every time point and AUC (Fig. 2).

Table 2 Baseline characteristics (mean ± SD or %)

	European American ($n = 31$)	African American ($n = 30$)	*p* value	*p* value adjusted for %Fat
Sex (% male)	54.8	40.0	0.183	
Diet (% Low-CHO/High-FAT)	58.1	53.3	0.455	
Age (years)	35.8 ± 8.0	34.4 ± 8.7	0.419	
Weight (kg)	96.3 ± 19.5	101.0 ± 18.8	0.339	
BMI (kg/m^2)	31.7 ± 3.9	32.9 ± 4.5	0.249	
Fat mass (kg)	38.5 ± 9.3	40.1 ± 8.3	0.479	
Lean mass (kg)	56.0 ± 13.6	57.0 ± 15.2	0.833	
Percent fat (%)	39.6 ± 6.4	40.4 ± 7.1	0.641	
Fasting ghrelin (pg/mL)	844.1 ± 304.9	524.5 ± 220.7	**<0.001**	**<0.001**
Fasting insulin (µU/mL)	11.1 ± 5.2	9.6 ± 6.4	0.238	0.249
Fasting glucose (mg/dL)	101.0 ± 10.9	98.2 ± 10.2	0.306	0.327

BMI body mass index, *Low-CHO/High-FAT* lower-carbohydrate/higher-fat diet

Bold values indicate statistically significant

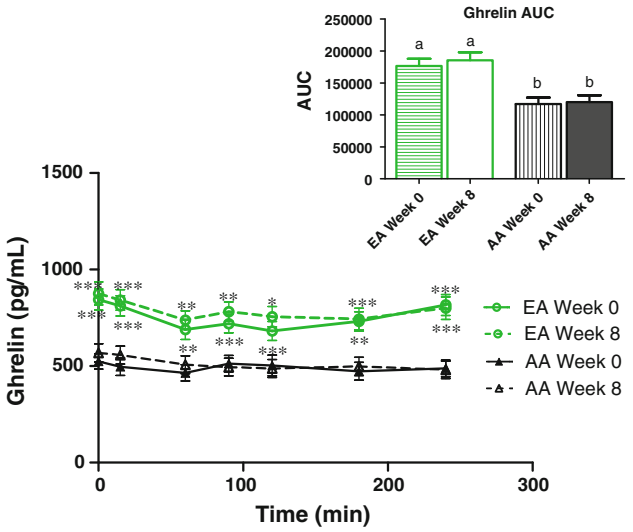

Fig. 2 Time courses for serum total ghrelin. *AUC* area under the curve, *EA* European American, *AA* African American, Ghrelin was higher among EA at every time point at week 0 and week 8 (***$p < 0.001$, **$p < 0.01$, *$p < 0.05$). Ghrelin AUC was greater among EA at week 0 and week 8 (**a**, **b**). Significant group differences at $p < 0.001$

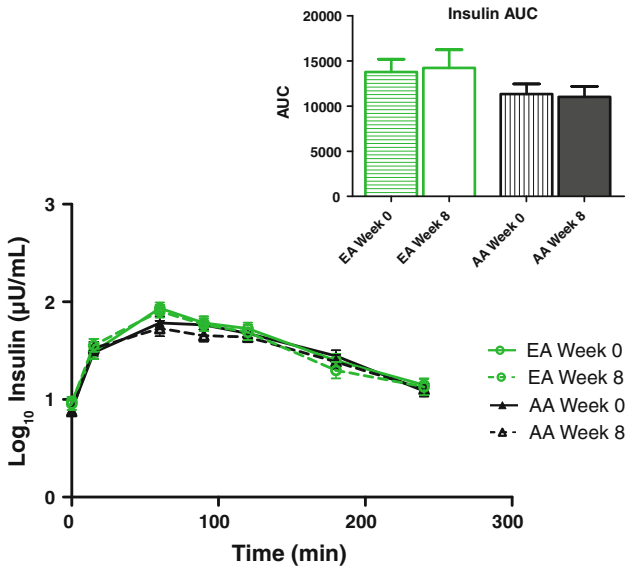

Fig. 3 Time courses for serum insulin. *AUC* area under the curve, *EA* European American, *AA* African American neither fasting insulin nor insulin AUC differed between ethnic groups at week 0 or week 8

Ghrelin concentrations did not differ by sex, and ethnic differences in ghrelin were independent of diet, sex, insulin, and percent body fat (Table 3). Repeated measures ANOVA revealed a main effect of ethnicity on fasting ghrelin and ghrelin AUC, but no effect of diet nor an ethnicity × diet interaction.

Figures 3 and 4 show post-meal responses of insulin and glucose. RM-ANOVA showed no effects of ethnicity or diet nor significant ethnicity*diet interactions for fasting insulin, insulin AUC (Fig. 3), or glucose AUC (Fig. 4). Results were similar after adjustment for percent fat (data not shown).

