Entero-insular axis and postprandial insulin differences in African American and European American children

Paul B Higgins, José R Fernández, W Timothy Garvey, Wesley M Granger, and Barbara A Gower

ABSTRACT
Background: African Americans have a greater insulin response after glucose challenge than do European Americans. Factors underlying this response are unknown.
Objective: We determined the insulin, C-peptide, and incretin responses to a mixed macronutrient meal in African American and European American children. We hypothesized that 1) African Americans would have greater postprandial insulin and C-peptide responses, 2) African Americans would have higher incretin responses, and 3) the greater β cell response among African Americans would be explained by greater incretin responses.
Design: Subjects were 34 African American and 18 European American children. Glucose, insulin, C-peptide, glucagon-like peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP) were measured after the subjects consumed a liquid mixed meal. Insulin, C-peptide, and incretin responses were derived from the area under the curve (AUC) for minutes 0–30 (early response) and minutes 30–180 (late response) after meal ingestion.
Results: The early insulin response was higher in African American (14 565 ± 6840 pmol/L × 30 min) than in European American (7450 ± 4077 pmol/L × 30 min; P < 0.01) children. Early C-peptide AUC did not differ by ethnicity (African Americans: 34.8 ± 12.5; European Americans: 28.6 ± 12.5 pmol/L × 30 min; P = 0.10). Early and late GLP-1 responses were lower in African Americans than in European Americans: 108.1 ± 56.4 compared with 160.5 ± 90.8 pmol/L × 30 min and 509.4 ± 286.9 compared with 781.9 ± 483.4 pmol/L × 150 min, respectively (P < 0.05 for both). The GIP response did not differ between groups.
Conclusions: The greater early insulin response in African Americans than in European Americans is not due to differences in circulating GLP-1 or GIP and may be due to lesser insulin clearance. Further research is needed to determine the physiologic implications of lower GLP-1 among African Americans.

INTRODUCTION
African American children and adults have significantly greater risk of type 2 diabetes than do their European American counterparts (1, 2). The cause of this risk disparity is incompletely understood. Recent findings indicate that these risk differences are not completely accounted for by differences in obesity, diet, physical activity, and other environmental risk factors (3). Hence, biologic factors may make an important contribution.

Numerous well-controlled studies have documented differences in glucose metabolism between African Americans and European Americans. Most prominent of these differences is the higher insulin response of African Americans (4). The higher insulin responses of African Americans (5–7) were found to be independent of differences in insulin sensitivity, body fat, diet, and other lifestyle variables (7, 8). It was suggested that this response may predispose African Americans to greater risk of type 2 diabetes relative to European Americans (8, 9). Hence, it will be important to fully characterize and determine the factors underlying this phenomenon.

To date, most studies designed to investigate and characterize higher postchallenge insulin among African Americans compared with European Americans have used only intravenous or oral glucose tolerance tests (9–12). Although protocols involving intravenous administration of glucose provide robust models for assessing insulin dynamics, such tests do not reflect the normal physiology of food intake and subsequent metabolic responses (13). In particular, the contribution of the entero-insular axis to insulin dynamics is not reflected in the response to an intravenous glucose challenge. In addition, the role of nutrients other than glucose is not assessed when an oral glucose tolerance test is administered. Hence, characterization of the postchallenge insulin response under physiologic conditions is warranted.

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are important determinants of postprandial insulin secretion. Combined, they are considered the principal components of the endocrine portion of the entero-insular axis (14, 15). To date, few investigations have quantified incretin responses in African Americans and European Americans. Obese African American adults were found to have significantly higher fasting and postchallenge GLP-1 concentrations.

