β-Cell Lipotoxicity in Response to Free Fatty Acid Elevation in Prepubertal Youth

African American Versus Caucasian Contrast

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Prepubertal African American (AA) youth compared with their Caucasian (C) peers have higher insulin secretion, which correlates positively with free fatty acid (FFA) concentration. In our continued efforts to explain the racial disparity in insulinemia, and because FFAs modulate insulin secretion, we hypothesized that AA youth would have a greater response to FFA-induced β-cell insulin secretion than C youth. We compared the short-term effects of FFA elevation on fasting and glucose-stimulated C-peptide–modeled insulin secretion in prepubertal normal-weight AA versus C peers during a 2-h hyperglycemic clamp (12.5 mmol/L) on two occasions: *1*) infusion of normal saline and *2*) infusion of 20% intralipid (IL). During IL infusion, insulin sensitivity (IS) declined comparably in AA and C youth. Glucose sensitivity of first- and second-phase insulin secretion showed a significant condition × race interaction being higher in AA youth. Disposition index, β-cell function relative to IS, declined with IL infusion in AA and C youth, with a significantly greater decrease in Cs compared with AAs. In conclusion, AA and C prepubertal youth both demonstrated a decline in β-cell function relative to IS during IL infusion, indicative of acute lipotoxicity. The greater decline in C youth compared with AAs may suggest that C youth are more susceptible to β-cell lipotoxicity than AA youth, or alternatively, that AA youth are hypersensitive to FFA stimulation of β-cell insulin secretion, consistent with our theory. ***Diabetes*** **62:2917–2922, 2013**

Short-term exposure to elevated free fatty acid (FFA) concentrations has a stimulatory effect on β-cell function in in vitro and in vivo animal and human studies (1–8). On the other hand, chronic exposure to sustained elevations in FFAs has been shown to decrease insulin secretion and result in β-cell lipotoxicity in in vitro rat and human islet experiments (4,9,10) and in vivo human experiments (1,5,7,11,12).

We previously found that prepubertal African American (AA) youth have higher insulin secretion and an upregulated β-cell function relative to insulin sensitivity (IS) compared with their Caucasian (C) peers (13). The hyperinsulinemic response in AA youth appears to be greater than the compensatory response to their lower IS, because the disposition index (DI), which is insulin secretion relative to IS, was ~75% higher in AA than in C youth (13). Furthermore, first- and second-phase insulin concentrations during the hyperglycemic clamp correlated positively and significantly with fasting FFA levels in AAs but not Cs, despite similar plasma FFA concentrations (13). We therefore hypothesized that the higher insulin secretion in AA prepubertal youth could be driven by a greater sensitivity to the stimulatory effects of FFA on insulin secretion and β-cell function. Thus, in the present investigation we examined the effects of FFA elevation on glucose-stimulated insulin secretion in AA and C prepubertal normal-weight children, expecting to observe a heightened response in AA children.

RESEARCH DESIGN AND METHODS

Twelve AA and 13 C prepubertal (Tanner I) normal-weight children participated in this study at the Pediatric Clinical and Translational Research Center (PCTRC) of Children's Hospital of Pittsburgh after institutional review board approval. Parental consent and child assent were obtained before study participation. Children were recruited through local newspaper advertisements and fliers posted on public transportation buses, the health campus, and the community. All children were in good health, as assessed by a medical history, physical examination, and hematological and biochemical tests (Table 1). None were taking any medications known to affect glucose metabolism. Family history of type 2 diabetes was obtained in all participants, and a positive history was defined as the presence of known cases of diagnosed diabetes in any of three generations, as described before (14).

Clamp studies. Participants were admitted twice within a 1- to 3-week period to the PCTRC for a control experiment of normal saline infusion plus hyperglycemic clamp and another for a 20% intralipid (IL) infusion plus hyperglycemic clamp, conducted in random order. Clamps were performed the morning after PCTRC admission, after a 10- to 12-h overnight fast, as previously described (13).

