Fasting Plasma Ghrelin Levels Are Reduced, But Not Suppressed During OGTT in Obese African American Adolescents

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Objective: Our study aimed to evaluate total plasma ghrelin (TGh) concentration and its correlation with leptin and insulin in obese African American (AA) adolescents with a family history of type 2 diabetes.

Participants and Methods: Insulin, leptin, and TGh were measured for 15 non-obese controls in fasted state and 19 obese AA adolescents on samples collected during oral glucose tolerance test (OGTT) using radioimmunoassay kits. The hormonal concentrations were compared at fasting levels between obese and non-obese AA adolescents. Insulin, leptin, and TGh concentrations were also compared during OGTT in the obese group.

Results: Fasting TGh was significantly lower in obese AA adolescents compared to non-obese controls, while fasting leptin and insulin were significantly higher in obese AA adolescents compared to non-obese controls. During OGTT, for the obese group, TGh increased significantly and plasma leptin decreased significantly. A significant negative correlation was found between TGh and leptin at 30 and 120 min, but at no other time points (0, 60, and 90 min). A significant positive correlation was found between TGh and insulin at 30 min during OGTT, but no other time points.

Conclusions: TGh was lower in obese AA adolescents with a family history of type 2 diabetes and a significant correlation occurred between TGh and leptin and TGh and insulin during OGGT at specific time points in our obese group. These findings indicate that insulin resistant obese AA adolescents have impaired ghrelin suppression. (*Ethn Dis.* 2013;23[4]:436–440)

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Key Words: Ghrelin, African American Adolescents, Insulin Resistance, Obesity, Diabetes Mellitus

Introduction

Ghrelin is an endogenous ligand of the growth hormone secretagogue receptor and a potent stimulator of growth hormone release in humans.[1–3] Ghrelin is synthesized primarily in the stomach, however current research suggests other sites of origin such as pancreatic β-cells, hypothalamus, and placenta.[3,4] Ghrelin has been reported to influence a number of metabolic functions, including initiating meal consumption, and promoting enlargement of adipocytes.[4–6] It is an orexigenic peptide. Total plasma ghrelin (TGh) levels reportedly increase preprandially and decrease after meal consumption.[2,7–9] The concentration of circulating TGh is influenced by changes in energy balance and nutritional intake.[4,10] TGh levels are also reported to be inversely related to body weight. A number of studies indicate that TGh is reduced in obesity.[2–4,7,11] Studies also suggest that ghrelin could play in a role in glucose homeostasis.

The incidence of obesity has increased in both developed and developing countries. This growing pandemic introduces a series of health and economic burdens, as the complications associated with obesity greatly alter the quality of life. The World Health Organization (WHO) reports that 700 million adults will be obese by 2015.[5] This increase is likely to be mimicked in the pediatric population. The American Heart Association reports that 23.9 million children (aged 2–19 years) are overweight or obese, with a disproportionate number of cases in non-Hispanic Blacks.[12] Thus, minority children and adolescents are at an increased risk for developing type 2 diabetes (T2D) and cardiovascular disease.[2] There is a need to identify causes of obesity and therapies in this population.

Previous studies have explored TGh concentration in obese Japanese and Chinese children and adolescents. These reports indicate that TGh is reduced in the obese group compared to normal weight controls.[1,4,7,11] Several studies have also explored differences in TGh concentration as a function of race.[2–4,13] One study found elevated TGh in Black women compared with White women. Reportedly, this finding was more pronounced in obese, Black women, suggesting that ghrelin does vary as function of race.[2]

The dynamics of ghrelin and leptin in obesity is of special interest, as leptin reportedly suppresses ghrelin secretion.[3] Failure to suppress ghrelin, especially after meal consumption, could drive the development of obesity. A previous study reported no correlation between TGh and leptin in obese Japanese children and adolescents.[11] Glucose and insulin metabolism have also been reported to be regulators of TGh concentration.[14] It is reported that patients with craniopharyngioma and hypothalamic obesity exhibit less ghrelin suppression during OGTT, which could contribute to obesity in these patients.[15] Failure to suppress ghrelin after meal consumption could explain, in part, the disproportionate number of obese African American adolescents.

The purpose of our study was to evaluate the TGh concentration in obese AA adolescents with a family history of T2D compared to normal, non-obese controls. We also sought to identify the possible correlation between leptin, insulin, and TGh in obese AA adolescents during OGTT to evaluate if ghrelin suppression is impaired in the obese cohort.

