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Activation of innate immunity modulates insulin sensitivity, glucose effectiveness and pancreatic β -cell function in both African ancestry and European ancestry healthy humans

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Abstract

Objective—Insulin resistance is a risk factor for type 2 diabetes, and is associated with inflammatory cardiometabolic disease. Given differences between African ancestry (AA) and European ancestry (EA) in the epidemiology of type 2 diabetes as well as in response to inflammatory stress, we investigated potential race differences in glucose homeostasis responses during experimental endotoxemia in humans.

Methods—Healthy volunteers (age 18–45 years, BMI 18–30 kg/m², 47% female, African-ancestry (AA, n=42) and European-ancestry (EA, n=106)) were recruited as part of the Genetics of Evoked Responses to Niacin and Endotoxemia (GENE) Study. Subjects underwent an inpatient endotoxin challenge (1ng/kg LPS) and two frequently-sampled intravenous glucose tolerance tests

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(FSIGTT). Insulin and glucose values obtained during FSIGTT pre- and 24-hours post-LPS were analyzed using the minimal model.

Results—FSIGTT derived insulin sensitivity index (S_I), disposition index (DI) and glucose effectiveness (S_G) decreased significantly following LPS ($p < 0.0001$) while the acute insulin response to glucose (AIR_g) increased ($p < 0.0001$). Although expected race differences were observed in glucose homeostasis parameters at baseline prior to LPS e.g., lower S_I (2.5 vs. 4.1 $\mu\text{U/L/min}$, $p < 0.0001$) but higher AIR_g (median 848 vs. 290 $\mu\text{U/L/min}$, $p < 0.0001$) in AA vs. EA, the changes in glucose homeostasis responses to LPS were directionally and proportionally consistent across race e.g., S_I median -35% in EA and -29% in AA and AIR_g median $+17\%$ in EA and $+26\%$ in AA.

Conclusion—Both EA and AA samples modulated glucose and insulin homeostasis similarly during endotoxemia.

Implications—Race differences in response to environmental inflammatory stress are unlikely to be a substantial contributor to the observed difference in diabetes incidence and complications between EA and AA.

Keywords

Insulin sensitivity; Glucose effectiveness; LPS; Race differences

INTRODUCTION

The global prevalence of type 2 diabetes is increasing rapidly and is characterized by the development of insulin resistance, a decrease in the efficiency of insulin to lower blood glucose levels, as well as failure of pancreatic β -cells to secrete adequate insulin to dispose of blood glucose [1]. This may be accompanied by reduced glucose effectiveness, i.e. a reduction in the ability of glucose to mediate its own disposal independent of insulin [2]. Overt diabetes typically develops over time, despite expansion of β -cells and enhanced insulin secretion, because this adaptation fails to compensate for increasing insulin resistance and decreasing glucose effectiveness, and the body can no longer maintain normal glucose homeostasis [3]. Risk factors for insulin resistance and β -cell failure are complex and include both genetic [4] and lifestyle, such as diet and lack of physical activity [5].

While the mechanisms remain incompletely understood, inflammation may play a role in the pathogenesis of type 2 diabetes [6]. Low-grade adipose and systemic inflammation coincide with the insulin resistant state found in obesity and diabetes [7]. Several lines of evidence suggest that maladaptive innate immune signaling, in response to genetic and environmental cues, may increase inflammatory macrophage and T-cell infiltration in adipose and liver, driving local and systemic insulin resistance [8, 9]. Ultimately, failure of the pancreatic β -cell to compensate under chronic inflammatory stress might precipitate overt diabetes [1, 3]. Circulating inflammatory markers have been found to differ by race, with higher baseline levels in AA compared with EA [10, 11].

