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Lipidomic profiling reveals soluble epoxide hydrolase as a therapeutic target of obesity-induced colonic inflammation

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Obesity is associated with enhanced colonic inflammation, which is a major risk factor for colorectal cancer. Considering the obesity epidemic in Western countries, it is important to identify novel therapeutic targets for obesity-induced colonic inflammation, to develop targeted strategies for prevention. Eicosanoids are endogenous lipid signaling molecules involved in regulating inflammation and immune responses. Using an LC-MS/MS–based lipidomics approach, we find that obesity-induced colonic inflammation is associated with increased expression of soluble epoxide hydrolase (sEH) and its eicosanoid metabolites, termed fatty acid diols, in colon tissue. Furthermore, we find that pharmacological inhibition or genetic ablation of sEH reduces colonic concentrations of fatty acid diols, attenuates obesity-induced colonic inflammation, and decreases obesity-induced activation of Wnt signaling in mice. Together, these results support that sEH could be a novel therapeutic target for obesity-induced colonic inflammation and associated diseases.

obesity | colonic inflammation | soluble epoxide hydrolase

Obesity is growing at an alarming rate in the United States: Currently, more than 35% of adults and nearly 17% of children are obese (1, 2). Obesity is associated with enhanced colonic inflammation (3–5), which is a major risk factor for developing colorectal cancer (6). Indeed, obese individuals have a 30 to 60% greater risk of developing colorectal cancer (7, 8). Considering the obesity epidemic and the potential lethal consequence of obesity-enhanced colorectal cancer, it is important to identify novel therapeutic targets for obesity-induced colonic inflammation.

Eicosanoids, which are metabolites of arachidonic acid (ARA; 20:4ω-6) produced by cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) enzymes, are autocrine and/or paracrine mediators regulating inflammation and hemostasis. Besides ARA, other polyunsaturated fatty acids (PUFAs), such as linoleic acid (LA; 18:2ω-6), α-linolenic acid (ALA; 18:3ω-3), dihomo-γ-linolenic acid (DGLA; 20:3ω-6), eicosapentaenoic acid (EPA; 20:5ω-3), and docosahexaenoic acid (DHA; 22:6ω-3), are also alternative substrates of these enzymes, leading to formation of a large array of oxylipin metabolites with diverse chemical structures and biological actions (9–11). The enzymes, receptors, and metabolites in the ARA cascade are important therapeutic targets of many drugs on the market, treating a variety of human disorders such as inflammation, fever, pain, cancer, and asthma (10, 12).

Emerging research supports the notion that eicosanoid signaling is deregulated in obesity and plays critical roles in the pathogenesis of obesity (13, 14). Previous studies showed that the tissue levels of COX-derived prostaglandin E_2 (PGE_2) and LOX-derived leukotriene B_4 (LTB_4) are increased in adipose tissues of obese subjects (15, 16). Besides the intensively studied COX and LOX pathways, recent research showed that soluble epoxide hydrolase (sEH), which is the enzyme that converts CYP-produced fatty acid epoxides to the corresponding fatty acid diols, is up-regulated in liver and adipose tissues of obese animals, and could contribute to various obesity-induced disorders (14, 17–24). However, the roles of eicosanoid signaling in obesity-induced colonic inflammation are unknown. In this study, we used a liquid chromatography tandem mass spectrometry (LC-MS/MS)-based lipidomics approach, which can analyze >100 eicosanoid metabolites produced by COX, LOX, and CYP enzymes from multiple PUFA substrates (*SI Appendix*, Table S1), to study the roles of eicosanoids in obesity-induced colonic inflammation in mice. We demonstrate that dietary administration of a high-fat diet (HFD) increases expression of sEH and its metabolites in colon tissues. In addition, pharmacological inhibition or genetic ablation of sEH abolishes HFD-induced colonic inflammation and activation of Wnt signaling.

Significance

Obesity is associated with enhanced colonic inflammation, which is a major risk factor for colorectal cancer. To date, the mechanisms by which obesity increases colonic inflammation are not well-understood, and there are few effective strategies for controlling obesity-induced colonic inflammation and associated diseases. Here, using LC-MS/MS–based metabolomics, we report that soluble epoxide hydrolase (sEH) could be a novel therapeutic target for obesity-induced colonic inflammation. This could lead to rapid human translation, as pharmacological inhibitors of sEH are being evaluated in human clinical trials targeting multiple disorders.

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These results support that sEH is a promising therapeutic target for obesity-induced colonic inflammation and associated diseases.

