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Epoxyeicosatrienoic acids and glucose homeostasis in mice and men

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ABSTRACT

Epoxyeicosatrienoic acids (EETs) are formed from arachidonic acid by the action of P450 epoxygenases (CYP2C and CYP2J). Effects of EETs are limited by hydrolysis by soluble epoxide hydrolase to less active dihydroxyeicosatrienoic acids. Studies in rodent models provide compelling evidence that epoxyeicosatrienoic acids exert favorable effects on glucose homeostasis, either by enhancing pancreatic islet cell function or by increasing insulin sensitivity in peripheral tissues. Specifically, the tissue expression of soluble epoxide hydrolase appears to be increased in rodent models of obesity and diabetes. Pharmacological inhibition of epoxide hydrolase or deletion of the gene encoding soluble epoxide hydrolase (*Ephx2*) preserves islet cells in rodent models of type 1 diabetes and enhances insulin sensitivity in models of type 2 diabetes, as does administration of epoxyeicosatrienoic acids or their stable analogues. In humans, circulating concentrations of epoxyeicosatrienoic acids correlate with insulin sensitivity, and a loss-of-function genetic polymorphism in *EPHX2* is associated with insulin sensitivity.

1. Introduction

Epoxyeicosatrienoic acids (EETS) are formed from arachidonic acid by the action of P450 epoxygenases (CYP2C and CYP2J) (Fig. 1) [1]. EETs act as potent vasodilators and have been identified as endothelium-derived hyperpolarizing factor [2]. In the kidney, EETs promote sodium excretion by inhibiting the translocation of the Na+/H+ exchanger (NHE3) in the proximal tubule [3]. EETs decrease inflammation by decreasing the activation of NFκB [4]. It follows that increasing the actions of EETs in rodent models protects against hypertension, endothelial dysfunction, cardiovascular remodeling and renal injury [1]. The effects of EETs are limited by hydrolysis by soluble epoxide hydrolase (sEH) to the less active dihydroxyepoxyeicosatrienoic acids (DHET)s and strategies to increase the action of EETs include both increasing the expression of epoxygenases and decreasing the activity of sEH [5].

Over the last decade, studies in rodent models have provided compelling evidence that EETs also exert favorable effects on glucose homeostasis either by enhancing pancreatic islet cell function or by increasing insulin sensitivity in peripheral tissues. More recently, studies measuring insulin secretion and insulin sensitivity in individuals with functional polymorphisms in the gene encoding for sEH (*EPHX2*) provide support for the concept that EETs modulate glucose homeostasis in humans. We review here the evidence from rodent models and human studies suggesting that EETs contribute to metabolic regulation and the implications for the development of pharmacologic strategies to increase EETs.

2. Type 2 diabetes results when insulin resistance exceeds the ability of the pancreas to secrete insulin

Type 2 diabetes (T2DM) affects an estimated 366 million adults worldwide and this number is predicted to grow to 552 million by 2030 due to a worldwide increase in the prevalence of obesity [6]. In obesity, increased inflammation and circulating free fatty acids, as well as endothelial dysfunction lead to insulin resistance in muscle, liver and adipose tissue. T2DM results when the insulin secretory capacity can no longer compensate for the degree of insulin resistance [7]. Studies in rodent models suggest that EETs affect both insulin sensitivity in peripheral tissues and the capacity of the islets to respond to insulin resistance.

3. Expression of sEH is increased in rodent models of glucose intolerance

Tissue-specific sEH expression and/or activity appears to be increased in rodent models of both T1DM and T2DM. For example, sEH activity was increased more than two-fold in microsomes prepared from the livers of Fisher rats treated with alloxan or streptozotocin (STZ) to induce T1DM [8]. sEH expression was also

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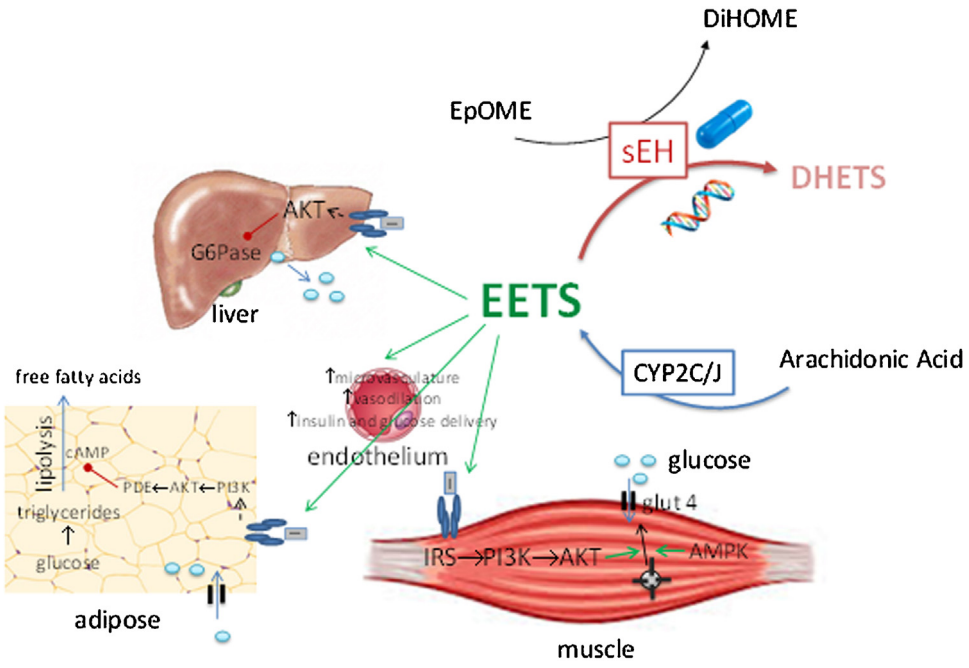

