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Arg287Gln VARIANT OF *EPHX2* AND EPOXYEICOSATRIENOIC ACIDS ARE ASSOCIATED WITH INSULIN SENSITIVITY IN HUMANS

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Abstract

Epoxyeicosatrienoic acids (EETs) protect against the development of insulin resistance in rodents. EETs are hydrolyzed to less biologically active diols by soluble epoxide hydrolase (encoded for by *EPHX2*). Functional variants of *EPHX2* encode for enzymes with increased (Lys55Arg) or decreased (Arg287Gln) hydrolase activity. This study tested the hypothesis that variants of *EPHX2* are associated with insulin sensitivity or secretion in humans. Subjects participating in metabolic phenotyping studies were genotyped. Eighty-five subjects underwent hyperglycemic clamps. There was no relationship between the Lys55Arg genotype and insulin sensitivity or secretion. In contrast, the *EPHX2* 287Gln variant was associated with higher insulin sensitivity index ($p=0.019$ controlling for body mass index and metabolic syndrome). Also, there was an interactive effect of *EPHX2* Arg287Gln genotype and body mass index on insulin sensitivity index ($p=0.029$). There was no relationship between *EPHX2* Arg287Gln genotype and acute or late-phase glucose-stimulated insulin secretion, but disposition index was higher in 287Gln carriers compared with Arg/Arg ($p=0.022$). Plasma EETs correlated with insulin sensitivity index ($r=0.64$, $p=0.015$ for total EETs) and were decreased in the metabolic syndrome. A genetic variant that results in decreased soluble epoxide hydrolase activity is associated with increased insulin sensitivity, as are higher EETs.

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Author Contributions

C.E.R. researched data, contributed to the discussion, wrote the manuscript, and reviewed and edited the manuscript. M.M.S. researched data, and reviewed the manuscript. K.G. researched data. H.N. data analysis, and reviewed the manuscript. C.Y. data analysis, and reviewed the manuscript. G.L.M. reviewed the manuscript. J.M.L. researched data, contributed to the discussion, and reviewed the manuscript. N.J.B. conceived the study, researched data, and reviewed and edited the manuscript. In addition, N.J.B. is the guarantor of this work, and as such, had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Keywords

soluble epoxide hydrolase; epoxyeicosatrienoic acids; insulin sensitivity; metabolic syndrome

Introduction

An estimated 366 million people over the age of 20 years have type 2 diabetes worldwide and this number is predicted to grow to 552 million by 2030.[1] Insulin resistance precedes the development of type 2 diabetes and can result from abnormalities in insulin signaling or from decreased perfusion of insulin-sensitive tissues. Type 2 diabetes results when insulin secretory capacity can no longer compensate for increased insulin requirements that result from resistance.[2]

Studies in rodent models suggest that epoxyeicosatrienoic acids (EETs), epoxygenase metabolites of arachidonic acid, protect against the development of insulin resistance.[3] Cytochrome P450s (CYP2C and CYP2J) oxygenate arachidonic acid to form four different regioisomers of EETs.[4,5] EETS are potent vasodilators,[6] exert anti-inflammatory properties,[7] and decrease sodium reabsorption in the kidney.[8] EETs are hydrolyzed to less biologically active dihydroxyeicosatrienoic acids (DHETs) by epoxide hydrolases, in particular soluble epoxide hydrolase (sEH) encoded for by the gene *EPHX2*. [9,10]

In rodent models of obesity and insulin resistance, Cyp2c expression and EET levels are decreased,[11] whereas adipose sEH expression is increased.[12] Conversely, treatment with a sEH inhibitor or disruption of *Ephx2* improves insulin sensitivity and hepatic insulin signaling in high fat- or carbohydrate-fed rodents.[3,13,14] Studies in streptozotocin-treated[15] and high fat-fed mice[3] suggest that inhibition or deletion of sEH can also improve insulin secretion.

