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Review article

Curcumin and dietary polyphenol research: beyond drug discovery

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Abstract

Numerous natural products available over the counter are commonly consumed by healthy, sub-healthy or ill people for the treatment and prevention of various chronic diseases. Among them, a few dietary polyphenols, including the curry compound curcumin, have been attracting the most attention from biomedical researchers and drug developers. Unlike many so-called "good drug candidates", curcumin and several other dietary polyphenols do not have a single known therapeutic target or defined receptor. In addition, the bioavailability of these polyphenols is usually very low due to their poor absorption in the gut. These recently debated features have created enormous difficulties for drug developers. In this review, I do not discuss how to develop curcumin, other dietary polyphenols or their derivatives into pharmaceutical agents. Instead, I comment on how curcumin and dietary polyphenol research has enriched our knowledge of insulin signaling, including the presentation of my perspectives on how these studies will add to our understanding of the famous hepatic insulin function paradox.

Keywords: curcumin; dietary polyphenols; insulin resistance; type 2 diabetes; dietary intervention; ChREBP; Fgf21; hepatic insulin function paradox

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Hepatic function of insulin and the paradox of hepatic insulin resistance

Insulin resistance is the common feature and the therapeutic target of type 2 diabetes (T2D) and its devastating vascular complications, as well as many other metabolic disorders. The liver is among the major organs that convey the function of insulin in maintaining metabolic homeostasis. Postprandial glucose elevation leads to the elevation of plasma insulin levels. Insulin exerts its glucose-lowering effect via a number of means, including the facilitation of glucose transport, the inhibition of hepatic gluconeogenesis and the stimulation of lipogenesis. As illustrated in Figure 1, in the liver, insulin exerts its regulatory effects via phosphoinositide 3-kinase (PI3K) mediated Akt (also known as protein kinase B, PKB) activation. Akt inactivates FoxO1, leading to the repression of the gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G6pase). Akt can also inactivate tuberous sclerosis proteins 1 and 2 (TSC1/2) and hence release their repression of mTOR signaling, resulting in the activation of sterol regulatory element-binding protein 1c (SREBP-1c) and increased lipogenesis. SREBP-1c and carbohydrate-responsive element-binding protein (ChREBP) are the two well-defined lipogenic transcription factors[1, 2].

Paradoxically, subjects with insulin resistance show elevated hepatic gluconeogenesis, as well as lipogenesis, in contrast to the physiological scenario that insulin should exert completely opposite effects on lipogenesis and gluconeogenesis (Figure 1). This is known as the paradox of hepatic insulin function[3-5]. The exploration of mechanisms underlying this paradox for the past two decades has been advancing our knowledge of the pathogenesis of metabolic diseases in general and further investigation will have a great impact on refining the overall strategies in the prevention and treatment of T2D and other insulin resistance-related disorders. For example, one may wonder whether insulin treatment or the utilization of insulin signaling sensitization therapeutic agents will, in turn, exacerbate hepatic lipogenesis, resulting in non-alcoholic fatty liver diseases (NAFLD). Evidently, elevated *de novo* lipogenesis (DNL) was shown in both humans with IR and in the insulin resistance mouse model[6-9].

It has been postulated by Brown and Goldstein that during

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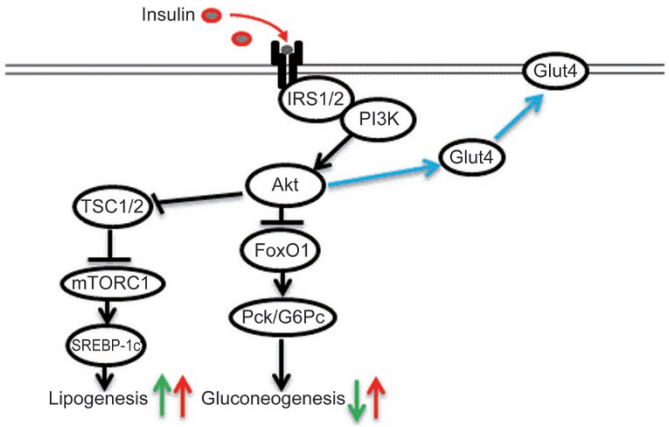