The prevalence of diabetes differs by race, suggesting that genetic and/or environmental differences may play a role in diabetes susceptibility [12]. For example, individuals of

African ancestry are ~2-times more likely to develop the disease than those of European ancestry and have a higher rate of diabetic complications, with increased morbidity and mortality [13]. Thus, heterogeneity in the incidence and complications of diabetes by race might be attributable to differences in underlying insulin resistance and β -cell homeostasis that emerge before the onset of disease, and these differences may be exacerbated by physiological influences including inflammatory stress. We hypothesized that mechanisms leading to higher chronic, basal inflammation but reduced ability to mount an immune response to an inflammatory stimulus in AA compared with EA may also influence insulin and glucose metabolism both acutely and chronically, leading to differences in development of insulin resistance and diabetes.

Experimental endotoxemia (lipopolysaccharide, LPS) is an established model for studying acute activation of innate immunity in humans [14]. Endotoxemia induces acute insulin resistance and adipose inflammation, characteristic of that observed chronically in clinical syndromes of metabolic syndrome and diabetes [15-17]. We analyzed frequently-sampled intravenous glucose tolerance tests (FSGITT) at baseline and after administration of intravenous endotoxin (1ng/kg LPS) in 148 young healthy, non-diabetic subjects of African and European ancestry, as part of the Genetics of Evoked Responses to Niacin and Endotoxemia (GENE) Study [18]. We hypothesized that FSIGTT-derived measures of insulin sensitivity, glucose homeostasis and β -cell function would be altered in response to LPS and that these responses might differ by race.

MATERIALS AND METHODS

Study Design and Sample

Healthy volunteers (N=294) recruited as part of the GENE Study [18] completed an endotoxin challenge and two FSIGTTs (Figure 1). Eligible individuals were invited to the Clinical and Translational Research Center at the University of Pennsylvania (UPenn) for a baseline visit (FSIGTT and dietary run-in) and returned ~2 weeks later for a standardized inpatient endotoxin protocol [18]. After overnight acclimatization, LPS (1ng/kg *E coli*-derived; U.S. standard reference lot No.CCRE-LOT-1+2, NIH, Bethesda MD) was administered intravenously at 6am. Serial clinical and blood samples were collected for 24 hours. A second FSIGTT was conducted 24-hours post-LPS, a time-point at which we have observed LPS-induced insulin resistance in a previous small study [17]. Participants provided written informed consent and the protocol was approved by the UPenn Institutional Review Board with oversight by an independent NIH-appointed data and safety monitoring board. The GENE Study was registered at clinicaltrials.gov (NCT00953667). The first 160 individuals enrolled (113 EA, 47 AA, age 18-45, BMI <30) who completed both the pre-LPS and post-LPS FSIGTT were selected for analysis, presented here. FSIGTT glucose and insulin levels were measured in this sequential subset selected to have sufficient power to address our hypothesis (power calculations below), while retaining the rest of the sample for future genetic and replication studies. Based on our prior LPS studies [16, 17], we required 32 subjects to detect LPS-induced changes in S_I (25% decrease) and 94 subjects to detect LPS-induced changes in AIRg (10% increase) at 80% power and one-sided test $\alpha < 0.05$ (given known directionality of changes). Further, our sample size was adequately powered

to detect a difference by race in Delta S_I (require $n=96$ EA and $n=38$ AA at $>80\%$ power and $\alpha < 0.05$ with effect size f 0.3). Power calculations were carried out using G*Power 3.1.9.2 [19]. Of the 160 participants, we included final FSIGTT data for 148 participants (106 EA, 42 AA) after exclusion of individuals with missing or poor quality insulin and glucose curves (as described below).

Frequently sampled intravenous glucose tolerance testing (FSIGTT)

FSIGTTs were performed and data analyzed as previously described by our group [20]. Briefly, a 50% glucose bolus (0.3 g/kg) was administered intravenously at baseline and an insulin bolus (0.03U/kg) injected 20 minutes post-glucose. Three baseline samples were collected (-10 , -5 , -1 min) as well as 21 samples after glucose (2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 25, 30, 40, 50, 70, 100, 140, and 180 min). Blood collected in EDTA tubes was placed on ice, centrifuged, and the plasma aliquots were stored at -80°C prior to insulin (radio-immunoassays, Linco Research, St Charles, MO) and glucose (Hitachi 912 automated chemistry system, Wako Diagnostics, Richmond VA) assays.