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MATERIALS AND METHODS

Study Participants

Participants were recruited from the Diabetes Treatment Center at the Howard University Hospital. The study population included 19 obese AA adolescents (female/male: 11/8; aged 10 to 20 years; mean body mass index (BMI) 37.07 ± 6.7 kg/m^2). The control population included 15 healthy aged matched AA adolescents and a BMI ≤ 85th percentile of BMI for age and sex. All participants had a family history of T2D in either parent or grandparent. Participants who were pregnant or had diabetes, heart, renal, liver, and thyroid disease were excluded. Patients who participated in competitive sports or were taking medication known to influence insulin, lipid and glucose metabolism were also excluded. Laboratory analyses were performed in the Howard University core laboratories (Beckman LX-20 and Beckman BXC autoanalyzers). All participants aged ≥ 18 years gave informed consent. Guardians gave consent for all participants aged <18 years. This study was approved by the Howard University institutional review board.

Radioimmunoassay

The samples were collected during an OGTT on participants BMI ≥ 85th percentile of BMI for age and sex.[16] All samples were stored at −70°C after collection and stored until analyses. The TGh concentration was measured for 19 healthy obese AA adolescents and 15 healthy non-obese AA adolescents. TGh concentrations were measured for each sample collected during OGTT at 0, 30, 60, 90, and 120 minutes on all obese participants (n=19). TGh concentrations were measured for control samples during the fasting period in the healthy controls because OGTT was not conducted for this group.

TGh assays were performed in the molecular endocrinology laboratory at the Howard University Hospital using commercially available radioimmunoassay (RIA) kits for TGh (Millipore, St. Charles, Missouri). The Millipore TGh RIA kit utilizes ^{125}I-labeled (Iodine-125) ghrelin and a ghrelin antiserum to determine the level of TGh by the double antibody/PEG technique. All counts were calculated and a standard curve was constructed with increasing concentrations of standard unlabeled antigen. The amount of unknown antigen was determined by extrapolation of the reference curve and values calculated to determine the TGh concentration (pM) in participants and controls. The assay kit for TGh had a precision of 14.7%–16.7% CV intra-assay and 4.4%–10.0% inter-assay. The assay kit for TGh had a high specificity for human ghrelin (100%). The limit of sensitivity for the human TGh assay was 93 pg/mL. Plasma leptin and insulin concentrations were measured in a previous study conducted by our laboratory exploring vitamin-D deficiency in African Americans using commercially available RIA kits for leptin and insulin.[16]

Fasting TGh levels were significantly lower in the obese group compared to non-obese controls.

Statistical Analysis

Analysis of data was performed using SPSS version 20.0. Average values were calculated as mean ± 1 standard error. Student's t-test was used for comparison of obese and non-obese groups. Spearman's Rho was used to assess statistical differences. Repeated measures ANOVA was used to compare hormonal concentrations at various time points of OGTT. The level of statistical significance was set at $P < .05$.

RESULTS

Obese participants presented with an average BMI 37.07±6.7 kg/m^2 compared to non-obese AA adolescents 21.33±2.16 kg/m^2 (P<.001). Laboratory data are summarized in Table 1. Fasting TGh levels were significantly lower in the obese group compared to non-obese controls, 181.2±17.04pM and 223±17.60pM (P=.04), respectively. Fasting insulin (obese: 33.00± 17.62 μU/mL; non-obese: 18.70± 7.65 μU/mL, P = .023) and leptin (obese: 44.01±13.22 ng/mL; non-obese: 12.00±13.25 ng/mL, P < .001) concentrations were significantly higher in the obese compared to the

Table 1. The relationship of fasting insulin, leptin, and TGh in obese (n=19) and non-obese (n=15) African American adolescents

Hormonal Parameters	Obese Participants n=19	Non-Obese Participants n=15	P[a]
Fasting Insulin, μU/mL	33.00±17.62	18.70±7.65	.023
Leptin, ng/mL	44.01±13.22	12.00±13.25	<.001
Ghrelin pM	181.26±74.30	223±68.14	.04

[a] P<.05 were considered significantly different.