In humans, functional variants in *EPHX2* encoding enzymes with increased (rs41507953 or Lys55Arg) or decreased (rs751141 or Arg287Gln) hydrolase activity have been associated with decreased and increased vasodilation, respectively.[16,17] This study tested the hypothesis that functional variants in *EPHX2* are associated with insulin sensitivity or secretion in individuals with and without the metabolic syndrome who underwent hyperglycemic clamp. We further examined the relationship between plasma EET concentrations and insulin sensitivity or the metabolic syndrome.

Material and Methods

Subjects

We report data for subjects who participated in studies of metabolic function (NCT00732160, NCT00872599, NCT01409993, NCT01103245) and donated genomic DNA. All studies were approved by the Vanderbilt University Institutional Review Board and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained for each study protocol and the collection of DNA.

Phenotyping

All subjects underwent a screening history and physical and blood was obtained after an overnight fast. Subjects were defined as having or not having the metabolic syndrome using the National Cholesterol Education Program criteria of 3 of the following: fasting plasma glucose of ≥ 100 mg/dL (5.5 mmol/L), serum triglycerides of ≥ 150 mg/dL (1.7 mmol/L), serum high-density lipoprotein cholesterol <40 mg/dL (1.04 mmol/L) in men or 50 mg/dL in women, untreated blood pressure of $\geq 130/85$ mm Hg, or waist girth of >102 cm in men or >88 cm in women. Subjects with significant cardiovascular (other than hypertension), renal, pulmonary, endocrine (other than insulin resistance or hyperlipidemia), or hematologic disease were excluded, as were pregnant women. Patients with diabetes mellitus, defined by a fasting glucose of ≥ 126 mg/dL (7 mmol/L) or medication use, were also excluded.

Hyperglycemic clamps

Eighty-five subjects underwent hyperglycemic clamps. Subjects fasted after midnight and clamps began between 0800 and 0900 on each study day. A catheter was inserted retrograde in a hand vein for blood sampling, and the hand was warmed throughout the study for blood arterialization. An antecubital catheter was inserted in the contralateral arm for glucose infusion. To account for pulsatile insulin secretion, baseline glucose, insulin, and C-peptide were measured at -20 , -10 , and -1 minutes before glucose infusion and the average value was used to calculate baseline values. Blood for plasma glucose was drawn every 5 minutes and immediately centrifuged and analyzed using the glucose oxidase method (YSI 2300 STAT Plus Glucose Analyzer YSI Life Sciences; Yellow Springs, IL). A standardized priming infusion of 20% dextrose (Hospira) was administered during the first 10 minutes (200 mg/kg body weight), and thereafter infusion rates were adjusted every 5 minutes to maintain plasma glucose at 200 mg/dL for 150 minutes, according to the method of DeFronzo *et al.*[18]

The acute glucose-stimulated insulin response was calculated as the maximum increase in insulin levels during the first 10 minutes above the average baseline value. The late-phase glucose-stimulated insulin response was calculated as the average of insulin levels between 90 and 120 minutes above the average baseline value. The insulin sensitivity index (ISI) was calculated by dividing the average glucose infusion rate (mg/kg body weight/min) by the average insulin concentration (μ U/mL) between 90 and 120 minutes multiplied by 100 (conversion factor). This ISI calculated from hyperglycemic clamps correlates well with the ISI obtained during hyperinsulinemic-euglycemic clamps ($r=0.816$). [18] The disposition index (DI) was calculated as acute glucose-stimulated insulin response multiplied by the ISI.

Genotyping

Genomic DNA was extracted from whole blood using the AutoPure LS extraction system (Qiagen, Valencia, CA, USA). *EPHX2* rs751141 (Arg287Gln) and rs41507953 (Lys55Arg) were genotyped using TaqMan assays (Applied Biosystems, Foster City, CA, USA). SDS v2.4 (Applied Biosystems, Foster City, CA, USA) was used for the creation of cluster plots to identify sample associated fluorescent markers for genotype call determination. A total of 126 subjects were genotyped for rs41507953 and 119 subjects were genotyped for rs751141.

