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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.

1. 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.

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2. Materials and methods

2.1. 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.

2.2. 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 ≥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.

2.3. 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 min before glucose infusion and the average value was used to calculate baseline values. Blood for plasma glucose was drawn every 5 min 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 min (200 mg/kg body weight), and thereafter infusion rates were adjusted every 5 min to maintain plasma glucose at 200 mg/dL for 150 min, 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 min above the average baseline value. The late-phase glucose-stimulated insulin response was calculated as the average of insulin levels between 90 and 120 min 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 (μU/mL) between 90 and 120 min 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.

2.4. 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.

2.5. Laboratory analysis

Blood samples were collected on ice and centrifuged immediately at 0 °C for 20 min. 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 min. 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 min. 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)-1β, 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).

2.6. 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, DHOME/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 x BMI was included for ISI and DI. Genotype × metabolic syndrome was included for ISI (Lys55Arg) and DI (Lys55Arg). BMI × metabolic syndrome was