FSIGTT data modeling and QC

Data were modeled using MINMOD Millennium [21, 22], implementing Bergman's minimal model [23, 24] which incorporates the insulin and glucose values obtained during FSIGTT to calculate parameters of glucose and insulin sensitivity including the insulin sensitivity index (S_I), glucose effectiveness (S_G), the acute insulin response to glucose (AIR_g), and the disposition index (DI). S_I approximates tissue glucose uptake capacity in response to insulin, as well as suppression of hepatic glucose production, and is calculated as the fractional glucose disappearance per insulin concentration unit [24]. FSIGTT-derived S_I correlates significantly with clamp-derived parameters in subjects with normal glucose tolerance [25]. AIR_g characterizes the first phase insulin response to glucose, calculated as the as the incremental insulin area under the curve in the ten minutes following glucose administration, and is representative of pancreatic β -cell function. S_G estimates the ability of glucose to mediate its own disposal, while the disposition index represents the degree to which the β -cell can increase insulin secretion as a consequence of insulin resistance, and is a product of S_I and AIR_g [22]. Relative to fasting measures, FSIGTT-derived parameters provide integrated physiological insights because they are more reflective of dynamic changes occurring during feeding, and capture both peripheral (muscle and adipose) and hepatic insulin sensitivity. Further, unlike clamps, FSIGTT allow simultaneous estimation of pancreatic β -cell function, an important parameter for consideration in our study. We focused primarily on S_I and AIR_g , as the FSIGTT measures of insulin sensitivity and β -cell function respectively. As a secondary focus permitting broader comparison with published epidemiological studies, we calculated pre- and post-LPS HOMA-IR and HOMA-B (Table 1) using fasting glucose and insulin samples [20].

Following MINMOD modeling, FSIGTT data were manually inspected by the authors (JFF, RYS, MRR, MPR). Exclusion criteria included clinical errors such as administration of the insulin bolus too early or too late, disturbances in the glucose bolus, or a poor best-fit line. For an individual to be included, reviewing authors had to reach consensus and both the baseline and LPS visit FSIGTT graphs had to pass these criteria and be appropriately

matched in terms of glucose and insulin timing and peaks and best fit. The r^2 fit index for model fit was generally >0.97 for all models passing QC, although four subjects were included with $r^2 > 0.94$ as their matched model was >0.97 , the response patterns were similar, and there were no obvious problems with the model. Determination was made blinded to any subject demographics including race. Of 113 EA subjects, 7 were excluded (2 for suspected mistiming in insulin administration, 5 for slow glucose administration); of 47 AA subjects, 5 were excluded (3 for mistiming in insulin and/or glucose administration, 2 for poor mathematical fit) yielding 106 EA and 42 AA with high-quality data pre- and post-LPS.

Laboratory Procedures

As described [18], plasma levels of interleukin-1 receptor agonist (IL-1RA) and interleukin-6 (IL-6), robust markers of innate immune activation, were measured by high-sensitivity ELISA (Quantikine, R&D Systems; Minneapolis, MN) according to manufacturer's instructions. The intra- and inter-assay coefficients of variation were 2.1% and 6.3% respectively for IL-1RA, and 6.4% and 10.5% respectively for IL-6. The lower limit of quantitation was 25.4pg/ml for IL-1RA and 0.154pg/ml for IL-6. Plasma levels of high-sensitivity C-reactive protein (CRP), a commonly used non-specific, acute phase protein measure of acute or chronic inflammation, were measured by latex particle-enhanced immunonephelometry on a Behring Nephelometer II Analyzer (Siemens Diagnostics; Munich, Germany). Plasma total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides (TG) were measured enzymatically on a Hitachi 912 Analyzer (Roche Diagnostics; Indianapolis, IN).