Laboratory Analysis

Blood samples were collected on ice and centrifuged immediately at 0°C for 20 minutes. All the plasma and serum were separated and stored at –80°C until the time of the assay. Plasma insulin concentrations were determined by radioimmunoassay (RIA; Millipore, St. Charles, MO). Plasma EETs, epoxy-12Z-octadecenoic acids (EpOMEs), and dihydroxy-12Z-octadecenoic acids (DiHOMEs) were quantified using ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS). Briefly, plasma (1 mL) lipids were extracted by the addition of 4 mL chloroform:methanol (2:1 v/v) following the addition of deuterated internal standards (Cayman Chemical, Ann Arbor, MI). The extraction mixture was centrifuged, the aqueous layer removed, and the organic layer was dried under a stream of nitrogen. The lipids were further purified using a silica Sep-Pak extraction cartridge (Waters, Milford, MA) and then analyzed by UPLC/MS/MS. The UPLC was equipped with an Acquity BEH C18 column (1.0 × 100 mm, 1.7 mm, Waters, Milford, MA); mobile phase A was 15 mM ammonium acetate pH 8.5 and mobile phase B was acetonitrile. Analytes were separated using gradient elution starting with 70% mobile phase A going to 40% mobile phase B over 5 minutes. The column was washed with 100% mobile phase B for an additional minute before returning to the starting mobile phase composition. The total run time was 8 minutes. Effluent from the UPLC was directed into a Thermo Scientific Quantum Vantage equipped with an electrospray source (ESI) operated in the negative ion-mode. Selected reaction monitoring (SRM) was used to monitor the analytes and the corresponding internal standards. The molar ratios of DiHOME to EpOME were used to estimate sEH activity, due to the greater stability and increased concentration of these lipids compared to EETs and DHETs.[19,20] Inflammatory cytokines interleukin (IL)-1 β , IL-6, IL-8, and tumor necrosis factor (TNF)- α were measured using the Cytometric Bead Array Human Soluble Protein Master Buffer Kit (BD Biosciences, San Jose, CA).

Statistical Analysis

Descriptive statistics are provided as median with interquartile range for continuous variables or frequencies (proportions) for categorical variables. Wilcoxon rank-sum test was used to compare various endpoints (insulin sensitivity/secretion, plasma EETs, DiHOME/EpOME ratio and inflammatory cytokines) between genotypes, or between subjects with or without metabolic syndrome. To further examine the relationship between *EPHX2* variants and insulin sensitivity or secretion, we fitted general linear regression models on Arg287Gln or Lys55Arg for ISI, acute insulin secretion, late-phase insulin secretion and DI separately. ISI was log-transformed and the other three variables were cube-root transformed in order to meet the model assumptions. BMI and metabolic syndrome were included in the models as covariates. A smooth relationship was assumed for BMI using restricted cubic regression splines with three knots. Two-way interaction between genotype, BMI and metabolic syndrome were also included in the models and later removed if corresponding p-values were greater than 0.2. In the final models, genotype × BMI was included for ISI and DI. Genotype × metabolic syndrome was included for ISI (Lys55Arg) and DI (Lys55Arg). BMI × metabolic syndrome was included for acute insulin secretion (Arg287Gln). Spearman's correlation coefficients were calculated to measure the association between EETs and ISI.

Two-sided p-values less than 0.05 were considered statistically significant. All the analyses were performed using R 3.0.1.[21]

Results

We found no relationship between *EPHX2* Lys55Arg genotype and ISI, acute or latephase glucose-stimulated insulin secretion, or DI (Supplemental Table 1). Thus, we focus on data analysis for *EPHX2* Arg287Gln genotype.

Subject characteristics

Table 1 provides the baseline characteristics of subjects with and without the metabolic syndrome. *EPHX2* Arg287Gln genotypes were in Hardy-Weinberg equilibrium in both groups. There was no significant difference in genotype frequencies between the two groups. Table 2 provides baseline characteristics of subjects who underwent hyperglycemic clamps by *EPHX2* Arg287Gln genotype.