Figure 1. A simplified illustration of hepatic insulin function and the paradox of hepatic insulin function. Insulin exerts its metabolic effects on glucose and lipid homeostasis via the common key molecule Akt. Postprandial insulin elevation leads to Akt activation (via IRS1/2 and PI3K). Insulin/Akt signaling inhibits the gluconeogenic enzymes (PEPCK and G6Pase as examples. They are encoded by the *Pck* and *G6pc* genes, respectively) and hence reduce gluconeogenesis. Akt can also activate mTORC1 and its target SREBP-1c (via TSC1/2 inactivation) and hence increase lipogenesis. Thus, physiologically, hepatic insulin signaling activation leads to increased lipogenesis and reduced gluconeogenesis (indicated by green arrows). Paradoxically, during insulin resistance, both gluconeogenesis and lipogenesis are elevated (indicated by red arrows). The stimulatory effect of Akt on Glut4 membrane translocation is also illustrated.

insulin resistance, insulin loses its capability to repress gluconeogenesis but continues to stimulate lipogenesis, resulting in hyperglycemia and hyperlipidemia, as well as hepatic steatosis[3, 4]. To test this "selective insulin resistant hypothesis", great efforts have been made to identify the branch point in insulin signaling where hepatic glucose and lipid metabolism diverge. Li *et al* reported that in rat hepatocytes, insulin treatment increased the expression of the gene that encodes the lipogenic transcription factor SREBP1c and repressed the expression of the gene that encodes the gluconeogenic transcription factor PEPCK. Both of these effects require intact PI3K and Akt signaling pathways[4]. Sub-nanomolar concentrations of the mTORC1 inhibitor rapamycin, however, blocked the effect of insulin in stimulating SREBP1c but not the effect of insulin in repressing PEPCK. This, along with the lack of blockage with S6 kinase inhibition, allowed the authors to suggest that mTORC1 is an essential component in the insulin-regulated pathway for hepatic lipogenesis but not for gluconeogenesis[4]. Shimomura *et al* found that chronic hyperinsulinemia led to reduced expression of the mRNA that encodes insulin receptor substrate 2 (IRS-2) and the development of insulin resistance. Furthermore, IRS-2 deficiency did not prevent stimulation by insulin of SREBP-1c production[10]. The differential effects of IRS-1 and IRS-2 on glucose and lipid metabolism, respectively, were also demonstrated with an siRNA-based gene silencing approach in human skeletal muscles[11]. Other investigations have led to the suggestion that insulin/mTORC regulated DNL can be independent of FoxO1 regulation[12] and that Akt2 phosphorylation is required for some but not all Akt activities[13].

Very recently, Vatner *et al* have reported that hepatic triglyceride synthesis can be stimulated by the substrate, independent of changes in hepatic insulin signaling[5]. The experiments were performed in normal or high fat diet (HFD) fed rats or in insulin receptor 2′-O-methoxyethyl chimeric antisense oligonucleotide-treated rats by infusing radioisotope labeled palmitate, followed by measuring the rate of fatty acid esterification into hepatic triglycerides. They found that the rate was dependent on plasma fatty acid infusion rates but appeared independent of changes in plasma insulin concentration or hepatocellular insulin signaling[5]. Taken together, these results obviate a paradox of selective insulin resistance, emphasizing the contribution of the lipogenic substrates.

Although these investigations have been advancing our understanding of hepatic functions of insulin in health and diseases, these investigations have not conceptually considered the existence of native compounds in our diet that can improve insulin signaling and prevent both hyperglycemia and dyslipidemia.

Dietary polyphenol and dietary polyphenol intervention

Nutrient sensing is known to affect insulin action in the liver and elsewhere, while dietary polyphenol intervention can effectively improve insulin signaling[14, 15]. Curcumin, anthocyanin, and resveratrol are perhaps the three most heavily studied dietary polyphenols[16]. They are available over the counter in both western societies and Asian countries and are commonly utilized by healthy, sub-healthy or ill people for the treatment and prevention of various chronic diseases.

Anthocyanins (also known as anthocyans) are water-soluble vacuolar pigments of plants. Depending on the pH, anthocyanins may appear red, blue, or purple. Dietary plants that are rich in anthocyanins include blueberries, raspberries, black rice, and black soybeans. Resveratrol is a stilbenoid, a hydroxylated derivative of stilbenes. Resveratrol can be found in the skin of grapes, blueberries and raspberries.

