REVIEW

Potential Mechanisms by Which Hydroxyeicosapentaenoic Acids Regulate Glucose Homeostasis in Obesity

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ABSTRACT

Dysregulation of glucose metabolism in response to diet-induced obesity contributes toward numerous complications, such as insulin resistance and hepatic steatosis. Therefore, there is a need to develop effective strategies to improve glucose homeostasis. In this review, we first discuss emerging evidence from epidemiological studies and rodent experiments that increased consumption of EPA (either as oily fish, or dietary/pharmacological supplements) may have a role in preventing impairments in insulin and glucose homeostasis. We then review the current evidence on how EPA-derived metabolites known as hydroxyeicosapentaenoic acids (HEPEs) may be a major mode of action by which EPA exerts its beneficial effects on glucose and lipid metabolism. Notably, cell culture and rodent studies show that HEPEs prevent fat accumulation in metabolic tissues through peroxisome proliferator activated receptor (PPAR)–mediated mechanisms. In addition, activation of the resolvin E1 pathway, either by administration of EPA in the diet or via intraperitoneal administration of resolvin E1, improves hyperglycemia, hyperinsulinemia, and liver steatosis through multiple mechanisms. These mechanisms include shifting immune cell phenotypes toward resolution of inflammation and preventing dysbiosis of the gut microbiome. Finally, we present the next steps for this line of research that will drive future precision randomized clinical trials with EPA and its downstream metabolites. These include dissecting the variables that drive heterogeneity in the response to EPA, such as the baseline microbiome profile and fatty acid status, circadian rhythm, genetic variation, sex, and age. In addition, there is a critical need to further investigate mechanisms of action for HEPEs and to establish the concentration of HEPEs in differing tissues, particularly in response to consumption of oily fish and EPA-enriched supplements. *Adv Nutr* 2022;13:2316–2328.

Statement of Significance: This review article covers the latest evidence on how EPA and hydroxyeicosapentaenoic acids (HEPEs) potentially improve aspects of glucose homeostasis, particularly in the context of obesity. The review also underscores critical areas for future research with EPA and HEPEs, which include the counterregulatory role of other dietary fatty acids on EPA-HEPE metabolism, the host genome, microbiome, and circadian rhythms.

Keywords: n–3 polyunsaturated fatty acids, glucose, insulin, obesity, type 2 diabetes, inflammation

Introduction

Diet-induced obesity drives impairments in insulin and glucose homeostasis, which contribute to a wide range of complications. These complications include type 2 diabetes (T2D), nonalcoholic fatty liver disease, cardiovascular diseases, and even cancers. Therefore, there is a need to develop effective intervention strategies for preventing impairments in glucose metabolism. This is vital to address, as the prevalence of obesity in adults has doubled in the past several decades and there is increasingly early onset of obesity compared with previous generations (1, 2).

Increased dietary intake of long chain n–6 and/or n–3 PUFAs, either as oily fish or as supplements, may be a means of controlling glucose and insulin metabolism by targeting differing inflammatory pathways. This review does not focus on aspects of n–6 PUFA metabolism and insulin sensitivity, which is elegantly covered in a recent review (3). Herein, we primarily focus on the n–3 PUFA EPA, with some discussion of its long-chain counterparts, docosapentaenoic acid (DPA; 22:5n−3) and DHA. EPA and DHA are of particular interest as they are routinely consumed as over-the-counter fish-oil supplements and are approved

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clinically in the United States and other countries for the treatment of elevated triglycerides. In addition, EPA ethyl esters (Vascepa®, Amarin) are also approved in the United States for lowering the risk of cardiovascular disease (4).

Increased Consumption of Long-Chain n–3 PUFAs May Reduce the Risk of T2D

There is considerable controversy about the role of EPA and DHA for the treatment or prevention of T2D. This controversy on the efficacy of long-chain n–3 PUFAs for the prevention or treatment of T2D comes from inconsistent results across model systems, which include epidemiological studies, randomized clinical trials, and experiments with rodents and cells. To exemplify, some very recent epidemiological studies suggest that intake of EPA and DHA is associated with a lower incidence of T2D. A study of 392,287 middle-aged and older participants who did not have T2D over a span of 10 y had a lower risk (up to 18%) of T2D in response to regular consumption of fish-oil supplements or consumption of oily fish (1 serving/wk) (5). The lowest risk of T2D was observed in participants who used fish-oil supplements continuously (5). Similarly, another study that utilized data from a global consortium of 20 prospective studies of 65,147 participants who were free of T2D reported that circulating concentrations of all long-chain n–3 PUFAs (EPA, DPA, and DHA) were associated with a lower incidence of T2D (6). There is also evidence that plasma n–3 and n–6 PUFA concentrations are inversely associated with risk of T2D in a study of 95,854 participants (7). Circulating n–3 PUFAs had significant interactions with a genetic predisposition to developing T2D; furthermore, the inverse association between plasma n–3 PUFAs and risk for T2D was for all total analyzed n–3 PUFAs but DHA (7).

Although very recent epidemiological studies, as described above, suggest a role for n–3 PUFAs as either dietary supplements or through consumption of oily fish in the prevention of T2D, there is considerable confusion in the field based on clinical trials. Clinical trials, many of which are underpowered, have not revealed any benefit of EPA/DHA intake for treating T2D (8, 9). Many clinical studies and trials have focused on n–3 PUFAs (as dietary or pharmacological supplements up to 4 g/d) as a treatment modality across differing patient populations (8–13). A meta-analysis of 83 randomized trials (some showing benefits, others showing no effect, and some even showing harm) concluded that long-chain n–3 PUFAs have no effects on the prevention or treatment of T2D (14). However, other investigators have argued that more data in this area of study are needed as only 6 trials in the previously mentioned meta-analysis had a low risk of bias (5). Furthermore, the meta-analysis was largely based on dietary supplement studies with little evidence with studies using oily fish (14).