Results

HFD Increases Body Weight and Colonic Inflammation in Mice. We treated 6-wk-old C57BL/6 male mice with a low-fat diet (LFD; 10 kcal% fat) or an HFD (60 kcal% fat) for 8 wk (*SI Appendix*, Fig. S1*A*). Compared with mice maintained on an LFD, HFD-fed mice had significantly increased body weight (*SI Appendix*, Fig. S1*B*). Regarding colonic inflammation, we found that HFD treatment increased gene expression of the proinflammatory cytokines *Il-1*β and *Tnf*-α in colon tissues, while the expression of other cytokines, such as *Il-6* and *Mcp-1*, was not changed (*SI Appendix*, Fig. S1*C*). In addition, HFD treatment increased infiltration of immune cells, including leukocytes ($CD45^+$) and macrophages ($CD45^+$ $F4/80^+$), into colon tissues (*SI Appendix*, Fig. S1*D*). These results demonstrate that HFD treatment increased colonic inflammation in mice, which is consistent with previous studies (3, 5).

HFD Increases sEH-Produced Fatty Acid Diols in Colon. To explore the roles of eicosanoids in obesity-induced colonic inflammation, we used LC-MS/MS–based lipidomics to compare the profiles of eicosanoid metabolites in the colon tissues of LFD- and HFD-fed mice. We quantified 50 eicosanoid metabolites in colon tissues of the treated mice (*SI Appendix*, Table S2; some eicosanoid metabolites were below the limit of detection or quantitation of the LC-MS/MS method). Partial linear square discriminant analysis showed that HFD treatment caused a significant change of the eicosanoid profiles in colon (*SI Appendix*, Fig. S2). Among the detected eicosanoids in colon, sEH-produced fatty acid diols, including ARA-derived 8,9-, 11,12-, and 14,15-dihydroxyeicosatrienoic acid (DHET), EPA-derived 17,18-dihydroxyeicosatetraenoic acid (DiHETE), and DHA-derived 7,8-, 10,11-, 16,17-, and 19,20-dihydroxydocosapentaenoic acid (DiHDPE), were the major compounds contributing to the difference between the LFD and HFD groups (Fig. 1*A* and *SI Appendix*, Fig. S3*A*). Notably, the colonic concentrations of 8,9-DHET in the LFD- versus HFD-fed mice were 3.72 ± 0.39 pmol/g (mean ± SEM) versus 6.42 ± 0.39 pmol/g ($P < 0.001$), the concentrations of 11,12-DHET were 6.16 ± 0.89 pmol/g versus 10.21 ± 0.75 pmol/g ($P = 0.003$), and the concentrations of 14,15-DHET were 6.91 ± 0.75 pmol/g versus 10.28 ± 0.69 pmol/g ($P = 0.005$) (Fig. 1*B*). Previous studies have shown that DHETs have proinflammatory effects (11, 25), and therefore increased concentrations of DHETs in colon are in agreement with HFD-induced colonic inflammation. Besides fatty acid diols, LC-MS/MS revealed that the concentrations of other metabolites, notably COX-derived prostaglandin E_2 (PGE_2), prostaglandin D_2 (PGD_2), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), were significantly ($P < 0.05$) increased in colon of HFD-treated mice (Fig. 1 *A* and *B* and *SI Appendix*, Table S2). Consistent with enhanced levels of prostaglandins, qRT-PCR showed that gene expression of *Cox-2* was increased in colon of HFD-treated mice (*SI Appendix*, Fig. S3*B*).

HFD Increases Expression of sEH in Colon. Given our findings that HFD increased concentrations of fatty acid diols in colon, we studied the expression of enzymes involved in fatty acid diol biosynthesis. The biosynthesis of fatty acid diols involves three enzymatic steps: The membrane-incorporated PUFA is released by phospholipase A_2 (PLA_2) to generate intracellular free PUFA, which is metabolized by CYP monooxygenases to generate fatty acid epoxides, followed by hydrolysis by sEH and other epoxide hydrolases to form the fatty acid diols (Fig. 2*A*) (11). GC-MS analysis showed that HFD treatment had no effect on the level of ARA or DHA in colon (*SI Appendix*, Table S3), and this result is in agreement with our previous study which showed that HFD had no impact on ARA levels in adipose tissues of HFD-treated