The lipophilic polyphenol curcumin is the principal curcuminoid of turmeric. As a traditional medicine, turmeric has been utilized in numerous Asian countries for over 3000 years in the treatment of various diseases, including hepatitis, arthritis, a variety of skin disorders, rashes, and burn injuries[16]. In today's modern society, dietary supplements with turmeric rhizome or its extracts have been employed in the treatment of arthritis.

Curcumin, constituting approximately 2%–5% of turmeric, is the most studied component of turmeric. Intensive experimental studies have shown that it possesses antimicrobial, insecticidal, larvicidal, antimutagenic, cardioprotective, radioprotective, and anticancer activities. A number of investigations have also demonstrated that curcumin can improve insulin signaling[13, 17-20]. Importantly, a recent clinical trial demonstrated that curcumin intervention in pre-diabetic human subjects lowered the number of individuals who eventually progressed to T2D[18]. Numerous *in vitro* investigations

have shown that curcumin can regulate various signaling cascades in different cell lineages, while most previous *in vivo* studies have attributed the insulin signaling sensitizing and other beneficial effects of curcumin to their anti-inflammatory and anti-oxidation actions[17, 20-26]. For example, Pan *et al* investigated the protective effects of the curcumin derivative C66 on diabetic cardiomyopathy. In the H9c2 cell line (a cardiomyocyte model) and in rat neonatal cardiomyocytes, C66 pretreatment reduced high glucose-induced overexpression of the inflammatory cytokines, possibly via the inactivation of nuclear factor-κB (NF-κB) and the inhibition of Jun NH_2-terminal kinase (JNK) phosphorylation. In mice with type 1 diabetes, C66 administration decreased the levels of plasma and cardiac tumor necrosis factor-α (TNFα), which is associated with a decreased risk of cardiac cell death[22]. Weisberg *et al* assessed the effect of native curcumin intervention in HFD fed C57BL/6J mice and the leptin-deficient (*ob/ob*) obesity mouse model. In addition to improvements in glucose disposal and insulin tolerance, they demonstrated an effect of curcumin intervention in reducing macrophage infiltration in white adipose tissue, which is associated with decreased expression of hepatic nuclear factor-kappaB (NF-κB) activity and various markers of hepatic inflammation. They hence suggested that oral curcumin ingestion reversed many of the inflammatory and metabolic derangements associated with obesity and improved glycemic control in mouse models of T2D [17].

My team has also evaluated the long-term effect of curcumin intervention in HFD-fed C57BL/6J mice. We showed that concomitant curcumin interventions attenuated the effect of HFD on glucose disposal, body weight and fat gain, as well as the development of insulin resistance. Furthermore, curcumin inhibited hepatic lipogenic gene expression and blocked the effects of HFD on macrophage infiltration and the inflammatory pathways in white adipose tissue[20]. In a separate investigation, we fed C57BL/6J mice with HFD for 16 weeks to induce obesity and insulin resistance. The mice were then fed curcumin (via gavage) for 15 days. We found that this short-term curcumin treatment effectively ameliorated muscular oxidative stress by activation of the anti-oxidative Nrf2 signaling cascade[27]. A recent study by Hao *et al* demonstrated that in the mouse pancreatic MIN6 β-cell line, curcumin attenuated palmitate-induced cell death, possibly via the activation of cell survival Akt signaling and the inhibition of nuclear translocation of FoxO1[26].

Insulin signaling sensitization effect of curcumin can be independent of its anti-inflammation and body weight lowering effects

In addition to its anti-inflammatory and anti-oxidative properties, curcumin has been shown to target various other signaling cascades in different cell lineages that are involved in cell proliferation, cell apoptosis, cell adhesion, and other cellular events. These properties have been heavily assessed for exploring its potential utilization in cancer treatment[28, 29]. In addition, a few recent studies have reported the potential effect of curcumin in promoting white adipocyte "browning"[30, 31]. Furthermore, curcumin has been shown to repress hepatic gluconeogenic gene expression via AMPK activation[32] and to activate the canonical Wnt signaling cascade in white adipose tissue to repress adipogenesis[33-35]. These effects, in the long run, may generate indirect effects on insulin sensitivity.