Perhaps EPA/DHA have benefits for only select clinical populations as a treatment modality. A meta-analysis of 7 randomized clinical trials concluded that n–3 PUFA supplementation reduced fasting plasma glucose and the HOMA-IR for gestational diabetes (15). Similarly, another meta-analysis with 1132 subjects showed that EPA/DHA-enriched fish oil had benefits for insulin resistance in children (16). Interestingly, a subgroup analysis in the same study revealed that the beneficial effects were pronounced when the intervention with fish-oil supplementation was at relatively low doses ($\leq$1.5 g EPA+DHA), short-term ($\sim$6 mo), and at high ratios ($\geq$1) of EPA to DHA. The notion of differences between EPA and DHA is further discussed toward the end of the review.

Overall, given the considerable debate in the field, there is an unmet need to understand how EPA, DPA, and DHA, consumed as dietary supplements, pharmacological supplements, or as oily fish, can potentially prevent or treat impairments in glucose metabolism. The confusion stems from studies using a wide range of doses of n–3 PUFAs, differing modes of delivery of n–3 PUFAs (mostly as varying fish oils), lack of discrimination between differing long-chain n-3 PUFAs, differing intervention durations, and a diverse study population with differing age, diet, sex, and genetics (10). Finally, underlying mechanisms of action for long-chain n–3 PUFAs on glucose homeostasis remain to be established. We now focus on studies at the rodent level to show that EPA, in particular, can improve varying aspects of glucose metabolism and insulin resistance, potentially through the biosynthesis of oxylipins known as hydroxyeicosapentaenoic acids (HEPEs).

EPA Improves Glucose Metabolism in Rodent Models, Which May Be Mediated through the Downstream Production of HEPEs

Several studies show that EPA at varying doses can prevent or treat varying aspects of T2D in rodent models (17–23). For instance, some show that doses less than the human equivalent of 4 g of fish oil/d can improve aspects of glucose homeostasis (17, 22). One study reported that EPA supplementation of a high-fat diet (1% by weight of the diet, $\sim$3 g/d for a human) led to an inhibition of adipocyte hypertrophy, improved glucose tolerance, and enhanced hepatic insulin signaling in obese mice fed a high-fat diet (17). Furthermore, relatively pure EPA ethyl esters at a pharmacological dose (equivalent of 4 g/d for humans) supplemented in a high-fat diet improved glucose tolerance, hyperglycemia, and hyperinsulinemia in male obese mice (24). The effects were sex specific as EPA ethyl esters did not improve glucose tolerance but did improve hyperglycemia, hyperinsulinemia, and body weight gain in female mice

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Abbreviations used: BLT1, leukotriene B4 receptor 1; ChemR23, chemokine-like receptor 1; COX, cyclooxygenases; DPA, docosapentaenoic acid; ERV1, human resolvin E1 receptor; GPR, G-protein coupled receptor; HEPE, hydroxyeicosapentaenoic acid; LO, lipoxygenase; LTB_4, leukotriene B_4; PMN, polymorphonuclear leukocyte; PPAR, peroxisome proliferator activated receptor; RvE, E-series resolvin; SPM, specialized pro-resolving mediator; T2D, type 2 diabetes; T_{reg}, regulatory T cell.

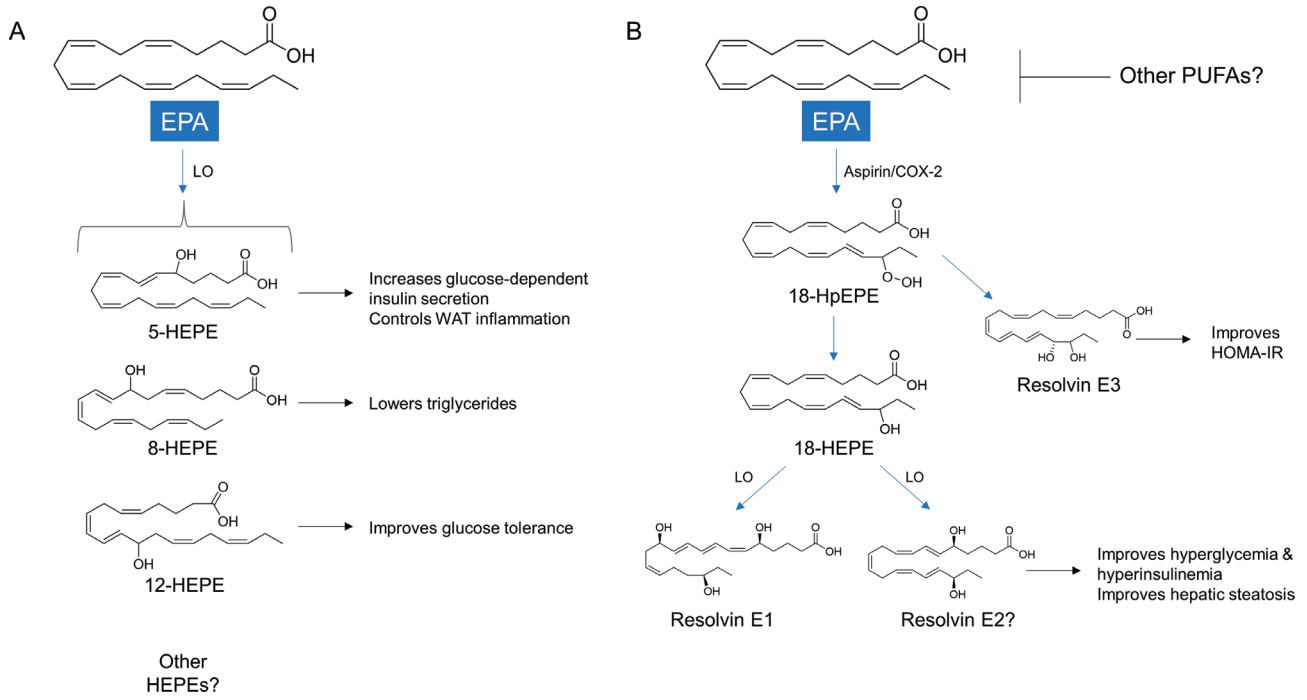

FIGURE 1 EPA and its downstream metabolites are leads for improving glucose homeostasis. (A) HEPEs are generated from EPA through LO. For simplicity, we present key HEPEs shown to improve aspects of glucose and lipid metabolism. Chemical structures are depicted and were constructed via ChemDraw 21.0.0. (PerkinElmer). (B) 18-HEPE and RvE1 biosynthesis from EPA. RvE1 has emerged to have insulin- and glucose-sensitizing properties in addition to improving hepatic steatosis. It remains unknown how other PUFAs may potentially impact EPA metabolism, which is an area for future research. In addition, the role for other HEPEs, RvE2, and RvE3 on glucose metabolism remains to be further investigated, although recent data suggest a role for RvE3 on improving insulin resistance. HEPE, hydroxyeicosapentaenoic acid; 18-HpEPE, 18-hydroperoxyeicosapentaenoic acid; LO, lipoxygenase; RvE1, resolvin E1; RvE2, resolvin E2; RvE3, resolvin E3; WAT, white adipose tissue.