Table 4 displays postprandial changes in ghrelin relative to fasting at weeks 0 and 8. Ghrelin suppression at minutes 60–120 was significantly lower in AA versus EA at week 0 ($p < 0.05$) but not week 8. Within EA, ghrelin suppression did not change at any time point from week 0 to 8; however, ghrelin suppression among AA at week 8 tended to be greater than week 0 at minutes 90 ($p = 0.05$) and 240 ($p = 0.06$).

Figure 5 illustrates reported hunger over the span of the test. No effect of diet group on hunger was observed, but both EA and AA reported greater fasting hunger at week 8 compared to week 0 ($p = 0.009$ for EA and $p = 0.003$ for AA). Fasting hunger and hunger AUC did not differ between ethnic groups, but EA reported greater hunger than AA one hour after test meal at week 0 ($p = 0.027$) and at minutes 15 of both meal tests ($p = 0.017$ at week 0 and $p = 0.040$ at week 8).

Discussion

Little is known about ethnic differences in circulating ghrelin. This study was designed to examine whether

Table 3 Ethnic differences fasting ghrelin and ghrelin response to the standard test meal (mean ± SD)

	European Americans ($n = 31$)	African Americans ($n = 30$)	p value (unadjusted)	p value (adjusted for sex, diet, insulin, and percent body fat)
Week 0				
Fasting ghrelin (pg/mL)	844.1 ± 304.9	524.5 ± 220.7	<0.001	<0.001
Ghrelin AUC	176,809 ± 62052	117,124 ± 56246	<0.001	<0.001
Week 8				
Fasting ghrelin (pg/mL)	876.0 ± 331.9	568.7 ± 258.9	<0.001	<0.001
Ghrelin AUC	185,563 ± 68958	120,184 ± 57524	<0.001	<0.001

AUC area under the curve mean ± SD

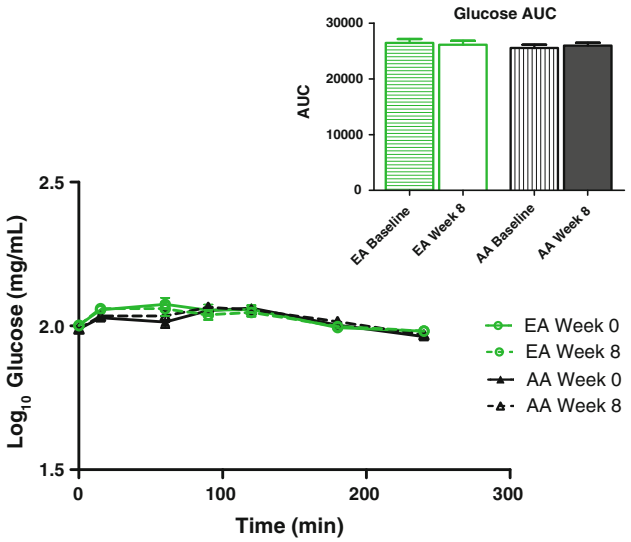

Fig. 4 Time courses for serum glucose. Neither fasting glucose nor glucose AUC differed between ethnic groups at week 0 or week 8

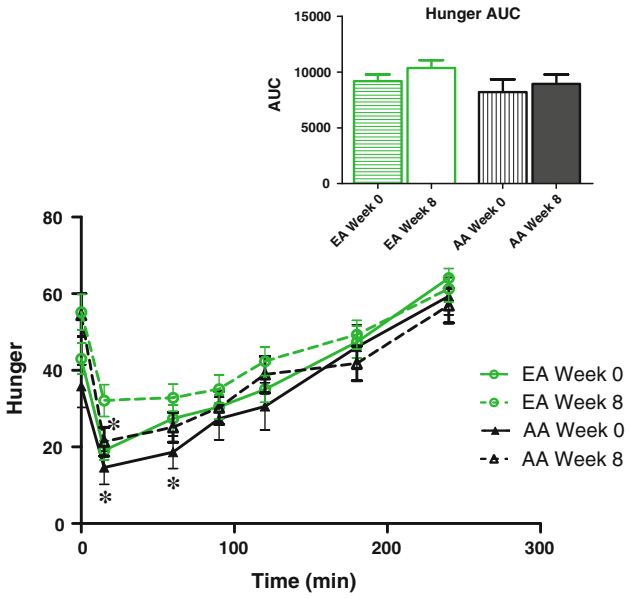

Fig. 5 Time courses for hunger. Neither fasting hunger nor hunger AUC differed between ethnic groups at week 0 or week 8. However, EA reported greater hunger at minute 15 of both meal tests and at minute 60 of the first meal test ($*p < 0.05$)

serum ghrelin would differ between EA and AA during the post absorptive period and in response to a standard mixed meal as well as whether circulating ghrelin would be affected by habituation to a low-CHO versus low-FAT diet. Secondary aims were to determine whether ethnic differences in ghrelin would relate to concomitant differences in insulin and subjective hunger. Our results revealed significantly higher serum ghrelin among EA versus AA in both postprandial and post absorptive states. Moreover, these differences were not affected by 8-week habituation to diets of dissimilar fat and carbohydrate content. EA exhibited greater ghrelin suppression, but also greater postprandial hunger, immediately following each test meal. Differences in ghrelin and hunger could not be attributed to differences in insulin concentration as circulating insulin did not differ between groups.