Relationship between *EPHX2* Arg287Gln genotype and insulin sensitivity

Eighty-five subjects underwent hyperglycemic clamps. Sixty-one of these met criteria for the metabolic syndrome. ISI was calculated in 82 subjects who completed hyperglycemic clamps up to minute 120. ISI was significantly lower in subjects with metabolic syndrome compared with those without metabolic syndrome (median ISI 5.3 vs. 14.7 mg glucose/kg/min per μU Insulin/mL*100, $P<0.001$) (Figure 1).

The ISI was significantly increased in carriers of the *EPHX2* 287Gln allele compared with Arg/Arg homozygotes in subjects without the metabolic syndrome ($p=0.03$, after controlling for BMI), but not in those with the metabolic syndrome ($p=\text{NS}$) (Figure 1). When the metabolic syndrome was incorporated in the model, however, the *EPHX2* 287Gln variant was associated with higher insulin sensitivity ($p=0.019$ after controlling for body mass index and metabolic syndrome), regardless of subjects' metabolic status. The relationship between *EPHX2* Arg287Gln genotype and ISI varied with body mass index, ($p=0.029$ for BMI $\times$ genotype interaction) in both groups (Figure 2). Thus, at a BMI of 30 kg/M² ISI was 40% higher in carriers of the *EPHX2* 287Gln allele compared to Arg/Arg homozygotes (95% CI: 0–103%, $p=0.05$) in individuals with or without the metabolic syndrome, while at a BMI of 25 kg/M², *EPHX2* 287Gln carriers had an ISI 180% higher than Arg/Arg homozygotes (95% CI: 45–439%, $p=0.002$).

Relationship between *EPHX2* Arg287Gln genotype and insulin secretion

Acute glucose-stimulated insulin secretion was not significantly different between subjects with and without the metabolic syndrome (median acute glucose-stimulated insulin secretion 42 and 39 $\mu\text{U}/\text{mL}$, respectively, $p=0.67$), whereas late phase glucose-stimulated insulin secretion tended to be higher in subjects with the metabolic syndrome compared with those without metabolic syndrome (median 82 versus 49 $\mu\text{U}/\text{mL}$, $P=0.06$). There was no relationship between *EPHX2* genotype and either the acute glucose-stimulated insulin response ($p=0.631$) or late phase glucose-stimulated insulin secretion ($p=0.496$) after

controlling for BMI and metabolic syndrome. The DI was higher in carriers of the *EPHX2* 287Gln allele compared with Arg/Arg homozygotes (Table 3).

Relationship between *EPHX2* Arg287Gln genotype and soluble epoxide hydrolase activity or inflammatory cytokines

Plasma EET concentrations were measured in 40 subjects (Figure 3). We were able to genotype 37 of those subjects for rs751145 (Table 4). Plasma EET concentrations were measured in 14 subjects who underwent hyperglycemic clamps (Figure 4). These subjects tended to be younger and had a lower BMI as compared with the remaining subjects in whom ISI was measured.

In 15 subjects we also measured plasma DiHOME and EpOME concentrations. There was no significant relationship between *EPHX2* Arg287Gln genotype and plasma EET concentrations. The ratio of DiHOME to EpOME, a measure of soluble epoxide hydrolase activity, tended to be lower in carriers of the 287Gln allele compared to in individuals homozygous for the Arg287 allele, but this was also not significant (Table 4).

IL-1b, IL-6, and TNF- α were statistically similar in carriers of the 287Gln allele and Arg287 homozygotes (all $p>0.05$). IL-8 concentrations were lower in 287Gln carriers (IL-8 medians 8.9 vs 15.0, $p=0.03$).

Relationship between plasma EET concentrations and insulin sensitivity

Plasma EET concentrations were significantly lower in those with the metabolic syndrome compared to controls (Figure 3). There was a significant correlation between ISI and plasma 14,15-EET, 8,9-EET, and total EET concentrations (Figure 4).