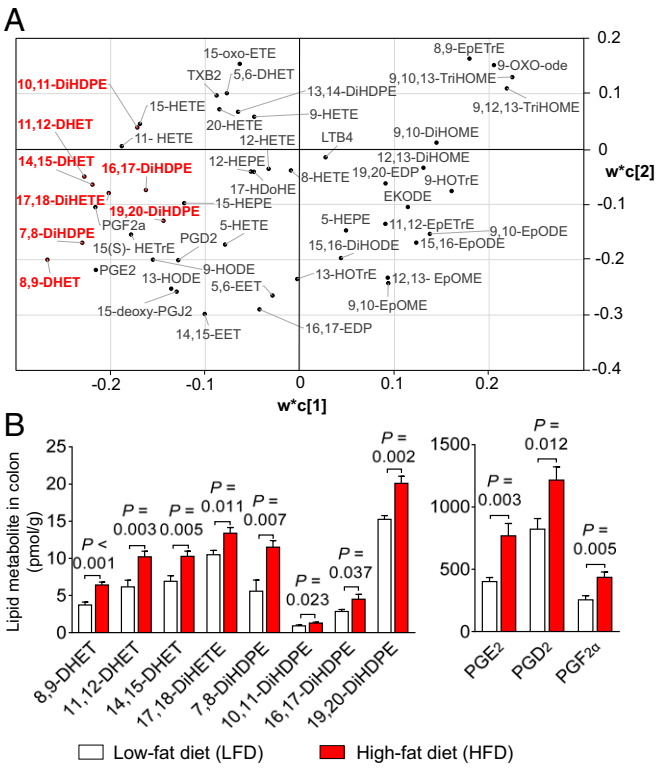

Fig. 1. LC-MS/MS–based lipidomics shows that HFD treatment increases concentrations of sEH-produced fatty acid diols in colon tissues. (*A*) Loading plot analysis shows that sEH-produced fatty acid diols contribute to the differentiation of eicosanoid profiles in LFD and HFD groups. (*B*) Concentrations of sEH-produced fatty acid diols and COX-produced prostaglandins in colon tissues. The results are mean ± SEM; $n = 8$ to 10 mice per group.

Pharmacological Inhibition of sEH Attenuates HFD-Induced Colonic Inflammation. We investigated the roles of sEH in obesity-induced colonic inflammation by testing the effect of pharmacological inhibition of sEH on HFD-induced colonic inflammation. We treated C57BL/6 mice with LFD or HFD as well as the sEH inhibitor TPPU or vehicle for 8 wk. TPPU is a potent sEH inhibitor, with an IC_{50} for human sEH of 3.7 nM and mouse sEH of 2.8 nM (27). TPPU treatment had no effect on HFD-induced body weight increase (*SI Appendix*, Fig. S4*A*). Regarding colonic inflammation, qRT-PCR analysis showed that HFD treatment increased gene expression of the proinflammatory cytokines *Il-1*β and *Tnf*-α and reduced expression of the antiinflammatory cytokine *Il-10* in colon, while such effects were abolished by TPPU treatment (Fig. 3*A*). In addition, flow cytometry analysis showed that HFD treatment increased infiltration of leukocytes, macrophages, and neutrophils into colon, while these effects were also abolished by TPPU treatment (Fig. 3*B* and *SI Appendix*, Fig. S4*B*).

To further study the effects of sEH inhibitors on HFD-induced colonic inflammation, we tested another sEH inhibitor, *t*-TUCB, a potent sEH inhibitor with an IC_{50} for human sEH of 0.9 nM and