The observation of higher circulating ghrelin among overweight EA adults compared to AA is of considerable interest. Previous studies have also reported higher fasting ghrelin among EA compared to other ethnic groups, including Pima Indians [20, 21] and Japanese adults [22]. On the other hand, in a prior study among EA and AA prepubertal children, higher fasting ghrelin among EA did

Table 4 Absolute ghrelin suppression (calculated as the difference from fasting at each time point) (pg/mL)

Minute	European American ($n = 31$)	African American ($n = 30$)	p value for race difference
Week 0			
15	−31.90 ± 21.27	−34.85 ± 15.38	0.663
60	−173.92 ± 30.28	−56.38 ± 14.21	**0.001**
90	−124.23 ± 38.58	−11.58 ± 21.33	**0.018**
120	−162.26 ± 35.96	−21.32 ± 30.06	**0.007**
180	−112.68 ± 34.09	−55.29 ± 18.24	0.246
240	−27.64 ± 33.79	−36.92 ± 19.00	0.559
Week 8			
15	−33.60 ± 21.50	−25.07 ± 13.03	0.842
60	−137.79 ± 26.74	−61.88 ± 17.30	0.078
90	−93.95 ± 28.56	−73.82 ± 20.43	0.480
120	−120.73 ± 31.54	−81.38 ± 20.91	0.445
180	−132.86 ± 27.37	−84.63 ± 22.78	0.309
240	−75.86 ± 21.33	−88.18 ± 20.61	0.708

Results are presented as mean ± SEM

Bold values indicate statistically significant

not reach statistical significance, nor were there significant differences in ghrelin AUC following an oral glucose tolerance test [23]. Another study comparing a single measure of 2-hour postprandial acylated ghrelin among EA versus AA pre- and post-menopausal women reported higher plasma ghrelin among AA at that time point [24]. Seemingly discordant results between these two studies and the present study could be due to differences in study design, reproductive status of participants, or form of hormone measured. Ghrelin is secreted as a pre-prohormone, cleaved to ghrelin, and further modified by variable addition of a fatty acid chain to yield either acylated or unacylated forms [25, 26]. The present study measured total (acylated + unacylated) ghrelin. Further research is needed to determine if age or ghrelin form affect ethnic differences in fasting and postprandial ghrelin concentrations.

In light of established inverse associations between ghrelin and insulin [5–7, 11, 27] and previous reports of higher post-challenge insulin among AA [8–10], we hypothesized that higher ghrelin among EA may be explained by lower insulin. However, we did not observe differences in fasting or postprandial insulin levels. The reason for the absence of an ethnic difference in insulin in this study is not clear, but by study design, our cohort included only overweight individuals. Obesity increases basal and post-challenge insulin concentrations [28], so the impact of obesity may have attenuated ethnic differences in insulin response. In any case, differences in circulating ghrelin could not be explained by differences in circulating insulin.

Immediately following the week 0 test meal, postprandial suppression of ghrelin was lower among AA. Two previous studies examining ghrelin differences between EA and AA likewise reported lower postprandial ghrelin suppression among AA children [23] and adults [24]. The reason for this ethnic difference is not clear. However, after 8-weeks of our diet intervention, blunted ghrelin suppression among AA was attenuated, regardless of diet assignment. Thus, another unknown aspect of the diet intervention, other than macronutrient profile, may have altered the processes that regulate ghrelin under free-living conditions. For example, it is well-established that EA have higher resting energy expenditure than AA [29, 30], so perhaps the fixed amount of daily energy provided during the intervention was a larger perturbation to EA, resulting in increased ghrelin and hunger. Additional research is needed to understand both the physiological basis and obesity implications for the observed ethnic difference in postprandial suppression of ghrelin.

In light of known associations between ghrelin and hunger, previous authors have speculated that lower postprandial ghrelin suppression in AA may lead to reduced suppression of hunger and thereby predispose AA to greater energy intake and consequent obesity [23, 24]. However, within our cohort, subjective hunger after the test meal was higher among EA compared to AA. Greater hunger among EA could relate to their higher ghrelin concentrations overall. Alternatively, because only acylated ghrelin binds to hypothalamic receptors to increase appetite [31], perhaps analysis of acylated versus unacylated forms specifically would yield different results.

Limitations of this study included analysis of total ghrelin rather than specific acylated and unacylated forms and the modest sample size. However, the study was strengthened by longitudinal design, and careful control of food intake with all food provided. In addition, the diets applied to the intervention are typical of a "real world" setting [32].

In conclusion, we observed higher fasting and postprandial ghrelin concentrations among overweight EA versus AA adults, and blunted postprandial suppression of ghrelin among AA. Higher concentrations of circulating ghrelin among EA were not influenced by dietary macronutrients; however, habitual diet appeared to attenuate the blunted suppression of ghrelin observed among AA. Despite greater suppression of ghrelin, EA reported greater hunger immediately following the test meal. Future studies are indicated to further explore these ethnic differences and how they may relate to obesity risk.

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Conflict of interest The authors declare that they have no conflict of interest.

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