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Short-Term Curcumin Gavage Sensitizes Insulin Signaling in Dexamethasone-Treated C57BL/6 Mice[1–3]

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Abstract

Background: Long-term dietary curcumin (>12 wk) improves metabolic homeostasis in obese mice by sensitizing insulin signaling and reducing hepatic gluconeogenesis. Whether these occur only secondary to its chronic anti-inflammatory and antioxidative functions is unknown.

Objective: In this study, we assessed the insulin sensitization effect of short-term curcumin gavage in a rapid dexamethasone-induced insulin resistance mouse model, in which the chronic anti-inflammatory function is eliminated.

Methods: Six-week-old male C57BL/6 mice received an intraperitoneal injection of dexamethasone (100 mg/kg body weight) or phosphate-buffered saline every day for 5 d, with or without simultaneous curcumin gavage (500 mg/kg body weight). On day 7, insulin tolerance tests were performed. After a booster dexamethasone injection and curcumin gavage on day 8, blood glucose and insulin concentrations were measured. Liver tissues were collected on day 10 for quantitative polymerase chain reaction and Western blotting to assess gluconeogenic gene expression, insulin signaling, and the expression of fibroblast growth factor 21 (FGF21). Primary hepatocytes from separate, untreated C57BL/6 mice were used for testing the in vitro effect of curcumin treatment.

Results: Dexamethasone injection impaired insulin tolerance ($P < 0.05$) and elevated ambient plasma insulin concentrations by ~2.7-fold ($P < 0.01$). Concomitant curcumin administration improved insulin sensitivity and reduced hepatic gluconeogenic gene expression. The insulin sensitization effect of curcumin was demonstrated by increased stimulation of S473 phosphorylation of protein kinase B ($P < 0.01$) in the dexamethasone-treated mouse liver, as well as the repression of glucose production in primary hepatocytes ($P < 0.001$). Finally, curcumin gavage increased FGF21 expression by 2.1-fold in the mouse liver ($P < 0.05$) and curcumin treatment increased FGF21 expression in primary hepatocytes.

Conclusion: These observations suggest that the early beneficial effect of curcumin intervention in dexamethasone-treated mice is the sensitization of insulin signaling, involving the stimulation of FGF21 production, a known insulin sensitizer. *J Nutr* 2015;145:2300–7.

Keywords: curcumin, FGF21, gluconeogenic gene, insulin signaling, hepatocytes

Introduction

The lipophilic polyphenol curcumin is a dietary heptanoid. It is the principal curcuminoid of turmeric, a member of the ginger family (1). Turmeric has been used as a medicine in numerous Asian countries for nearly 3000 y in treating inflammatory diseases, including hepatitis, arthritis, and various types of skin disorders, as well as rash, hives, and burn injuries (2). In our modern society, dietary supplements with turmeric rhizome and extracts have been used for the treatment and prevention of arthritis (2).

Curcumin is perhaps the most studied component of turmeric, which constitutes ~2–5% of turmeric. It was found to possess antimicrobial, insecticidal, larvicidal, antimutagenic, cardioprotective, radioprotective, and anticancer activities. Experimental animal studies have shown the capability of curcumin to mitigate inflammatory diseases, cancer, neurodegenerative diseases, depression,

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diabetes, obesity, and atherosclerosis. Importantly, extensive experimental animal studies and a handful of recent clinical studies have shown the effect of curcumin in improving insulin signaling (3–7). Curcumin can also lower plasma glucose concentrations and body weight in obese animals (3, 7). A recent small scale clinical trial indicated that a 9 mo curcumin intervention in prediabetic subjects substantially lowered the number of individuals who eventually developed type 2 diabetes (T2D)[10] (5). Thus, the nutraceutical curcumin possesses great potential not only in treatment but also prevention of diabetes, obesity, and other metabolic disorders in which insulin resistance plays a fundamental role.

Intensive mechanistic investigations have revealed that curcumin up- and down-regulates complicated cell signaling networks. We and others have shown that long-term curcumin intervention in high-fat diet (HFD)–fed mice attenuates body weight gain and the development of insulin resistance, involving the suppression of the inflammatory response in the liver and adipose tissue (3, 7). An important question that remains to be addressed is whether or not the improvement of insulin signaling by curcumin consumption occurs only secondary to its long-term anti-inflammatory and antioxidant effects.

To identify the early beneficial effect of curcumin in vivo, we previously conducted a relatively short-term curcumin gavage in an HFD-induced insulin resistant mouse model, showing that 15 d curcumin gavage started to reduce body weight and fat weight gain, improved glucose intolerance, and elevated skeletal muscle nuclear factor erythroid 2-related factor 2 concentrations (8). In the HFD mouse model, we found that curcumin also improved the pyruvate tolerance test (7). Because pyruvate intolerance indicates increased hepatic gluconeogenesis, this observation suggested that the liver is an important target organ for curcumin to exert its beneficial metabolic function (7). In this study, to avoid its anti-inflammatory action, we assessed the effects of curcumin intervention at a very early stage in a rapid dexamethasone-induced insulin resistant C57BL/6 mouse model, focusing on its hepatic effect.