Statistical Analysis

To detect differences in baseline variables by race and sex, we compared groups using incremental linear regression models. Model 1 included race, sex, age, BMI and a race by sex interaction term. If there was no significant interaction, the interaction term was removed and Model 2 was adjusted for race, sex, age and BMI. A similar modeling approach was used to look at differences in variables post-LPS, and differences in the delta from pre to post. Correlations between baseline measures and LPS-induced change in insulin sensitivity and inflammatory variables were assessed using Spearman's rho correlation coefficient (r_s). A two sided p-value <0.05 was considered to indicate statistical significance. We did not adjust for multiple testing because of correlations between variables and because our secondary analyses were performed to complement and inform the primary outcomes i.e., change in S_I and AIR_g in response to LPS. Analysis was carried out using SPSS Statistics 19 (IBM, Armonk, NY).

RESULTS

Baseline characteristics

Baseline descriptive data for the study sample are shown in Table 1A. By design, the sample was young, lean and healthy with normal blood pressure and cardiovascular risk factors. There was no clinical or biochemical evidence of glucose homeostasis disorders. The AA sample was slightly older (median age 26 vs. 24 years, $p=0.02$) and had higher BMI, albeit

not obese (median 25.6 vs. 23 kg/m², $p=0.005$). Age, sex and BMI were included as covariates in subsequent analytical models to control for their potential confounding influence; however inclusion of these covariates did not change the results. Differences by race or sex in circulating lipids and blood pressure were consistent with expectations [26]. As reported previously, there were race and sex differences in inflammatory markers at baseline, with higher pro-inflammatory IL-6 but lower anti-inflammatory IL-1RA in AA compared with EA [18]. Fasting insulin, HOMA-IR and HOMA-B values were significantly higher in AA than EA (Table 1B), consistent with larger population studies [27].

At baseline FSIGTT, African Americans have lower S_I , higher AIR_g and higher DI

Pre-LPS (Table 2), AA subjects had significantly lower (47.1%) S_I values compared to EA (Figure 2A, $p<0.0001$) consistent with published data for race differences in fasting and glucose tolerance test parameters of insulin sensitivity [28-30]. However, AIR_g was ~95% higher in AA than EA (Figure 2B, $p<0.0001$). AA subjects also had a significantly higher DI than EA subjects (Figure 2C; $p<0.0001$), but no significant difference in S_G (Figure 2D). Analysis of FSIGTT data 24-hours post-LPS (Table 2) showed a similar race pattern with lower S_I (Figure 2A, $p=0.001$), higher AIR_g (Figure 2B, $p<0.0001$), higher DI (Figure 2C, $p<0.0001$), and a small difference by race in S_G (Figure 2D, $p=0.02$) in AA compared to EA subjects.

Endotoxemia induces insulin resistance and increases insulin secretion in both EA and AA

As previously shown in these individuals [18], endotoxemia induced a robust inflammatory response with median(IQR) peak IL-1RA (54.2(41) vs. 33.3(45) ng/ml in EA vs. AA, $P=0.008$), IL-6 (153(220) vs. 145(160) pg/ml in EA vs. AA, $P=0.1$) and CRP (18(14) vs. 15(11) mg/L in EA vs. AA, $P=0.03$). In our prior work, involving much smaller samples, we reported LPS-induced insulin resistance at 24-hours post-LPS [16, 17] but these studies were not powered to detect modest differences in insulin secretion (i.e., AIR_g) and not designed to address the influence of race. In the GENE study, we found that endotoxemia induced insulin resistance and increased insulin secretion in both EA and AA. There was a significant decrease in S_I (Figure 2A, $p<0.0001$), increase in AIR_g (Figure 2B, $p<0.0001$), decrease in DI (Figure 2C, $p<0.0001$), and decrease in S_G (Figure 2D, $p<0.0001$) in all subjects from the pre-LPS to the post-LPS FSIGTT.

