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ENDOTHELIN-1 RESPONSE TO GLUCOSE AND INSULIN AMONG AFRICAN AMERICANS

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Abstract

Background—Endothelin-1 (ET-1) is implicated in the pathogenesis of hypertension. *In vitro* studies demonstrate that ET-1 is up-regulated by insulin and glucose. The purpose of this study was to determine the effects of insulin and glucose upon ET-1 levels in young adult African Americans, a population with a high burden of hypertension and diabetes.

Methods—Plasma and urine ET-1 levels were measured before and after an oral glucose tolerance test (OGTT) and insulin clamp procedure in 288 participants. Subjects were classified according to glucose tolerance and blood pressure (BP) status.

Results—Plasma and urine ET-1 were not significantly different among the glucose tolerance groups. There was a trend toward increased plasma ET-1 among DM compared to IGT and NGT, however this was not statistically significant ($p=0.085$). According to BP status, plasma ET-1 was highest among the high BP group compared to the normal BP group ($p=0.01$). Following glucose challenge, plasma ET-1 levels decreased and urine ET-1 increased in all three BP groups ($p=0.037$).

Conclusions—Our data show that plasma ET-1 is higher among young adult Black Americans with hypertension compared to normotension. Urine ET-1 levels increased in response to glucose challenge possibly indicating early renal injury.

Keywords

Endothelin-1; Hypertension; Black Americans; Hyperglycemia

Introduction

African Americans have a higher prevalence of hypertension and greater burden of related vascular complications compared to Caucasians. Endothelin-1 (ET-1) is an endothelial cell-derived peptide that acts as a potent vasoconstrictor. ET-1 has been implicated as a key mediator in pathogenesis of hypertension and vascular disease among African Americans (1,2). Higher

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plasma endothelin levels have been described in hypertensive compared to normotensive individuals (3). African Americans have higher ET-1 levels compared to Caucasians (4). ET-1 has also been implicated in the pathway for salt sensitive hypertension, which is highly prevalent among African Americans (5). As a result of these clinical observations, ET receptor blockade is of increasing interest as a target for antihypertensive therapy.

Previous reports implicate insulin and glucose in regulating ET-1 activity. *In vitro* studies, using both bovine and human endothelial cells, report that both glucose (6) and insulin (7) stimulate ET-1 secretion. Further, *in vitro* studies of aorta from insulin-treated diabetic rats, demonstrate increased ET-1 induced vasoconstriction compared to non-diabetic controls. Diabetes, impaired glucose tolerance and central obesity are all associated with higher plasma ET-1 compared to lean and non-diabetic individuals (8). Diabetics with renal involvement, as evidenced by albuminuria, also have higher plasma ET-1 levels. ET-1 has also been linked to the pathogenesis of kidney disease primarily via vasoconstriction-related injury.

We sought to determine if plasma ET-1 concentration and urine ET-1 excretion are modified by changes in glucose and/or insulin levels among African Americans. Further, we determined if these effects are modified by the presence of abnormal glucose metabolism or hypertension.

Methods

Participants in this study were healthy young adults enrolled in a longitudinal study of blood pressure and cardiovascular risks. The participants were all self-identified African Americans, who were recruited in Philadelphia, Pennsylvania, and were initially examined between 1994 and 1999. Exclusion criteria at the time of initial enrollment included known type 1 and 2 diabetes, polycystic ovarian syndrome, and chronic kidney disease. The participants were re-enrolled for this project between August 2001 and July 2007. Individuals who developed type 2 diabetes subsequent to the initial enrollment were not excluded. Written informed consent was obtained from each participant. The study protocol and consent form were approved by the institutional review board of Thomas Jefferson University.