Methods

Mice, dexamethasone, and curcumin administration. Twenty-four male mice aged 5 wk were purchased from Charles River. After an acclimatization period of 1 wk, they were randomly assigned to the following 4 groups, with 6 mice per group: a PBS control group, a PBS plus curcumin group (PBS/CUR group), a dexamethasone group (for the generation of insulin resistance), and a dexamethasone plus curcumin group (DEX/CUR group). All mice were fed standard mouse feed (Harlan Teklad LM-485 Mouse, code 7912, equivalent to 7012 except for the irradiation procedure) (9).

These mice received daily intraperitoneal injections of either PBS or dexamethasone (100 mg/kg body weight per day) (9) for 5 consecutive days (**Supplemental Figure 1**). Mice in the 2 curcumin groups (PBS/CUR group and DEX/CUR group) received curcumin gavage (500 mg/kg body weight per day for 5 d) (3, 7), whereas the other 2 groups received a gavage of the same amount of solvent for curcumin (sesame oil). After a 1 d rest, an intraperitoneal insulin tolerance test (ITT) was performed (day 7). A booster intraperitoneal dexamethasone injection and curcumin gavage were performed on day 8, and all mice were killed by cervical dislocation on day 10 for blood and liver tissue collection. The dexamethasone sodium phosphate was from Omega (10), and the curcumin was purchased from Sigma Aldrich (no. C1385) (7). Mice were maintained at room temperature and relative humidity of 50%, with free access to food and water under a 12 h light/dark cycle. The protocol for animal use and euthanasia were approved by the University Health Network Animal Care Committee and were performed in accordance with the guidelines of the Canadian Council of Animal Care.

Cell cultures. To further verify the in vivo findings in the liver of mice receiving the curcumin intervention, we also conducted an in vitro curcumin treatment study against primary hepatocytes from untreated mice. Mouse primary hepatocytes were isolated as described (11). Briefly, the hepatic portal vein was cannulated with a 25G evacuated tube butterfly needle in anesthetized 8–12-wk-old C57BL/6 male mice. Anterograde perfusion of the liver with the use of a peristaltic pump with HBSS was followed by perfusion with DMEM containing 5.5 mmol/l glucose, 15 mmol/L HEPES, 1% penicillin/streptomycin, and type IV collagenase (100 collagen digestion units/mL). Hepatocytes extracted from digested livers were filtered through a 200 μm membrane, washed 3 times with DMEM, and resuspended in DMEM containing 25 mmol/L glucose, 1 mmol/L sodium lactate, 15 mmol/L HEPES, 1% penicillin/streptomycin, 100 nmol/L dexamethasone, and 10% FBS. HepG2 hepatocarcinoma cells were cultured in DMEM containing 10% FBS. Fibroblast growth factor 21 (FGF21) recombinant protein was purchased from NovoProtein.

ITT, glucose, and insulin measurement. An ITT was performed as described (11). Mice were deprived of food for 4 h before intraperitoneal injection of insulin (1 unit/kg). Plasma glucose concentrations were determined by a glucometer (Roche) with the use of tail vein blood. Plasma insulin concentrations were measured with the use of the Advanced Ultra-Sensitive Mouse Insulin Immunoassay Kit (Antibody and Immunoassay Services, no. 32392) according to the manufacturer's instructions.

Real-time PCR. RNA was isolated from the mouse liver tissue with the use of TRI Reagent (Sigma), as described (12). Real-time PCR was performed with the use of Power SYBR Green Mix (Applied Biosystems) with a 7900 Fast Real-Time PCR System (Applied Biosystems). Primers used in this study and sizes of the PCR products are listed in **Supplemental Table 1**. Levels of given mRNA were normalized to the levels of β-actin.

Antibodies and Western blotting. Whole-cell lysates from mouse liver tissue, mouse hepatocytes, or HepG2 cells were harvested and subjected to SDS-PAGE as described (13). The antibody for phosphorylated protein kinase B (Akt) (Ser473, no. 21054) was from Signalway Antibody. Antibodies for Akt (no. 9272), phosphorylated glycogen synthase kinase (GSK) 3β (Ser9, no. 9331), GSK3β (no. 9315), phosphorylated forkhead box protein O1 (FoxO1) (Ser256, no. 9461), FoxO1 (no. 2880) and phosphorylated tyrosine (no. 9411) were purchased from Cell Signaling Technology. FGF21 antibody (no. ab171941) was purchased from Abcam. The antibodies for β-actin (sc69879) and GAPDH (sc25778) were purchased from Santa Cruz Biotechnology. Densitometric analysis was performed with the use of ImageJ software, as described (14).

In vitro glucose production assay. The method for in vitro glucose production in mouse primary hepatocytes has been described previously, and was normalized to cellular protein content (13). FGF21 recombinant protein was purchased from NovoProtein.

Statistical analysis. All data are presented as means ± SEMs. Overall significant differences between multiple groups were determined by 2-factor ANOVA, followed by a Bonferroni post-test for pairwise comparisons and adjusted P values. Comparison between 2 sets of samples was analyzed by Student's t test. Statistical significance was determined at $P < 0.05$.

[10] Abbreviations used: Akt, protein kinase B; DEX/CUR group, dexamethasone plus curcumin group; *Fbp1*, fructose-1,6-bisphosphatase 1; FGF21, fibroblast growth factor 21; FoxO1, forkhead box protein O1; *G6pc*, glucose-6-phosphatase, catalytic subunit; GSK, glycogen synthase kinase; *Gys2*, glycogen synthase 2; HFD, high-fat diet; ITT, insulin tolerance test; PBS/CUR group, PBS plus curcumin group; *Pck1*, phosphoenolpyruvate carboxykinase 1; *Ppargc1α*, peroxisome proliferator-activated receptor γ coactivator 1α; T2D, type 2 diabetes.

