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Targeting the Gut Microbiota–FXR Signaling Axis for Glycemic Control: Does a Dietary Supplement Work Magic?

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Glucose-dependent organs such as the brain are sustained through periods of fasting by glucose production in the liver, called hepatic gluconeogenesis. Normally, rising blood glucose levels homeostatically suppress gluconeogenesis. In obesity-related type 2 diabetes, however, insulin resistance and elevated glucagon inhibit the suppression of gluconeogenesis, contributing to hyperglycemia. Therapeutic intervention to suppress hepatic gluconeogenesis is therefore clinically important for controlling glucose in type 2 diabetes.

Recent studies have suggested that the bile acid−farnesoid X receptor (FXR) signaling axis is a potential therapeutic target for metabolic disorders, including hyperglycemia caused by elevated hepatic gluconeogenesis (1,2). Bile acids are synthesized in the liver from cholesterol as conjugates of taurine (in mice) or glycine (in humans) and delivered to the gut to facilitate solubilization of dietary lipids and vitamins. Bile acids and their metabolites also function as endogenous ligands of FXR, a ligand-activated transcription factor highly expressed in the liver and the intestine. Bile acid−FXR signaling provides negative feedback control of bile acid production and transport to maintain bile acid homeostasis (3). Moreover, the bile acid−FXR signaling axis has been shown to regulate fat and glucose metabolism (4–7). Comparative analysis of global, liver-specific, and intestine-specific FXR knockout mice revealed a complex signaling network, wherein activation of the liver FXR and intestinal FXR result in distinct metabolic outcomes in diet-induced or genetic obesity models (6,8–12). In particular, several recent studies indicate that selective inhibition of intestinal FXR improves metabolic phenotypes in obese animals (9–11). Although mechanisms underlying the tissue-specific FXR metabolic effects remain to be unraveled, these studies suggest that tissue-specific manipulations of FXR signaling may be exploited to combat obesity-related metabolic syndrome and type 2 diabetes (1,8,13).

The gut microbiota serves as a metabolic "organ" that actively participates in host metabolism (14), in part by regulating bile acid metabolism and FXR signaling (15,16). Critically, primary bile acids are liberated from their conjugated amino acids by microbe-derived bile salt hydrolase (BSH), a necessary step prior to further modification by additional microbial enzymes that generate diverse bile acid metabolites and influence host physiology. Profiling of bile acid metabolites in germ-free and conventionally raised mice revealed tauro-β-muricholic acid (T-β-MCA) as a major bile acid that is elevated in germ-free mice and also showed that T-β-MCA is normally metabolized to β-MCA through the action of BSH produced by the gut microbiota (15). T-β-MCA is a strong FXR antagonist. Thus, conversion of T-β-MCA to β-MCA by gut microbiota relieves FXR inhibition and favors FXR agonism (15). The effect of gut microbiota on FXR signaling appears to be restricted to the intestine, leaving hepatic FXR signaling relatively unchanged (11,15). Interestingly, in germ-free mice fed a high-fat diet (HFD), FXR antagonism due to elevated T-β-MCA rendered the mice less prone to obesity and metabolic disorders (16). These studies point to BSH and microbiota as therapeutic targets to help contain metabolic disorders through intestine-specific FXR inhibition.

In this issue of *Diabetes*, Xie et al. (17) took advantage of a recent chemical screening study showing that the dietary supplement caffeic acid phenethyl ester (CAPE) inhibits bacterial BSH (18). Using CAPE to elevate levels of intestinal T-β-MCA for intestinal FXR inhibition, they found that CAPE reduced hepatic gluconeogenesis and alleviated hyperglycemia in mice fed an HFD. In contrast, CAPE had no effect on intestine-specific *Fxr* knockout

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See accompanying article, p. 613.

($Fxr^{\Delta IE}$) mice, which already exhibited resistance to HFD-induced hyperglycemia. Extensive metabolic and lipid profiling revealed that inhibition of intestinal FXR correlated with reduced intestinal ceramide production and reduced serum ceramide levels, which resulted in reduced pyruvate carboxylase (PC) activity and increased citrate synthase (CS) activity in hepatocytes. Increased CS (which converts mitochondrial acetyl-CoA to citrate for the tricarboxylic acid cycle) and decreased PC (which converts pyruvate to oxaloacetate and is the first enzyme in gluconeogenesis) provide likely mechanisms by which CAPE was able to reduce hepatic gluconeogenesis. Finally, surveying mRNAs of genes involved in endoplasmic reticulum (ER) stress, ER-mitochondria coupling, and calcium transport led to the conclusion that elevated ceramides in obese animals induce ER stress and increase ER-mitochondria tethering and calcium transport, which together increase gluconeogenesis, whereas intestinal FXR antagonism counters these effects by reducing ceramide levels (Fig. 1).

