

# Effects of an Intravenous Lipid Challenge and Free Fatty Acid Elevation on In Vivo Insulin Sensitivity in African American Versus Caucasian Adolescents

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**OBJECTIVE**— African American youth have lower insulin sensitivity than their Caucasian peers, but the metabolic pathways responsible for this difference remain unknown. Free fatty acids (FFAs) are associated with insulin resistance through the Randle cycle. The present investigation determined whether elevating FFA is more deleterious to insulin sensitivity in African American than in Caucasian adolescents.

**RESEARCH DESIGN AND METHODS**— Insulin sensitivity (3-h hyperinsulinemic-euglycemic clamp) was evaluated in 22 African American and 21 Caucasian adolescents on two occasions: 1) infusion of normal saline and 2) infusion of 20% intralipid.

**RESULTS**— During intralipid infusion, fasting insulin and C-peptide concentrations increased while fasting glucose and basal glucose turnover did not change in either group. Insulin sensitivity decreased similarly in African American (normal saline  $7.65 \pm 0.61$  vs. intralipid  $5.15 \pm 0.52 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  per pmol/l) and Caucasian subjects (normal saline  $8.97 \pm 0.85$  vs. intralipid  $5.96 \pm 0.56 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  per pmol/l) ( $P < 0.001$ ).

**CONCLUSIONS**— African American and Caucasian adolescents respond to FFA elevation similarly through increased fasting insulin secretion to maintain fasting glucose homeostasis and reduced peripheral glucose uptake and insulin resistance. Thus, African American adolescents are not more susceptible to FFA-induced insulin resistance than Caucasian youth.

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Free fatty acids (FFAs) are implicated in insulin resistance development through the Randle cycle (1,2). In adults, elevating plasma FFA by infusing lipid emulsion inhibits glucose disposal and oxidation in normal-weight, obese, and type 2 diabetic patients (3–5). The only pediatric study was performed in Caucasian children and demonstrated that intralipid infusion decreased insulin sensitivity in pubertal adolescents, with no such effect in prepubertal children (6). Such data support that pubertal insulin

resistance is driven by the Randle cycle/substrate competition (7,8).

Insulin sensitivity is lower in African American children than in their Caucasian peers (9,10), but the metabolic pathways responsible for this contrast remain unknown. Lower insulin sensitivity correlates inversely with an increased fat-to-carbohydrate ratio in the diet of African American children (10,11). Thus, FFAs may be detrimental to insulin sensitivity in African American children. If race-related differences in the susceptibility to

FFA-induced insulin resistance exist, then this is of increased importance during puberty when increased FFAs decrease glucose disposal (6). The present investigation, therefore, aimed to compare the effects of FFA elevation on fasting and insulin-stimulated glucose metabolism in African American and Caucasian adolescents. We hypothesized that increased concentrations of circulating FFAs result in a greater decrement in insulin sensitivity in African American than in Caucasian adolescents.

## RESEARCH DESIGN AND METHODS

Twenty-two African American and twenty-one Caucasian adolescents, recruited through advertisements, participated in this study at the Pediatric Clinical and Translational Research Center of Children's Hospital Pittsburgh after institutional review board approval. Participants and their parents gave written informed consent. Participants were in good health as assessed by medical history, physical examination, and hematological and biochemical tests (Table 1). All participants were in Tanner stages II–V of puberty and were weight stable before study entry. None were dieting, and none were receiving medications including contraceptive pills. Total body composition and abdominal adiposity were assessed by dual-energy X-ray absorptiometry and computed tomography, respectively, as previously described by us (10).

## Study design

Participants maintained free-living conditions during the experimental period. None took part in organized sports. We prescribed a weight-maintaining diet containing 55% carbohydrate, 30% fat, and 15% protein during the investigations. Each participant undertook a 3-h hyperinsulinemic-euglycemic clamp on two occasions after an overnight fast during infusion of normal saline or infusion of 20% intralipid in random order and 1–2 weeks apart.