in response to a high-fat diet (18). A very recent study reported that administration of prescription EPA ethyl esters (Vascepa®, Amarin) at 190.7 mg/(kg · d) (which corresponds to the human equivalent of 1 g/d) for 7 d improved glucose homeostasis when administered prior to administering a murine high-fat diet, suggesting a role for EPA ethyl esters as a prevention modality (25).

The mechanisms by which EPA exerts its physiological actions are highly pleiotropic (26–30). One mechanism by which EPA may exert its effects is through the downstream biosynthesis of HEPEs (**Figure 1**). These metabolites are synthesized in response to EPA serving as a substrate for cytochrome P450, lipoxygenases (LO), or cyclooxygenases (COX). This mechanism of action may be of particular relevance as there is some evidence that obesity drives a deficiency in HEPEs. To exemplify, female C57BL/6J mice fed a high-fat diet show a significant reduction in 8-HEPE in white adipose tissue, whereas male C57BL/6J mice display a strong reduction in 12-HEPE and 18-HEPE in white adipose tissue and liver (18, 24) A reduction in white adipose tissue 18-HEPE was also reported in another study (31); however, not all studies show this reduction as *db/db* mice, a genetic model of obesity driven by leptin receptor deficiency, showed an increase in white adipose tissue concentrations of 18-HEPE (32).

It is important to acknowledge that there are limitations of this line of mouse research and investigators must be careful in making conclusions about deficiencies in HEPEs across tissues. For instance, the values reported for differing HEPEs are normalized to the wet weight of the tissue; however, the analyses do not account for potential increase in tissue weight with increased adiposity. Thus, the concentrations of reported HEPE may not reflect the total amount of a given HEPE in differing tissues of lean and obese mice. Another limitation is the use of inbred mouse models, which can vary considerably in their metabolic profiles. Given that *db/db* mice are a model of leptin receptor deficiency, their baseline metabolism is different from a C57BL/6J mouse fed a high-fat diet. In fact, even C57BL/6J mice from differing vendors vary in their metabolic profiles (33). Thus, interpretation of data across mouse models and its applications to humans will require more work, perhaps with the use of humanized mouse models and population-based approaches such as diversity outbred mice.

There is compelling evidence that 18-HEPE concentrations are decreased in plasma and leukocytes of individuals

TABLE 1 Summary of recent experimental studies to show differing effects and underlying mechanisms by which HEPEs and EPA-derived resolvins may improve outcomes in obesity[1]

EPA-derived metabolite	Model system	Findings	Reference
5-HEPE	Culture studies (1–10 μM) with insulinoma cells and intestinal adenocarcinoma cells	Increased glucose-dependent insulin secretion via a cAMP-mediated mechanism	(40)
	Primary T cells in the presence of macrophages treated with 100 μM 5-HEPE	Induction of T_{regs}	(41)
8-HEPE	8-HEPE purified from krill oil and delivered to mice for 4 wk at 10 mg/kg	Reduction in plasma and liver triglycerides in obese mice via activation of PPARα; similar effects were also observed with LDL receptor–deficient knockout mice in which 8-HEPE improved hepatic steatosis	(43,44)
	3T3-L1 cells treated with 5 μM 8-HEPE	Reduction in triglycerides	(18)
12-HEPE	Mice administered 200 μg/kg 12-HEPE for 2 wk	Improvement in glucose tolerance by driving glucose uptake into skeletal muscle and brown adipose tissue	(35)
	Adipocytes treated with 0.01–1 μM 12-HEPE	Improved glucose uptake in adipocytes	(35)
	Serum samples from lean individuals as well as those with obesity and overweight	Negative correlation between 12-HEPE and HOMA-IR	(35)
	Subcutaneous white adipose tissue samples from lean individuals and those with obesity	Negative correlation between 12-HEPE and insulin resistance	(56)
	12-HEPE administered 100 ng/mouse for 4 consecutive days	Improved pulsatility and resistive indexes in common carotid artery in an obesity mouse model	(58)
	Isolated peritoneal cells from mouse and treated for at least 24 h with 30 μM 12-HEPE	Inhibited foam cell formation induced by oxidized LDL	(58)
15-Hydroxy-EPA ethyl esters	Placebo-controlled randomized phase 2 trial with synthetic 15-hydroxy-EPA ethyl esters (2 g/d for 16 wk)	Improved glycemic control, circulating triglycerides, inflammatory markers, and HbA1c in adults with NAFLD	(38)
18-HEPE	Obese mice administered 300 ng/mouse for 4 consecutive days	No effect on hyperglycemia or hyperinsulinemia	(24)
RvE1	*Ob/ob* mice administered RvE1 at a dose of 1.2 ng/g body weight for 4 consecutive days	Increase in adipose tissue mRNA expression of adiponectin, GLUT-4, PPARγ, IRS-1; decrease in liver triglycerides	(30)
	Obese mice administered RvE1 2 ng/g body weight, twice a week for 4 wk of RvE1	Increased polarization to M2-like macrophages and lowering of hepatic inflammatory transcripts	(81)
	Obese mice administered RvE1 at 300 ng/mouse for 4 consecutive days	Improved hyperglycemia and hyperinsulinemia in a ChemR23-dependent manner; improved hepatic inflammatory and metabolic transcripts	(24,82)
	Obese diversity outbred mice administered RvE1 at 21.4 ng/g fat mass for 4 consecutive days	Positive and negative responders for metabolic outcomes such hyperglycemia and hyperinsulinemia are identified in response to RvE1	(24,87)
	1.2 ng/g body weight of RvE1 for 3 times/wk to mice after 11 wk of a high-fat diet	Improved glucose tolerance but not insulin tolerance or HOMA-IR	(93)
RvE3	Administration of RvE3 (150–300 ng/mouse for 4 consecutive days) or 1.2 ng/g body weight 3 times for 1 wk to mice after 11 wk of a high-fat diet	Improved glucose tolerance, hyperglycemia, and HOMA-IR score	(93)
	3T3-L1 cells treated with 100 nM RvE3 or RvE1	RvE3, but not RvE1, increased insulin-stimulated glucose update and Akt phosphorylation	(93)