Results

ITT and gluconeogenic gene expression. With this short-term dexamethasone treatment and curcumin intervention protocol in mice, we first assessed insulin tolerance. ITT data did not differ between groups (**Figure 1**A). The lack of significant impairment in the dexamethasone group is partially due to the relatively lower basal glucose concentration in this group (**Table 1**). However, if we compare the percentage changes, dexamethasone was shown to impair insulin tolerance significantly, and this was restored by curcumin gavage (Figure 1B). The development of insulin resistance by dexamethasone treatment was also demonstrated by the elevation of ambient plasma insulin concentrations (from ~314 pM to ~837 pM) (Table 1). However, this short-term curcumin gavage did not reduce the concentrations of plasma insulin provoked by dexamethasone injection (Table 1). In the absence of dexamethasone, curcumin generated no appreciable effect on plasma insulin concentrations (Table 1). We did not observe any effect from dexamethasone or curcumin on body weight within the 10 d experimental period (**Supplemental Figure 2**).

Because the aim of this study was to determine the initial hepatic beneficial effect of short-term curcumin gavage, the mice were killed for liver tissue and blood collection. Dexamethasone injection increased mRNA levels of a few gluconeogenic genes, including phosphoenolpyruvate carboxykinase 1 (*Pck1*) (Figure 1C), fructose-1,6-bisphosphatase 1 (*Fbp1*) (Figure 1D), and peroxisome proliferator-activated receptor γ coactivator 1α (*Ppargc1α*) (Figure 1E). Curcumin blocked the increase in the expression of these 3 genes (Figure 1C–E). We did not see a significant stimulatory effect of dexamethasone on the expression of glucose-6-phosphatase, catalytic subunit (*G6pc*), although curcumin inhibited its expression in the presence or absence of dexamethasone (Figure 1F). The expression levels of glycogen synthase 2 (*Gys2*) were inhibited by curcumin in the presence but not in the absence of dexamethasone (Figure 1G). These observations collectively suggest that in this short-term dexamethasone mouse model, simultaneous curcumin gavage had already generated the sensitization effect on insulin signaling, although the mice still maintained a hyperinsulinemic state.

Akt/GSK3 signaling. We then directly examined the phosphorylation status of several known downstream targets of insulin signaling in mouse liver tissue. Dexamethasone or curcumin on their own did not affect S473 Akt or GSK3β (Ser9) phosphorylation levels (**Figure 2**A and B). In mice that received the dexamethasone injection, however, curcumin significantly increased their phosphorylation levels (Figure 2A and B). The stress signaling mediator FoxO1 positively regulates gluconeogenesis, whereas phosphorylation at its S256