1 From the Departments of Nutrition Sciences (PBH, JRF, WTG, and BAG) and Critical Care (WMG), and the Clinical Nutrition Research Center (PBH, JRF, WTG, WMG, and BAG), University of Alabama at Birmingham, Birmingham, AL.
2 Portions of this study were published on-line at Proquest/UMI dissertation publishing.
3 Supported by grants from the National Institutes of Health (R01-DK067426, P30-DK56336, and M01-RR-00032) and the American Heart Association (0515149B). PBH was supported in part by a predoctoral fellowship from the American Heart Association and a partial scholarship from the Diabetes Trust Fund of Alabama.
4 Address reprint requests and correspondence to PB Higgins, Department of Genetics, Southwest Foundation for Biomedical Research, PO Box 760549, San Antonio, TX 78245-0549. E-mail: phiggins@sfbgenetics.org. Received May 1, 2008. Accepted for publication July 24, 2008.

In the morning each child underwent a mixed meal tolerance test to minimize physical activity. Children did not receive or consume prescription medications known to affect glucose metabolism or body composition, and had normal glucose tolerance (2-h oral glucose tolerance test) on entry into the study. The Institutional Review Board of the University of Alabama at Birmingham approved all procedures. Parents and children provided written informed consent and assent, respectively, before entry to the study. Parental and child ethnicity was assigned according to parental report. Each child classified as African American or European American had both parents classified as African American or European American, respectively. From adult data (16), a sample size of n = 50 provided 80% power to detect an ethnic difference in GLP-1 at a 2-tailed α level of 0.05.

Study design

Study design was cross-sectional and observational. Data were collected during an overnight in-patient visit to the General Clinical Research Center at the University of Alabama at Birmingham. All children arrived between 1600 and 1800. Each child underwent a physical examination, including pubertal assessment by an experienced pediatrician according to the criteria of Marshall and Tanner (18, 19). Children received a standard meal (hamburger and fried potatoes), which they consumed by 2000. Each child and parent were assigned a room and instructed to minimize physical activity. Children did not receive or consume any caloric or caffeinated beverages or snacks after 2000. In the morning each child underwent a mixed meal tolerance test that was administered at 0700. After completion of the mixed meal tolerance test, children and parents were escorted to the Webb Nutrition Sciences building for body composition analysis. Physical activity and dietary questionnaires were administered during this visit.

Mixed meal tolerance test

A flexible intravenous catheter was placed in the antecubital space of the left arm. Two blood samples were taken over a 15-min period to determine basal glucose and insulin (the average of the values is used for basal “fasting” concentrations). At time “0,” a liquid meal was administered (Ensure; Abbott Laboratories, Abbott Park, IL; 250 kcal, 6 g fat, 40 g carbohydrate, and 9 g protein). Children were instructed to consume the meal within 5 min of administration. Blood was drawn at baseline and at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, and 180 min after the start of meal ingestion. Dipeptidyl peptidase IV inhibitor (10 μL/mL; Linco Research, St Charles, MO) was added to all blood samples at collection to inhibit incretin degradation. Plasma samples were stored at −85 °C until assay. For data analysis, postprandial responses were determined as the area under the curve (AUC) with the use of the trapezoidal method (20). Early responses were defined as AUC for the first 30 min, and late phase responses as the AUC for the last 150 min after challenge (21).

Body composition

Total body composition (fat and lean mass) was determined with the use of a Lunar Prodigy densitometer (GE/Lunar Radiation Corp, Madison, WI). Subjects were scanned in the supine position with hands placed at their sides. Height was measured to the nearest centimeter with the use of a wall-mounted stadiometer, and body weight was measured on an electronic scale while children wore light clothing.

