The Wnt signaling pathway effector TCF7L2 is upregulated by insulin and represses hepatic gluconeogenesis

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Ip W, Shao W, Chiang YA, Jin T. The Wnt signaling pathway effector TCF7L2 is upregulated by insulin and represses hepatic gluconeogenesis. Am J Physiol Endocrinol Metab 303: E1166–E1176, 2012.—TCF7L2 expression is regulated by nutrient availability and whether TCF7L2 and Wnt regulate hepatic gluconeogenesis. We examined hepatic Wnt activity in the TOPGAL transgenic mouse, assessed hepatic TCF7L2 expression in mice upon feeding, determined the effect of insulin on TCF7L2 expression and β-catenin Ser675 phosphorylation, and investigated the effect of Wnt activation and TCF7L2 knockdown on gluconeogenic gene expression and glucose production in hepatocytes. Wnt activity was observed in pericentral hepatocytes in the TOPGAL mouse, whereas TCF7L2 expression was detected in human and mouse hepatocytes. Insulin and feeding stimulated hepatic TCF7L2 expression in vitro and in vivo, respectively. In addition, insulin activated β-catenin Ser675 phosphorylation. Wnt activation by intraperitoneal lithium injection repressed hepatic gluconeogenic gene expression in vivo, whereas lithium or Wnt-3a reduced gluconeogenic gene expression and glucose production in hepatic cells in vitro. Small interfering RNA-mediated TCF7L2 knockdown increased glucose production and expression of the key rate-limiting gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase) (20). These observations suggest that Wnt signaling and TCF7L2 are negative regulators of hepatic gluconeogenesis, and TCF7L2 is among the downstream effectors of insulin in hepatocytes.

transcription factor 7-like 2; β-catenin; glucose production; liver; phosphoenolpyruvate carboxykinase; Wnt-3a

TRANSCRIPTION FACTOR 7-LIKE 2 (TCF7L2, previously known as TCF-4) belongs to the T cell factor (TCF) family of high-mobility group box transcription factors and is a major effector of the canonical Wnt signaling pathway (defined as the Wnt pathway hereafter unless further clarification is necessary) (18, 24). Upon stimulation by Wnt ligands, a TCF protein binds to nuclear β-catenin (β-cat) to form a bipartite transcription factor cat/TCF, leading to the stimulation of Wnt target gene expression (14). cat/TCF may also serve as an effector of other signaling molecules, including certain peptide hormones that utilize cAMP as a second messenger, IGF-I, insulin, and the lipid metabolite lysophosphatidic acid (LPA) (2, 5, 14, 15, 37).

Previous studies have established the fundamental role of TCF7L2 and Wnt in embryogenesis and tumorigenesis. However, the involvement of Wnt signaling and TCF7L2 in hormone gene expression and metabolic homeostasis has been recognized only recently (15, 21, 25, 26, 41).

During the past few years, extensive genome-wide association studies have revealed that certain single nucleotide polymorphisms (SNPs) within intronic regions of TCF7L2 are strongly associated with the risk of type 2 diabetes (9, 10). This finding fueled many efforts to investigate the role of TCF7L2 and Wnt signaling in pancreatic β-cells (22, 33, 34). Several studies have demonstrated the beneficial effect of TCF7L2 on β-cell proliferation, insulin secretion, and the expression of incretin hormone receptors (3, 33, 34). These findings, however, are in contradiction with the suggestion that TCF7L2 exerts deleterious effects on β-cells by other investigations (22, 30). It has also been demonstrated that TCF7L2 type 2 diabetes risk SNPs are associated with elevated hepatic glucose production in individuals even during a hyperinsulinemic euglycemic clamp as well as during hepatic insulin resistance (22, 28, 39). Moreover, hepatic β-catenin deficiency in mice resulted in alterations in glucose production and expression of the key rate-limiting gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G-6-Pase) (20). These observations prompted us to assess whether TCF7L2 is regulated by nutrient availability as well as the role of TCF7L2 and Wnt signaling in hepatic glucose production.

Here, we show the detection of hepatic Wnt activity in the TOPGAL Wnt/TCF-reporter transgenic mouse and the expression of TCF7L2 in both human and mouse hepatic cells. We have also found that insulin and feeding stimulated TCF7L2 expression in vitro and in vivo, respectively, and that insulin is able to stimulate the activity of β-catenin, the partner of TCF7L2 in mediating Wnt activity. Moreover, we have defined the repressive effect of both the Wnt ligand Wnt-3a and TCF7L2 on hepatic glucose production.