For each condition, one catheter was inserted into a forearm vein for infusion of normal saline or IL and 20% dextrose, and a second catheter was inserted into the contralateral heated hand vein for sampling of arterialized venous blood. At −180 min before the start of the hyperglycemic clamp, the infusion of 0.9% normal saline or 20% IL (20% soybean oil, 1.2% egg yolk phospholipids, and 2.25% glycerol; Kabi Pharmacia, Clayton, NC) was initiated at 0.02 mL/kg/min and continued until the end of the 2-h clamp. At 0 min, a 2-h hyperglycemic (12.5 mmol/L) clamp was performed to assess first- and second-phase insulin and C-peptide, as previously described (13), together with measurement of FFA and triglycerides (TG).

Body composition and abdominal adiposity. Height and weight were assessed to the nearest 0.1 cm and 0.1 kg, respectively. Dual-energy X-ray absorptiometry was used to determine fat-free mass (FFM), fat mass (FM), and percent body fat (% BF). Assessment of subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) was done using magnetic resonance imaging, as previously described (15). SAT and VAT data are missing for one AA subject due to technical difficulties.

Biochemical measurements. Blood samples from each sampling point were placed on ice, and plasma was immediately separated in a refrigerated centrifuge. Plasma samples were divided into aliquots and stored at −80°C until analysis. Plasma glucose was determined, at the bedside, by the glucose oxidase method using a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH) and plasma insulin and C-peptide by commercially available

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TABLE 1
Physical characteristics of the participants

	AA	C	*P*
n	12	13	
Age (years)	9.5 ± 0.3	9.9 ± 0.4	NS
Sex			NS
Male	6	5	
Female	6	8	
Family history of T2DM			NS
Yes	7	6	
No	4	6	
Height (cm)	138.8 ± 2.4	136.5 ± 2.1	NS
Weight (kg)	31.9 ± 1.3	32.8 ± 1.6	NS
BMI (kg/m^2)	16.5 ± 0.4	17.5 ± 0.4	NS
BMI (%)	47.6 ± 5.8	59.0 ± 5.3	NS
FFM (kg)	23.8 ± 0.7	23.2 ± 1.1	NS
FM (kg)	6.2 ± 0.7	7.8 ± 0.7	NS
BF (%)	19.5 ± 1.5	24.1 ± 1.7	NS
VAT (cm^2)	11.9 ± 2.0	19.5 ± 2.3	0.03
SAT (cm^2)	49.2 ± 4.6	73.5 ± 7.1	0.01
VAT-to-SAT ratio	0.26 ± 0.05	0.27 ± 0.03	NS

T2DM, type 2 diabetes mellitus. Continuous data are presented as mean ± SEM and categoric data as *n*. One AA is missing family history of T2DM and data for VAT and SAT. All subjects are Tanner I puberty.

radioimmunoassay (Linco catalog no. 1011, St. Charles, MO), as reported by us before (16). FFA and TG were determined using enzymatic colorimetric methods with a Wako nonesterified fatty acid (NEFA) C test kit (Wako, Osaka, Japan).

Calculations. Fasting data were expressed as the means of samples at −30, −15, and 0 min. First-phase glucose, insulin, and C-peptide were the means of 2.5-, 5-, 7.5-, 10-, and 12.5-min samples. First-phase FFA was the mean of 5- and 10-min samples. Second-phase insulin and C-peptide were the means of eight determinations every 15 min, from 15 to 120 min. Second-phase glucose was the mean of 22 determinations every 5 min, from 15 to 120 min. Second-phase FFA was the mean of 30-, 60-, 90-, and 120-min samples, and second-phase TG was the mean of 60- and 120-min samples. IS was calculated as the rate of glucose disposal during the last 60 min of the hyperglycemic clamp minus urinary glucose excretion, divided by the mean insulin concentration during the same period and multiplied by 100 and expressed as μmol/kg/min per pmol/L (13). An index of basal insulin clearance (units: $L \cdot min^{-1} \cdot m^{-2}$ body surface area) was computed as the ratio of the model-derived (see below) basal insulin secretion rate divided by the basal insulin concentration (17). An index of insulin clearance during the hyperglycemic clamp was computed as the ratio of the model-derived area under the curve of the insulin secretion rate divided by the area under the curve of the insulin concentration during the clamp (18).