Assays

Glucose was assayed using the glucose oxidase method on a Sirrus analyzer (Stanbio, Boerne, TX). Insulin was assayed in duplicate 100-μL aliquots by radioimmunoassay (Linco Research). In our laboratory, this assay has a sensitivity of 3.35 μIU/mL, a mean intraassay CV of 3.49%, and a mean interassay CV of 5.57%. C-peptide was assayed in duplicate 25-μL aliquots with a double antibody radioimmunoassay (Diagnostic Products Corp, Los Angeles, CA). In our laboratory this assay has a sensitivity of 0.318 ng/mL, a mean intraassay CV of 3.57%, and a mean interassay CV of 5.59%. Intact GLP-1 was measured in duplicate 100-μL aliquots with the use of enzyme-linked immunoabsorbent assay (ELISA; Linco Research). This ELISA is highly specific for the immunologic measurement of the active forms of GLP-1 (7–36 amide and 7–37). In our laboratory, this assay has a sensitivity of 2 pmol/L, a mean intraassay CV of 7.4%, and a mean interassay CV of 10.7%. Total GIP was measured in duplicate 20-μL aliquots by ELISA (Linco Research). In our laboratory, this assay has a sensitivity of 8.2 pg/mL, a mean intraassay CV of 4.5%, and a mean interassay CV of 7.2%. The antibody has 100% cross-reactivity with both forms of GIP (1–42 and 3–42).

Dietary intake and physical activity assessment

Twenty-four-hour dietary recalls were administered by trained technicians according to the multiple pass method, as previously described (22), on the evening of the overnight visit. Recall data were entered into the NUTRITION SYSTEM FOR RESEARCH (version 5; University of Minnesota, Minneapolis, MN) to determine daily macronutrient intake. The Physical Activity Questionnaire for Children was developed by the Centers for Disease Control and Prevention, and it is designed to assess physical activity in the week before the interview (23, 24). This questionnaire was administered on the evening of the overnight
visits. The scoring range is 1 to 5, with 5 being the highest physical activity level.

Statistical analyses

Two-factor analysis of variance was used to assess potential effects of ethnicity and sex on the continuously distributed variables analyzed. The Mann-Whitney U test was used to determine differences in the categorical variables of the Tanner stage. Independent samples t tests were used to compare postprandial responses between African American and European American children. Relations between insulin and C-peptide and incretin responses were assessed with the use of Pearson’s correlation analysis. Multiple linear regression analyses were used to determine whether ethnicity was independently related to insulin and C-peptide responses after adjusting for glucose and incretin responses. Variable distributions that deviated from normal were log transformed before analysis. Statistical significance was set at \( P < 0.05 \). Data were analyzed using SPSS version 10.0 (SPSS Inc, Chicago, IL). Power calculations were performed with the use of POWPAL5 software (Gorman, Primavera, and Allison, 1993).

RESULTS

Descriptive data

Descriptive data are presented in Table 1. African American and European American children did not differ with respect to sex distribution between the groups \(( P = 0.30 \) ). Neither sex nor the sex-by-ethnicity interaction was significantly related to any of the variables described in Table 1.

Postprandial responses

Postprandial insulin, C-peptide, and glucose responses are depicted in Figure 1. Mean early insulin response was approximately 2-fold higher in African American \((14,565 \pm 6,840 \text{ pmol/L} \times 30 \text{ min}) \) than in European American \((7,450 \pm 4,077 \text{ pmol/L} \times 30 \text{ min}; \ P < 0.01 \) ) children. The early insulin response remained higher in African American children after adjustment for the early glucose response \(( P < 0.01 \) ). The early C-peptide response did not differ between African American and European American children before \((34.8 \pm 12.5 \text{ pmol/L} \times 30 \text{ min}; \ P = 0.10 \) ) and after adjustment for the early glucose response \(( P = 0.28 \) ). Responses for late insulin \((41,140 \pm 18,000 \text{ pmol/L} \times 150 \text{ min}; \ P = 0.69 \) ), late C-peptide \((166.6 \pm 38.9 \text{ pmol/l} \times 150 \text{ min}; \ P = 0.34 \) ), and early \((172.3 \pm 17.4 \text{ pmol/L} \times 30 \text{ min}; \ P = 0.17 \) ) and late \((744.2 \pm 89.1 \text{ pmol/L} \times 150 \text{ min}; \ P = 0.59 \) ) glucose did not differ by ethnicity. Early and late postprandial incretin responses are depicted in Figure 2. Responses for mean early \((108.1 \pm 56.4 \text{ pmol/L} \times 30 \text{ min}; \ P = 0.03 \) ) and late \((509.4 \pm 286.9 \text{ pmol/L} \times 30 \text{ min}; \ P = 0.04 \) ) GLP-1 were significantly lower in the African American than in the European American children. Responses for early \((5505.9 \pm 2350.3 \text{ pmol/L} \times 30 \text{ min}; \ P = 0.12 \) ) and late \((28,107 \pm 11,796 \text{ pmol/L} \times 150 \text{ min}; \ P = 0.17 \) ) GIP did not differ between groups.