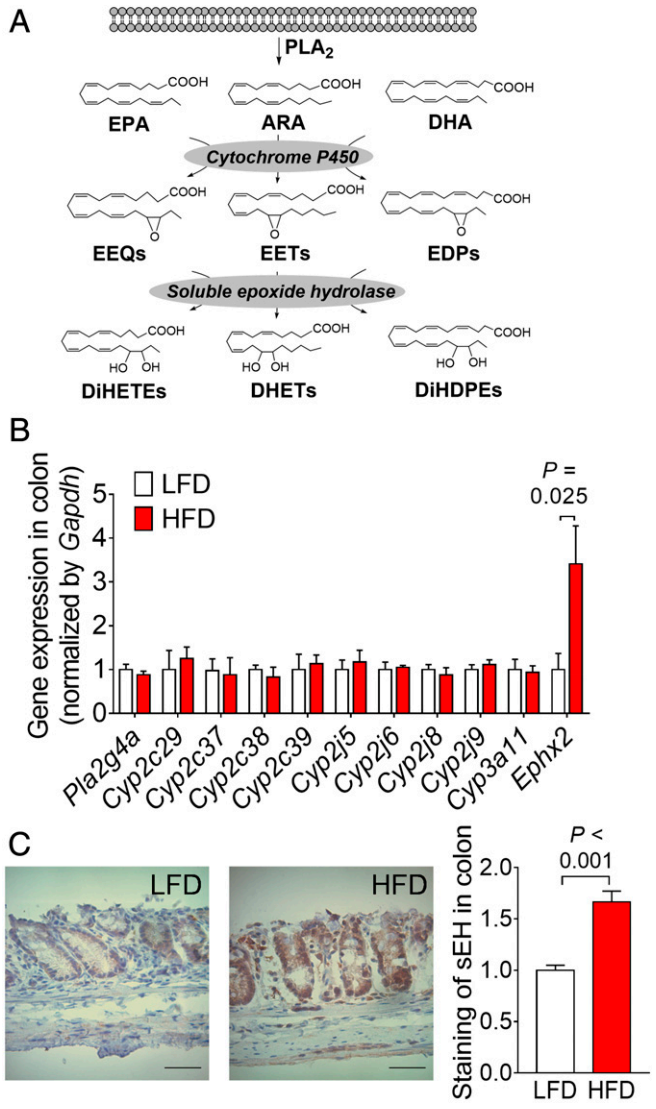

Fig. 2. HFD treatment increases expression of the DHET-producing enzyme sEH in colon tissues. (*A*) Biochemistry for biosynthesis of fatty acid epoxides and diols. (*B*) qRT-PCR analysis of gene expression in colon tissues ($n = 4$ or 5 mice per group for *Pla2g4a*, *Cyp3a11*, *Cyp2c29*, *Cyp2c38*, *Cyp2c37*, *Cyp2c39*, *Cyp2j5*, *Cyp2j6*, *Cyp2j8*, and *Cyp2j9*; $n = 7$ mice per group for *Ephx2*). (*C*) Immunohistochemical staining showing increased sEH in colon tissues from HFD mice (magnification 600×; $n = 5$ for LFD; $n = 8$ for HFD). The results are mean ± SEM. (Scale bars: 50 μm.)

mouse sEH of 1.3 nM (28). Consistent with the above result, treatment with *t*-TUCB had no effect on HFD-induced body weight increase (*SI Appendix*, Fig. S4*C*) but abolished HFD-induced colonic inflammation, as assessed by the levels of cytokines (Fig. 3*C*) and immune cells in colon (Fig. 3*D* and *SI Appendix*, Fig. S4*D*). Together, these results demonstrate that treatment with two different sEH inhibitors abolished HFD-induced colonic inflammation, suggesting that sEH plays a critical role in obesity-induced colonic inflammation.

Genetic Ablation of sEH Attenuates HFD-Induced Colonic Inflammation. To validate the roles of sEH in obesity-induced colonic inflammation, we studied the effect of genetic ablation of sEH on HFD-induced colonic inflammation. To this end, we treated sEH knockout (KO) mice and wild-type (WT) control mice with LFD or HFD for 8 wk. We found that HFD treatment increased colonic inflammation in WT mice, while such an effect was abolished in sEH KO mice. Indeed, in WT mice, HFD treatment increased the expression of the proinflammatory cytokines *Il-1*β and *Tnf*-α and reduced the expression of the antiinflammatory cytokine *Il-10*, while such effects were abolished in sEH KO mice (Fig. 4*A*). These results confirm a critical role of sEH in HFD-induced colonic inflammation.

Genetic Ablation of sEH Attenuates HFD-Induced Elevation of Fatty Acid Diols in Colon. We used LC-MS/MS to analyze eicosanoid profiles in colon of HFD-treated WT and sEH KO mice (*SI Appendix*, Table S2). Compared with HFD-treated WT mice, the colonic concentrations of sEH products (such as 5,6-, 8,9-, 11,12-, and 14,15-DHET) were reduced, while the concentrations of sEH