Insulin secretion was modeled during the hyperglycemic clamp and described as a summation of three components: *1*) a basal (postabsorptive) secretion rate, *2*) a dynamic secretion component (glucose sensitivity of first-phase insulin secretion), presented here as the amount of insulin secreted in response to a rate of increase in glucose concentration of 1 mmol/L per 1 min, and *3*) a static or proportional secretion component (glucose sensitivity of second-phase secretion) further boosted by a gain factor (17), presented here as the steady-state insulin secretion rate in response to a step increase in glucose concentration of 1 mmol/L (19–22).

Parameters were estimated by implementing this minimal model of C-peptide secretion in SAAM-II 1.2 software (SAAM Institute, Seattle, WA). Numerical values of the unknown parameters were estimated by using nonlinear least squares. Weights were chosen optimally; that is, equal to the inverse of the variance of the measurement errors, which were assumed to be additive, uncorrelated, with zero mean, and a constant coefficient of variation of 13%. Data are expressed per kg body weight. Results of the analyses did not change when data were expressed relative to body surface area (m^2) or FFM. β-Cell function relative to IS (i.e., DI) was calculated as the product of IS × glucose sensitivity of first- or second-phase insulin secretion (23).

Statistical analyses. Data are presented as mean ± SEM. Statistical significance was set at $P < 0.05$. PASW Statistics 18 software (SPSS Inc., Chicago, IL) was used for the statistical analyses. Because no prior studies have examined the effects of IL infusion on insulin secretion in AA versus C, adult or youth, our sample size was estimated using our previous data that demonstrated a 150% higher first-phase insulin in AA compared with C children (13). Power calculations indicated that to detect a 100% difference between AA and C in the response to IL infusion, 11 subjects would need to complete the study assuming 80% power and 5% α error. To detect a 120 or a 150% difference in the response to IL infusion, 11 or 8 subjects, respectively, would need to complete the study. This assumed a 90% power and 1% α error. Student *t* tests were used to determine differences in subject characteristics between the two groups. Nonparametric statistics were used when appropriate. Categorical variables were compared using the χ^2 analysis. A 2 × 2 repeated-measures ANOVA (within-subjects factor of condition, i.e., normal saline vs. IL; between-subjects factor of race, i.e., AA vs. C; condition × race interaction) was used to analyze hormone and substrate concentrations. Adjustments for VAT were made in the primary outcome variables of basal insulin secretion rate, β-cell glucose sensitivity of first- and second-phase insulin secretion, IS, and DI.

RESULTS

Participant characteristics. Prepubertal AA and C children were similar in age, BMI, FFM, FM, and %BF. AA youth had lower VAT, as reported before (13,24), but a similar VAT-to-SAT ratio (Table 1).

Baseline postabsorptive and hyperglycemic clamp hormone and substrate profiles. Consistent with prior findings, AA youth had higher fasting insulin concentration (13) and lower fasting TG concentrations compared with C youth (15). In response to IL infusion, fasting glucose, insulin, C-peptide, FFA, and TG increased in both groups. First-phase glucose concentrations were slightly higher in C compared with AA youth during normal saline and IL conditions. IL infusion increased first- and second-phase FFA and TG concentrations similarly in AA and C children. First- and second-phase insulin and C-peptide concentrations increased with IL infusion, but the increase in all was greater in AA than C youth (Table 2).

Model-derived parameters of insulin secretion. The basal insulin secretion rate (Fig. 1*A*–*C*) increased significantly and similarly with IL infusion in AAs and Cs (Fig. 1*A*). During normal saline infusion, first-phase insulin secretion (Fig. 1*B*) was significantly higher in AAs versus Cs, consistent with prior findings (13). During IL infusion, there was a significant condition × race interaction, with the increase in glucose sensitivity of first- and second-phase insulin secretion greater in AAs (Fig. 1*B* and *C*). After adjusting for VAT, the condition × race interactions remained significant.

Consistent with prior observations of lower insulin clearance in AA youth (13), basal and clamp insulin clearance were both significantly lower in AA than in C children (Table 2). During IL infusion, no significant changes in insulin clearance were detected in the basal state or during the clamp (Table 2).