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Table 1—Physical and biochemical characteristics of the participants

	African American	Caucasian	P
n	22	21	
Age (years)	13.3 ± 0.2	13.9 ± 0.3	0.09
Sex (male/female)	11/11	13/8	0.43
Family history of type 2 diabetes (+/−)	16/6	10/11	0.09
Tanner stage			
II–III	8	9	—
IV–V	14	12	—
Height (cm)	159.8 ± 2.1	163.3 ± 2.1	0.25
Weight (kg)	58.7 ± 2.5	61.4 ± 3.5	0.53
BMI (kg/m <sup>2</sup> )	22.9 ± 0.7	22.8 ± 1.0	0.97
BMI percentile	72.8 ± 5.6	68.3 ± 6.3	0.59
Fat mass (kg)	13.9 ± 1.7	15.8 ± 2.2	0.50
Body fat %	23.6 ± 2.2	25.4 ± 2.5	0.61
Visceral adipose tissue (cm <sup>2</sup> )	26.2 ± 3.5	37.7 ± 4.6	0.052
Subcutaneous adipose tissue (cm <sup>2</sup> )	131.7 ± 17.8	187.2 ± 26.8	0.09
Testosterone (nmol/l)*	11.7 ± 2.2	13.6 ± 2.2	0.56
Estradiol (pmol/l)*	93 ± 19	88 ± 18	0.87
A1C (%)	5.2 ± 0.1	5.2 ± 0.1	0.85

Data are means ± SEM unless otherwise indicated. Sex and family history of type 2 diabetes compared using  $\chi^2$  analysis. All other variables compared using an independent Student's *t* test. \*Testosterone in males only and estradiol in females only.

For each condition, one intravenous catheter was inserted into a forearm vein for administration of test infusions and a second in a vein of the contralateral hand, after being heated, for sampling of arterialized venous blood (8). After blood samples were obtained at 0700 h (−180 min from start of hyperinsulinemic-euglycemic clamp), a primed (2.2  $\mu\text{mol/kg}$ ), constant infusion of [6, 6-<sup>2</sup>H<sub>2</sub>] glucose (0.22  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was administered from 0700 to 1300 h (6). At 0700 h, either a triglyceride emulsion of 20% intralipid (20% soybean oil, 1.2% egg yolk phospholipids, and 2.25% glycerol; Kabi Pharmacia, Clayton, NC) or 0.9% saline was infused at 0.02 ml  $\cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  until 1300 h. Blood was sampled at −180 min and every 10 min from −30 to 0 min (basal period) for determination of plasma glucose, insulin, C-peptide, FFA, and isotopic enrichment. Plasma triglycerides were determined at −30 and 0 min of the basal infusion period.

After basal evaluation (0700–1000 h), insulin-stimulated glucose metabolism was investigated by a 3-h hyperinsulinemic-euglycemic clamp (8) starting at 1000 h. Intravenous crystalline insulin (Humulin; Lilly, Indianapolis, IN) was infused at a constant rate of 40 mU/m<sup>2</sup> per min for 3 h. Plasma glucose was clamped at 5.5 mmol/l with a variable rate infusion of 20% dextrose, enriched with [6, 6-<sup>2</sup>H<sub>2</sub>] glucose (6). The glucose infusion

rate was adjusted based on arterialized plasma glucose measurements every 5 min. Blood was sampled every 10 min over the last 30 min of the clamp (150–180 min) for determination of insulin, FFA, and isotope enrichment. Triglycerides were determined at 150 and 180 min of the clamp.

For each condition, indirect calorimetry was performed using a ventilated hood system (Deltatrac Metabolic Monitor; SensorMedics, Anaheim, CA) for a 30-min period before starting (−30 to 0 min) (basal period) and ending the (150–180 min) clamp (6). Urine was collected overnight from 2300 h until 0700 h for urinary nitrogen measurement for calculation of baseline substrate oxidation and from 0700 h until 1300 h for calculating substrate oxidation during the clamp.

#### Biochemical measurements

At each sampling point, blood samples were placed immediately on ice and plasma was separated within 15 min in a refrigerated centrifuge. Plasma samples were divided into aliquots and stored at −80°C until analysis. Plasma glucose was measured by the glucose oxidase method using a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin and C-peptide were determined by radioimmunoassay (12). Enzymatic colorimetric methods were used to determine FFA (Wako nonesterified fatty

acid [NEFA] C test kit; Wako, Osaka, Japan) and triglyceride concentrations. Urinary nitrogen was measured by the Kjeldahl method (8). Deuterium enrichment of plasma glucose was determined on a gas chromatography mass spectrometer (model 5985A; Hewlett Packard, Palo Alto, CA) (6,13). Standard curves of known enrichments were performed with each assay.