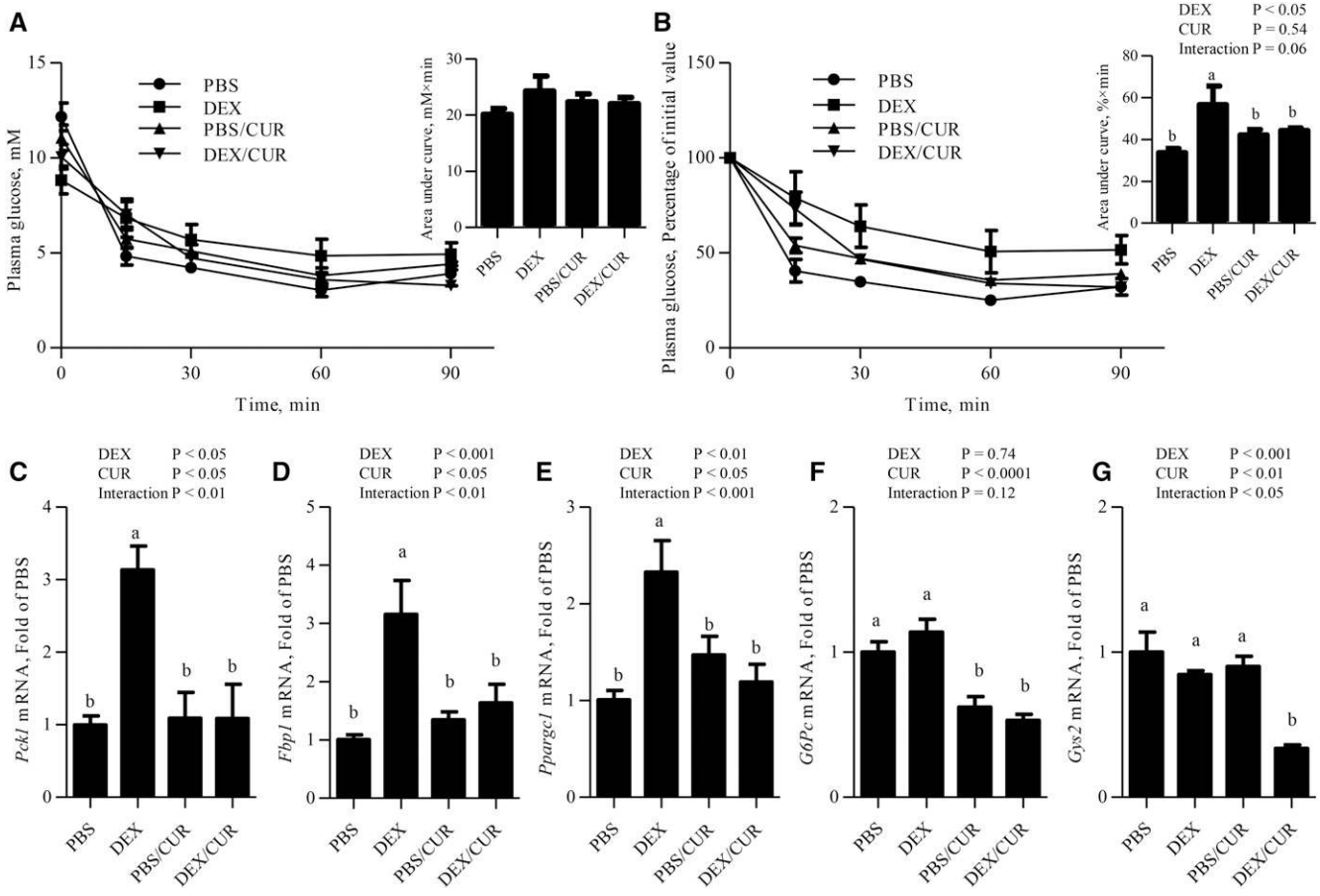

FIGURE 1 Simultaneous short-term curcumin gavage improves insulin tolerance in dexamethasone-treated mice, associated with reduced hepatic gluconeogenic gene expression. Comparison of ITT results among the 4 groups of mice (A). Comparison of ITT results in terms of percentage changes normalized to the initial plasma glucose value (B). qRT-PCR analysis for 4 gluconeogenic genes (C–F) and *Gys2* (G) in indicated mouse liver tissue. Values are means ± SEMs; $n = 4$–5 for panels A and B, and $n = 6$ for panels C–G. Labeled means without a common letter differ, $P < 0.05$. CUR, curcumin; DEX, dexamethasone; *Fbp1*, fructose-1,6-bisphosphatase 1; *G6pc*, glucose-6-phosphatase, catalytic subunit; *Gys2*, glycogen synthase 2; ITT, insulin tolerance test; *Pck1*, phosphoenolpyruvate carboxykinase 1; *Ppargc1α*, peroxisome proliferator-activated receptor γ coactivator 1α.

TABLE 1 Plasma glucose and insulin concentrations in mice treated with dexamethasone, curcumin, or both[1]

	Group				*P*		
Variable	PBS	DEX	PBS/CUR	DEX/CUR	DEX	CUR	Interaction
Glucose, mM	12.2 ± 1.27[a]	8.82 ± 1.62[b]	11.1 ± 1.16[a]	10.1 ± 1.13[a]	< 0.05	0.68	0.21
Insulin, pM	314 ± 131[b]	837 ± 111[a]	140 ± 57.1[b]	915 ± 243[a]	< 0.01	0.99	0.65

[1] Values are means ± SEMs, n = 6. Means without a common letter differ, $P < 0.05$. CUR, curcumin; DEX, dexamethasone.

residue leads to its inactivation. We have also observed a stimulatory effect of dexamethasone on FoxO1 S256 phosphorylation (Figure 2C). In dexamethasone-injected mice, curcumin also showed a trend of increasing FoxO1 S256 phosphorylation (P = 0.06, Figure 2C), supporting the notion that curcumin sensitizes insulin signaling.

In vitro Akt signaling and glucose production. We then verified the effect of curcumin on insulin signaling in vitro in mouse primary hepatocytes. **Supplemental Figure 3**A shows that 25 μM curcumin did not stimulate Akt S473 phosphorylation, and generated no additive effect with 100 nM insulin on Akt S473 phosphorylation. We wondered whether the potential additive effect was masked because of the use of a high concentration of insulin (100 nM), and hence tested whether preincubation of hepatocytes with curcumin would increase the stimulatory effect of insulin at lower dosages (1 and 10 nM) on Akt S473 phosphorylation. In addition, we checked the potential stimulatory effect at 15 and 30 min, in case the additive effect occurs in a transient manner. Although 1 or 10 nM of insulin stimulated Akt S473 phosphorylation, curcumin preincubation generated no additive effect (**Figure 3**A). We then did the preincubation of curcumin for 6 h. The cells were then incubated with 1 nM of insulin in the presence and absence of curcumin. With these experimental conditions, we observed a statistically significant additive effect from curcumin and insulin on Akt S473 phosphorylation (Figure 3B).