IS and β-cell function relative to IS, DI. During IL infusion, IS declined comparably in AA and C children (Fig. 2*A*). During normal saline infusion, first-phase DI was significantly higher in AAs than in Cs, consistent with prior observations (13). During IL infusion, DI, β-cell function relative to IS calculated from modeled first- or second-phase insulin, decreased (Fig. 2*B* and *C*), with a significantly greater decline in second-phase DI in Cs compared with AAs (Fig. 2*C*). After adjusting for VAT, the IL-induced decline in DI and the condition × race interaction remained significant.

VAT and IL-induced β-cell function and IS. To assess whether the amount of VAT per se, irrespective of race, could predict IL-induced changes in β-cell function and IS, we analyzed the whole cohort with AAs and Cs combined. Bivariate relationships revealed that VAT correlated with

TABLE 2
Hormone and substrate concentrations

	AA		C		*P* (ANOVA)		
	Normal saline	Intralipid	Normal saline	Intralipid	Condition	Race	Interaction
Fasting measurements							
FFA (mmol/L)	0.23 ± 0.04	0.72 ± 0.10	0.20 ± 0.04	0.89 ± 0.10	<0.001	NS	NS
Glucose (mmol/L)	5.0 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	0.009	NS	NS
Insulin (pmol/L)	77.5 ± 6.9	104.1 ± 9.0	59.9 ± 7.7	72.1 ± 6.7	<0.001	0.02	NS
C-peptide (nmol/L)	0.43 ± 0.04	0.56 ± 0.04	0.48 ± 0.05	0.56 ± 0.05	<0.001	NS	NS
TG (mmol/L)	0.63 ± 0.12	3.2 ± 0.3	0.81 ± 0.08	4.4 ± 0.46	<0.001	0.04	0.04
Basal insulin clearance*	0.95 ± 0.10	0.93 ± 0.08	1.43 ± 0.10	1.40 ± 0.12	NS	0.0001	NS
Clamp measurements							
First phase							
FFA (mmol/L)	0.21 ± 0.04	0.61 ± 0.10	0.21 ± 0.04	0.84 ± 0.10	<0.001	NS	NS
Glucose (mmol/L)	12.3 ± 0.2	12.3 ± 0.1	12.8 ± 0.2	12.9 ± 0.1	NS	0.006	NS
Insulin (pmol/L)	495.2 ± 44.6	679.0 ± 73.8	330.8 ± 42.9	332.9 ± 70.9	0.032	0.002	0.035
C-peptide (nmol/L)	1.45 ± 0.1	1.78 ± 0.2	1.30 ± 0.1	1.32 ± 0.1	0.017	NS	0.041
TG (mmol/L)	0.63 ± 0.13	3.0 ± 0.27	0.78 ± 0.08	4.3 ± 0.54	<0.001	NS	NS
Second phase							
FFA (mmol/L)	0.08 ± 0.02	0.53 ± 0.10	0.04 ± 0.02	0.66 ± 0.10	<0.001	NS	NS
Glucose (mmol/L)	12.4 ± 0.05	12.4 ± 0.04	12.3 ± 0.04	12.5 ± 0.04	0.03	NS	NS
Insulin (pmol/L)	595.8 ± 65.8	738.4 ± 77.9	418.3 ± 63.2	417.1 ± 74.9	0.014	0.016	0.013
C-peptide (nmol/L)	2.19 ± 0.2	2.53 ± 0.3	2.11 ± 0.2	2.13 ± 0.2	0.019	NS	0.037
TG (mmol/L)	0.57 ± 0.10	3.0 ± 0.3	0.79 ± 0.12	4.2 ± 0.57	<0.001	NS	NS
Clamp insulin clearance*†	0.81 ± 0.03	0.74 ± 0.04	1.16 ± 0.08	1.16 ± 0.08	NS	0.0001	NS

Data are presented as mean ± SEM. *Measured as $L \cdot min^{-1} \cdot m^{-2}$ body surface area. †$P < 0.01$ clamp insulin clearance vs. basal insulin clearance.