Although starting at different levels, glucose homeostasis responses to LPS were largely similar in both AA and EA; S_I median -35% in EA and -29% in AA (Figure 2A), AIR_g median +17% in EA and +26% in AA (Figure 2B), DI median -24% in EA and -17% in AA (Figure 2C), and S_G -18% in EA and -14% in AA (Figure 2D).

Inflammatory marker correlations with markers of insulin sensitivity

We hypothesized that insulin sensitivity parameters would correlate with inflammatory variables. Baseline levels of CRP correlated modestly but significantly and inversely with S_I both pre- and post-LPS ($r_s = -0.225$, $p=0.006$ pre-LPS; $r_s = -0.215$, $p=0.009$ post-LPS), and positively with AIR_g ($r_s = 0.271$, $p=0.001$ pre-LPS; $r_s = 0.317$, $p<0.0001$ post-LPS). There was a significant relationship between baseline interleukin-1 receptor agonist (IL-1RA) and

S_I pre-LPS ($r_s = -0.209$, $p = 0.01$) but not post-LPS ($r = -0.13$, $p = 0.1$). This relationship had similar patterns in EA and AA, although only reached significance in EA alone, likely due to limited power of the smaller AA sub-sample.

In contrast to the association between baseline inflammatory levels and markers of insulin sensitivity, there was no correlation between the change in inflammatory markers and the change in insulin sensitivity markers.

DISCUSSION

Diabetes incidence and outcomes differ by race; however the underlying mechanisms for these disparities are poorly understood. We focused on glucose homeostasis and insulin resistance in healthy EA and AA subjects before and after an inflammatory stimulus. At both pre and post-LPS FSIGTTs, AA subjects had significantly lower S_I and higher AIR_g and DI than EA subjects. There was no difference in baseline S_G by race. This suggests that AA subjects may have increased insulin secretion, either as part of a compensatory mechanism for lower peripheral insulin sensitivity or differences in hepatic insulin sensitivity or clearance to maintain glucose homeostasis. Endotoxemia induced a similar decline in insulin sensitivity and glucose effectiveness and increase in pancreatic β -cell function in both EA and AA with no significant race differences in the degree of LPS-induced changes to this innate immune inflammatory stress.

To our knowledge, this is the largest study ever conducted to examine the effects of evoked endotoxemia on dynamic measures of glucose homeostasis and includes representative samples of young healthy EA and AA individuals. Although previous smaller studies, including our own, [15-17] have demonstrated decreased insulin sensitivity in response to LPS, we show for the first time that activation of innate immunity induces an increase in indices of pancreatic β -cell function (AIR_g) as well as a reduction in glucose effectiveness (S_G). Despite modest race differences in the inflammatory cytokine response to LPS as we reported [18], we did not observe race differences in LPS-induced insulin resistance. Indeed, the insulin-related changes in response to endotoxemia were equivalent and highly consistent across race. Thus, race-related differences in response to environmental inflammatory stress are not likely to be a major driver of race differences in diabetes incidence and complications. Further, we observed only weak correlation between baseline inflammatory markers and markers of insulin resistance, and saw no relationship between the induced inflammatory response and the induced insulin resistance. Therefore, the mechanisms which regulate systemic cytokine release in response to LPS may not be responsible for regulating the insulin response to LPS; for example localized tissue-specific inflammatory signaling in the pancreatic islet [31] and other tissues might be of greater relevance.

Race differences at baseline in insulin sensitivity in our study are consistent with published reports [30, 32] and are potentially attributable to increased basal and first phase insulin secretion and β -cell sensitivity to glucose or to decreased hepatic insulin extraction and decreased insulin clearance [27-30, 32-36]. While the overall risk of diabetes is greater in AA than EA, the risk for diabetes within individuals with impaired glucose tolerance does

not appear to differ by race [12]. Thus, genetic and race-specific factors that modulate the progression from normal to impaired glucose tolerance may have the greatest impact on diabetes risk in AA populations.