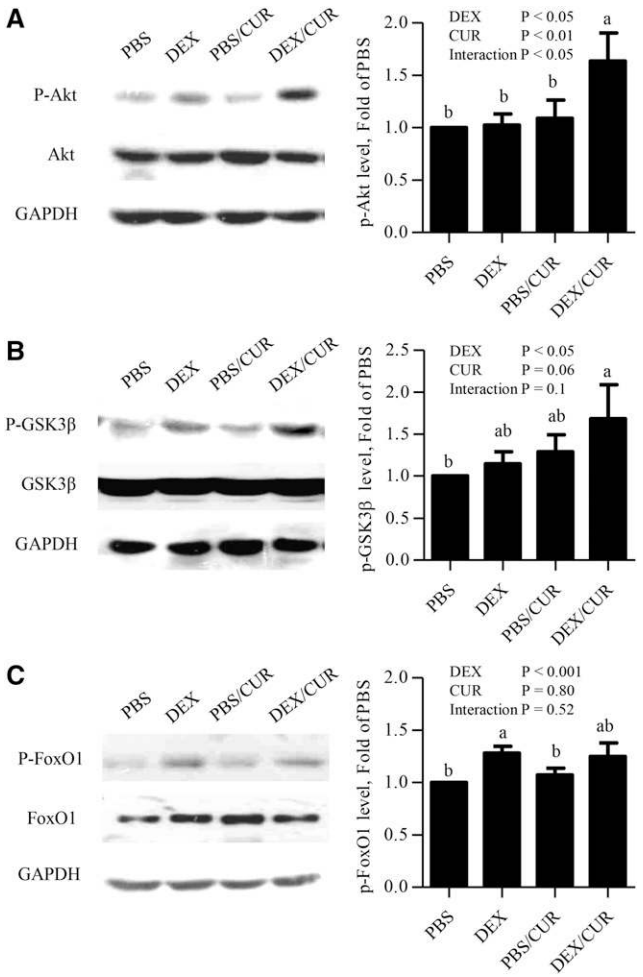

FIGURE 2 Curcumin administration in dexamethasone-treated mice improves the Akt/GSK3 signaling cascade. Representative Western blotting results for p-Akt (A), p-GSK3β (B) and p-FoxO1 (C). Right panels show results of densitometric analysis. Values are means ± SEMs, n = 6 per group. Labeled means without a common letter differ, $P < 0.05$. Akt, protein kinase B; CUR, curcumin; DEX, dexamethasone; FoxO1, forkhead box protein O1; GSK3β, glycogen synthase kinase 3β; p, phosphorylated.

Because curcumin intervention reduced gluconeogenic gene expression in dexamethasone-injected mice, we asked whether it directly inhibited glucose production in primary hepatocytes. Mouse primary hepatocytes were incubated with 25 μM of curcumin in the absence of FBS and glucose. The culture medium was withdrawn at 1, 2, and 4 h after the addition of curcumin to assess the amount of glucose produced. At each of the 3 time points, curcumin inhibited glucose production (Figure 3C). We also observed the additive effect of curcumin and insulin in repressing glucose production when primary hepatocytes were treated with these 2 reagents together for both 2 h (Figure 3D) and 4 h (Figure 3E) experimental settings.

FGF21 expression. The observations that curcumin sensitizes insulin signaling in dexamethasone mice, and that curcumin reduced the expression of gluconeogenic genes only in the presence of dexamethasone, made us wonder whether these 2 agents had a common target. Because dexamethasone was shown to increase the expression of FGF21, we assessed FGF21 expression in mouse livers and in primary hepatocytes treated with curcumin. Curcumin gavage on its own significantly increased liver FGF21 concentrations (**Figure 4**A). In the presence of dexamethasone injection, curcumin gavage also showed a strong tendency toward stimulating FGF21 concentrations (P = 0.06) (Figure 4B). The stimulatory effect of curcumin on FGF21 expression was then demonstrated in vitro in both the human hepatic carcinoma cell line HepG2 (Figure 4C) and mouse primary hepatocytes (Figure 4D).

We then tested the effect of FGF21 on Akt S473 phosphorylation in mouse primary hepatocytes. Although 1 nM of FGF21 stimulated Akt S473 phosphorylation, at higher concentrations, FGF21 showed no stimulation (**Supplemental Figure 4**). We hence tested the effect of 50, 200, and 1000 pM of FGF21 on Akt S473 phosphorylation, in the presence and absence of 1 nM of insulin. FGF21 at all 3 concentrations increased the stimulatory effect of 1 nM insulin (Figure 4E). FGF21 on its own at concentrations of 200 and 1000 pM, but not at 50 pM, also stimulated Akt