Clinical measurements

Clinical assessment consisted of anthropometric measurements (height and weight), blood pressure (BP) and fasting blood samples. BP measurements were obtained on each subject, in the right arm, following a 10-minute rest period in a seated position using auscultation with a mercury column sphygmomanometer. The average of two successive readings of systolic and diastolic (Korotkoff phase V) was used as the BP values of each visit. All measurements were performed by trained research nurses and medical assistants. Hypertension (HBP) was defined as a BP $\geq 140/90$ mmHg and/or taking antihypertensive medication. Controlled BP (CBP) was defined as BP $< 140/90$ mmHg and taking antihypertensive medication. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2).

Oral glucose tolerance test (OGTT)

Each participant was examined on two separate visits, 4–6 weeks apart. An oral glucose tolerance test (OGTT) was performed on the first visit. The OGTT was conducted after a 12-hour overnight fast. The fasting blood sample was obtained for plasma insulin and glucose concentrations before the ingestion of 75 g of glucose solution (Glucola; Ames Diagnostics, Elkhart, IN). Blood samples were then obtained at 30, 60 and 120 minutes post-ingestion and assayed for plasma insulin and glucose concentrations. Plasma glucose concentration was analyzed with the glucose oxidase technique (YS Model 27; Glucostat, Yellow Springs, OH). Plasma insulin concentration was determined with a solid phase radioimmunoassay (Coat-a-

Count; Diagnostic Products Corp, Los Angeles, CA). Coefficients of variation for intra- and inter-assay variability for glucose and insulin assays were <5%.

Based on the results of the OGTT, individuals were classified as normal glucose tolerance (NGT, average fasting glucose <110 mg/dL or two-hour glucose <140 mg/dL), impaired glucose tolerance (IGT, two hour glucose 140–199 mg/dL), or diabetic (DM, fasting glucose $\geq$ 126 mg/dL or two-hour glucose $\geq$ 200 mg/dL). The current definition of NGT is now average fasting glucose <100 mg/dL. We continued to use <110 mg/dL as the upper limit for NGT because we have found that among individuals in this cohort fasting plasma glucose concentration between 100 and 110 tends to decrease on repeat measurement, with average fasting glucose levels less than 100 mg/dL. (9).

Euglycemic hyperinsulinemic clamp

A euglycemic hyperinsulinemic clamp was carried out on the second visit. The euglycemic hyperinsulinemic clamp was not performed on all participants with diabetes. The euglycemic hyperinsulinemic clamp was carried out as previously described (10–12). Briefly, all subjects were required to have a 12-hour overnight fast before the insulin clamp procedure. Blood samples were obtained for baseline plasma glucose and insulin concentration.

Hyperinsulinemia was established with a primed constant infusion of insulin (Eli Lilly, Indianapolis, IN) at a concentration of 1000 mU/mL in normal saline according to the method of Rizza et al (11). The primed infusion rate was sufficient to achieve steady-state hyperinsulinemia at 80–120 μ U/mL above fasting insulin levels with the goal of suppressing hepatic glucose production. Hyperinsulinemia was maintained for 120 minutes, during which time sustained euglycemia was achieved using a variable infusion of 20% dextrose in water (Abbott Lab, Abbott Park, IL). For participants with elevated fasting glucose greater than 110 mg/dl, the baseline glucose was set at 100 mg/dl as the target euglycemic level. The glucose infusion rate was adjusted by the negative feedback equation of DeFronzo et al (10) according to plasma glucose sampled every 10 minutes. The insulin-stimulated glucose uptake (M) was computed as the mean glucose infusion during the final 60 minutes of the procedure and expressed as mg per kg per min. Higher values of M were indicative of greater insulin sensitivity. Since the level of steady-state hyperinsulinemia achieved during the clamp procedure varied slightly among the participants, an index of insulin sensitivity (M/I) was calculated by dividing the glucose infusion rate (M) by the mean insulin level (I) achieved during the final 60 minutes of steady-state hyperinsulinemia.