Both insulin resistance and pancreatic β -cell dysfunction have been implicated in the pathogenesis of diabetes [37]. These parameters are intimately connected, with a hyperbolic relationship existing between S_I and AIR_g ; increased insulin secretion can compensate for low insulin sensitivity and vice versa in maintenance of a normal level of glucose [38]. We observed significantly lower S_I in AA compared with EA subjects. However, AA subjects' higher AIR_g appeared to compensate for this lower S_I , coupled with higher DI, which potentially suggests a higher requirement of insulin in the liver for regulation of hepatic glucose production and maintenance of normal glucose levels [39]. In both EA and AA, the decrease in S_I post-LPS was accompanied by an increase in AIR_g . However DI was significantly decreased in both EA and AA post-LPS, suggesting incomplete compensation, potentially due to a failure to reach steady state by the time of our post-LPS FSIGTT at 24 hours post-LPS. The absence of any race difference in the change in DI suggests no effect of race differences in inflammatory responses on inflammation-induced insulin resistance. We also observed a decrease in S_G post-LPS, indicating a reduction in the ability of glucose to mediate its own disposal. While glucose effectiveness contributes significantly to glucose uptake, the regulatory mechanisms remain poorly understood [40]. Whether this change was mediated through a central effect of LPS [2], or through intermediates such as hormonal changes or increased free fatty acid release post-LPS remains unclear [41].

Our study has several strengths and unique findings. This is a large study using FSIGTT to give an integrated view of glucose homeostasis across race, and is unique in examining the impact of inflammation on these parameters across a biracial sample. Application of our low-dose experimental endotoxemia protocol revealed an inflammatory-mediated reduction in S_I with a partially compensatory increase in pancreatic β -cell insulin secretion. However, as evidenced by the decrease in DI post-LPS, the increase in AIR_g was not sufficient to restore normal glucose homeostasis, at least at 24 hours post-LPS. Further, we observed a decrease in glucose effectiveness, indicating that LPS-induced inflammation affects both insulin-mediated and insulin-independent glucose uptake. Our data suggest strongly that despite race differences in LPS-induced cytokines [18], inflammatory modulation of glucose homeostasis does not differ meaningfully across race. Our study also has limitations; we did not use clamps (ideally with tracer to directly assess hepatic insulin sensitivity) in these subjects, and thus could not directly compare the differences in measures of glucose homeostasis using both methods. We were unable to schedule FSIGTTs to coincide with menstrual cycle phases in female volunteers; however stratified analyses did not reveal differences by sex. The AA sample was slightly older and of modestly greater BMI than the EA group, potentially contributing to some race-differences in baseline parameters of glucose homeostasis, however adjustment for age and BMI did not alter the results, and the baseline race differences were consistent with the literature [28, 30, 32]. Due to limited sample resources, we have not measured all inflammatory proteins and molecules that may affect insulin sensitivity (e.g. leptin, adiponectin) [42-45], and thus may present an incomplete picture of adipocytokine modulation by and influence on endotoxemia-induced changes in insulin signaling. While evoked endotoxemia has been shown to be a powerful

model of inflammation, of relevance to inflammatory cardiometabolic disease [14, 15, 46], it does not directly approximate the chronic inflammatory pathogenic state found in obesity and sedentary states.

In conclusion, our study revealed that acute activation of innate immunity induced insulin resistance, decreased glucose effectiveness and decreased the disposition index in humans and that this inflammatory effect occurred equally in both EA and AA healthy volunteers. Thus, in healthy normoglycemic individuals, inflammation reduces glucose uptake both through insulin-mediated and insulin-independent mechanisms and the pancreatic β -cell appeared to provide only a partial compensation in response by increasing insulin secretion. Further, although the expected race differences were observed in glucose homeostasis parameters at baseline prior to LPS, the similarities in response to inflammation in EA and AA samples indicate that race differences in response to environmental inflammatory stress are unlikely to contribute to the observed race difference in diabetes incidence and complications.