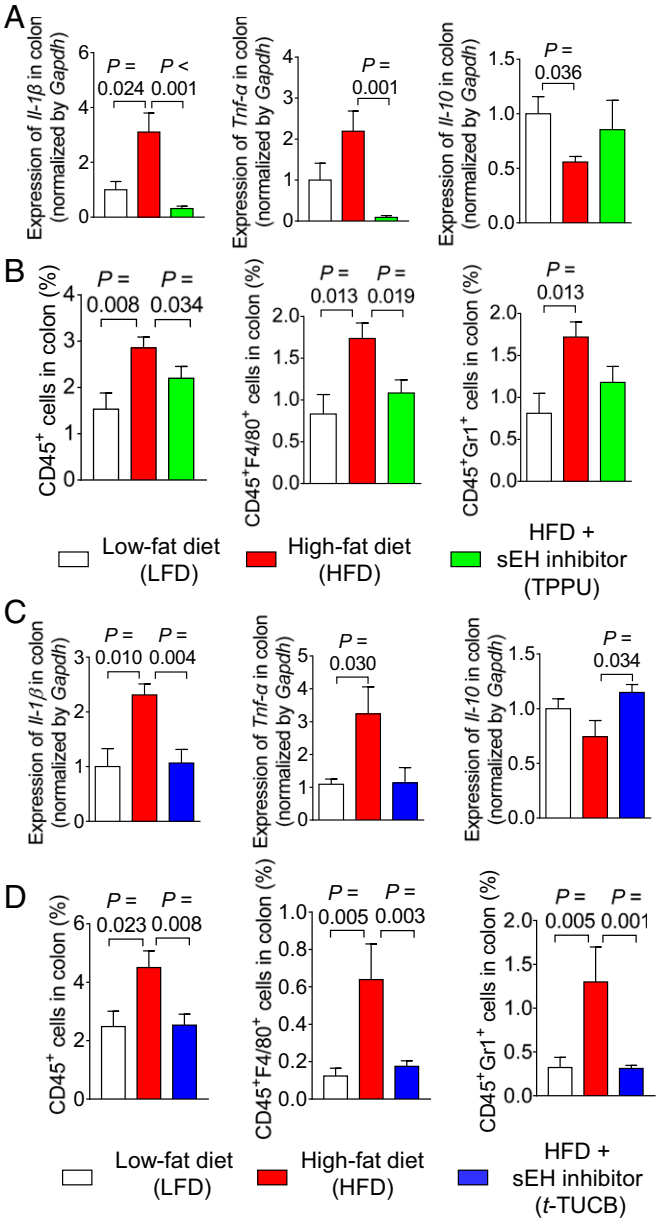

Fig. 3. Pharmacological inhibition of sEH attenuates HFD-induced colonic inflammation. (*A*) qRT-PCR analysis of cytokine expression in colon. (*B*) FACS quantification of immune cells in colon. (*C*) qRT-PCR analysis of cytokine expression in colon. (*D*) FACS quantification of immune cells in colon. The results are mean ± SEM; $n = 8$ to 12 mice per group.

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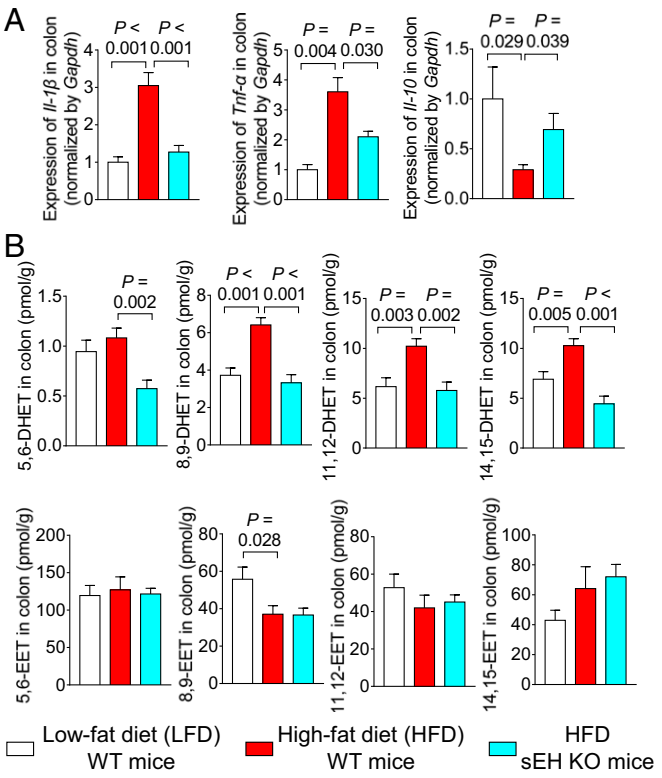

Fig. 4. Genetic ablation of sEH attenuates HFD-induced colonic inflammation. (*A*) qRT-PCR analysis of gene expression of *Il-1β*, *Tnf-α*, and *Il-10* in colon. (*B*) LC-MS/MS analysis of DHETs and EETs in colon. The results are mean ± SEM; $n = 8$ to 10 mice per group.

substrates [such as 5,6-, 8,9-, 11,12-, and 14,15-epoxyeicosatrienoic acid (EET)] were not changed, in HFD-treated sEH KO mice (Fig. 4*B*). This result supports that the effect of sEH inhibition is through reducing its metabolites (fatty acid diols) in colon.