Discussion

Soluble epoxide hydrolase, encoded for by *EPHX2*, hydrolyzes EETs to less biologically active DHETs. We report that carriers of a loss-of-function *EPHX2* 287Gln variant exhibit enhanced insulin sensitivity compared with carriers of the common allele. In addition, we report for the first time that circulating EET concentrations are decreased in individuals with the metabolic syndrome, and that EET concentrations correlate with insulin sensitivity. Taken together these data suggest that, as observed in rodent models,[3,13] EETs protect against the development of insulin resistance.

Insulin sensitivity reflects both the perfusion of insulin-sensitive tissues[22,23] and insulin signaling in those tissues.[24] We have previously reported that the *EPHX2* 287Gln variant is associated with increased vasodilation in the forearm vasculature under basal conditions, [17] suggesting that increased muscle perfusion could contribute to enhanced insulin sensitivity. In addition, Luria *et al* have reported that *EPHX2* null mice exhibit enhanced insulin signaling characterized by increased tyrosyl phosphorylation of the insulin receptor and insulin receptor substrate (IRS-1), increased association between phosphatidylinositol 3-kinase and IRS-1, and the downstream cascade,[3] consistent with a local effects of EETs.

When the insulin secretion by pancreatic islets is insufficient to compensate for insulin resistance, type 2 diabetes ensues. The effect of EETs on insulin secretion is uncertain. Falck *et al.* reported that 5,6-EET doubled insulin release from isolated rat pancreatic islets, compared with control values.[25] In contrast, Klett *et al* have reported that unesterified EETs decrease glucose-stimulated insulin secretion in isolated islets,[26] and we have found that glucose-stimulated insulin secretion is increased in *Cyp2c44* null mice, the equivalent of CYP2C8/9 in humans (unpublished data). In the current study, we do not find any relationship between either *EPHX2* genotype or EETs and glucose-stimulated insulin secretion in humans. On the other hand, DI, a metric which reflects the composite of the acute glucose-stimulated insulin response and insulin sensitivity was significantly higher in carriers of the loss-of-function *EPHX2* 287Gln allele.

We found a significant relationship between *EPHX2* Arg287Gln genotype and insulin sensitivity in individuals of lean-to-overweight body mass, but not in obese individuals. Decreased EET production in obesity could diminish the importance of genetic variation in EET hydrolysis. Cyp2c expression and EETs are decreased in rodent models of obesity,[11] whereas sEH expression in adipose tissue is increased.[12] We did not observe a relationship between BMI and EET concentrations or sEH activity in the present study (data not shown); however, Theken *et al* reported an inverse relationship between total plasma EET concentrations and BMI.[27] The group also reported that the ratio of 14,15-EET to 14,15-DHET was decreased in obese individuals with coronary artery disease compared to non-obese individuals. Alternatively, the lack of relationship between *EPHX2* genotype and insulin sensitivity in these subjects may reflect the well-described defects in insulin signaling pathways in obesity.[28]

We found that circulating EET concentrations were significantly lower in individuals with the metabolic syndrome even though EETs did not correlate with BMI. This is consistent with the finding that EETs correlated significantly with ISI. This observed relationship between EETs and insulin sensitivity provides supportive evidence that increased insulin sensitivity in carriers of the *EPHX2* 287Gln allele results from decreased hydrolysis of EETs. It is worth noting, however, that the relationship between ISI and EET concentrations was measured in subjects who tended to be younger, and who had a lower BMI than the rest of our cohort, which constitutes one of the limitations of the study.

Of note, we did not find a significant relationship between *EPHX2* Arg287Gln genotype and either EET concentrations or sEH activity as reflected by the ratio of DiHOME to EpOME. This likely reflects the small number of samples available for analysis. Alternatively, circulating concentrations may not reflect tissue concentrations. Przybyla-Zawislak *et al* reported that the activity of the enzyme encoded by the *EPHX2* 287Gln variant is 25 to 58% of that of the wild-type enzyme based on homozygous variant proteins.[16] It is also possible that an up-regulation of allelic expression in heterozygotes may compensate for the less active allele. Because we found no Gln/Gln homozygous and we did not measure the abundance of sEH mRNA, we are not able to exclude this possibility.