Data are means ± SE of TGh, leptin, and insulin. Blood samples were drawn before oral glucose administration in both obese and non-obese cohort.

non-obese group. (Table 1). The pubertal status did not affect IR values significantly in the obese or non-obese group. During OGTT, TGh increased significantly after oral glucose load, 0 to 60 minutes (Figure 1A). TGh levels were statistically different for all time points during OGTT in the obese group. Plasma leptin concentration decreased significantly after oral glucose load, measurements were statistically different for all time points during OGTT (Figure 1B). Plasma insulin increased during OGTT, but all time points were not significant, 60 min (P=.39) and 120 min (P=.63) (Figure 1C). In comparing the hormones during OGTT, there was a significant negative correlation between TGh and leptin at 30 min (r = −.41, P=.04) (Figure 2A) and TGh and leptin at 120 min (r=−.45, P=.02) (Figure 2B). However, no significant correlation was found between TGh and leptin in the obese group for 0, 60, and 90 min. There was a significant positive correlation between TGh and insulin at 30 min during OGTT in obese AA adolescents (r=.42, P=.03) (Figure 2C). Conversely, no significant correlation was identified between TGh and insulin at 0, 60, 90, and 120 min in the obese group during OGTT.

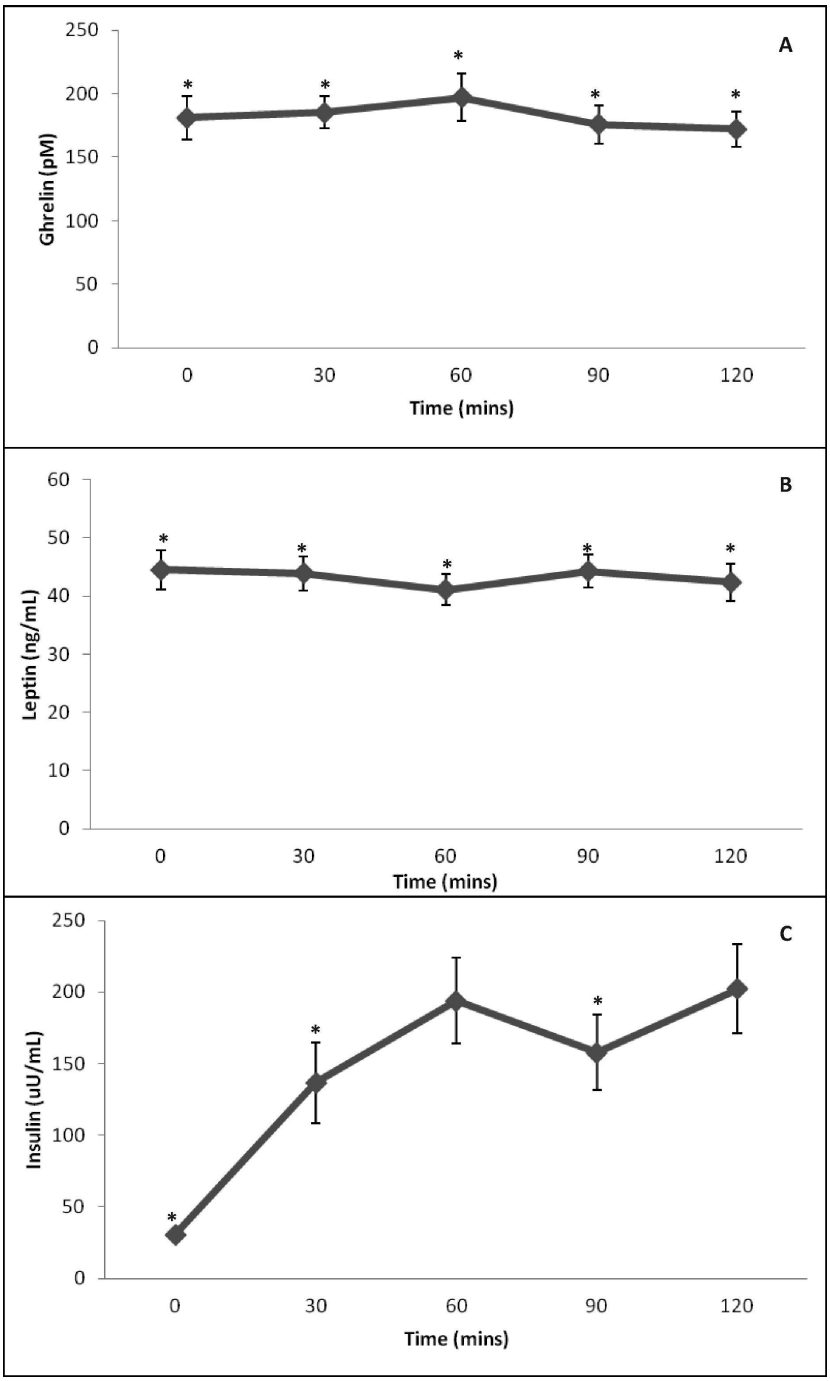

Fig 1. Hormone concentrations during OGTT in obese AA adolescents. A) TGh concentration (pM) during OGTT in obese AA adolescents. B) Plasma leptin concentration (ng/mL) during OGTT in obese AA adolescents. C) Plasma insulin concentration (μU/mL) during OGTT in obese AA adolescents. Data are means ± SE for TGh of 19 obese AA adolescents. Oral glucose was administered in a dose of 1.75g/kg body weight to a maximum of 75g. Blood samples were assayed using RIA kits for TGh for each collected sample during OGTT at 0, 30, 60, 90, and 120 min, *P<.05 considered significantly different