IL-induced change in glucose sensitivity of the second-phase insulin secretion ($r = -0.43$, $P = 0.04$), with no significant correlations observed with any of the other outcome measures. Additionally, we performed multiple regression analyses with VAT, age, race, and sex as independent variables and the change in each of IS, glucose sensitivity of first- and second-phase insulin secretion, and DI as dependent variables. VAT, age, and sex did not contribute to the variance in any of the dependent variables; however, race contributed 20.2% ($P = 0.03$) to the variance in the IL-induced change in glucose sensitivity of first-phase and 21.5% ($P = 0.02$) of second-phase insulin secretion.

DISCUSSION

In our efforts to explain the hyperinsulinemia and the upregulated β-cell function relative to IS in AA youth, we hypothesized that the pancreatic β-cell in AA children is more sensitive to the stimulatory effect of FFA on IS compared with Cs. Our hypothesis was based on our previous observations that for the same degree of IS, glucose-stimulated IS is significantly higher in AA versus C children and correlated with plasma FFA concentrations (13,25). In the current study, using C-peptide modeling during a hyperglycemic clamp, a 5-h IL infusion resulted in a significant decline in DI, β-cell function relative to IS, in AA and C youth, indicative of acute lipotoxicity. However, there was a significant racial contrast in absolute β-cell response to IL infusion, with a greater first- and second-phase insulin secretion in AA youth compared with their C peers and a lesser decline in DI in AA youth. This may suggest that C youth are more susceptible to β-cell lipotoxicity than AA youth, or alternatively, that AA youth are hypersensitive to FFA stimulation of β-cell insulin secretion, consistent with our theory.

Most in vitro (4) and in vivo (2,3,6–8) studies, but not all (26), demonstrate that acute FFA elevations increase glucose-stimulated insulin secretion and are an important component for basal insulin secretion (27,28). In the current study, we demonstrate a 32% increase in basal insulin secretion in response to FFA elevation. This is in agreement with our previous observation of FFA elevation significantly increasing basal C-peptide by ~28%, with no differences between AA and C adolescents (16). Among lean men and women, 6 h of IL and heparin infusion increased glucose-stimulated insulin secretion by 37%, as assessed by an intravenous glucose tolerance test (6). In lean women, FFA elevation increased glucose-stimulated (7 mmol/L) plasma insulin concentrations by 30%, with further increases observed at higher plasma glucose concentrations (11 mmol/L), although the latter increase was driven by a reduction in endogenous insulin clearance (8). Similarly, during a two-step hyperglycemic clamp in healthy men, acute IL infusion increased glucose-stimulated insulin secretion ~3.5-fold during the 10 mmol/L plasma glucose concentration, with further increases observed during the 20 mmol/L plasma glucose concentration (7).

We are not aware of literature reports on race-related differences in response to IL infusion and β-cell function. Our investigation demonstrates that whereas AAs responded to IL infusion with a 15 and 32% increase in first- and second-phase insulin secretion, Cs demonstrated a decrease of 7 and 15% in first- and second-phase insulin secretion. The difference in the response to IL infusion between AA and Cs occurred despite adjusting for their level of VAT. In further support of a role of FFA in β-cell insulin secretion is the observation that treatment of fasted adults with nicotinic acid, which suppresses lipolysis and FFA, resulted in a decline in the stimulated-insulin area under the curve (28).

The greater decline in obese (70%) versus normal-weight individuals (40–50%) was suggestive of a greater dependency on circulating FFAs for glucose-stimulated insulin