#### Calculations

Basal rate of appearance of endogenous glucose in the plasma or hepatic glucose production was calculated during the last 30 min of the postabsorptive period (−30 to 0 min) using steady-state tracer dilution equations (8). Under basal conditions, rate of appearance equals glucose rate of disposal ( $R_d$ ). Calculations were made over the last 30 min of the 3-h clamp (150–180 min) for insulin sensitivity and clearance (6,10).

Basal and insulin-stimulated substrate oxidation rates were calculated from indirect calorimetric data during each period (14). Basal and insulin-stimulated nonoxidative glucose  $R_d$  was estimated by subtracting the rate of glucose oxidation from the total  $R_d$  during the last 30 min of the basal period (−30 to 0 min) and clamp (150–180 min).

#### Statistical analysis

Statistics were analyzed using SPSS 15.0 for Windows. Based on our previous data (6,10), the current study was powered (80% power with 22 participants in each group) to test the primary hypothesis that the decrement in insulin sensitivity with intralipid infusion would be 60% greater in African American adolescents than in Caucasian adolescents. A 2 × 2 mixed ANOVA (repeated measures within subjects based on factor of condition, i.e., normal saline vs. intralipid; between subjects based on factor of race, i.e., African American vs. Caucasian; and condition × race interaction) was used to analyze glucose; insulin; C-peptide; triglycerides; FFAs; fat oxidation; total, oxidative, and nonoxidative glucose disposal; and insulin sensitivity. Sex-related differences were analyzed with a 2 × 2 mixed ANOVA (repeated measures within subjects based on factor of condition, i.e., normal saline vs. intralipid; between subjects based on factor of sex, i.e., male vs. female; and condition × sex interaction), combining African American and Caucasian data. Data are presented as means ± SEM with significance set at  $P < 0.05$ .

Table 2—Hormone and substrate concentrations during the last 30 min of the 3-h baseline period in the normal saline vs. intralipid infusion conditions

	African American		Caucasian		P		
	Normal saline	Intralipid	Normal saline	Intralipid	Condition	Race	Interaction
Glucose (mmol/l)	5.25 ± 0.05	5.27 ± 0.06	5.35 ± 0.09	5.40 ± 0.07	0.340	0.198	0.728
C-peptide (nmol/l)	0.66 ± 0.04	0.86 ± 0.06	0.73 ± 0.05	0.93 ± 0.06	<0.001	0.364	0.878
Triglycerides (mmol/l)	0.93 ± 0.08	5.42 ± 0.35	1.32 ± 0.20	6.03 ± 0.64	<0.001	0.219	0.162

Data are means ± SEM.