Pharmacological Inhibition or Genetic Ablation of sEH Attenuates HFD-Induced Activation of Wnt Signaling in Colon. Wnt signaling plays an important role in colorectal tumorigenesis: About 90% of sporadic colorectal cancers have activating mutations within the Wnt pathway (29). Previous research showed that HFD treatment increased the activation of Wnt signaling in colon (5), supporting a potential role of Wnt signaling in obesity-enhanced colorectal tumorigenesis. We investigated the role of sEH in HFD-induced activation of Wnt signaling. Glycogen synthase kinase 3β (GSK3β) is a key component within the Wnt signaling cascade; phosphorylation of GSK3β activates the Wnt signaling, leading to increased expression of downstream genes such as *Axin2* (29). Western blot and immunohistochemistry showed that HFD treatment increased expression of phosphorylated GSK3β in colon tissues, while such an effect was attenuated in sEH KO mice, or by treatment with sEH inhibitors (Fig. 5 *A* and *B*). Consistent with this result, HFD treatment increased gene expression of *Axin2*, and such an effect was attenuated by genetic ablation or pharmacological inhibition of sEH (Fig. 5*C*). Together, these results showed that pharmacological inhibition and genetic ablation of sEH attenuated HFD-induced activation of Wnt signaling in colon.

Discussion

Obesity is associated with enhanced colonic inflammation (3–5), which is a major risk factor for developing colorectal cancer (6). It is important to identify novel therapeutic targets of obesity-enhanced colonic inflammation, to develop targeted strategies for prevention. The central finding of our research is that sEH could be a promising target for obesity-induced colonic inflammation. We find that obesity-induced colonic inflammation is associated with increased expression of sEH and its eicosanoid metabolites, termed fatty acid diols, in colon tissues. Furthermore, we find that pharmacological inhibition or genetic ablation of sEH abolishes obesity-induced colonic inflammation and activation of Wnt signaling in mice. These results demonstrate that sEH plays a critical role in obesity-induced colonic inflammation.

We found that HFD treatment increased expression of sEH and concentrations of sEH-produced eicosanoid metabolites in colon tissues. Our results are largely in agreement with previous studies. Bettaieb et al. (17) showed that after 5 to 10 months of dietary feeding of HFD, there is a significant increase of sEH expression in liver and adipose tissues of treated mice. In addition, it was discovered that saturated fatty acids, which were enriched in the HFD used in our study, could directly increase sEH expression in HepG2 cells. Liu et al. (21) showed that a 16-wk dietary feeding of HFD increased expression of sEH in liver. It should be noted that there are inconsistent results, which