In 2015, my team proved the existence of the anti-inflammation and anti-oxidation effects of curcumin, independent of insulin sensitizing[36]. For this purpose, we have adopted a dexamethasone-induced insulin resistance mouse model[37]. Briefly, C57BL/6J mice fed a regular diet were administered daily intraperitoneal dexamethasone injections with or without daily curcumin gavage for 5 days. After a one-day rest, intraperitoneal insulin tolerance tests (IPITT) were performed on day 7, followed by an additional curcumin gavage on day 8. Mice were then sacrificed for serum and liver tissue collection on day 10. We defined this protocol as the "6-day curcumin intervention". Dexamethasone injection induced insulin resistance while concomitant curcumin gavage improved insulin tolerance, indicating that insulin resistance attenuating effect of curcumin can be dissociated from its anti-inflammatory effects, and it may not necessarily be secondary to its bodyweight lowering effects[36].

Dietary polyphenols regulate hepatic Fgf21 expression and curcumin intervention attenuates high-fat-diet-induced Fgf21 resistance

Fibroblast growth factor 21 (Fgf21) is a newly recognized hepatic hormone[38-41]. It is mainly produced and released by hepatocytes in response to fasting, although its expression has also been demonstrated in white adipose tissues and in pancreatic islets[38, 42, 43]. In both rodents and humans, *Fgf21/FGF21* gene transcription is positively regulated by the nuclear receptor peroxisome proliferator-activated receptor α (PPARα), which is also the defined pharmacological target of the hypercholesterolemia drug fibrates[44]. During the adaptive starvation response period, the PPARα/Fgf21/PGC-1α axis can facilitate fatty acid oxidation, tri-carboxylic acid cycle flux and gluconeogenesis[45]. The insulin-sensitizing effect of Fgf21 has been well documented, and this hormone and its homologs have been intensively studied in pre-clinical studies and in clinical trials[38, 46-54].

Fgf21 expression and its circulation level in rodents can be robustly stimulated by a ketogenic diet[39], while in human subjects, a ketogenic diet does not induce an appreciable elevation of circulating FGF21 levels[55]. Recently, a study showed that fructose ingestion acutely stimulated circulating FGF21 levels in human subjects [56]. A few studies, including one conducted by our team, suggested a regulatory effect of dietary polyphenols, including resveratrol and curcumin, on hepatic Fgf21 expression[36, 57, 58]. We have also observed the stimulatory effect of anthocyanin on Fgf21 expression (unpublished). Recently, a study demonstrated that white pitaya (*Hylocereus undatus*) juice attenuated insulin resistance and hepatic steatosis in HFD-induced obese mice and this effect was associated with increased expression levels of Fgf21-related genes[59]. White pitaya juice is rich in various polyphenols, flavonoids,

and vitamin C. Furthermore, dietary betaine supplementation has also been shown to increase plasma Fgf21 levels and is associated with improved glucose disposal and reduced hepatic steatosis in HFD-fed mice[60].

Although Fgf21 was shown to exert a beneficial metabolic effect, in murine and human subjects with obesity or IR, serum Fgf21 levels were found to be elevated. Hence, Fisher and colleagues, as well as a number of other investigators, have suggested that obesity and IR represent Fgf21 resistant status[50, 61-66]. Indeed, Fisher *et al* found that when obese mice (either after HFD consumption or due to a genetic defect) were treated with human recombinant FGF21, they showed an attenuated response to ERK1/2 phosphorylation and impaired induction of FGF21 target genes[61]

As stated above, we found that a "6-day curcumin intervention" attenuated dexamethasone-induced insulin resistance[36]. In conducting this study, we found that in the absence or presence of dexamethasone treatment, a curcumin intervention can increase hepatic Fgf21 mRNA and protein levels. We hence conducted further investigation into the role of curcumin in regulating Fgf21 both *in vitro* and *in vivo*. We showed that in mice on a low fat diet (LFD), daily curcumin gavage (4 or 8 days) increased serum and hepatic Fgf21 levels. Using the quantitative chromatin immunoprecipitation (ChIP) approach, we observed increased PPARα interactions with the Fgf21 promoter after curcumin treatment. HFD feeding also increased serum and hepatic Fgf21 levels, while the increase was attenuated by a 12 week concomitant curcumin intervention in association with partial restoration of hepatic expression of the genes that encode the Fgf21 receptor and co-receptor (FGFR1 and βKlotho), and a battery of lipolysis genes[58]. Importantly, hepatocytes from mice on a HFD showed an attenuated response to *ex vivo* recombinant FGF21 treatment, and the attenuation was effectively abolished by concomitant curcumin intervention[58].