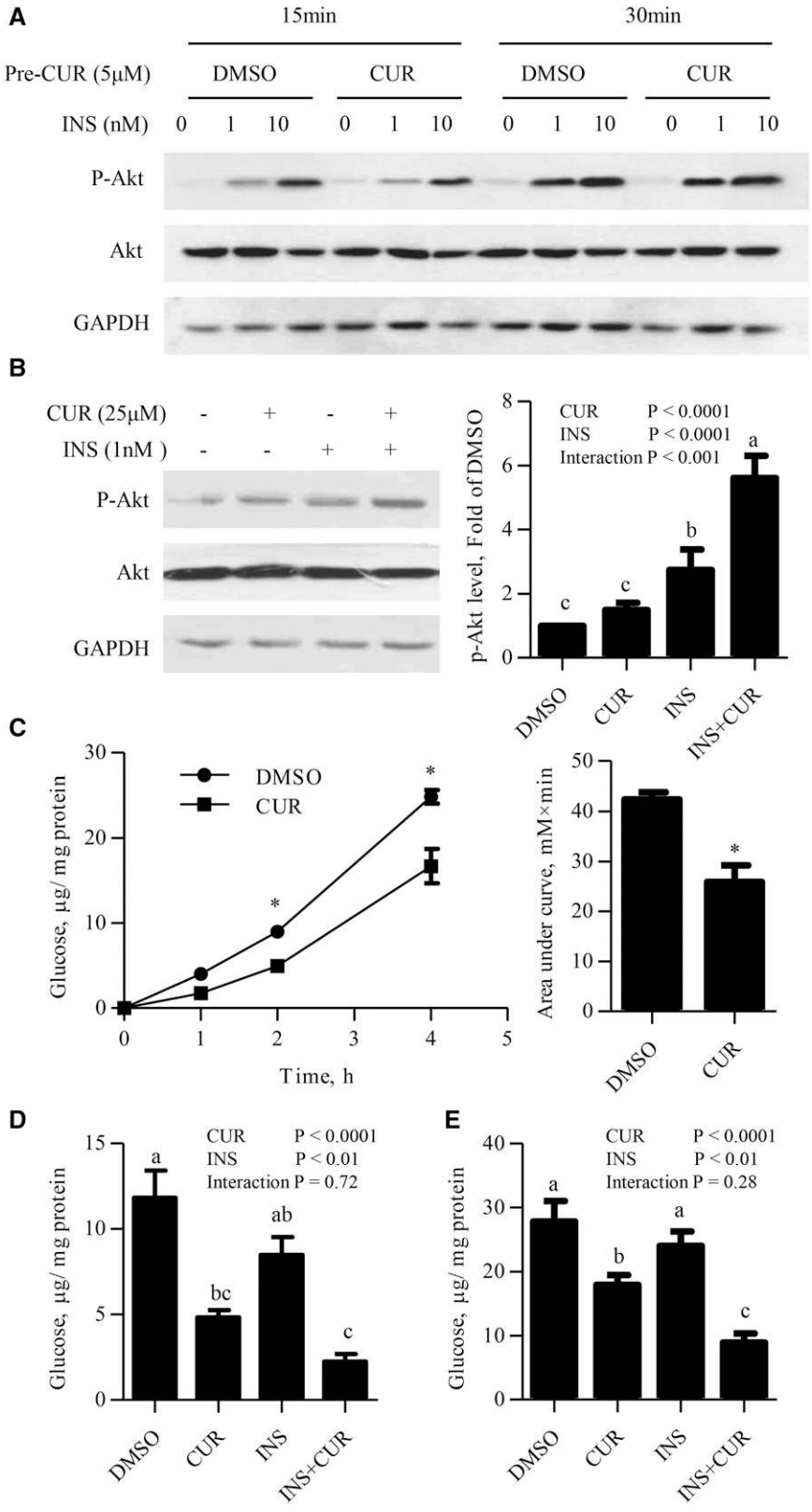

FIGURE 3 Curcumin treatment sensitizes insulin signaling in mouse primary hepatocytes. Hepatocytes were pretreated with the indicated concentration of curcumin for overnight (A) or 4 h (B), followed by treatment with the indicated concentration of insulin for 15 or 30 min (A) or 5 min (B), and Western blotting analysis. Densitometric analysis (B, right panel). Glucose production assays were performed in the presence of curcumin (25 μM), vehicle (DMSO), or insulin (100 nM), or insulin plus curcumin, as indicated (C–E). For D and E, the assay was performed for 2 and 4 h, respectively. Values are means ± SEMs, n = 3. Labeled means without a common letter differ, $P < 0.05$. *Different from DMSO, $P < 0.05$. Akt, protein kinase B; CUR, curcumin; INS, insulin; p, phosphorylated.

phosphorylation. Figure 4F shows that 1 nM FGF21 or 100 nM insulin was able to repress glucose production in mouse primary hepatocytes. A combination of FGF21 and insulin at these 2 concentrations did not show an additive effect. It may be noted, however, that in this in vitro cell culture model, concentrations of insulin below 100 nM do not inhibit glucose production, limiting the study of combinations of FGF21 with lower insulin (15).

Discussion

As an anti-inflammatory drug, dexamethasone has been broadly used in generating insulin-resistant models, which showed various outcomes based on dosages and durations (10, 16–18). Because the aim of this study was to determine the early beneficial effect of curcumin on insulin resistance, we chose to induce insulin resistance