[1]ChemR23, chemokine-like receptor 1; GLUT-4, glucose transporter type 4; HbA1c, glycated hemoglobin; HEPE, hydroxyeicosapentaenoic acid; IRS-1, insulin receptor substrate 1; NAFLD, nonalcoholic fatty liver disease; PPAR, peroxisome proliferator activated receptor; RvE1, resolvin E1; RvE3, resolvin E3; T_{reg}, regulatory T cell.

with obesity [BMI (kg/m^2) >30] relative to nonobese controls (BMI <30) (34). There are also data that 12-HEPE concentrations are decreased in subjects with obesity (BMI >30) or those who are overweight (25 < BMI < 30) compared with lean controls (BMI <25) (35). Interestingly, 12-HEPE concentrations were negatively correlated with insulin resistance, as measured by HOMA-IR (35). The same study also showed a negative association between 14-hydroxydocosahexaenoic acid and HOMA-IR. Although we do not focus on DHA-derived metabolites, there is evidence for a reduction in DHA-derived metabolites in obese mice and humans with obesity, which may also be important for insulin sensitivity (31, 32, 36, 37).

Potential Mechanisms by Which Key HEPEs Control Glucose and Insulin Metabolism

There are several lines of evidence to suggest that hydroxylated metabolites of EPA have a role in controlling differing aspects of glucose metabolism (Figure 1A). Here we review data from key studies, which are summarized in **Table 1**, in

the context of obesity and its associated complications. The bulk of the research is with the use of mice. However, 1 recent clinical trial highlights the importance of hydroxylated EPA. A placebo-controlled, randomized, phase 2 multicenter study with the 15-hydroxy EPA ethyl ester known as Epeleuton (2 g/d for 16 wk) administered to adults with nonalcoholic fatty liver disease showed an improvement in glycemic control, a reduction in triglycerides, decreased glycated hemoglobin (HbA1c), and a reduction in key inflammatory markers relative to those on placebo (38). Thus, there is a need to understand how HEPEs work at the whole animal, cellular, and molecular level to drive future precision clinical trials. As described below, data thus far indicate that specific HEPEs improve glucose metabolism through distinct mechanisms. We now review the current evidence for specific HEPEs.

5-HEPE

In culture studies using mouse MIN6 insulinoma cells and human HuTu80 intestinal adenocarcinoma cells, 5-HEPE increases glucose-dependent insulin secretion, which is mediated by a cAMP-mediated mechanism dependent on the receptor known as G-protein coupled receptor-119 (GPR-119) (39). This study is of interest given that GPR-119 agonists control multiple mechanisms of glucose homeostasis such as incretin levels and beta-cell insulin secretion (40). A potential limitation of this study was that the concentration of 5-HEPE that elicited a response was in the 1–10-μM range. It is unknown if this dose range is physiologically relevant for differing metabolic tissues.

5-HEPE may also have a role in controlling white adipose tissue inflammation, which is a major driver of glucose dysregulation. A study using C57BL/6J mice showed that administration of 5% EPA into the diet of *ob/ob* mice (leptin-deficient genetic model of obesity on a C57BL/6J background) led to an increase in the number of regulatory T cells (T_{regs}) in epididymal adipose tissue (41). Follow-up mechanistic studies using primary cells showed that 100 μM 5-HEPE, but not other HEPEs, induced the formation of T_{regs} in the presence of macrophages, suggesting a potential role for 5-HEPE in controlling the white adipose tissue inflammatory microenvironment (41). The mechanistic data provided proof-of-concept regarding the role of 5-HEPE on T_{regs}; however, a limitation is that the dose of 5-HEPE used in this study is likely more than what is produced in response to EPA intervention as fish-oil or pharmacological supplements. To exemplify, a study showed that 5-HEPE concentrations were in the nanomolar range, which was a 5.7-fold increase in 5-HEPE in response to consumption of 4 g/d of long-chain n–3 PUFAs in an intervention in healthy volunteers for 4 wk (42). Thus, future mechanistic studies will need to address how 5-HEPE controls T_{reg} function at doses in the nanomolar range.

8-HEPE

The metabolite 8-HEPE, purified from krill and administered at a dose of 10 mg/kg with a high-fat diet for 4 wk to C57BL/6J mice, can lower plasma and liver triglycerides in obese mice by activating peroxisome proliferator activated receptor (PPAR) α (43, 44). There is also evidence for a benefit of 8-HEPE–containing krill oil for improving hepatic steatosis in LDL-receptor knockout mice (43). PPARα is important given its role in controlling lipid biosynthesis (45). PPARα promotes the expression of a variety of metabolic enzymes such as those related to driving fatty acids toward the mitochondria and thereby β-oxidation (46). PPARα also has an important and well-described role in controlling inflammation by interacting with other transcription factors (47). Thus, activation of PPARα may be an important mechanism by which 8-HEPE exerts its beneficial effects. Overall, the PPAR superfamily of nuclear receptors is important as they have several critical roles in regulation of lipid storage and catabolism (48). PUFAs also serve as PPAR ligands for these nuclear receptors regulating PPAR-dependent transcription activity (49–53). PUFAs bind to all 3 types of PPAR (50). The highest affinity of PPAR for PUFAs is essentially equivalent to concentrations found in blood (54).