DISCUSSION

Fasting TGh levels were significantly lower in the obese group compared to normal controls. This finding supports the idea that TGh is reduced in the obese state. In 2001, Tschop et al proposed that reduced ghrelin concentration in obesity is a result of a physiological adaptation to excessive positive energy balance, as in the case of obesity.[13] The findings of our study support this argument, suggesting that ghrelin does not function to promote obesity, but rather to maintain energy homeostasis.

Our findings reveal that fasting plasma leptin and insulin are found at significantly higher levels in the obese

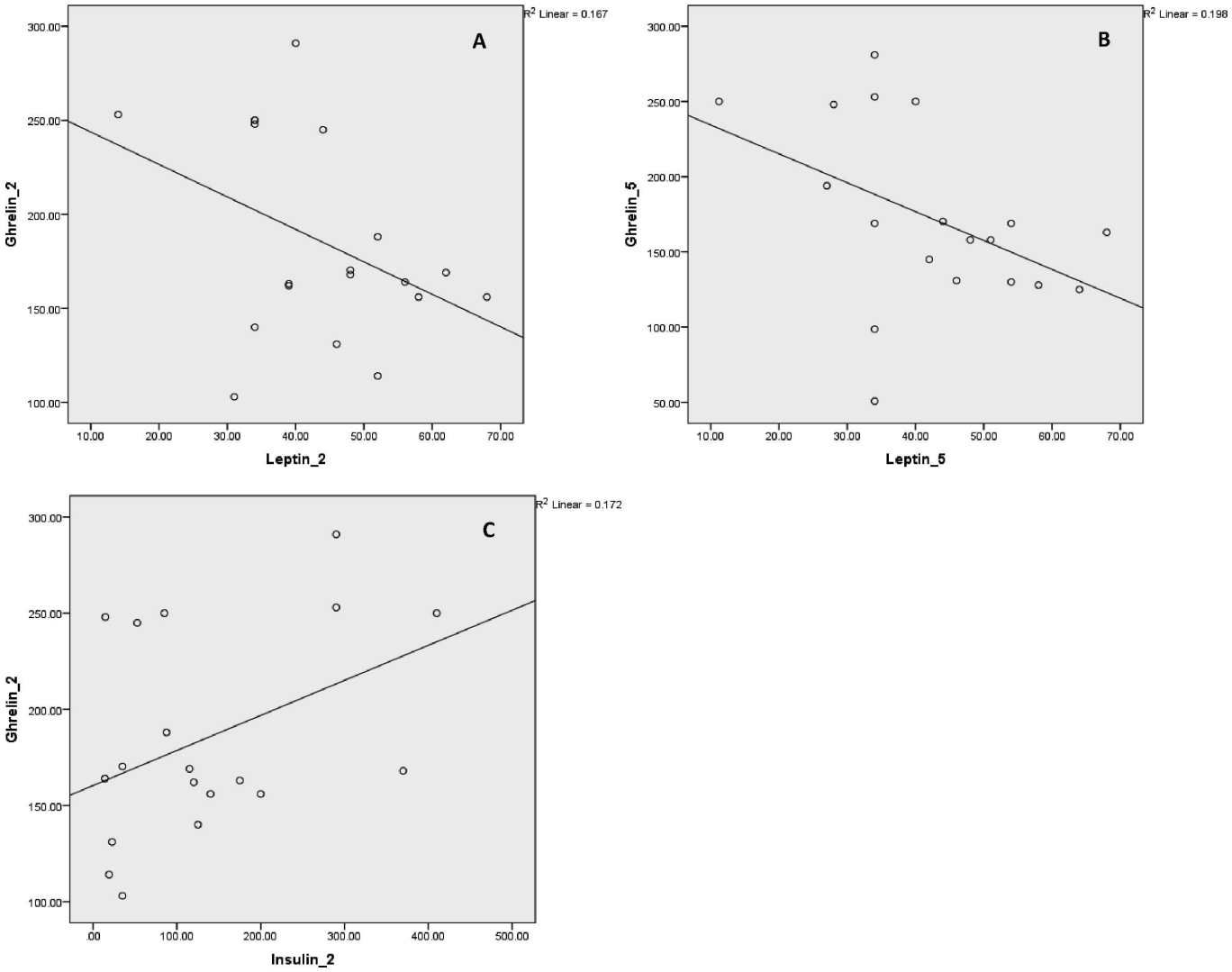

Fig 2. Correlation between TGh, leptin, and insulin during OGTT in obese AA adolescents. A) Correlation between TGh and leptin at 30 min during OGTT, *P*=.04. B) Correlation between TGh and leptin at 120 min during OGTT, *P*=.02. C) Correlation between TGh and insulin at 30 min during OGTT, *P*=.03

group compared to normal controls. The adipocyte-specific hormone leptin is known to tonically suppress ghrelin. Much of the literature reports that leptin is inversely associated with ghrelin. For instance, TGh concentration significantly decreases after leptin administration, indicating an inverse relationship between these hormones.[17] Our study, however, did not reveal any significant correlation between fasting TGh and leptin in obese AA adolescents. This discrepancy could be attributed to the heterogeneity of obesity and insulin resistance in our obese group. Additionally, a study conducted in Japanese children and adolescents found similar results, reporting no correlation between fasting plasma ghrelin and leptin in this cohort.[11]

During OGTT, TGh increased significantly from 0 to 60 min in obese AA adolescents, an unexpected finding. Much of the literature reports that TGh is suppressed after oral glucose administration in adults and children.[18–21] We speculate that the increased TGh during OGTT in our study may be indicative of impaired ghrelin suppression. A study exploring differences in ghrelin concentration as a function of race, proposed that higher TGh found in Black women was a result of subnormal postprandial ghrelin suppression.[2] In addition, another study demonstrated similar findings in AA children. The researchers of this study found that AA children, compared to White children, have lower suppression of ghrelin levels in response to OGTT.[14] Our findings support previous reports of reduced ghrelin suppression in AA children and adolescents during OGTT.

During OGTT, there was also a significant increase in plasma insulin from 0 to 60 min in obese AA