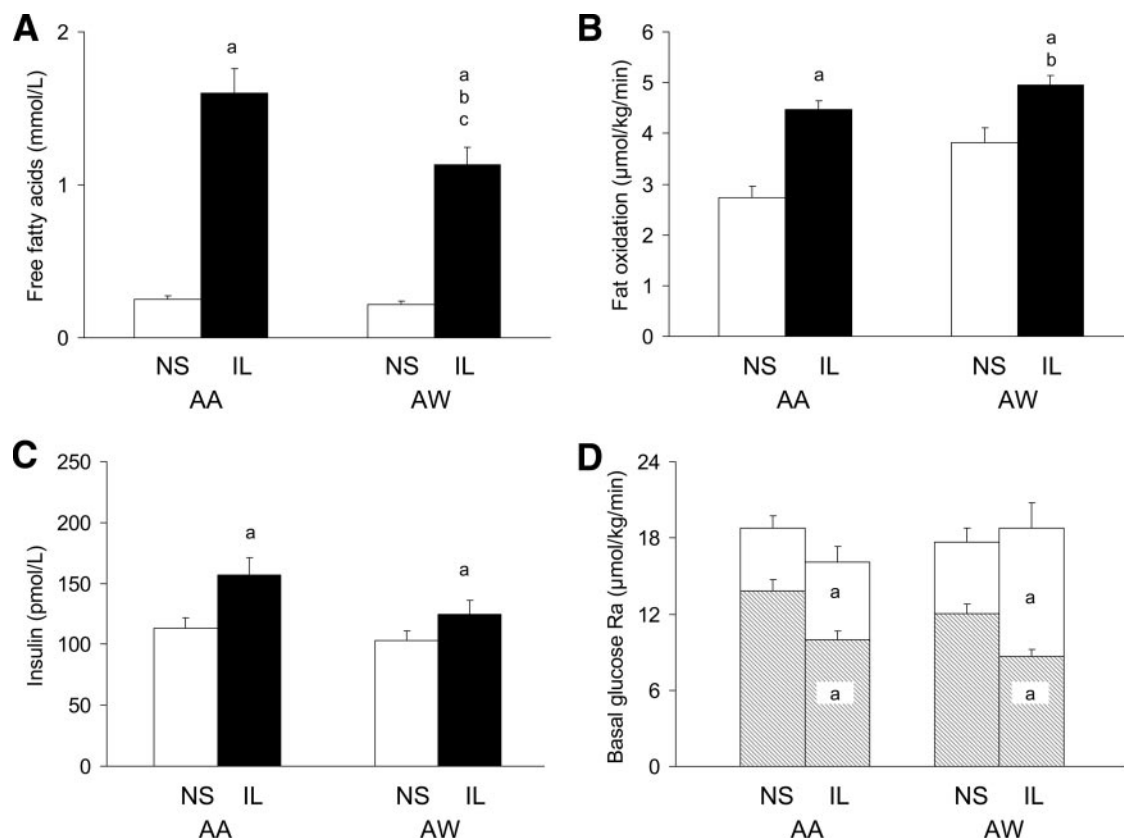
## RESULTS

### Basal period

Hormone and substrate concentrations during the final 30 min of the baseline period are presented in Table 2 and Fig. 1. Insulin, C-peptide, and triglyceride concentrations all increased with intralipid infusion (main effect of condition for all variables;  $P < 0.001$ ) (Table 2, Fig. 1C) with no differences between African American and Caucasian adolescents. Concentrations of FFA significantly increased with intralipid

infusion (main effect of condition;  $P < 0.001$ ) (Fig. 1A) and, overall, were significantly higher in African American than in Caucasian subjects (main effect of race;  $P = 0.010$ ). The increase in FFA concentrations between conditions was greater in African American than in Caucasian subjects (condition × race interaction;  $P = 0.016$ ). Despite higher FFAs with intralipid infusion, there were no differences in fasting glucose concentrations between conditions and no differences between African American and Caucasian subjects (Table 2).

Fat oxidation was significantly greater with intralipid infusion (main effect of condition;  $P < 0.001$ ) (Fig. 1B). Overall, fat oxidation was greater in Caucasian than in African American subjects (main effect of race;  $P = 0.008$ ). Oxidative glucose disposal decreased with intralipid infusion (main effect of condition;  $P < 0.001$ ), whereas nonoxidative glucose disposal increased (main effect of condition;  $P = 0.012$ ) (Fig. 1D), with no difference between African American and Caucasian subjects.



**Figure 1**—FFA concentration (A), fat oxidation (B), insulin concentration (C), and glucose rate of appearance (Ra) (D) in African American (AA) ( $n = 22$ ) and Caucasian (AW) ( $n = 21$ ) adolescents during infusion of normal saline (NS) (empty bars) or 20% intralipid (IL) (filled bars) in the final 30 min of the baseline postabsorptive period. D: Hatched bars represent glucose oxidation, and blank bars represent nonoxidative glucose disposal. <sup>a</sup>Main effect of condition, normal saline vs. intralipid ( $P < 0.05$ ). <sup>b</sup>Main effect of race, African American vs. Caucasian ( $P < 0.05$ ). <sup>c</sup>Condition × race interaction ( $P < 0.05$ ).

Table 3—Hormone and substrate concentrations during the last 30 min of the 3-h hyperinsulinemic-euglycemic clamp during normal saline vs. intralipid infusion

	African American		Caucasian		P		
	Normal saline	Intralipid	Normal saline	Intralipid	Condition	Race	Interaction
Glucose (mmol/l)	5.57 ± 0.02	5.57 ± 0.03	5.61 ± 0.03	5.59 ± 0.02	0.689	0.193	0.848
Insulin (pmol/l)	676.9 ± 27.7	688.4 ± 24.4	606.4 ± 25.0	650.2 ± 24.8	0.130	0.091	0.373
Triglycerides (mmol/l)	0.65 ± 0.06	6.17 ± 0.44	1.12 ± 0.22	6.93 ± 0.94	<0.001	0.190	0.028

Data are means ± SEM.