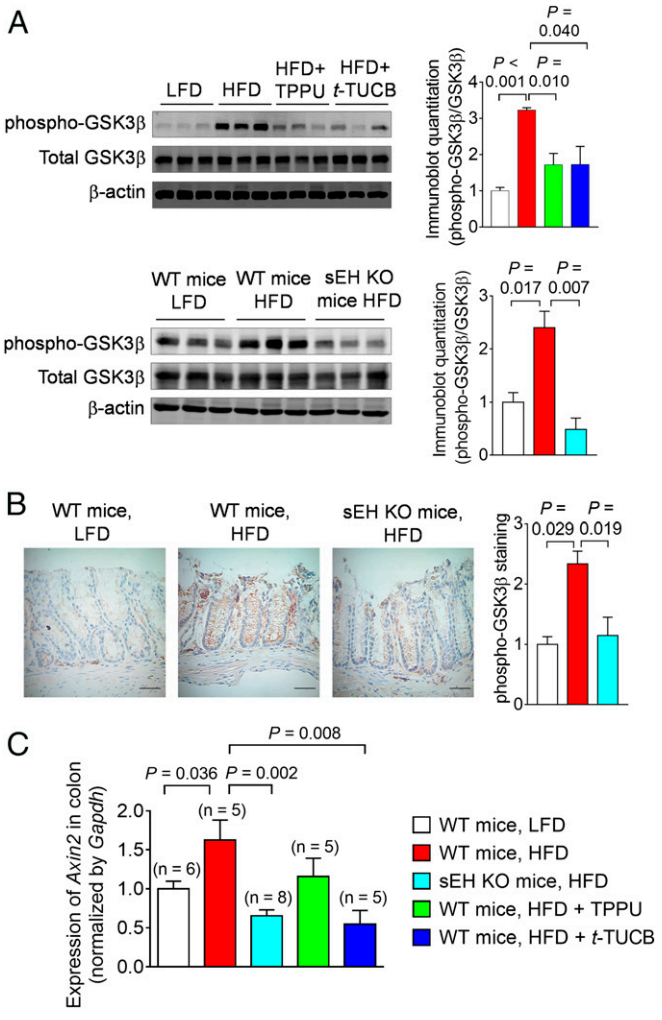

Fig. 5. Pharmacological inhibition or genetic ablation of sEH attenuates HFD-induced activation of Wnt signaling in colon. (*A*) Immunoblotting analysis of phosphorylated and total GSK3β in colon ($n = 3$ per group). (*B*) Immunohistochemical staining of phosphorylated GSK3β in colon ($n = 4$ per group). (*C*) qRT-PCR analysis of *Axin2* expression in colon. The results are mean ± SEM. (Scale bars: 50 μm.)

showed that HFD treatment did not increase sEH expression, but increased total sEH activity, in the fat pads of treated mice (30). sEH is the major enzyme to convert the antiinflammatory eicosanoids EETs to DHETs that are usually biologically inactive or proinflammatory (11, 25). Therefore, enhanced expression of sEH in tissues could lead to an increased inflammatory state, which is consistent with obesity-induced inflammation in tissues, and evaluation of recent literature suggests the sEH enzyme itself can be considered an inflammatory marker (11).

We further found that pharmacological inhibition or genetic ablation of sEH abolished HFD-induced colonic inflammation in mice, demonstrating a critical role of sEH in obesity-induced colonic inflammation. These results support that obesity increases colonic inflammation in part through up-regulating sEH in colon tissues, and inhibition of sEH could be a novel strategy to attenuate obesity-induced colonic inflammation and associated diseases. This finding is in agreement with previous studies of the beneficial effect of sEH inhibition on obesity and inflammation. Previous studies showed that pharmacological inhibition or genetic ablation of sEH suppressed various adverse consequences of obesity, including endoplasmic reticulum stress, metabolic syndrome, fatty liver, hepatic steatosis, inflammation, and endothelial dysfunction (14, 17–24). In addition, inhibition of sEH has been shown to suppress inflammatory responses in other disease states (11). In our studies, we found that compared with HFD-treated WT mice, the colonic concentrations of several sEH products [such as 9,10- and 12,13-dihydroxyoctadecenoic acid (DiHOME), 15,16-dihydroxyoctadecadienoic acid (DiHODE), 5,6-, 8,9-, 11,12-, and 14,15-DHET, and 10,11-DiHDPE] were significantly reduced, while the concentrations of many sEH substrates (such as 5,6-, 8,9-, 11,12-, and 14,15-EET) were not changed, in HFD-treated sEH KO mice. These results support the notion that the effect of sEH is mediated by its products. The concentrations of some sEH products, such as 16,17- and 19,20-DiHDPE, were not significantly changed in colon of HFD-treated sEH KO mice compared with HFD-treated WT mice. This could be because sEH has varied activity to hydrolyze different fatty acid epoxides to form fatty acid diols (31), or other types of EHs such as microsomal epoxide hydrolase (mEH) could also be involved in the biosynthesis of fatty acid diols (32). Previous research showed that mEH expression was found in 46% of normal human colon tissues (33). Together, these results support that inhibition of sEH could be a promising strategy for prevention and/or treatment of obesity-caused human disorders.

Colonic inflammation is a major risk factor for colorectal cancer (6). Individuals with obesity have a 30 to 60% greater risk of developing colorectal cancer (7). A recent Colorectal Adenoma/Carcinoma Prevention Program 2 (CAPP2) study showed obesity is associated with substantially increased risk of colorectal cancer in patients with Lynch syndrome but that this enhanced risk is abrogated in those taking aspirin (34), suggesting that targeting inflammation is a promising strategy to reduce the risks of obesity-enhanced colorectal cancer. Previous studies support that inhibition of sEH has beneficial effects on colonic inflammation and associated colorectal cancer (35–37). Compared with WT mice, dextran sodium sulfate (DSS)-induced colonic inflammation is reduced in $sEH^{-/-}$ mice (35). In an interleukin 10 (IL-10) deficiency-induced colon cancer model, $sEH^{-/-}$ $IL\text{-}10^{-/-}$ mice have reduced colon carcinoma compared with $IL\text{-}10^{-/-}$ mice (36, 37). In this study, we also found that pharmacological inhibition or genetic ablation of sEH attenuated HFD-induced activation of protumorigenic Wnt signaling in colon. These results support that sEH might be a potential therapeutic target for obesity-enhanced colorectal tumorigenesis.