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ABBREVIATIONS

LPS	Lipopolysaccharide
FSIGTT	Frequently-Sampled Intravenous Glucose Tolerance Test
GENE	Genetics of Evoked Responses to Niacin and Endotoxemia
EA	European Ancestry
AA	African Ancestry
S_I	Insulin Sensitivity
S_G	Glucose effectiveness
AIR_g	Acute insulin response to glucose
DI	Disposition index
HOMA	Homeostasis Model Assessment
IL-1RA	Interleukin 1 receptor agonist
CRP	C-reactive Protein

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Figure 1. Study Design

Post-screening, subjects underwent a frequently-sampled intravenous glucose tolerance test (FSIGTT) at baseline. 2-4 weeks later, subjects underwent a second FSIGTT 24-hours post-administration of LPS. Paired pre- and post-LPS FSIGTT data for the first 160 individuals to complete the study were analyzed here. Results from the niacin portion of the study are not discussed here.

Figure 2.

European ancestry (EA) subjects had significantly higher S_I than African ancestry (AA) subjects at baseline ($p<0.0001$) and post-LPS ($p<0.001$) (A). S_I was reduced by LPS in both EA and AA ($p<0.0001$). EA subjects had significantly lower AIR_G than AA subjects at baseline ($p<0.0001$) and post-LPS ($p<0.0001$) (B). AIR_G was increased by LPS in both EA and AA ($p<0.0001$). The Disposition Index (DI) was higher in AA than EA subjects pre-LPS ($p<0.0001$) and post-LPS ($p<0.0001$) (C). DI declined in both AA and EA following LPS ($p<0.0001$). Glucose effectiveness (S_G) was not significantly different by race at baseline ($p=0.6$) but was slightly different post-LPS ($p=0.02$) (D). S_G decreased post-LPS in both EA and AA ($p<0.0001$). There were no differences by sex in any parameters. * $P<0.05$; ** $P<0.001$; *** $P<0.0001$ (P values for race difference). Data presented as mean and SD.

Table 1

Baseline Characteristics of the GENE Study FSIQTT sample

	EA		AA		P values	
	Male (n=54)	Female (n=52)	Male (n=19)	Female (n=23)	EA vs. AA	Females vs. Males
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)		
A Clinical and Biochemical						
* Age (years)	24.0 (9.0)	24.0 (8.0)	27.0 (18.0)	26.0 (9.0)	0.019	0.33
BMI (kg/m)	23.1 (4.3)	22.6 (3.65)	26.2 (6.3)	25.2 (5.8)	0.005	0.07
Waist Circumference (cm)	84.5 (9.5)	83.0 (13.0)	89.0 (16.5)	85.0 (14.5)	0.15	0.39
Body Fat % (Trunk)	14.0 (8.6)	22.8 (8.9)	18.1 (12.8)	22.0 (15.3)	0.52	<0.0001
Cholesterol (mg/dl)	146.0 (45.0)	148.0 (40.0)	162.0 (37.0)	152.0 (56.0)	0.22	0.40
LDL-C (mg/dl)	79.8 (34.2)	71.9 (32.8)	103.8 (27.2)	82.2 (42.6)	0.23	0.009
HDL-C (mg/dl)	47.0 (16.0)	52.5 (20.0)	47.0 (18.0)	58.0 (22.0)	0.035	<0.0001
Triglycerides	75.0 (53.0)	68.0 (39.0)	61.0 (30.0)	48.0 (24.0)	0.005	0.27
Systolic blood pressure (mmHg)	112.0 (15.0)	105.0 (13.0)	116.0 (16.0)	111.0 (20.0)	0.009	0.006
Diastolic blood pressure (mmHg)	64.0 (11.0)	59.0 (13.0)	71.0 (10.0)	65.0 (9.0)	0.002	0.004
*IL-1RA (pg/ml)	106.3 (40)	117.3 (55)	86.6 (52)	109.6 (82)	0.023	<0.0001
*IL-6 (pg/ml)	1.7 (1.8)	2.2 (1.8)	2.6 (1.7)	2.9 (1.4)	0.025	0.013
*CRP (mg/L)	0.32 (0.7)	0.6 (1.2)	0.6 (1.0)	0.7 (1.8)	0.62	0.014
B Glucose Homeostasis						
Fasting Glucose (mmol/l)	5.1 (0.5)	4.7 (0.5)	5.1 (0.5)	4.8 (0.4)	0.42	<0.0001
*Fasting Insulin (pmol/l)	62.7 (31.5)	66.5 (29.5)	72.9 (56.7)	77.3 (29.9)	0.039	0.48
*HOMA-IR	1.2 (0.6)	1.2 (0.5)	1.4 (1.1)	1.4 (0.6)	0.047	0.68
HOMA-β	102.2 (25.5)	111.8 (53.3)	117.4 (46.5)	131.6 (34.2)	0.004	0.0003