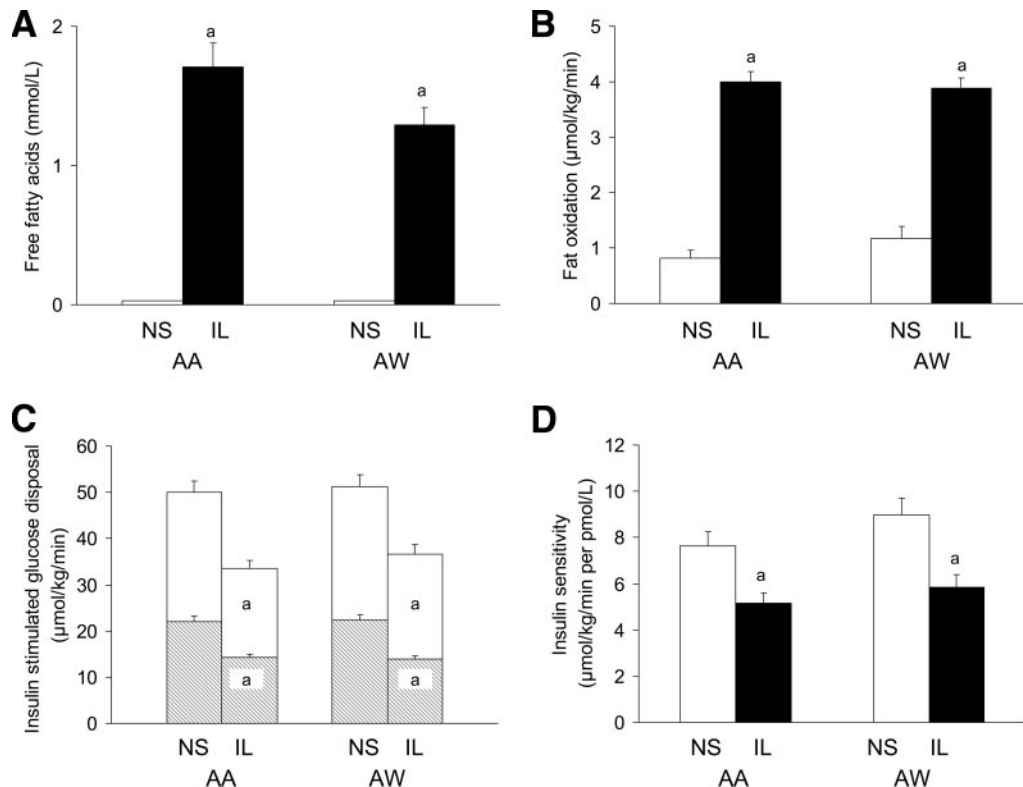
### Hyperinsulinemic-euglycemic clamp

Hormone and substrate concentrations during the final 30 min of the hyperinsulinemic-euglycemic clamp are presented in Table 3 and Fig. 2. Steady-state plasma glucose and insulin concentrations did not differ between conditions or groups during the clamp (Table 3). Mean within-subject coefficient of variation of blood glucose during the clamp was  $3.6 \pm 1.8\%$ . Plasma triglyceride concentrations were significantly greater with intralipid infusion but did not differ overall between the African American and Caucasian adolescents. However, the increase in plasma triglyceride concentrations between conditions was greater in African

American than Caucasian subjects (condition × race interaction;  $P = 0.028$ ) (Table 3). FFA concentrations (main effect of condition;  $P < 0.001$ ) (Fig. 2A) and fat oxidation (main effect of condition;  $P < 0.001$ ) (Fig. 2B) were increased with intralipid, but there was no difference between African American and Caucasian subjects. Both oxidative (main effect of condition;  $P < 0.001$ ) and nonoxidative (main effect of condition;  $P < 0.001$ ) glucose disposal decreased with intralipid infusion, with the changes similar between the African American and Caucasian groups (Fig. 2C). Insulin sensitivity was lower during intralipid infusion (main effect of condition;  $P <$

0.001), but the change in insulin sensitivity between conditions did not differ between the African American and Caucasian youth (Fig. 2D).