Other HEPEs may also activate PPARs, although 8-HEPE appears to be the most potent compared with EPA and 5-, 9-, 12-, and 18-HEPEs based on a luciferase reporter assay in cultured cells (55). A limitation of the previous work is that the relation between differing HEPE concentrations in specific cells and tissues in response to EPA intervention is not well established. In turn, the concentration of HEPEs that is relevant for PPAR activation needs to be determined. The lowest concentration of a HEPE, which is 8-HEPE, that can activate PPARα, PPARγ, and PPARδ is 5 μM (55). Thus, the studies to demonstrate that HEPEs, particularly 8-HEPE, can activate PPARs need to be tested under physiological conditions in response to interventions with differing levels of oily fish intake (i.e., differing servings per week), over-the-counter fish-oil supplements, and pharmacological supplements.

The effects of 8-HEPE are not limited to fat accumulation in the liver. Administration of a high-fat diet to female C57BL/6J mice led to a significant reduction in the concentration of gonadal white adipose tissue 8-HEPE, which was reversed upon EPA ethyl ester intervention (2% of energy, which models human consumption of ~4 g/d of either over-the-counter fish-oil or pharmacological supplements). Interestingly, EPA ethyl esters prevent an increase in fat mass in response to the high-fat diet and supporting in vitro studies demonstrate 5 μM 8-HEPE prevents the accumulation of triglycerides, as measured with Oil Red O staining, in 3T3-L1 cells (18). Again, the biological relevance of 5 μM remains to be established, which is further discussed toward the end of the review under the Future Directions section.

12-HEPE

Very recently, 12-HEPE has emerged to have an important role in glucose metabolism. A combination of murine and human studies reveal that 12-HEPE is significantly increased in response to cold exposure or β_3-adrenergic stimulation (35). The source of 12-HEPE is brown adipocytes

based on studies with C57BL/6J mice. Interestingly, intraperitoneal administration of 200 μg/kg 12-HEPE for 2 wk improves glucose tolerance by driving glucose uptake into skeletal muscle and brown adipose tissue. Supporting in vitro studies reveal that 12-HEPE (dose range of 0.01–1 μM) controls glucose uptake in adipocytes, although this was not observed in vivo with white adipose tissue (34). It was recently reported that 12-HEPE in subcutaneous white adipose tissue was negatively associated with insulin resistance (56). Future studies will need to establish the role of 12-HEPE across differing tissues. One starting point is the brain, where EPA (2% of energy) dramatically increased 12-HEPE concentrations in the context of a murine high-fat diet (57).

There is also emerging evidence that 12-HEPE can improve aspects of atherosclerosis using a mouse obesity model. In this study, the investigators demonstrated that linseed oil, which increases the circulating concentrations of 12-HEPE in the nanogram/milliliter range, improved measures of atherosclerosis (notably foam cell formation) driven by a high-fat diet. Mechanistically, a combination of add-back (100 ng/mouse, 3 times/wk) and cell culture studies (using nanomolar levels) with 12-HEPE inhibited foam cell formation through a PPARγ-dependent manner (58).

Potential Mechanisms by Which the 18-HEPE and Resolvin E1 Biosynthetic Pathways Control Aspects of Glucose Homeostasis

Here we will focus on a key pathway of EPA, which is the downstream biosynthesis of 18-HEPE (Figure 1B), the precursor for resolvin E1 (RvE1). RvE1 belongs to a family of immunoresolvent oxylipins known as specialized pro-resolving mediators (SPMs) that act in concert to modulate varying aspects of epithelial, endothelial, and immune cell function for the restoration of homeostasis (31, 59–63). As discussed below, the resolution circuits are dysregulated in human and murine obesity and associated morbidities, including T2D, potentially through reduced production, increased clearance, and/or diminished action of SPMs, which can be rescued by therapeutic SPM delivery or upregulation of SPM receptors.

RvE1 has potent pro-resolving and insulin-sensitizing actions mediated by 2 distinct shared receptors, leukotriene B4 receptor 1 (BLT1) and human resolvin E1 receptor (ERV1), in metabolic organs. Based on rodent studies, RvE1 mediates an increase in protective adipokines such as adiponectin in white adipose tissues, the enhancement of monocyte/macrophage shift to a pro-resolution phenotype, as well as macrophage-clearing functions that all improve metabolic control in obesity-related conditions [reviewed in (64)]. RvE1-enhanced resolution in obesity prevents dysbiosis of the gut microflora and resultant increased gut permeability (64, 65). These functions suggest that RvE1 has therapeutic potential for immunometabolic pathologies associated with obesity.

RvE1 synthetic pathways

RvE1 was first identified in vivo in inflammatory exudates in mice after treatment with aspirin and EPA (66). The RvE1 synthetic pathways depend upon EPA by acetylation by aspirin or S-nitrosylation by statins of COX-2 to produce a new 15-LO, cytochrome P450, or 15-LO-1 in endothelial cells to metabolize 18R-HEPE, which is further processed in leukocytes through a 5-LO pathway (67,68). Importantly, aspirin via acetylated COX-2, statins via S-nitrosylated COX-2, and thiazolidinediones via phosphorylation of 5-LO all promote RvE1 synthesis (68–70).

RvE1 actions

Two GPRs chemokine-like receptor 1 (ChemR23 or ERV1 in humans) and BLT1 signal in response to high-affinity binding by RvE1 (**Figure 2**A) (60). Monocyte/macrophages express ERV1, as do osteoclasts, osteoblasts, and other stromal cells (67, 71, 72). RvE1 signals through BLT-1 on resting neutrophils (73), but neutrophils isolated from inflamed subjects with T2D also express functional ERV1 (74). ERV1 binds to ChemR23 with high affinity (Kd = 11.3 $\pm$ 5.4 nM) with a Bmax of 4200 $\pm$ 1050 binding sites/cell (67). ERV1 is also expressed in cardiovascular tissues, brain, kidney, and gastrointestinal and oral (periodontal) tissues (67). Neutrophils expressing functional BLT-1 bind RvE1 with a Kd = 45 nM, where it acts as a partial agonist for leukotriene B_4 (LTB_4) (73).