Besides sEH, we also found that the expression of COX-2 and its metabolites, notably PGE_2, was increased in colon of HFD-treated mice. Considering the critical importance of COX-2/PGE_2 in colonic inflammation and colon tumorigenesis, it is likely that the COX-2 pathway could also contribute to obesity-induced colonic inflammation and associated diseases. Dual inhibition of COX-2 and sEH might be a promising strategy to suppress obesity-induced colonic disorders. Our previous studies showed that coadministration of sEH inhibitors and COX-2 inhibitors synergistically suppressed inflammation, pain, primary tumor growth, and tumor metastasis, with reduced COX-2 inhibition-induced cardiovascular toxicities (38, 39). In addition, we have designed first-in-class COX-2/sEH dual inhibitors, and showed they have potent anticancer and antimetastatic effects (39). It would be important to test whether targeting both COX-2 and sEH could generate more effective suppression of obesity-induced colonic diseases.

In summary, here our studies support that pharmacological inhibition of sEH could be a novel strategy to suppress obesity-induced colonic inflammation. A pharmacological inhibitor of sEH has been in phase II human clinical trials targeting hypertension (12). Currently, GlaxoSmithKline is conducting human clinical trials to test the effect of an sEH inhibitor, GSK2256294, on chronic obstructive pulmonary disease, and the double-blind placebo-controlled clinical trial showed that this compound is well-tolerated and causes sustained inhibition of sEH activity in human (40). In addition, other novel classes of sEH inhibitors are being considered for human trials (41). These resources could help in the translation of sEH inhibitors for the prevention of obesity-induced inflammation and associated diseases.

Materials and Methods

Details of the experimental protocols are given in *SI Appendix, Materials and Methods*.

Animal Experiments. The animal experiments were conducted in accordance with the protocols approved by the Institutional Animal Care and Use Committees of UMass Amherst and UC Davis. C57BL/6 male mice were purchased from Charles River and maintained in a standard animal facility. The experimental diets, including the high-fat diet (60 kcal% fat; D12492) and low-fat diet (10 kcal% fat; D12450J), were purchased from Research Diet.

Animal protocol 1: HFD on colonic inflammation. C57BL/6 male mice (6 wk old) were randomly assigned to two equal groups ($n = 11$ or 12). One group was maintained on the LFD diet, and the other group was maintained on the HFD diet. After 8 wk, the mice were killed to dissect the colon tissues for analysis.

Animal protocol 2: Effect of pharmacological inhibition of sEH on HFD-induced colonic inflammation. C57BL/6 male mice (6 wk old) were randomly assigned to three groups: The first group ($n = 8$) was maintained on the LFD diet, with drinking water containing 0.2% (vol/vol) polyethylene glycol 400 (PEG 400) as vehicle; the second group ($n = 8$) was maintained on the HFD diet, with drinking water containing 0.2% PEG 400; and the third group ($n = 12$) was maintained on the HFD diet, with drinking water containing 10 mg/L *N*-[1-(1-oxopropyl)-4-piperidinyl]-*N*′-[4-(trifluoromethoxy)phenyl]-urea (TPPU) or *trans*-4-{4-[3-(4-trifluoromethoxyphenyl)ureido]cyclohexyloxy}benzoic acid (*t*-TUCB), and 0.2% PEG 400. It is estimated the dose of sEH inhibitors is ~1 $mg{\cdot}kg^{-1}{\cdot}d^{-1}$, with an average water intake of 3 mL/d. After 8 wk, the mice were killed for analysis.

Animal protocol 3: Effect of genetic ablation of sEH on HFD-induced colonic inflammation. C57BL/6 WT male mice and sEH KO male mice (age 12 to 13 wk) were maintained on the LFD or HFD for 8 wk, and then killed for analysis.

Data Analysis. All data are expressed as the mean ± SEM. For the comparison between treatment groups, the Shapiro–Wilk test was used to verify the normality of data. When data were normally distributed, statistical significance was determined using the two-sided *t* test; otherwise, significance was determined by the Mann–Whitney *U* test. Partial linear square discriminant analysis was implemented using MetaboAnalyst (www.metaboanalyst.ca/). The data were scaled using autoscaling before the analysis. *P* values less than 0.05 are reported as statistically significant.

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