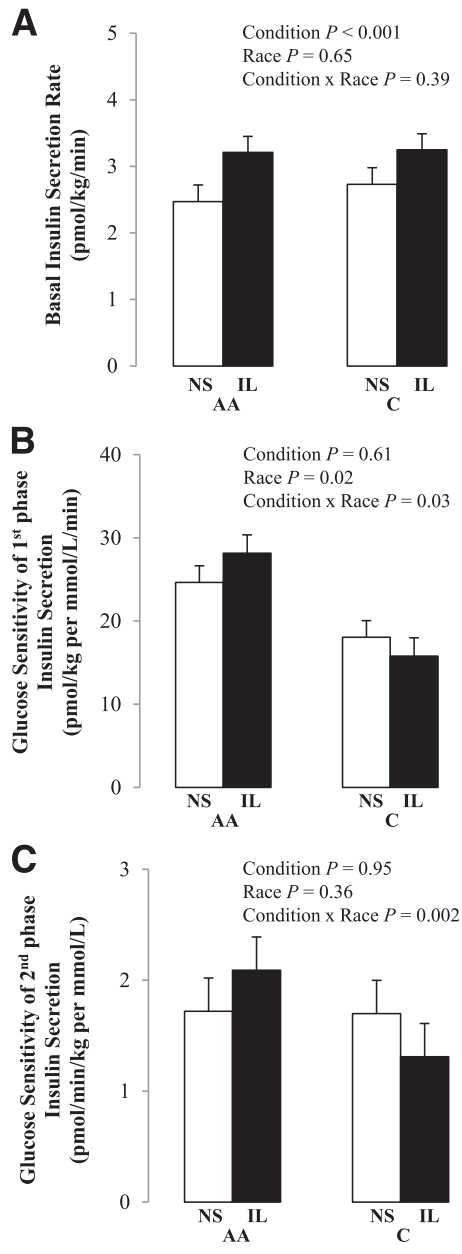

FIG. 1. Basal insulin secretion rate (*A*), glucose sensitivity of first-phase insulin secretion (*B*), and glucose sensitivity of second-phase insulin secretion (*C*) in AA and C children during infusion of normal saline (NS) or 20% IL. Condition = main effect of condition, normal saline vs. IL. Race = main effect of race, AA vs. C. Condition × race = condition × race interaction.

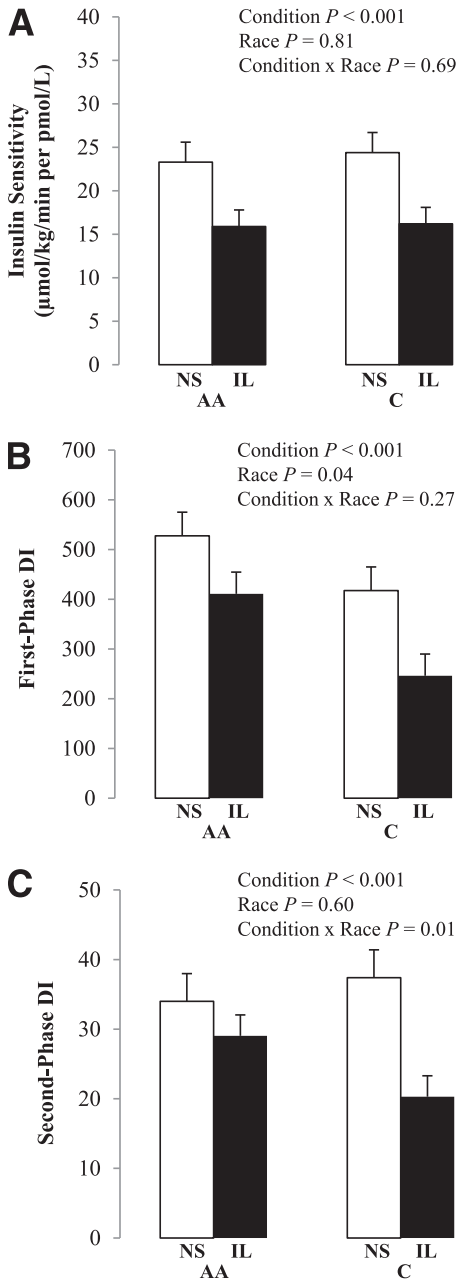

FIG. 2. IS (*A*), DI from first-phase insulin secretion (*B*), and DI from second-phase insulin secretion (*C*) in AA and C children during infusion of normal saline (NS) or 20% IL. Condition = main effect of condition, NS vs. IL. Race = main effect of race, AA vs. C. Condition × race = condition × race interaction.

secretion in obese than in normal-weight adults. Our present findings would suggest that this could be the case in AA youth, who might have a greater dependency on circulating FFA for glucose-stimulated insulin secretion compared with Cs. Furthermore, our prior findings of a significant positive relationship between fasting FFA concentrations and first-phase insulin secretion in AAs, with no such relationship in Cs (13), lend further support to such a theory. Whether suppression of FFA levels may demonstrate a racial contrast in FFA dependency of β-cell insulin secretion remains to be determined.