adolescents. Normally, ghrelin is suppressed after oral glucose load. This effect is in part dependent on insulin.[2] Insulin is known to inhibit ghrelin secretion.[19] It is likely that the suppression of ghrelin is impaired in obese and insulin-resistant individuals after meal intake. The impaired suppression of ghrelin, coupled with increased insulin during OGTT, suggests the inability of insulin to suppress ghrelin in the obese group, causing ghrelin to continually rise and stimulate appetite in obese AA adolescents.[14] This effect is potentially maximized in the event that obese individuals do not respond to leptin, a ghrelin antagonist. The findings of our study support our previous findings that the obese group was insulin resistant.[16] We also sought to identify possible correlation between TGh, leptin, and insulin during OGTT. Our data does reveal significant correlation between TGh and leptin, and TGh and insulin at specific time points during OGTT in obese AA adolescents.

CONCLUSIONS

Our results are novel and interesting, in spite of the limited study size (n=19) and that we did not attempt to age and sex match obese and non-obese controls.

Our study supports the idea that TGh is reduced in the obese state. Our findings also suggest that ghrelin suppression is impaired in insulin-resistant obese AA adolescents, which could contribute to the disproportionate number of obese AA adolescents. It is also likely that impaired ghrelin suppression precedes insulin resistance, which suggests ghrelin as a potential biomarker of obesity and T2D. This warrants additional research to determine if impaired ghrelin suppression precedes insulin resistance or a manifestation of insulin resistance. Moreover, further investigation is needed in a larger sample size to identify correlations between TGh, leptin, and insulin during OGTT, to evaluate the mechanisms of ghrelin suppression, and other hormones known to play a role in regulating appetite and glucose homeostasis.

ACKNOWLEDGMENTS

Dr. Kanwal K. Gambhir is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of data and the accuracy of data analyses. The authors thank the General Clinical Research Center, Dr. Verle E. Headings, MD/PhD, Raj Sridhar, PhD, Camelia Saddler, and Rhea Green for their assistance and support of this study. These studies were supported by the Baltimore DC Health Disparity Project 2P20MD30198-06, Dr. Gail Nunlee-Bland Principal Investigator. These studies are part of the master's thesis submitted by MBF as partial fulfillment for requirements to the degree, July 2011 (Howard University).

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Administrative: Gambhir, Nunlee-Bland, Odonkor
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