* Log-transformed for analysis. BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; IL-1RA, interleukin 1 receptor agonist; CRP, C-reactive protein; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-β, homeostatic model assessment of β-cell function

Table 2
Frequently-sampled intravenous glucose tolerance test (FSIGTT) Parameters preand post-LPS

FSIGTT parameters changed significantly in both EA and AA following LPS, while fasting insulin, glucose, HOMA-IR and HOMA-B did not change significantly.

		EA	AA	P value
		Median (IQR)	Median (IQR)	(EA vs. AA)
A. FSIGTT Parameters				
S_I (μU/L/min)	Pre-LPS	4.12 (2.46)	2.47 (1.1)	<0.0001
	Post-LPS	2.42 (1.7)	1.82 (0.99)	<0.001
	Delta [†]	-1.23 (2.03)	-0.72 (0.89)	0.058
* AIR_g (μU/L/min)	Pre-LPS	289.9 (253)	848.0 (1051)	<0.0001
	Post-LPS	365.8 (276)	1006.2 (1206)	<0.0001
	Delta [†]	54.6 (189)	202.9 (607)	0.053
* DI	Pre-LPS	1307.5 (1089)	2076.1 (1681)	<0.0001
	Post-LPS	883.2 (821)	1722.5 (1208)	<0.0001
	Delta [†]	-252.2 (1016)	-345.6 (1699)	0.94
S_G (U/min)	Pre-LPS	0.019 (0.01)	0.022 (0.01)	0.64
	Post-LPS	0.015 (0.01)	0.018 (0.01)	0.024
	Delta [†]	-0.003 (0.01)	-0.003 (0.01)	0.31
B. Fasting Parameters				
Fasting Glucose (mmol/l)	Pre-LPS	4.96 (0.49)	4.89 (0.55)	0.416
	Post-LPS	4.94 (0.44)	4.81 (0.67)	0.064
	Delta	0.01 (0.37)	-0.06 (0.51)	0.372
* Fasting Insulin (pmol/l)	Pre-LPS	63.62 (30.19)	75.7 (35.46)	0.039
	Post-LPS	65.17 (24.04)	80.6 (51.72)	0.018
	Delta	-0.12 (18.42)	2.63 (30.37)	0.827
* HOMA-IR	Pre-LPS	1.19 (0.55)	1.42 (0.65)	0.047
	Post-LPS	1.22 (0.46)	1.48 (0.97)	0.025
	Delta	-0.0003 (0.36)	0.07 (0.6)	0.88
HOMA-B	Pre-LPS	110.05 (30.4)	126.2 (41.73)	0.004
	Post-LPS	110.25 (34.4)	133.1 (50.48)	<0.0001
	Delta	-0.05 (25.35)	3.4 (32.5)	0.226

* Log-transformed for analysis. P values obtained from linear regression or RM-ANCOVA analysis by race, adjusted for sex, age, and BMI.

[†] Significant difference from Pre to Post-LPS, P < 0.0001 by paired t-test. HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-β, homeostatic model assessment of β-cell function; S_I, insulin sensitivity index; S_G, glucose effectiveness; AIR_g, acute insulin response to glucose; DI, disposition index.