A timed overnight urine sample was collected from each participant at both visits. The volume and collection time were recorded for each urine sample. On the first visit, each participant was asked to void before ingesting the glucose load for the OGTT. Two additional urine samples were then collected at 2 hours (2-hr OGTT) and 4 hours (4-hr OGTT) after the glucose ingestion. On the second visit, each participant was asked to void before starting the insulin clamp. One additional urine sample was collected at the end of the insulin clamp study. All urine samples were assayed for albumin, creatinine and ET-1. Urine albumin concentration was analyzed by enzyme-linked immunosorbent assay (ELISA) utilizing an Albuwell kit (Exocell, Philadelphia, PA) according to the manufacturer's instructions. Urine creatinine concentration was assayed using a NOVA analyzer. Urine albumin excretion (UAE) was computed in μ g/min and in mg/gm creatinine.

Plasma and urine endothelin-1 (ET-1) measurements

The methodology to quantify ET-1 in plasma and urine samples avoids cross-reaction with big-ET-1, a precursor of ET-1, that is more abundant in human plasma than authentic ET-1. An extraction procedure with SepPack Vac/3ccC18 extraction cartridge is performed on each sample. ET-1 is assayed in plasma and urine samples using a commercial ELISA kit (R&D

Systems, Minneapolis, MN). The assay is performed using standard quality control procedures to achieve a low coefficient of variation (<5%). To avoid variations in plasma levels of ET-1 during menstrual cycle, all samples in women were obtained during the mid-follicular phase of the menstrual cycle. Plasma concentrations of ET-1 were expressed as pg/ml. ET-1 concentrations in the urine were corrected for creatinine and expressed as pg/mg creatinine.

Statistical Methods

We analyzed levels of plasma and urine ET-1, as well as changes following OGTT and insulin clamp. The analyses compared glucose tolerance groups and blood pressure groups which were stratified as previously described in the Methods section. Baseline clinical and demographic data were summarized via means and standard deviations for continuous variables, and frequency counts and percentages for categorical variables. Comparisons across groups were based on ANOVA for continuous variables and Fisher's exact test for categorical variables.

Because of right skew of the plasma and urine ET-1 distributions, the main analyses were performed after log transformation and results are expressed in terms of geometric means; comparisons across different subgroups are expressed in terms of ratios of geometric means (geometric mean ratios, GMRs), along with associated confidence intervals and p-values. The analyses were based on mixed-effects linear regression, an extension of ordinary linear regression that appropriately accounts for the correlated nature of the repeated urine ET-1 measurements on the same subject (up to 3 measurements during the OGTT and 2 measurements during the insulin clamp).

Results

Data were available for analysis on 288 participants. The distribution of plasma and urine ET-1 values in the entire study cohort, as measured during the OGTT and insulin clamp procedures, is presented in Figure 1.

Baseline characteristics of the entire cohort and glucose tolerance subgroups are presented in Table 1. The cohort consisted of subjects between the ages of 28 and 52 years, with 63% being women. The majority of the subjects (n=151, 52%) had a body mass index (BMI) in the obese range ($>30 \text{ kg/m}^2$), and the mean BMI was greater in DM and IGT than NGT. The mean blood pressure for the entire group was 125/74 mmHg, and both systolic and diastolic blood pressure were higher in DM compared to the other two groups. Compared to NGT, M/I was lower in the IGT and lowest in DM indicating greater insulin resistance in IGT and DM.

Table 2 provides the results of the ET-1 measures before and following the OGTT and insulin clamp procedures across the glucose tolerance groups. Compared to NGT and IGT, baseline plasma ET-1 levels were somewhat higher in DM (geometric mean, GM = 2.7, 2.7, and 3.5, respectively), but the difference was not statistically significant ($p=0.085$, Table 2). There was a statistically significant decrease in plasma ET-1 following OGTT in each glucose tolerance group (-14% for NGT, $p=0.001$; -19% , for IGT, $p=0.001$; -27% for DM, $p=0.018$), but the magnitude of the change was not different between the three groups ($p=0.425$, Table 2). Plasma ET-1 values following the insulin clamp did not differ between groups ($p=0.788$, Table 2).