Contrary to our findings, Ohtoshi *et al* reported that the *EPHX2* 287Gln variant is associated with insulin resistance in Japanese patients with T2DM. The group found no association

between *EPHX2* Arg287Gln genotype and (HOMA-IR) in healthy controls and no association between the genotype and diabetes. The investigators did not report EET concentrations. Factors that might explain these disparate findings include differences in the ethnic groups studied, in the presence and absence of diabetes, and in body mass index.[29]

Conclusions

The observation that a loss-of-function variant of *EPHX2* and EETs are associated with higher insulin sensitivity has important clinical implications. Pharmacological inhibition of sEH increases cellular and circulating EET levels, potentiates vasodilation, and lowers blood pressure in preclinical models. Because of these properties, sEH inhibitors are currently under clinical development in humans. Studies are needed to determine whether pharmacological sEH inhibition improves insulin sensitivity in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Relationship between *EPHX2* Arg287Gln genotype and insulin sensitivity index (ISI) in individuals with and without the metabolic syndrome (MetSyn). ISI was calculated from hyperglycemic clamps.

* $P < 0.001$ versus not MetSyn. † $P = 0.03$ versus Arg/Arg.

Figure 2.

A linear regression model was fitted for log-transformed ISI on genotype with adjustment for BMI, metabolic syndrome as well as the interaction between genotype and BMI. A smooth relationship was assumed for BMI using restricted cubic regression splines with three knots. Presented are predicted ISI as a function of BMI for Arg/Gln (solid line) and Arg/Arg (dashed line) with metabolic syndrome set to overall mean; shaded area are 95% confidence intervals. Solid and empty circles are observed data for individual subjects.

Figure 3. Plasma epoxyeicosatrienoic acids (EETs) in individuals without (N=28) and with (N=12) the metabolic syndrome (MetSyn). EETs were measured using UPLC/MS/MS as described in the methods. *P<0.05 versus not MetSyn.

Figure 4.

Correlation between plasma epoxyeicosatrienoic acid (EET) concentrations and insulin sensitivity index (ISI) calculated from hyperglycemic clamp. Solid circles depict individuals with metabolic syndrome (MetSyn) and open circles those without MetSyn.

Table 1**Subject Characteristics**

Characteristic	Metabolic Syndrome	No Metabolic syndrome	P-values
Number	69	50	
Age, years	51 (43–60)	32 (21–43)	<0.001
Gender, N (%) females	42 (60.9%)	27 (54%)	0.56
Race			
Black American	17	16	
White American	51	34	0.49
More than one	1	0	
BMI, Kg/M2	33 (29–38)	27 (24–31)	<0.001
Fasting Glucose, mg/dL	97 (88–106)	89 (82–95)	<0.001
Systolic Blood Pressure, mm Hg	129 (118–140)	125(110–139)	<0.001
Diastolic Blood Pressure, mm Hg	75(68–83)	73 (63–84)	<0.001
HDL, mg/dL	39 (33–45)	54 (42–65)	<0.001
Triglycerides 150 mg/dL	108 (65–152)	78 (39–117)	<0.001
Waist circumference, cm	113 (106–121)	95 (85–105)	<0.001
rs751141 (Arg287Gln)			
Arg/Arg	57 (82.6%)	39 (78%)	
Arg/Gln	12 (17.4%)	11 (22%)	0.53
Gln/Gln	0 (0%)	0 (0%)	

Numbers in parenthesis represent interquartile range unless otherwise stated. P-values were based on Wilcoxon rank-sum test for continuous variables and Chi-square test for categorical variables.