Both curcumin and insulin stimulate hepatic ChREBP expression

As illustrated in Figure 1, SREBP-1c and ChREBP are the two key lipogenic transcription factors[1, 2]. Although the function of ChREBP is known to be controlled by glucose-induced nuclear translocation[1, 67], our team has demonstrated previously that insulin can stimulate *ChREBP* gene transcription[68]. Early investigations showed that *ChREBP* deletion led to reduced lipogenesis[69] while *ob/ob;ChREBP*$^{-/-}$ mice exhibited lower expression of mRNAs for all hepatic lipogenic enzymes, decreased fatty acid synthesis and normalized plasma free fatty acid and triglyceride levels, as well as reduced weight gain and decreased adiposity[70]. These observations led to a suggestion that ChREBP inhibition may serve as a novel therapeutic tool for metabolic syndromes[70]. A few recent studies, however, have presented a completely opposing view. First, although hepatic over-expression of active ChREBP induced lipogenic programs, the mice with ChREBP over-expression remained insulin sensitive[71] and, when placed on a HFD, showed improved insulin and glucose tolerance, despite the persistence of diet-induced hepatic steatosis. Thus, increased hepatic ChREBP expression can dissociate hepatic steatosis from IR, with improvements in both lipid and glucose homeostasis[71]. Second, a second ChREBP isoform, ChREBPβ, that predicts insulin sensitivity, has been identified in white adipose tissue, and the loss of adipose-ChREBP was shown to be sufficient to cause insulin resistance in mice[72, 73]. Third, FA binding protein 4 (FABP4)-Cre mediated expression of constitutively active ChREBP in mice was shown to improve insulin sensitivity and glucose tolerance in response to HFD challenge[74]. Finally, very recent studies demonstrated that ChREBP protected the liver from fructose-induced injury[75, 76].

We recently found that both curcumin and insulin stimulated *ChREBPa* transcription via Akt-independent activation of MEK/ERK signaling, leading to the inactivation of the transcriptional repressor Oct-1[77]. Further exploration revealed the involvement of p21-activated protein kinase 1 (Pak1). *Pak1*$^{-/-}$ mice are glucose and insulin intolerant, as demonstrated previously by my team and by other investigators[78-81]. Importantly, *Pak1*$^{-/-}$ mice showed reduced ChREBPα protein levels and an ~70% reduction of *ChREBPa* and *ChREBPβ* mRNA levels[77]. Furthermore, these mice exhibited a reduced body fat volume. Indeed, *Pak1*$^{-/-}$ hepatocytes lack the normal ChREBP response to *in vitro* insulin or curcumin treatment[77]. We thus discovered a novel Pak1/MEK/ERK/Oct-1 signaling cascade that mediated insulin or curcumin-induced ChREBP expression. It needs to be noted that this novel signaling cascade (Figure 2) revealed by our study regulates *ChREBPa* but not *ChREBPβ* transcription, as there is no functional Oct-1 binding motif on the *ChREBPβ* promoter. Nevertheless, *ChREBPβ* expression is known to be positively regulated by ChREBPα because its promoter contains the binding motif for ChREBPα[72].

Summary and perspectives

As mentioned above, curcumin (and many other dietary polyphenols) can target multiple organs or cell lineages without a known receptor or a defined target. This, along with other chemistry features of curcumin, has generated enormous difficulties in the detailed dissection of mechanistic pathways underlying its bio-medical functions. Indeed, these features have been causing some confusion among drug developers and have been heavily debated recently[82-85]. However, investigations presented by numerous investigators, including our team, suggest that the implications of dietary polyphenol research are beyond the scope of drug development. Utilizing curcumin as a tool, scientists have now linked dietary curcumin consumption with many functions of insulin signaling. The insulin signaling improvement effect of curcumin (and possibly other dietary polyphenols) can be attributed, in the long term, to its anti-inflammation, anti-oxidation and body weight lowering effects. The body weight lowering effect of this compound can be attributed to not only the attenuation of adipose tissue macrophage infiltration[20] but also to its potential adipocyte "browning" effect, leading to an increase in energy expenditure[30].