Baseline urine ET-1 values were similar for the three groups (GM = 7.1, 6.0, and 7.3, respectively; $p=0.180$, Table 2), as were the values at 2 hours ($p=0.528$, Table 2) and 4 hours post-OGTT ($p=0.470$, data not shown). The increase in urine ET-1 following OGTT was comparable in the three groups (23% for NGT, $p=0.001$; 34% , for IGT; $p=0.0021$; 28% for DM, $p=0.148$; $p = 0.743$ for their difference). Changes in urine ET-1 following the insulin clamp procedure were small ($6\text{--}16\%$) and comparable across the three glucose tolerance groups ($p=0.739$). Overall, the data demonstrate a decrease in plasma and an increase in urine ET-1

following glucose and insulin challenge (OGTT). However, the changes are comparable across the three glucose tolerance groups.

Baseline geometric means for UAE ranged from 0.8 to 1.4 and were not significantly different among the three glucose tolerance groups ($p=0.415$). UAE increased during the OGTT to similar extent in all three glucose tolerance groups ($p=0.870$) but showed little change during the clamp procedure. Changes in plasma and urine ET-1 and UAE before and during the OGTT and insulin clamp procedures according to glucose tolerance status are presented in Figure 2 (Panels a-c, respectively). The results were essentially unchanged after controlling for age, sex, smoking, alcohol use, obesity and BP status.

Data were then analyzed with the cohort stratified according to BP status. Table 3 provides the characteristics of the cohort according to blood pressure status. Hypertensives, both CBP and HBP, had greater BMI compared to NBP.

Table 4 provides plasma and urine ET-1 measures before and following the OGTT according to blood pressure classification. Plasma ET-1 increased with increasing blood pressure ($p=0.010$). At 2-hours post-glucose ingestion, there was a significant decrease in plasma ET-1 (-14% for NBP, $p=0.001$; -17% for CBP, $p=0.075$; and -24% for HBP, $p=0.001$), although the degree to which plasma ET-1 decreased was not significant between the BP groups ($p=0.322$). Changes in plasma ET-1 values for the three blood pressure groups, are presented in Figure 3a. Further analysis to examine the effects of antihypertensive therapy versus blood pressure level on plasma ET-1 detected no independent effect of drug treatment on plasma ET-1 level.

Urine ET-1 values at baseline were comparable across the three blood pressure groups ($p=0.252$, Table 4). However, by 2 hours post-glucose ingestion, urine ET-1 had increased by 25% in the NBP group ($p=0.001$) and by 54% in the HBP group ($p=0.001$), and remained essentially unchanged in the CBP group ($p=0.037$ for the comparison of these changes between the three groups). From 2 hours to 4 hours post glucose ingestion, urine ET-1 values decreased by a similar amount in all three groups ($p=0.168$, data not shown). The results were unchanged after adjustment for age, sex, smoking, alcohol use, obesity, and glucose tolerance classification (data not presented). Urine ET-1 values for the three blood pressure groups, are depicted in Figure 3b.

UAE data are provided in Table 4 and Figure 3. Baseline UAE was significantly lower among the NBP, compared to the CBP and HBP groups ($p=0.002$). At 2-hours post glucose ingestion, UAE had almost doubled in both the NBP and CBP groups, and there was a modest increase in the HBP group. UAE values for the three blood pressure groups are provided in Figure 3c. No effect was detected for age, BMI, smoking, or alcohol use on either plasma or urine ET-1 excretion. However, women were found to have about 15% lower urine ET-1 excretion than men ($p=0.015$), although a sex difference was not found for plasma ET-1 ($p=0.723$).