Findings presented in the article by Xie et al. (17) extend previous studies from this group documenting the effects of intestine-specific FXR inhibition on lipid and glucose metabolism through downregulation of ceramide production in the intestine by genetic deletions as well as compounds such as tempol or glycine-β-muricholic acid that cause intestine-selective FXR antagonism (9,10,19). Identification of CS and PC as two enzymes whose activities are significantly altered by intestinal FXR inhibition offers a molecular explanation for the previously reported effects on hepatic gluconeogenesis. Of note, changes in CS and PC activities were shown to be independent of body weight or hepatic insulin signaling and were readily reversed by direct ceramide administration in $Fxr^{\Delta IE}$ mice, although whether this is also the case in CAPE-treated wild-type mice was not addressed.

The study raises the interesting possibility that properly regimented administration of an over-the-counter dietary supplement would help control type 2 diabetes–associated hyperglycemia. However, several questions need to be addressed before clinical translation. Not least is the contradictory finding from a different study showing that biased activation of the intestinal FXR using a gut-restricted FXR agonist, fexaramine, improves rather than aggravates metabolic profiles in obese mouse models (8). Comprehensive analysis to determine in detail the extent of FXR signaling in different tissues, as well as how these signals integrate under the various FXR manipulation conditions in these studies, is needed to fully resolve the discrepancies. Beyond these issues remains the question of mouse versus man. Humans differ from mice in their bile acid composition, gut microbiota, and FXR signaling. It is far from certain whether results from studies conducted in mice to evaluate the effects of FXR signaling on metabolic diseases, including the current study, will immediately apply to humans. Finally, CAPE, a potent anti-inflammatory and antioxidant, has a wide range of reported bioactivities, including neuroprotective, hepatoprotective, and cardioprotective effects (20); it also has antibacterial effects that could

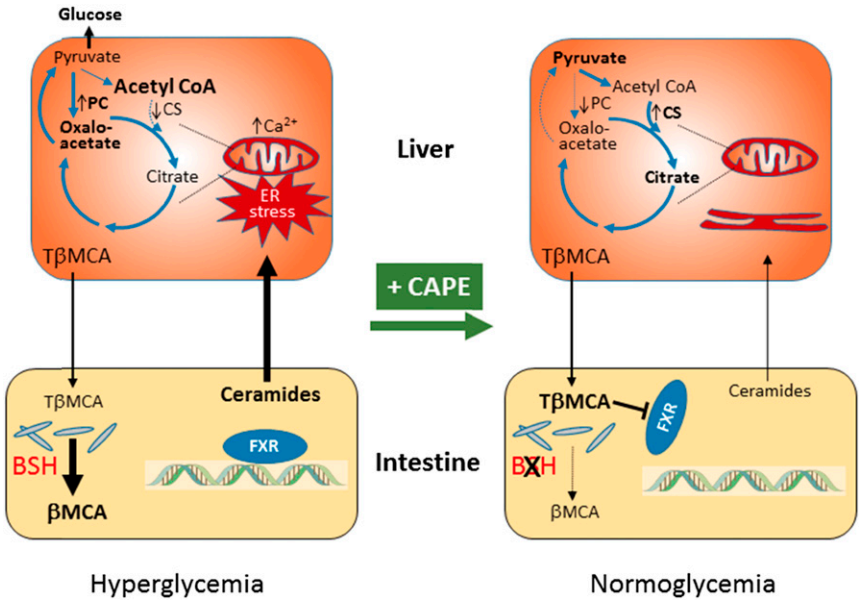

Figure 1—CAPE reverses hyperglycemia in mice fed an HFD by inhibiting BSH from the gut microbiota. In HFD-fed animals, conversion of T-β-MCA to β-MCA by microbial BSH favors intestinal FXR signaling to increase ceramide production, which causes ER stress in hepatocytes and changes in enzymes and metabolites involved in gluconeogenesis. In particular, reduced CS activity (↓CS), accompanied by elevated acetyl-CoA levels and increased PC activity (↑PC), promotes gluconeogenesis. By inhibiting BSH, CAPE leads to accumulation of T-β-MCA and inhibition of intestinal FXR to reduce ceramide synthesis, thereby relieving ER stress, increasing CS activity (↑CS), and reducing PC activity (↓PC) to suppress gluconeogenesis and normalize blood glucose.

modify gut microbiome composition. Further study will be needed before a firm conclusion can be made regarding the mechanism by which CAPE reduces hepatic gluconeogenesis.

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