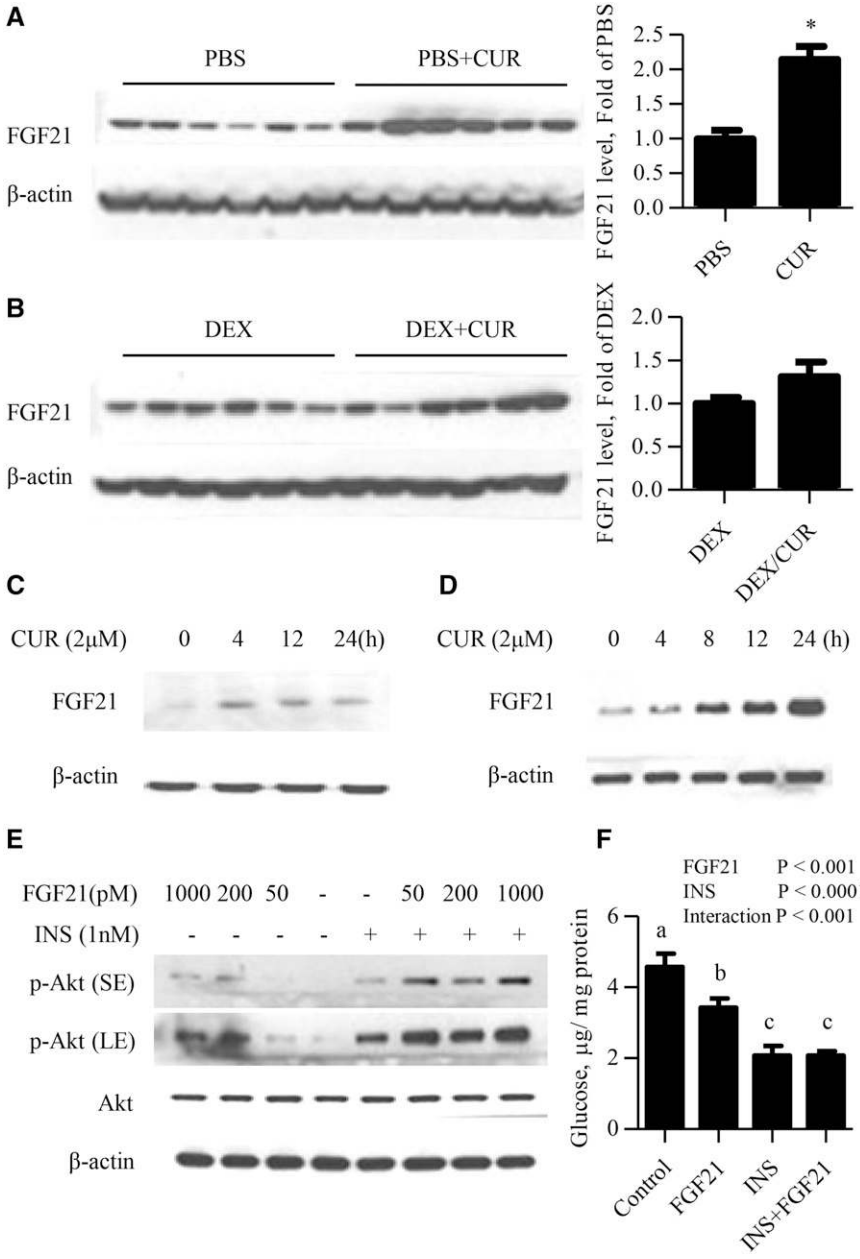

FIGURE 4 Curcumin stimulates the expression of FGF21, which sensitizes insulin signaling in primary hepatocytes. Western blotting shows liver FGF21 expression from 4 groups of mice (A, B). Right panels show the densitometric analysis. Western blotting of curcumin treated HepG2 hepatocarcinoma cells (C) and mouse primary hepatocytes (D). Primary hepatocytes were pretreated with the indicated concentration of FGF21 for 4 h, followed by 5 min of insulin treatment and Western blotting analysis (E). Glucose production assay was performed on primary hepatocytes treated with 1 nM FGF21, 100 nM insulin, or both (F). Values are means ± SEMs, $n = 6$ for panels A, B, and F. Labeled means without a common letter differ, $P < 0.05$. *Different from PBS, $P < 0.05$. Akt, protein kinase B; CUR, curcumin; DEX, dexamethasone; FGF21, fibroblast growth factor 21; INS, insulin. LE, long time exposure; p, phosphorylated; SE, short time exposure.

rapidly with a short-term dexamethasone administration along with simultaneous curcumin gavage (10). Our observations suggest that short-term curcumin gavage improved insulin sensitivity in the context of dexamethasone-induced insulin resistance. The sensitization effect of curcumin on insulin signaling in hepatocytes was then confirmed by assessing Akt and GSK3β phosphorylation. We also observed stimulation by dexamethasone of FoxO1 S256 phosphorylation, which could be due to the activation of serum- and glucocorticoid-induced kinase 1 (19). To explore the mechanism underlying the insulin sensitization effect, we assessed whether FGF21, a known insulin sensitizer that was shown to be stimulated by dexamethasone (18), could also be stimulated by curcumin. Indeed, we found that curcumin stimulated FGF21 expression both in vivo and in vitro. We hence suggest that the early beneficial effect of curcumin in the liver is the sensitization of insulin signaling, likely involving enhanced FGF21 production. It appears that this occurs ahead of its chronic effect to attenuate inflammation in adipose tissue and liver, which contributes to the improvement of insulin signaling in long-term HFD models (3, 7).

The development of insulin resistance is common in T2D, obesity, and other metabolic disorders (20–23). Although it is difficult to normalize, therapeutic agents such as metformin and rosiglitazone can improve insulin sensitivity. Curiously, metformin was shown to increase FGF21 production in various cell lineages, including mouse primary hepatocytes (24). These drugs, however, may not always be effective in the clinic for certain patients (25, 26).

The role of inflammation and oxidative stress in the development of insulin resistance has been broadly recognized (27–29). Curiously, many plant compounds, including curcumin, possess anti-inflammatory and antioxidative effects and hence improve insulin signaling (3, 7, 30–35). An intriguing question is whether improved insulin signaling by dietary consumption of these native plant compounds could be a direct effect or only secondary to their chronic anti-inflammatory and antioxidative effect.