Associations among postprandial incretin, C-peptide, and insulin responses

The early GLP-1 response was not correlated with the early insulin \((r = -0.16, \ P = 0.33 \) ) or early C-peptide \((r = -0.13, \ P = 0.36 \) ) responses. In contrast, the early GIP response was significantly related to the early insulin response \((r = 0.54, \ P < 0.001 \) ) and the early C-peptide response \((r = 0.56, \ P < 0.001 \) ). The late GLP-1 and GIP responses were not associated with the late insulin or C-peptide responses (data not shown). These relations were similar when the data were analyzed within each ethnic group. In multiple regression analyses, ethnicity remained a significant predictor of the early insulin response after adjustment for early glucose and early GIP responses (Table 2). Only the early GIP and glucose responses were found to be significant predictors of the early C-peptide response (Table 2).

DISCUSSION

The purpose of this study was to determine the responses of insulin, C-peptide, and incretin to a mixed macronutrient test meal in a group of African American and European American children.
FIGURE 1. Insulin, C-peptide, and glucose responses in African American (●, n = 34) and European American (○, n = 18) children after ingestion of a mixed meal. Data are means ± SEs. Early response is defined as the area-under-the curve (AUC) for minutes 0–30 and late response as the AUC for minutes 30–180 after challenge. Means were compared by independent samples t tests. African Americans had a higher mean early insulin response (P < 0.001). African American and European American children did not differ in late insulin response. Mean early and late C-peptide responses did not differ in African American and European American children. Mean early and late glucose responses did not differ in African American and European American children.
children. We tested the specific hypotheses that 1) African Americans would have greater postprandial insulin and C-peptide responses, 2) African Americans would have higher incretin responses, and 3) the greater \( \beta \) cell response among African Americans would be explained by greater incretin responses. As expected, we found that African American children had a significantly higher early insulin response; however, early and late C-peptide responses did not differ statistically by ethnicity. In contrast to our hypothesis, the postprandial GLP-1 response was lower in African Americans and was not associated with the early postprandial insulin response. Notably, GIP was significantly positively associated with the early insulin response but did not differ significantly by ethnicity. Taken together, these results suggest that incretins do not explain greater postchallenge insulin among African Americans compared with European Americans, and they suggest that greater postchallenge insulin among African Americans is due to lesser insulin clearance.

Our data showed that African Americans had greater postprandial insulin responses than did European Americans of similar age, body composition, dietary intake, and physical activity. These results agree with those of previous studies that have documented greater first phase or acute insulin responses to

**TABLE 2**

Multiple linear regression analyses for early insulin response and early C-peptide response (n = 52)

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early insulin response (( R^2 = 0.48 ))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model(^1)</td>
<td>-5.09</td>
<td>2.03</td>
<td>0.016</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>0.19</td>
<td>0.06</td>
<td>0.004</td>
</tr>
<tr>
<td>Early glucose response</td>
<td>2.03</td>
<td>0.61</td>
<td>0.002</td>
</tr>
<tr>
<td>Early GIP response</td>
<td>0.46</td>
<td>0.14</td>
<td>0.002</td>
</tr>
<tr>
<td>Early C-peptide response (( R^2 = 0.46 ))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model(^1)</td>
<td>-5.02</td>
<td>1.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>0.003</td>
<td>0.05</td>
<td>0.95</td>
</tr>
<tr>
<td>Early glucose response</td>
<td>1.63</td>
<td>0.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Early GIP response</td>
<td>0.36</td>
<td>0.10</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^1\) All variables were log transformed. Ethnicity was entered as a categorical variable: 1 = European American; 2 = African American. Early response is defined as the area under the curve for minutes 0–30 after challenge. GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulino tropic polypeptide.