ERV1 binding reduces IL-12 production by dendritic cell signaling through Akt, mTOR, and rS6 (Figure 2B) (75), whereas RvE1 binding to BLT-1 competitively blocks LTB_4 to neutrophils (73). In a murine model of zymosan-induced peritonitis, RvE1 (300 ng, intraperitoneally) reduced leukocyte infiltration and upregulated C-C Motif Chemokine Receptor 5 (CCR5) expression on apoptotic polymorphonuclear leukocytes (PMNs), stimulating macrophage phagocytosis of apoptotic PMNs and clearance through lymph nodes and spleen (76–78). RvE1 is metabolized by enzymatic conversion to inactive or less potent compounds with 1 exception, 20-hydroxy-RvE1, in blood, lung, liver, kidney, and spleen (79, 80).

RvE1 actions in obesity

Systemic activation of RvE1-mediated pathways prevents morbid obesity and hyperglycemia despite dietary overload. Table 1 summarizes studies related to RvE1 administration and obesity. For simplicity, we do not cover data related to resolvins and T2D, which are reviewed elsewhere (64). In gain-of-function studies, ERV1 overexpressing transgenic mice fed a high-fat diet exhibited reduced inflammation, reduced body weight gain, and were protected from developing hyperglycemia (81). Mechanistically, this was accompanied by monocyte/macrophage phenotypic shifts to an increased M2/M1-like ratio. Administration of a natural ERV1 agonist, RvE1 (2 ng/g body weight, twice a week for 4 wk), recapitulated the pro-resolving actions of ERV1 overexpression. The metabolic impact of RvE1 was a result,

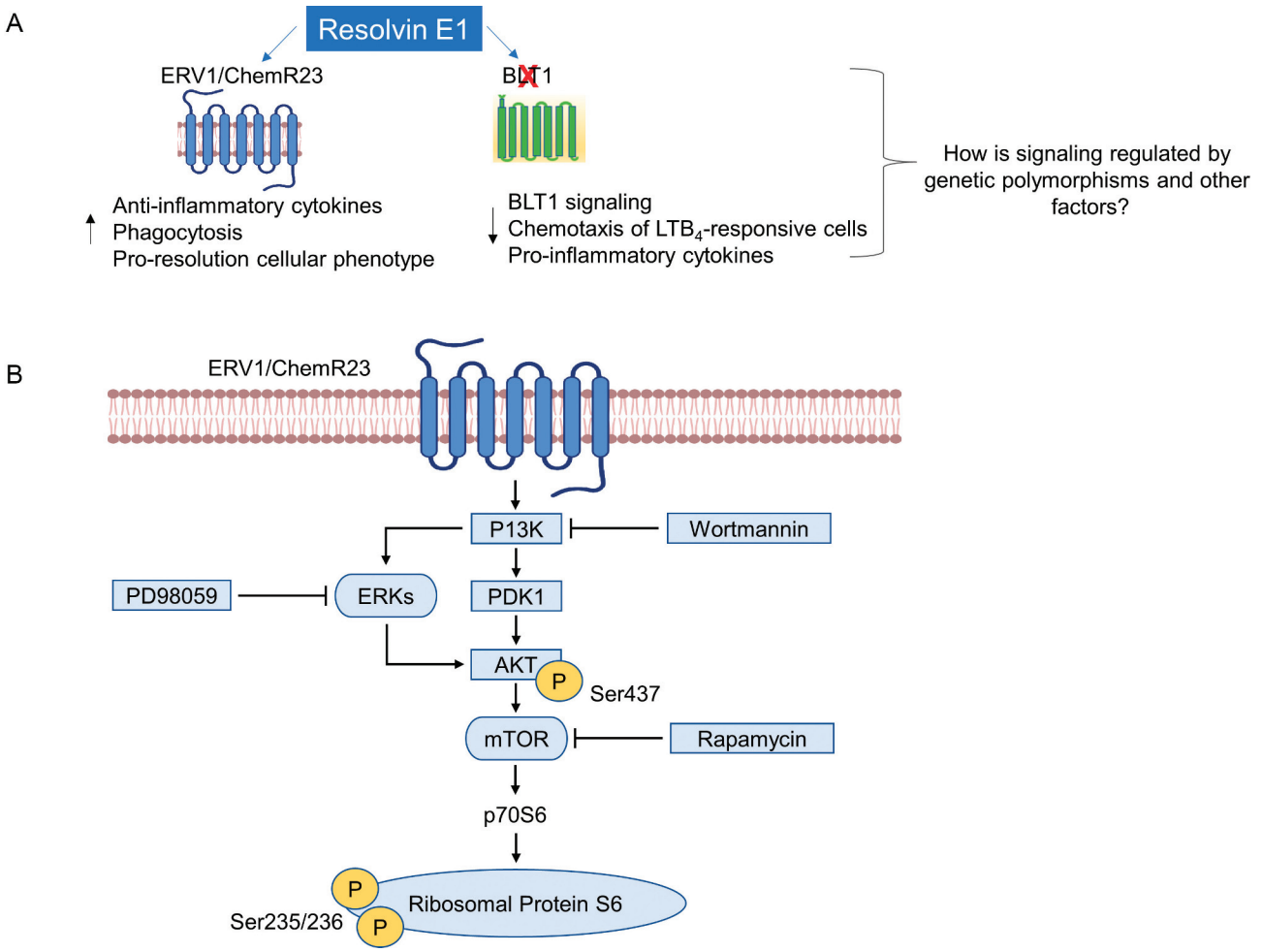

FIGURE 2 Mechanisms by which RvE1 limits inflammation and promotes resolution. (A) RvE1 is an agonist for ERV1/ChemR23. Signaling through this receptor drives the release of anti-inflammatory cytokines, enhances phagocytosis of dying cells, and drives a phenotypic switch toward pro-resolution immune cell phenotypes. RvE1 is also an antagonist for BLT1. This inhibits LTB_4-mediated signaling and thereby a reduction in recruitment of immune cells that secrete proinflammatory cytokines. A key question is whether the host genome, in addition to other factors such as age and sex, controls signaling through these receptors in response to RvE1 binding. (B) The signaling pathway for RvE1 has been defined and is represented in this scheme of key phosphorylation components and the points of action of specific inhibitors used to define the pathway (75). BLT1, leukotriene B4 receptor 1; ChemR23, chemokine-like receptor 1; ERV1, human resolvin E1 receptor; LTB_4, leukotriene B_4; RvE1, resolvin E1.

in part, of systemic activation of resolution programs leading to increased synthesis of other SPM classes.