Sex-related differences were analyzed combining African American and Caucasian data. During intralipid infusion, insulin sensitivity declined significantly in both female (normal saline  $7.95 \pm 0.56$  vs. intralipid  $4.73 \pm 0.38 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  per pmol/l) and male subjects (normal saline  $8.58 \pm 0.83$  vs. intralipid  $6.20 \pm 0.59 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  per pmol/l; main effect of condition,  $P < 0.001$ ), with no sex differences. Both oxidative (main effect of condition;  $P < 0.001$ ) and nonoxidative (main effect of



**Figure 2**—FFA concentration (A), fat oxidation (B), insulin-stimulated glucose disposal (C), and insulin sensitivity (D) in African American (AA) ( $n = 22$ ) and Caucasian (AW) ( $n = 21$ ) adolescents during infusion of normal saline (NS) (empty bars) or 20% intralipid (IL) (filled bars) in the final 30 min of the 3-h hyperinsulinemic-euglycemic clamp. C: Hatched bars represent glucose oxidation, and blank bars represent nonoxidative glucose disposal. <sup>a</sup>Main effect of condition, normal saline vs. intralipid ( $P < 0.05$ ).



condition;  $P = 0.019$ ) glucose disposal decreased significantly with intralipid infusion, but there was no difference between male and female adolescents. Concentrations of FFA increased significantly with intralipid in both male (normal saline  $0.04 \pm 0.00$  vs. intralipid  $1.64 \pm 0.18$  mmol/l) and female (normal saline  $0.03 \pm 0.00$  vs. intralipid  $1.34 \pm 0.10$  mmol/l; main effect of condition,  $P < 0.001$ ) subjects and were similar between sexes.

**CONCLUSIONS**— We hypothesized that increasing FFA concentrations results in a greater decrement in insulin sensitivity in African American than in Caucasian adolescents. This was based on observations that African American youth have lower insulin sensitivity than Caucasian youth (9,10) and that an increased fat-to-carbohydrate ratio in the diet of African American youth correlates negatively with insulin sensitivity and clearance (10,11) and positively with fasting FFA and insulin secretion (10). Contrary to our hypothesis, we found that FFA-induced insulin resistance is similar between African American and Caucasian adolescents. It does not appear, therefore, that the glucose–fatty acid (Randle) cycle (1) explains insulin sensitivity differences between African Americans and Caucasians. A number of secondary observations from this study, however, deserve brief elaboration. Some caution is required in interpreting these secondary observations because our study was not powered to test them.

The present study is the first to examine race-related differences in susceptibility to FFA-induced insulin resistance. Consistent with adult studies (3–5) and our prior pediatric investigation examining the Randle cycle (6), we found decreased insulin-stimulated glucose disposal and induction of insulin resistance with intralipid infusion. In addition, the present study and our previous pediatric investigation found that intralipid infusion did not alter basal glucose turnover or concentration. Rather, FFA elevation resulted in increased fasting insulin and C-peptide concentrations in both African American and Caucasian adolescents—a finding consistent with FFA's short-term stimulatory effect on insulin secretion in Caucasian adults (15). Because of the supraphysiological levels of FFAs achieved with intralipid infusion, the relevance of these findings may be limited to extremely insulin-resistant or hyperinsulinemic subjects with hypertriglyceridemia.

During the baseline period, intralipid infusion resulted in a greater increase in plasma FFA concentrations in African American than in Caucasian subjects. Whether the lower fat oxidation observed in the African American group during the postabsorptive period could potentially explain that group's greater increase in FFA levels cannot be surmised from the current data. Our study design did not completely control for the antecedent diet and physical activity of the participants, factors which influence postabsorptive substrate oxidation. Therefore, between-group differences in fat oxidation cannot be confidently attributed to ethnic factors. Despite higher FFA concentrations in the African American group, however, glucose turnover was similar at baseline between the two groups of adolescents.

We did not find any sex differences with intralipid-induced insulin resistance. Previous adult studies examining sex-related differences in FFA-induced insulin resistance are disparate (16–18). One study (16) found that FFA elevation significantly decreased insulin-stimulated total and nonoxidative glucose disposal in men, with no change in women. Conversely, another study (17), with similar conditions and participants, found FFA-impaired glucose disposal in women. Insulin sensitivity was not reported, however, in either study. A recent investigation (18) comparing obese male and female subjects found glucose disposal similar between sexes with intralipid infusion but found insulin sensitivity lower in male than in female participants. Disparity among the results of these adult studies and our data could be related to age, maturational stage, adiposity state, or number of participants studied.

In conclusion, our study demonstrates that African American adolescents are not more susceptible to FFA-induced insulin resistance than Caucasian youth. Data on long-term elevations in FFA concentrations in adolescents, induced by prolonged lipid-rich diets, are needed to assess an elevation's full impact on insulin resistance. It also remains to be determined whether the increased insulin secretion previously reported in healthy African American youth (10,19) is a stimulatory phenomenon of FFA on the  $\beta$ -cell.

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