\(^2\) \( R^2 \) excluding ethnicity = 0.39.

\(^3\) \( R^2 \) excluding ethnicity = 0.48.

**FIGURE 2.** Incretin responses in African American (AA; ■; \( n = 34 \)) and European American (EA; □; \( n = 18 \)) children after ingestion of a mixed meal. Data are means ± SDs. Early response is defined as the area-under-the-curve (AUC) for minutes 0–30 and late response as the AUC for minutes 30–180 after challenge. Means were compared by independent samples \( t \) tests. *AAs had lower early (\( P = 0.03 \)) and lower late (\( P = 0.04 \)) glucagon-like peptide-1 (GLP-1) responses compared with EAs. Mean early (\( P = 0.12 \)) and late (\( P = 0.17 \)) glucose-dependent insulino tropic polypeptide (GIP) responses did not differ in AA and EA children.
intravenous glucose in African Americans and European Americans (6, 7, 9–11). Insulin responses quantified from the peripheral circulation reflect both the β cell response and the extent of hepatic insulin clearance. Hence, it is important to also consider C-peptide concentrations; insulin and C-peptide are secreted in equimolar concentrations, but hepatic C-peptide clearance is negligible relative to that of insulin (13). In contrast to findings from intravenous glucose challenge tests, with the meal test we did not find greater postchallange C-peptide concentrations in African American children. Hence, our data suggest that, after a mixed macronutrient test meal, a greater β cell response is not the primary cause of higher insulin responses in African Americans. Therefore, the greater insulin response of African Americans compared with European Americans to a meal stimulus is probably due to lesser hepatic insulin clearance.

Differences among studies in whether an ethnic-specific C-peptide response is detected may be due to the stimulus used, i.e., oral meal test compared with intravenous glucose test (13). Specifically, the rate at which the β cells are exposed to nutrients and the dynamics of the secretory response differ with test type (13). Thus, intravenous and oral tests are likely to capture unique aspects of the insulin response. From a cellular perspective, the immediate release of previously docked insulin secretory granules is probably responsible for the higher β cell response observed in African Americans in the first few minutes after an intravenous glucose challenge (10, 13). However, rapid depletion of these immediately available granules and subsequent reliance on granule movement and other processes during the extended early phase after the meal test may impair the ability to detect the acute C-peptide response. Thus, we propose that the higher C-peptide or β cell response of African Americans compared with European Americans is apparent only during the acute response to a challenge, which is most easily observed during an intravenous glucose tolerance test. Importantly, our meal test data are compatible with previous data showing that C-peptide concentrations after oral glucose did not differ among adult and adolescent African Americans and European Americans, despite significantly higher postchallengue insulin concentrations in African Americans (12, 25). Understanding test-related differences in the assessment of insulin dynamics is of critical importance to future studies designed to address β cell function, particularly in heterogeneous populations.

Regardless of the physiology underlying the response, compared with European Americans, African Americans exhibit greater circulating insulin in the postprandial period. In a recent longitudinal analysis, greater insulin responses in African American children were shown to account for their lower adiponectin concentrations compared with European American children (26), indicating that excessive circulating insulin may indirectly compromise insulin sensitivity. In addition, hyperinsulinemia was shown to be an independent risk factor for type 2 diabetes and coronary heart disease (27, 28), and chronic hyperinsulinemia was shown to directly induce insulin resistance in vitro (29). The potential long-term consequences of postprandial hyperinsulinemia among African Americans deserve further study.