Weisberg et al. (3) demonstrated that long-term dietary curcumin attenuated the development of diabetes in both HFD-fed

and leptin-deficient obese mice. Curcumin intervention reduced plasma glucose and glycated hemoglobin concentrations, attenuated insulin intolerance (decreased insulin resistance), decreased macrophage infiltration in adipose tissue, and inhibited hepatic NF-κB activity (3). We found that dietary curcumin started to exert its body weight–lowering effect in HFD-fed mice at wk 16 (8). Importantly, we showed in a previous study that curcumin-treated HFD-fed mice exhibited a better response to pyruvate challenge, indicating decreased hepatic gluconeogenesis, suggesting that the liver is an important target (7). Indeed, curcumin-treated HFD-fed mice showed an enhanced response to insulin intraperitoneal injection of liver Akt phosphorylation (7). However, in these long-term intervention models, one cannot determine whether the effect was secondary to the attenuation of inflammation and oxidative stress. Dexamethasone, as an anti-inflammatory drug, has been used in generating rodent insulin resistance (10, 16–18, 36). We chose this short-term protocol with one intraperitoneal injection per day without sesame oil as vehicle that would prolong its effect (36), because we were testing the short-term effect of curcumin intervention. This treatment did not change the body weights of the animals. In addition, we found that neither dexamethasone nor curcumin affected hepatic inducible nitric oxide synthase concentrations (**Supplemental Figure 5**). Thus, we conclude that the insulin sensitization effect of curcumin observed in this mouse model is independent of its anti-inflammatory effect.

FGF21 is a novel hepatic hormone with great potential in the treatment of metabolic disorders (37–41). A few recent studies indicated a correlation between FGF21 and insulin sensitivity. Kim et al. (24) showed that metformin increases FGF21 production in mouse primary hepatocytes and other cell lineages. Liu et al. (42) demonstrated the effect of adiponectin in stimulating autophagy and reducing oxidative stress in HFD-fed mice, associated with insulin-signaling sensitization and the elevation of FGF21 production. Moreover, Jiang et al. (43) found that the repressive effect of diabetes-induced male germ cell apoptosis by FGF21 is attributed to Akt activation. To our knowledge, we show here for the first time that curcumin stimulates FGF21 both in vivo and in vitro, and that FGF21 represses glucose production in primary hepatocytes. It remains to be determined how this initial effect of curcumin on FGF21 expression contributes to its long-term effect in metabolic homeostasis and the body weight–lowering effect.

Several recent clinical investigations have revealed the beneficial metabolic effect of dietary curcumin intervention in T2D patients and prediabetic subjects. Na et al. (44) have shown that a 3 mo curcumin intervention improved fasting glucose and glycated hemoglobin concentrations and the HOMA-IR index in overweight and obese diabetes patients. As mentioned above, a small-scale clinical trial in a population in Thailand showed that a 9 mo curcumin intervention prevented the development of T2D in subjects with prediabetic syndromes (5). Studies in healthy subjects, however, showed that curcumin had very limited beneficial effects (6, 45). These observations are in agreement with our findings that the insulin sensitizing effect of curcumin was observed in the DEX/CUR group only, but not in mice from the control PBS group. Our observations further support the notion that curcumin intervention can be used for the prevention of the development of T2D and other metabolic disorders in subjects with insulin resistance.

It is worth pointing out that, in this study, we observed a decreased, instead of increased, ambient plasma glucose concentration with dexamethasone treatment (Figure 1B). A previous study performed in Wistar rats found that a 24 d dexamethasone treatment resulted in overt diabetes in only 16% of rats, although all of them developed insulin resistance (46). Another study indicated that, in addition to inducing insulin resistance, dexamethasone can also stimulate pancreatic β cell proliferation (47). Thus, the reduction in ambient glucose concentrations, along with increased ambient insulin concentrations, in dexamethasone-treated mice (Figure 1D) could be a transient event in response to compensatory growth and/or insulin secretion of pancreatic β cells.

An overall suggested scheme of the effect of curcumin intervention on hepatic metabolic homeostasis is summarized in **Supplemental Figure 6**. The effect of dexamethasone administration via glucocorticoid receptor activation is the development of insulin resistance. The initial effect of curcumin in this model is the restoration of insulin sensitivity, which involves the stimulation of FGF21 production and the activation of insulin receptor substrate 1. It is worth further pointing out that dexamethasone injection can also stimulate FGF21 expression, demonstrated recently by Patel et al. (18). This could also be part of a compensatory negative feedback in response to insulin resistance. The improved insulin signaling, elevated plasma insulin concentration, and increased FGF21 production are responsible for the attenuation of hepatic gluconeogenesis by curcumin. Whether and how this initial insulin sensitizing effect of curcumin consumption contributes to the long-term effect of curcumin in repressing body weight gain needs further investigation.

As a steroid medication, dexamethasone is widely used in treating inflammatory and autoimmune diseases. Weight gain and the development of insulin resistance are among the important side effects when taking this category of drugs over the long term. Whether curcumin, a plant compound with anti-inflammatory properties, can be used to attenuate these side effects is worthy of further investigation.

Acknowledgments

LT and TJ designed the research; LT, KZ, and WS conducted the experiments; LT, BBY, IGF, JW, and TJ analyzed the data; LT and TJ wrote the manuscript; and TJ had primary responsibility for the final content. All authors read and approved the final manuscript.

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