Discussion

In our cohort of young adult African Americans, there appeared to no increase in plasma ET-1 in response to hyperinsulinemia (insulin clamp). There was also no significant increase in plasma ET-1 in response to glucose challenge (OGTT). There were also no differences according to glucose tolerance status. Results from this study indicate that circulating plasma ET-1 is higher among African Americans with HBP compared to those with CBP and NBP. The lower plasma ET-1 in the CBP compared to HBP group appears to be due to the lower blood pressure levels, because an independent effect of antihypertensive therapies could not be detected in the data analysis. We also found a modest decrease in plasma ET-1 and a corresponding increase in urine ET-1 excretion in response to the increases in glucose and

endogenous insulin during the OGTT. UAE increased during the OGTT in parallel with the increase in urine ET-1 and decrease in plasma ET-1.

Based upon observations from *in vitro* studies we expected to find an increase in plasma ET-1 in response to hyperinsulinemia, both endogenous hyperinsulinemia during the OGTT and exogenous hyperinsulinemia, during the insulin clamp. *In vitro* studies, using bovine and human endothelial cells, demonstrated that increases in glucose promote ET-1 secretion from endothelial cells [13]. Additionally, direct *in vitro* exposure of insulin to endothelial cells stimulates ET-1 gene expression and ET-1 secretion (14,15). However, the results from *in vivo* studies in humans have not been consistent with *in vitro* results. In a study of healthy subjects, Leyva et al, found no change in plasma ET-1 levels following an intravenous glucose tolerance test (14). Similarly, Metsarinne et al, demonstrated no change in plasma ET-1 levels among 23 healthy, normotensive men during hyperinsulinemia with and without hyperglycemia (15). In our study, involving a substantial cohort of African American young adults, with varying degrees of glucose tolerance, we observed a modest decrease in plasma ET-1 in response to glucose, but we found no changes in plasma or urine ET-1 following the insulin clamp procedure. Given the disparity in findings between *in vitro* and *in vivo* studies, it is plausible that insulin and glucose promote ET-1 secretion from endothelial cells. However, the changes in ET-1 concentration in human plasma cannot accurately quantify the changes in ET-1 at the tissue level.

Elevated plasma ET-1 levels are associated with diabetes especially in the setting of endothelial dysfunction (7,16). In a study by Ferri et al, middle-aged men with type II diabetes had a significant increase in plasma ET-1 following a euglycemic hyperinsulinemic clamp procedure (7). In another cross-sectional study of middle-aged type II diabetics, plasma ET-1 levels were linked to UAE, with highest ET-1 levels seen among the subjects with macroalbuminuria (17). We found only a marginally statistical difference in plasma ET-1 levels according to glucose tolerance status, and the changes in plasma and urine ET-1 following OGTT were comparable for subjects of differing glucose tolerance. However, known diabetics were not enrolled in the study; subjects identified as diabetic were few and newly diagnosed as a result of the study OGTT, and all subjects with proteinuria were excluded from the analyses.

ET-1 has also been linked to the pathogenesis of kidney disease (18,19). Particularly, ET-1 is implicated in the pathogenesis of renal injury via vasoconstriction, cellular proliferation and fibrosis. Adults with chronic kidney disease (CKD) have increased urine ET-1 excretion that correlates inversely with GFR (20). In a study of adults with sickle cell disease, urine ET-1 excretion was greater compared to controls, particularly among those with microalbuminuria, another marker of renal injury (21). Similarly, children with chronic kidney disease (CKD) from various causes have increased urinary ET-1 excretion compared to healthy controls (22). Subjects in our study had normal renal function and no evidence of intrinsic renal disease. Baseline urine ET-1 levels were similar across the blood pressure groups. Following glucose challenge a somewhat greater increase in urine ET-1 level was detected in the HBP group compared to NBP and CBP groups.