This is the first study to characterize the main components of the endocrine portion of the entero-insular axis and their potential role in insulin response in healthy African American and European American children. We did not find significant ethnic differences in GIP responses. Unexpectedly, we found that early and late phase GLP-1 responses were lower in African American than in European American children. Hence, our findings indicate that circulating incretins are unlikely to be responsible for higher postchallange insulin among African Americans than among European Americans.

Our GLP-1 findings agree with those of Velasquez-Meyer et al (17) who found that, among severely obese adolescents, African Americans had lower GLP-1 responses to oral glucose. Importantly, our results extend those findings to show for the first time that ethnic differences in GLP-1 are also present in healthy children with normal glucose tolerance. Hence, lower GLP-1 in African American compared with European American children is probably not due to obesity or glucose intolerance and may be an inherent metabolic characteristic of this population. Given the many extra-pancreatic effects of GLP-1, including improvements in insulin-mediated glucose uptake, reductions in gastric emptying, and inhibition of food intake (30), lower GLP-1 in African Americans may predispose them to type 2 diabetes or other metabolic diseases. Clearly, it will be important to determine the contribution, if any, of lower GLP-1 to the risk of metabolic disease in African Americans in future studies.

We did not find an association between the postprandial GLP-1 response and the insulin response. This finding appears to conflict with previous findings in which GLP-1 infusion was shown to elicit an increase in insulin response under steady state glycemia (31–36). However, in those infusion studies, the concentrations of GLP-1 achieved were higher than those measured after the meal in the present study. Indeed, our findings are in agreement with data from experiments in humans (37) and in dogs (38) that showed that physiologic concentrations of circulating GLP-1 do not seem to have an effect on insulin secretion. Moreover, a recent study in adults also failed to show an association between postprandial insulin and GLP-1 responses (39). Those findings do not, however, preclude a role for GLP-1 in postprandial insulin secretion. Given the short circulating half-life of GLP-1, it is likely that peripheral venous concentrations of intact GLP-1 do not adequately reflect that which is secreted from the intestinal L cell. Hence, these concentrations do not reflect local or portal vein peptide concentrations that may indirectly promote insulin secretion (30). In contrast to GLP-1, the early postprandial GIP response was strongly correlated with the early insulin response. Previous studies undertaken in rodents support the notion that GIP is the dominant circulating incretin peptide (40). Although the African American children in our study had higher GIP responses, these differences were not statistically significant and suggest that GIP is unlikely to contribute to the higher insulin response of African Americans. Considering both the GIP and GLP-1 data presented, results from this study do not support a major role for circulating incretins in the higher insulin response of African American compared with European American children.

In conclusion, African American children had a higher postprandial early insulin response relative to their European American counterparts. This difference was not explained by differences in circulating concentrations of the incretin peptides, but it may have been due in part to lower hepatic insulin clearance. Importantly, we observed that African Americans had lower GLP-1 concentrations than did European Americans. This finding, if confirmed, could have important implications for type 2 diabetes or other metabolic disease prevention and treatment in African Americans.
ETHNICITY AND THE ENTERO-INSULAR AXIS

We thank Amanda Willig, Alexandra McPherson, Fernando Gomez, Paul Zuckerman, Buddy Sirikul, PhD, David Bryan, Betty Darnell, Mia Amaya, MD, John Paul Clancy, MD, Jamy Ard, MD, and Frank Franklin, MD, for their support and efforts. We also thank the Nursing, BioNutrition, and Metabolic Kitchen staff (Yolanda Guyton, Cluster Boyd, Dena Jackson, Cassandra Thomas, and Karen Thomas) for additional recruitment efforts and other staff members of the General Clinical Research Center for their efforts in support of this study.

The author’s responsibilities were as follows—PBH and BAG: conceived and designed the study and wrote the manuscript; and JRF, WMG, and WGT: aided in statistical analyses, data interpretation, and edited the manuscript. None of the authors had a personal or financial conflict of interest.

REFERENCES