Table 2

Subject characteristics of subjects who underwent hyperglycemic clamps

Characteristic	rs751141 (Arg287Gln)		P-values
	GG	GA	
Number	69	16	
Age, years	48 (36–60)	37 (29–45)	0.07
Gender, N (%) females	42 (61%)	7 (44%)	0.21
Race			
Black American	14	6	
White American	54	10	0.32
More than one	1	0	
BMI, Kg/M ²	31 (27–36)	31 (26–36)	0.64
Fasting Glucose, mg/dL	93 (84–102)	91 (82–100)	0.23
Systolic Blood Pressure, mm Hg	124 (112–136)	121 (108–134)	0.72
Diastolic Blood Pressure, mm Hg	73 (65–81)	71 (62–81)	0.17
HDL, mg/dL	41 (33–49)	41 (36–47)	0.99
Triglycerides, mg/dL	94 (51–138)	77 (39–114)	0.19
Waist circumference, cm	108 (99–118)	105 (95–115)	0.67
rs41507953 (Lys55Arg)			
Lys/Lys (AA)	49 (71%)	12 (75%)	
Lys/Arg (AG)	17 (25%)	4 (25%)	0.78
Arg/Arg (GG)	2 (2.4%)	0	
Undetermined	1 (1.6%)	0	

GG=Arg/Arg, GA=Arg/Gln. Numbers in parenthesis represent interquartile range unless otherwise stated. P-values were based on Wilcoxon rank-sum test for continuous variables and Chi-square test for categorical variables.

Table 3

Relationship between *EPHX2* Arg287Gln genotype and acute and late-phase glucose-stimulated insulin secretion

	Arg/Arg	Arg/Gln	P-value	All
No Metabolic Syndrome				
Acute GSIS, μ U/mL	34 (24–47)	73 (39–74)	0.29	39 (13–64)
Late-phase GSIS, μ U/mL	49 (31–74)	56 (33–134)	0.81	49 (19–78)
Disposition index	705 (363–835)	1233 (711–1476)	0.06	716 (486–945)
Metabolic Syndrome				
Acute GSIS, μ U/mL	44 (20–60)	34 (27–56)	0.81	42 (23–61)
Late-phase GSIS, μ U/mL	81 (53–123)	96 (48–111)	0.84	82 (47–118)*
Disposition index	188 (140–472)	212 (122–338)	0.99	197 (74–321)
All				
Acute GSIS, μ U/mL	42 (22 – 59)	38 (27 – 73)	0.631	40 (20–59)
Late-phase GSIS, μ U/mL	73 (48–115)	84 (35–117)	0.496	73 (35–112)
Disposition index	252 (148–470)	435 (161–760)	0.022	265 (37–493)

Numbers in parenthesis represent interquartile range. GSIS, glucose-stimulated insulin secretion. P-values were calculated by Wilcoxon rank-sum test for subjects with and without the metabolic syndrome. P-values depicted for all subjects were calculated using fitted general linear regression models that included BMI and metabolic syndrome as covariates.

* P=0.06 versus no metabolic syndrome.

Table 4

Relationship between *EPHX2* Arg287Gln genotype and soluble epoxide hydrolase activity and epoxyeicosatrienoic acid concentrations

	Arg/Arg	Arg/Gln	P-value
	<i>N</i> = 26	<i>N</i> = 11	
14,15-EET, ng/mL	0.29 (0.21–0.61)	0.41 (0.39–0.44)	0.26
11,12-EET, ng/mL	0.19 (0.13–0.30)	0.35 (0.21–0.40)	0.15
8,9-EET, ng/mL	0.40 (0.27–0.56)	0.44 (0.41–0.57)	0.27
Total EETs, ng/mL	0.85 (0.70–1.56)	1.16 (1.11–1.33)	0.27
	<i>N</i> = 8	<i>N</i> = 7	
9,10-DiHOME/9,10-EpOME	1.66 (1.30–1.95)	1.34 (1.16–1.54)	0.22
12,13-DiHOME/12,13-EpOME	1.30 (1.11–1.61)	1.12 (1.00–1.60)	0.58
total-DiHOME/total-EPOME	1.46 (1.15–1.69)	1.12 (1.09–1.54)	0.37

Numbers in parenthesis indicate interquartile range. Total-, 12,13-, and 9,10- DiHOME/EpOME represent molar ratios.