Mechanisms for clearance of ET-1 in humans are controversial. Local clearance appears to be mediated by ET-B receptors (23–25). Previous studies indicate whole body clearance of ET-1 via kidney, liver and lung (26,27). Our data show modest increases in urine ET-1 in response to glucose challenge. The increase in urine ET-1 corresponds with a concurrent increase in UAE and decrease in plasma ET-1. These changes likely reflect a renal contribution to ET-1 clearance. Alternatively, glucose challenge may have stimulated an increase in renal ET-1 production. Previously published reports support evidence of renal production of ET-1 (22, 28). Further, urinary ET-1 may represent shedding of the peptide from podocytes as a result of stimulation by a host of factors including angiotensin II, hyperglycemia, insulin and obesity

(28,29). However, it was not the intent of this study to quantify renal clearance of ET-1 or to consider the clinical utility of renal ET-1 clearance. The increases in urine ET-1 activity seen in response to the OGTT may suggest a link between glucose handling and insulin activity and possibly indicate early renal injury.

ET-1 has been implicated in the pathogenesis of hypertension among African Americans. ET-1 is also associated with salt-sensitive hypertension, more commonly seen in African Americans compared to Whites. Prior investigations have noted increased circulating levels of ET-1 among African Americans compared to Whites among both normotensives and hypertensives (2,30). Similar to our findings, a small study of hypertensive African Americans noted a reduction in plasma ET-1 levels following antihypertensive therapy with agents of various classes (31). Not surprisingly, treatment with an ET-1 receptor blocker results in decreases in ET-1-mediated vasoconstriction. Campia et al observed greater vasodilation, as measured by forearm blood flow, in response to selective ET-1 receptor blockade among hypertensive African Americans compared to Whites (1). ET-1 may be an important target for antihypertensive therapy in African Americans.

This study was limited by the lack of plasma ET-1 measurements prior to the insulin clamp procedure. Additionally, the decreases in plasma ET-1 levels seen during the OGTT may not fully represent the ET-1 activity at the tissue level. Finally, our cohort consisted of young adult African Americans and therefore the results may not be generalizable to other age groups and ethnicities.

Plasma ET-1 is higher among African Americans with HBP compared to CBP and NBP. Urine ET-1 excretion increases in response to glucose, but not insulin, challenge among both hypertensives and normotensives regardless of glucose tolerance status. These findings suggest the ET-1 mediates renal injury in the setting of hypertension and hyperglycemia among African Americans.

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Figure 1. Boxplots of plasma ET-1 (left panel) and urine ET-1 (right panel), during the OGTT and insulin clamp

Each box extends from the 25th to the 75th percentile (interquartile range), with the middle line representing the median. The whiskers extend to a distance of 1.5 times the interquartile range. OGTT visit: N = 288 for urine ET-1 and 279 for plasma ET-1. Clamp visit: N = 265 for urine ET-1 and 176 for plasma ET-1. ET-1, endothelin-1; OGTT, oral glucose tolerance test

Figure 2. Estimated geometric means and associated 95% confidence intervals for plasma ET-1 (pg/ml) (Panel a), urine ET-1 (pg/mg creatinine) (Panel b), and UAE (mg/gm creatinine) (Panel c), during the oral glucose tolerance test (OGTT) and the insulin clamp, by glucose tolerance group ET-1, endothelin-1; UAE, urine albumin excretion; NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetes mellitus.

Figure 3. Estimated geometric means and associated 95% confidence intervals for plasma ET-1 (pg/ml) (Panel a), urine ET-1 (pg/mg creatinine) (Panel b), and UAE (mg/gm creatinine) (Panel c), during the oral glucose tolerance test (OGTT) and the insulin clamp, by blood pressure group ET-1, endothelin-1; UAE, urine albumin excretion; NBP, normal blood pressure; CBP, controlled blood pressure; HBP, high blood pressure.

Table 1

Summary of baseline participant characteristics according to glucose tolerance status.