As illustrated in Figure 2, we have demonstrated that Fgf21

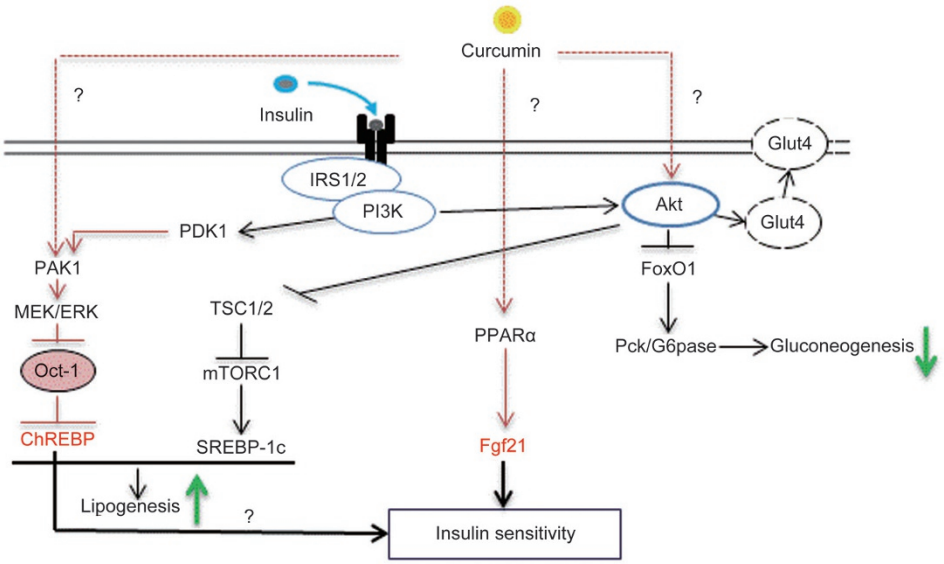

Figure 2. Summary of hepatic effects of curcumin intervention. Curcumin has been shown to repress glucose production in hepatocytes. Furthermore, curcumin treatment stimulates Fgf21 expression via a PPARα dependent mechanism. In addition, curcumin intervention attenuates HFD induced Fgf21 and insulin resistance (not illustrated in this figure). Finally, both insulin and curcumin can stimulate *ChREBPα* transcription via inactivating the transcriptional repressor Oct-1, which is involved in Akt-independent PAK1/MEK/ERK activation. It remains unknown mechanistically how curcumin can activate Akt, PPARα and PAK1 and hence regulate gluconeogenesis, as well as Fgf21 and ChREBP expression (indicated with the question marks). We speculate that ChREBP and Fgf21 form a network whereby the liver exerts its regulatory effects on energy homeostasis in response to nutrient sensing and dietary intervention.

and ChREBP are two novel hepatic targets of curcumin; both of them are known as positive regulators of hepatic insulin signaling. Curiously, Fgf21 is a "starvation" hormone, and its circulating level is elevated after fasting, while ChREBP activity is facilitated by hyperglycemia and its production is likely stimulated by hyperinsulinemia. At this stage, the connection between these two hepatic metabolic regulators is largely unknown. I speculate that Fgf21 and ChREBP form the two "arms" of a yet to be fully recognized "large" network whereby the liver exerts its regulatory effects on energy homeostasis, such that impairment of either one of the "arms" will lead to the loss of response to curcumin intervention or to dietary intervention in general.

The stimulation of hepatic Fgf21 expression has been shown with curcumin, two other dietary polyphenols[36, 57, 58], and white pitaya or dietary betaine supplementation[59, 60]. It is necessary to determine whether ChREBP expression can be regulated by anthocyanin, resveratrol, and other dietary polyphenols. More importantly, it is urgent to investigate whether the simultaneous regulation of hepatic Fgf21 and ChREBP expression is a common feature of dietary polyphenols. It is also important to investigate whether Fgf21 signaling restoration by dietary polyphenol intervention is a pre-requirement for improving insulin signaling. If yes, the investigation will lead to a therapeutic approach for improving hepatic insulin signaling from a novel angle. Furthermore, detailed investigations are needed to explore whether a defect in either one of the two "arms" will lead to the loss of response of the subject to dietary polyphenol intervention for insulin signaling improvement. These investigations will likely bring about a "concept shift" in the metabolic field, adding to our understanding of the hepatic insulin function paradox.

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