RvE1, at 1.2 ng/g body weight for 4 consecutive days, was identified to have a role in improving hepatic triglycerides and transcripts of white adipose tissue metabolism [i.e., glucose transporter type 4 (GLUT-4)] in *ob/ob* mice (30). In subsequent studies, RvE1 administration (300 ng/mouse) to obese mice for 4 consecutive days prior to being sacrificed improved hyperinsulinemia and hyperglycemia (24). The effects of RvE1 were mitigated in ChemR23 knockout mice, underscoring the critical role of this receptor in the mechanism of action for RvE1 (Figure 2). In this model system, RvE1's mechanism of action was independent of controlling the enrichment of white adipose tissue inflammatory cells, notably monocytes or B cells. Instead, RvE1 (300 ng/mouse for 4 consecutive days) upregulated hepatic transcripts in pathways related to insulin sensitivity while downregulating inflammatory pathways such as those related to transforming growth factor β (TGF-β) signaling (82). The effects of RvE1 on the hepatic transcriptional landscape were generally mitigated upon knockout of ChemR23. Interestingly, in this line of work, 18-HEPE did not influence hyperinsulinemia and hyperglycemia of obese mice, although it is important to note that 18-HEPE is reported to have a beneficial role in cardiometabolic outcomes, which is reviewed elsewhere (83, 84).

As described above for HEPEs, there is a need to establish the concentration of RvE1 in tissues/cells in response to EPA at doses that are achievable in humans through either oily fish intake or as supplements. There are data to show

that marine oil supplementation can increase RvE1 concentrations in a dose-dependent manner within hours (≥4 h) of consumption at a high dose (4.5 g marine oil enriched in SPM precursors) in healthy subjects (61). Moreover, RvE1 concentrations are increased (in the ng/ml range) in adults with obesity by nearly 3-fold in response to an over-the-counter marine oil supplement administered at a dose of 2 g/d (85).

An interesting question remains open as to whether RvE1 and HEPE deficiencies in disease are due to lack of sufficient production or excess metabolism. Experiments that add back excess RvE1 or HEPEs with clinical outcomes would argue that metabolism is normal, but production is reduced. This notion is further supported by human data that demonstrate a lack of production of precursors (HEPEs) and active SPMs in specific inflammatory diseases (86).

Interestingly, the effects of RvE1 are dependent on the host genome, based on studies with diversity outbred mice, which model human genetic heterogeneity unlike inbred mice (24, 87). These results suggest that future experiments with RvE1 will require establishing which genotypes control RvE1 synthesis, bioavailability, and degradation. A recent study highlights the importance of the host genome as specific HEPE concentrations were higher in *APOE4* carriers compared with controls in response to 1 y of intervention with a marine oil supplement (88).

Obesity also induces gut dysbiosis and increased intestinal permeability (leaky gut) that leads to metabolic endotoxemia and inflammatory macrophage activation that amplifies insulin sensitivity, obesity, and T2D (89, 90), which is reversed, in part, by overexpression of the RvE1 receptor in a gain-of-function study (64). Obesity-associated gut dysbiosis is characterized by an increase in the ratio of Firmicutes to Bacteroidetes phyla, which results in altered microbial metabolism, including a reduction in SCFA production (e.g., butyrate) and a loss of mucus secretion by intestinal epithelial cells, T_{reg} differentiation, IgA expression by B cells, and inhibition of NF-κB (91, 92).

It is important to note that the effects of other E-series resolvins on glucose metabolism remain to be investigated. However, data are emerging to suggest a role for these metabolites. A very recent study shows that resolvin E3 (RvE3) dose-dependently (from 150–300 ng/mouse administered via intraperitoneal injection for 4 consecutive days or given 1.2 ng/g body weight 3 times in a week) improved hyperglycemia, glucose tolerance, and/or the HOMA-IR score in C57BL/6J mice fed a high-fat diet (93). Interestingly, RvE3 improved insulin signaling through the PI3K/Akt pathway independent of targeting inflammatory pathways in 3T3L1 cells (93).

Gaps in Knowledge and Future Directions

There are several major gaps in the knowledge as they pertain to future dietary or pharmacological studies with EPA, downstream HEPEs, and their underlying mechanisms of action. One gap in knowledge is the role of other PUFAs on EPA metabolism. A good starting point is to focus on DHA. A recent study, discussed above, suggested that DHA may negate the effects of EPA in a prevention model. Specifically, mice administered EPA ethyl esters (Vascepa®, Amarin) at 190.7 mg/(kg · d) (human equivalent of 1 g/d) for 7 d via oral gavage 1 wk before the administration of a high-fat diet showed a decrease in hyperglycemia, hyperinsulinemia, glucose tolerance, and insulin resistance (25). Mechanistically, the actions of Vascepa were mediated through enhanced beta-cell function, remodeling of the gut microbiome, and a reduction in liver triglycerides (25). Strikingly, administration of a prescription EPA/DHA mixture (Lovaza®, GSK) at 410 mg/(kg · d) (human equivalent of 2 g/d) for 7 d via oral gavage did not display the same effects as Vascepa, suggesting that DHA may negate the beneficial effects of EPA (25).