	ALL (N = 288)	NGT (N = 184)	IGT (N = 78)	DM (N = 26)	P*
Age (years), mean $\pm$ sd	40 $\pm$ 3.5	40 $\pm$ 3.4	40 $\pm$ 3.6	41 $\pm$ 3.8	0.853
Female , n (%)	182 (63)	113 (41)	54 (69)	15 (58)	0.404
BMI (kg/m ²), mean $\pm$ sd	31.5 $\pm$ 7.4	30.5 $\pm$ 7.0	32.7 $\pm$ 7.8	35.3 $\pm$ 7.3	0.002
Smoking , n (%)					0.089
Yes	143 (50)	100 (54)	31 (40)	12 (46)	
SBP (mm Hg), mean $\pm$ sd	125 $\pm$ 19	124 $\pm$ 20	125 $\pm$ 18	134 $\pm$ 13	0.054
DBP (mm Hg), mean $\pm$ sd	74 $\pm$ 13	73 $\pm$ 13	72 $\pm$ 10	80 $\pm$ 11	0.020
HDL cholesterol (mg/dL), mean $\pm$ sd	48 $\pm$ 14	49 $\pm$ 14	47 $\pm$ 14	44 $\pm$ 12	0.181
LDL cholesterol (mg/dL), mean $\pm$ sd	117 $\pm$ 34	118 $\pm$ 36	115 $\pm$ 32	116 $\pm$ 32	0.877
Triglycerides (mg/dL), mean $\pm$ sd	89 $\pm$ 57	83 $\pm$ 55	97 $\pm$ 60	108 $\pm$ 61	0.051
M/I x 100 , mean $\pm$ sd	9.1 $\pm$ 6.5	10.2 $\pm$ 6.8	7.3 $\pm$ 5.5	4.0 $\pm$ 3.1	0.001

NGT, normal glucose tolerance; IGT, impaired fasting glucose; DM, diabetic; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; M/I, mean clamp insulin/mean glucose infusion rate (insulin sensitivity).

* P-values compare the NGT, IGT, and DM groups.

Table 2

Summary of plasma ET-1 and urine ET-1 measurements before and after the OGTT and insulin clamp, according to glucose tolerance status.

	NGT (N = 184)	IGT (N = 78)	DM (N = 26)	P*
Plasma ET-1 (pg/ml)				
Baseline, GM (95% CI)	2.7 (2.5, 2.9)	2.7 (2.4, 3.1)	3.5 (2.8, 4.4)	0.085
2 hours post OGTT, GM (95% CI)	2.3 (2.1, 2.6)	2.2 (1.9, 2.6)	2.6 (2.0, 3.5)	0.610
<i>Change in plasma ET-1, GMR (95% CI)</i>	0.86 (0.79, 0.92)	0.81 (0.71, 0.92)	0.73 (0.56, 0.95)	0.425
Post clamp, GM (95% CI)	2.1 (1.8, 2.4)	1.9 (1.5, 2.4)	1.9 (1.2, 3.1)	0.788
Urine ET-1 (pg/mg cr)				
Baseline, GM (95% CI)	7.1 (6.4, 7.9)	6.0 (5.0, 7.0)	7.3 (5.4, 9.8)	0.180
2 hours post OGTT, GM (95% CI)	8.8 (7.9, 9.8)	8.0 (6.8, 9.4)	9.3 (7.1, 12.3)	0.528
<i>Change in urine ET-1, GMR (95% CI)</i>	1.23 (1.09, 1.39)	1.34 (1.11, 1.62)	1.28 (0.92, 1.80)	0.743
Pre-clamp, GM (95% CI)	7.0 (6.2, 7.8)	8.1 (6.7, 9.7)	9.6 (6.4, 14.4)	0.172
Post-clamp, GM (95% CI)	8.1 (7.2, 9.0)	8.5 (7.1, 10.1)	10.9 (7.2, 16.6)	0.373

NGT, normal glucose tolerance; IGT, impaired glucose tolerance; DM, diabetic; ET-1: endothelin 1; OGTT, oral glucose tolerance test.

GM: geometric mean. GMR: geometric mean ratio. CI: confidence interval.

Table 3

Summary of baseline participant characteristics according to blood pressure status.