Fig. 1. Possible mechanisms through which epoxyeicosatrienoic acids (EETs) improve glucose homeostasis. EETs are produced by the actions of epoxygenases CYP2C and CYP2J on arachidonic acid. EETs improve insulin signaling in muscle, liver and adipose tissue. Genetic deletion of *Ephx2* or pharmacologic inhibition of soluble epoxide hydrolase increases insulin-induced tyrosyl phosphorylation of the insulin receptor and tyrosyl phosphorylation of (IRS)-1 as well as AKT phosphorylation in insulin-sensitive tissues [13,15,22–33]. EETs also improve insulin sensitivity by increase capillary volume and microvascular blood flow in insulin sensitive tissues such as muscle [33]. The increase in capillary blood volume appears to be nitric oxide-independent, whereas increases in microvascular blood flow are nitric oxide-dependent. Not shown, EETs also preserve islet cell function and increase insulin secretion, particularly in rodent models of type 1 diabetes. The actions of EETs are limited by hydrolysis by soluble epoxide hydrolase (sEH) to dihydroxyeicosatrienoic acids (DHETs). sEH activity is often measured as the ratio of dihydroxyoctadecenoic acid (DiHOME) to epoxy-9Z-octadecenoic acid (EpOME). AKT, protein kinase B; IRS, insulin receptor substrate; G6Pase, glucose 6 phosphatase; glut 4, glucose transporter type 4; PDE, phosphodiesterase; PI3K, phosphoinositide 3-kinase.

increased in the heart and gastrocnemius muscle of Akita mice, a murine model of T1DM, but not in the liver; the investigators did not assess sEH activity [9]. Contradicting these findings, Oguro et al. reported that STZ treatment decreased sEH mRNA and protein expression in liver and kidney of mice, but the investigators did not measure sEH activity [10]. Insulin restored sEH expression in this model. The same investigators reported that high glucose concentrations reduced sEH expression in a hepatocarcinoma cell line, an effect that was mediated by Sp1 [11].

In a model of T2DM, Liu et al. reported that sEH expression was increased 2.6-fold in the liver of C57Bl6 mice after long-term (16 weeks) high-fat feeding, but not after a shorter duration (8 week) of high fat feeding; sEH activity was increased approximately 35% in the liver regardless of the duration of high fat feeding [12]. Schäfer et al. reported that high fat diet significantly decreased protein expression of epoxygenases, as well as the ω-hydroxylase Cyp4a12, in the liver by 21 days and up-regulated soluble epoxide hydrolase three-fold [13]. sEH activity has also been reported to be increased in epididymal fat in mice fed a high-fat diet [14]. In this study, sEH was expressed in cultured adipocytes and expression increased with differentiation of preadipocytes (3T3-L1 cells) into mature adipocytes [14]. Conversely, EET concentrations have been reported to be higher in preadipocytes or mesenchymal stem cells (MSCs) compared to mature adipocytes [14,15].

4. Effects of EETs on insulin secretion and islet cell apoptosis

Epoxygenases of both the CYP2C and CYP2J families have been reported to be present in rodent and human islets [16,17]. Studies in isolated islets or beta cells provide conflicting data on the effect of EETs on insulin secretion. In 1983, Falck et al. reported that P450 inhibition decreased arginine-stimulated insulin and glucagon release and glucose-stimulated insulin release from isolated rat pancreatic islets [18]. The group reported that 5,6-EET stimulated insulin-secretion from islets, whereas 8,9-, 11,12-, and 14,15 EET stimulated glucagon secretion. Turk did not find an effect of EETs on glucose-stimulated insulin secretion in isolated islets [19]. In contrast, Klett et al. reported that unesterified EETs decrease insulin secretion in isolated INS 832/13 beta cells [20]. In this model, esterification of EETs by acyl-CoA synthetase 4 resulted in their sequestration into glycophospholipd and increased glucose-stimulated-insulin secretion [20].

Luo et al. reported that *Ephx2*-null mice demonstrated preserved glucose-stimulated insulin secretion and were protected against islet cell apoptosis, measured by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining, following STZ treatment [21]. The group also found that treatment with the sEH inhibitor *trans*-4-[4-(3-adamantan-1-ylureido)-cyclohexyloxy]-benzoic acid (t-AUCB) prevented apoptosis in pancreatic islets from rats, measured five days after treatment with low-dose STZ [16]. Insulin release in response to glucose or potassium channel inhibition was increased in isolated islets from *Ephx2*-null mice and mice treated with t-AUCB [21]. There was no effect of t-AUCB on the ratio of EETs to DHETs measured in the pancreas or in plasma of the STZ-treated mice [16].

EETs also appear to influence pancreatic islet cell number or size in models of T2DM. In high fat-fed mice, for example, targeted deletion of *Ephx2* or selective epoxide hydrolase inhibition using 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-trifluoro methoxy-phenyl)-urea (TUPS) increased islet size and vascular density measured by staining for CD31 [22]. In another study, t-AUCB increased the number of β cells without increasing islet size in high carbohydrate-, high fat-fed rats [23].