Support for the notion that DHA may negate the effects of EPA on glucose homeostasis in humans (or even rodents, for the most part) is completely lacking, although there is the suggestion, based on analyses of NHANES data, that consumption of EPA, but not DHA, was associated with improved glucose tolerance in a sex-specific manner for individuals with obesity (BMI >30) (24). The potential for DHA to impact EPA metabolism has also emerged in the cardiovascular literature, where there is some debate on the efficacy of EPA compared with EPA+DHA therapy for differing outcomes (94, 95). This is clearly an area for future investigation as there are studies to show that DHA and its downstream metabolites have benefits for differing aspects of glucose metabolism (22, 36, 96, 97). Perhaps, EPA and DHA can work synergistically at some concentrations and are competing with one another at other doses.

Differences between EPA and DHA may be driven by differing structures and metabolism (98–102). Perhaps EPA is just more effective than DHA at specific doses, similar to the suggestion in the literature on depression (103). A recent review from our research group highlights potential mechanisms by which EPA and DHA may compete against each other. These mechanisms include competition for occupancy of EPA and DHA into membrane phospholipids (and thereby its impact on membrane biophysical organization and downstream oxylipin biosynthesis), in addition to DHA lowering the rate of EPA conversion to DPA (99).

An additional area for investigation is the potential role for n–6 PUFAs on EPA metabolism. In particular, linoleic acid (18:2n–6), the most abundant PUFA consumed in the diet, can bind some of the same enzymes that are used for the biosynthesis of SPMs (104, 105); therefore, there is a need to understand if excess n–6 PUFAs such as linoleic acid can also inhibit the benefits of EPA. Again, some NHANES data suggest that high linoleic acid intake was associated with a reduction in the benefit of EPA in individuals with obesity on glucose concentrations (24). There is also a rodent study demonstrating that linoleic acid is associated with a reduction in the concentrations of metabolites of the SPM family (106). However, these findings are limited as they do not demonstrate causality.

Translation of key metabolites derived from EPA into clinical practice will also require extensive knowledge in other key areas. In particular, there is a need to account for the heterogeneity of obesity in studies of dietary EPA and its metabolites. Obesity is not a single disease but rather a culmination of "obesities" that are dependent on host microbiome status, age, circadian rhythm, sex, baseline fatty acid profile (and other macro- and micronutrients that impact metabolism), and many other variables (107–109). Therefore, translation from the mouse to human level will require accounting for many of these variables and understanding their role in glucose metabolism in the presence and absence of EPA/DHA. For instance, there are known single nucleotide polymorphisms associated with the metabolic pathways of PUFAs, which will impact bioavailability of metabolites synthesized from EPA (88, 110–112). As an example, individuals with obesity who have a C allele in the rs1878022 polymorphism of ERV1/ChemR23 display a reduction in white adipose tissue inflammation (113). In addition, there is evidence from a recent epidemiological study that participants carrying specific fatty acid synthesis and elongation alleles that relate to DPA synthesis had a greater reduction in the risk of T2D (7). Furthermore, the role of circadian rhythms is generally unknown on PUFA metabolism in obesity. There is evidence that time of day of n–3 PUFA consumption may have a role in controlling circulating concentrations of EPA/DHA (114). n–3 PUFAs such as DHA may control circadian timekeeping and associated inflammation (115).

There is a critical need to further investigate how intake of dietary over-the-counter and pharmacological supplements in addition to consumption of oily fish (perhaps across a range of servings per week) can control the concentration of HEPEs in circulation and in specific cell types (116). For example, a randomized clinical trial quantified the concentration of differing plasma PUFA-derived oxylipins in response to providing healthy subjects n–3 PUFA triglycerides that corresponded to consumption of 0, 1, 2, or 4 fatty fish meals/wk, with 1 serving defined as 3.3 g EPA+DHA. The results showed that HEPEs (largely in the nanomolar range), along with many other oxylipins, were elevated within 3 mo of intervention and the increase in the concentration of oxylipins was linear with dose. This study notably identified 5-, 8-, 9-, 11-, 12-, 15-, 18-, 19-, and 20-HEPEs, which is in contrast to a recent study with a fish-oil supplement that only showed an increase in 5-, 11-, and 15-HEPEs in adults with obesity (85). Furthermore, the concentrations of HEPEs measured in differing human populations must then be related to concentrations in rodent models and in culture. Establishing Kds for HEPEs will also be of great utility. As discussed above, many of the cell culture based mechanistic studies relied on a micromolar concentration of HEPEs, which is unlikely to be physiologically relevant. Thus, mechanistic studies will need to use far lower concentrations of HEPEs in culture and potentially in rodents.

Finally, there is considerable controversy around the detection of oxylipins of the SPM family, with many intervention studies with fish-oil supplements showing no change in metabolites such as resolvin E3 (117, 118). Thus, future studies that link intake of EPA as oily fish or supplements in select populations with the concentration of SPMs in specific tissues and cells are critical and will also guide mechanistic studies at appropriate doses in cell culture and rodents.

Conclusions

In summary, there are compelling data from very recent epidemiologic studies to suggest that increased dietary intake of long-chain n–3 PUFAs, either as oily fish or as supplements, is associated with a reduction in the risk of developing T2D. Some of these studies suggest a unique role for EPA. Experiments with rodent models of obesity and supporting in vitro studies show that EPA, in particular, can improve glucose homeostasis. EPA's mechanism of action is mediated, in part, through the downstream biosynthesis of HEPEs. The current evidence suggests that 5-HEPE, 8-HEPE, 12-HEPE, 18-HEPE, and RvE1 can improve glucose and insulin metabolism through differing mechanisms that largely relate to improvements in chronic inflammation. There is also a potential role for RvE3, albeit the mechanism of action may be independent of inflammation. Finally, the translation and clinical development of these metabolites and their parent compound EPA will require accounting for a variety of factors that are responsible for the heterogeneity of obesity and the response to PUFAs. These variables, as examples, include host genetics, age, circadian rhythm, sex differences, and host microbiome status. Furthermore, it remains unknown if other PUFAs such as DHA may interfere with EPA's metabolism, which is an area of investigation that remains in its infancy. Finally, dose–response studies in humans with oily fish and supplements as they relate to the concentration of HEPEs and resolvins in specific tissues will guide subsequent mechanistic experiments in cell culture and rodents.

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