We and others have shown that infusion of IL and/or elevation of plasma FFA concentration is associated with a decline in in vivo IS (2,3,7,16). In a prior study using a hyperinsulinemic-euglycemic clamp together with IL infusion, we found that the decline in IS was similar between AA and C adolescents (16). The findings from the current study in prepubertal children are consistent with those in adolescents. Because FFAs induce insulin resistance, expressing first- and second-phase insulin secretion in absolute terms, without consideration of IS, may not provide a complete picture of the dynamic relationship between the decline in IS and the change in β-cell function consequent to IL infusion. Therefore, in addition, we elected to express insulin secretion relative to IS, or the DI. We originally anticipated that DI would remain comparable to the normal saline condition because insulin

secretion would compensate for the decline in IS. Contrary to our expectation, the present data demonstrate that β-cell function relative to IS declines during IL versus normal saline infusion in AA and C youth, suggestive of a lipotoxic phenomenon. This seems to be more pronounced in C (39 and 44% decline in first- and second-phase DI) than in AA youth (22 and 12% decline). This is the first demonstration that a β-cell lipotoxic phenomenon might be operational in young prepubertal youth. The potential ramification of such an observation is the adverse effect that high-fat foods might have on β-cell function starting early in life and progressing with aging and on racial predisposition to β-cell dysfunction driven by dietary factors. We previously demonstrated that an increased fat-to-carbohydrate ratio in the diet of prepubertal youth is associated positively with first-phase insulin secretion and negatively with IS (13).

The strengths of this investigation include *1*) the paired design of IL versus normal saline conditions in the same youth participant, *2*) the use of the gold standard hyperglycemic clamp to determine in vivo insulin secretion, and *3*) modeling of insulin secretion from C-peptide data, which overcomes race-related differences in insulin clearance (13,29). A potential limitation could be that IS and insulin secretion, as well as DI, were derived from a single hyperglycemic clamp instead of two separate clamps: a hyperinsulinemic-euglycemic clamp for IS and a hyperglycemic clamp for insulin secretion. However, we recently demonstrated, in 330 youth, that IS from the hyperglycemic clamp correlates with IS from the euglycemic clamp ($r = 0.90$, $P < 0.001$) and that DI derived from a single hyperglycemic clamp correlates with DI from two clamps: a euglycemic clamp and a hyperglycemic clamp ($r = 0.85$, $P < 0.001$) (23), thus supporting the use of a single hyperglycemic clamp (23). Further, a single-clamp approach is similar to the intravenous glucose tolerance test approach from which IS and acute insulin response are both measured and DI is calculated (30).

Another potential limitation of our study is that we could not assess the effect of family history of type 2 diabetes on β-cell response to FFA elevations because our study was not powered to address this. However, there was no difference between AAs and Cs with respect to family history of type 2 diabetes (Table 1). Others have demonstrated that family history of diabetes is associated with an enhanced susceptibility to lipotoxicity in adults (12). Additional studies in youth are needed to assess if this familial risk is present early in life.

In conclusion, in the current study, AA and C prepubertal youth both demonstrate a decline in β-cell function relative to IS with IL infusion, indicative of acute lipotoxicity. The greater decline in C compared with AA youth may suggest that C youth are more susceptible to β-cell lipotoxicity than AA youth, or alternatively, that AA youth are hypersensitive to FFA stimulation of β-cell insulin secretion, consistent with our theory. Whether the latter is reflective of a greater dependency of glucose-stimulated insulin secretion on circulating FFA in AAs remains to be determined. Lastly, it remains to be investigated if dietary FFAs might induce similar adverse changes in β-cell function in youth.

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S.F.M. was first author of the manuscript and contributed to the data analyses and interpretation. R.C.B. provided C-peptide modeling analysis and critically reviewed and edited the manuscript. L.A.S. and S.L. contributed to data interpretation and reviewed and edited the manuscript. L.F. maintained the database and contributed to data analysis. S.A.A. provided the study concept and design; acquired data; obtained funding; provided administrative, technical, and material support; supervised the study; and critically reviewed and edited the manuscript. S.A.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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