	NBP (N = 195)	CBP (N = 32)	HBP (N = 61)	P*
Age (years), mean $\pm$ sd	40 $\pm$ 3.3	40 $\pm$ 4.2	41 $\pm$ 3.4	0.136
Female , n (%)	120 (62)	29 (91)	33 (54)	0.001
BMI (kg/m ²), mean $\pm$ sd	30.6 $\pm$ 7.2	33.4 $\pm$ 5.7	33.5 $\pm$ 8.2	0.008
Smoking , n (%)				0.335
Yes	91 (47)	17 (53)	35 (57)	
SBP (mm Hg), mean $\pm$ sd	117 $\pm$ 12	124 $\pm$ 11	152 $\pm$ 18	
DBP (mm Hg), mean $\pm$ sd	69 $\pm$ 9	76 $\pm$ 8	89 $\pm$ 11	
Glucose tolerance status , n (%)				0.083
Normal	129 (66)	16 (50)	39 (64)	
Impaired	54 (28)	10 (31)	14 (23)	
Diabetes	12 (6)	6 (19)	8 (13)	
HDL cholesterol (mg/dL), mean $\pm$ sd	49 $\pm$ 15	49 $\pm$ 10	44 $\pm$ 12	0.037
LDL cholesterol (mg/dL), mean $\pm$ sd	114 $\pm$ 35	128 $\pm$ 33	118 $\pm$ 33	0.101
Triglycerides (mg/dL), mean $\pm$ sd	82 $\pm$ 48	91 $\pm$ 51	109 $\pm$ 78	0.007

NBP, normal blood pressure; CBP, controlled blood pressure; HBP, high blood pressure; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

* P-values compare the NBP, CBP, and HBP groups.

Table 4

Summary of plasma ET-1, urine ET-1, and UAE measurements before and after the OGTT according to blood pressure status.

	NBP (N = 195)	CBP (n = 32)	HBP (N = 61)	P*
Plasma ET-1 (pg/ml)				
Baseline, GM (95% CI)	2.6 (2.4, 2.8)	2.9 (2.4, 3.5)	3.3 (2.9, 3.8)	0.010
2 hours post OGTT, GM (95% CI)	2.2 (2.1, 2.5)	2.4 (1.9, 3.1)	2.5 (2.1, 3.0)	0.430
<i>Change in plasma ET-1, GMR (95% CI)</i>	0.86 (0.80, 0.93)	0.83 (0.67, 1.02)	0.76 (0.66, 0.87)	0.322
Urine ET-1 (pg/mg cr)				
Baseline, GM (95% CI)	6.8 (6.1, 7.6)	8.1 (6.2, 10.5)	6.2 (5.1, 7.4)	0.252
2 hours post OGTT, GM (95% CI)	8.5 (7.7, 9.4)	7.8 (6.1, 10.0)	9.5 (7.9, 11.3)	0.425
<i>Change in urine ET-1, GMR (95% CI)</i>	1.25 (1.11, 1.40)	0.97 (0.72, 1.29)	1.54 (1.24, 1.90)	0.037
UAE (mg/gm cr)				
Baseline, GM (95% CI)	0.7 (0.5, 0.9)	1.5 (0.8, 2.6)	1.5 (1.0, 2.3)	0.002
2 hours post OGTT, GM (95% CI)	1.2 (1.0, 1.5)	2.5 (1.5, 4.2)	1.9 (1.3, 2.7)	0.008
<i>Change in UAE, GMR(95% CI)</i>	1.74 (1.36, 2.22)	1.74 (0.95, 3.20)	1.25 (0.80, 1.94)	0.427

NBP, normal blood pressure; CBP, controlled blood pressure; HBP, high blood pressure; ET-1: endothelin 1; UAE: urine albumin excretion; OGTT, oral glucose tolerance test.

GM: geometric mean. GMR: geometric mean ratio. CI: confidence interval.

* P-values compare the NBP, CBP, and HBP groups.