MATERIALS AND METHODS

Cell culture and treatment. Human HepG2 and mouse Hepa-1–6 hepatoma cell lines (ATCC) were cultured in DMEM (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Gibco). Mouse primary hepatocytes were isolated by liver perfusion of 12-wk-old C57BL/6 mice, as described previously (40). Hepatocytes or hepatic cell lines were starved of serum overnight prior to treatment with 100 nM insulin (Eli Lilly), 10 μM forskolin (Sigma), 10 nM glucagon (Sigma), 10 mM lithium chloride (Bioshop), or 2.5 nM Wnt ligand, either Wnt-11 or Wnt-3a (R & D Systems) for the indicated times. The chemical inhibitor Akti1/2 (10 μM) was obtained from Sigma, whereas the inhibitors LY-294002 (50 μM) and PD-98059 (50 μM) were obtained from Calbiochem. Transfection of 5 nM small inter
ferring RNA (siRNA) recognizing either a scrambled sequence or TCF7L2 (Ambion) was achieved using Lipofectamine RNAiMAX (Invitrogen) per the manufacturer’s instructions. Transfection of luciferase (LUC) reporter plasmids (2.0 μg/well in a 12-well plate) was achieved using 3 μg of polyethylenimine (Sigma).

Animals. Twelve-week-old male C57BL/6 mice (Jackson) were fasted overnight prior to refeeding or a defined treatment. Mice were intraperitoneally (ip) injected with lithium chloride (BioShop) at a final dosage of 3 mmol/kg body wt or PBS as the control 4 h prior to extraction of liver tissue for reverse transcription PCR (RT-PCR) or Western blot analysis. TOPGAL transgenic mice (4) were purchased from Jackson (004623, Tg-Fos-lacZ-34Efu/J).

RNA isolation, RT-PCR, and real-time RT-PCR. Total RNA was isolated from HepG2, Hepa1–6, mouse primary hepatocytes, or liver tissue using TRI reagent (Sigma) and reverse transcribed using a complimentary DNA reverse transcription kit (Applied Biosystems), following the instructions of the manufacturers. For RT-PCR detection of TCF7, LEF1, TCF7L1, TCF7L2, and Wnt-3a, specific transcript sequences were amplified by Taq DNA polymerase (New England BioLabs) using the S1000 Thermal Cycler (Bio-Rad) per the instructions of the manufacturers. A reaction lacking any DNA template served as a negative control. For quantitative real-time RT-PCR analysis, specific transcript sequences were amplified using Taq SYBR Green (Bio-Rad) and the Rotor-Gene 3000 machine (Corbett) as described previously (41).

LCR reporter analysis. The generation of the TCF7L2 (3.1 kbp)-LCR fusion gene construct was described previously (38). The rat Signalway Antibody and for PEPCK was that of Abcam. The LUC fusion gene construct was described previously (38). The rat Signalway Antibody and for PEPCK was that of Abcam.

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Western blotting and antibodies. Methods for whole cell lystate preparation and Western blotting have been described previously (42). Protein samples (30 μg/sample) were separated by SDS-PAGE and transferred to nitrocellulose membrane. Antibodies for TCF7L2, TCF7L1, β-catenin, and cyclin D1, Akt, and phosphorylated ERK1 (Tyr204) were purchased from Santa Cruz Biotechnology. Antibodies for phosphoAKT (Ser9), and phosphorylated CREB (Ser133) were purchased from Cell Signaling Technology. Antibodies for phospho-β-catenin (Ser675), were purchased from Cell Signaling Technology.

Glucose production assay. Mouse primary hepatocytes and hepatoma cell lines were cultured in six-well plates (1.0 g of polyethylenimine (Sigma)). After 4 h, the medium was collected and assayed for glucose content using a colorimetric glucose assay kit (Sigma). Glucose content was normalized to total protein content measured in whole cell lysates.

Statistical analysis. All experiments were performed independently as a minimum of three replicates or mice. Quantitative results are expressed as means ± SE relative to the indicated control group. Statistical analyses were performed using the two-tailed Student’s t-test or one-way ANOVA as appropriate for single or multiple comparisons, respectively. Differences were considered significant when P < 0.05.

RESULTS

Detection of Wnt signaling and TCF7L2 expression in hepatocytes. To assess Wnt activity in hepatocytes, we took advantage of the availability of TOPGAL transgenic mice (4).

Table 1. Primers used for RT-PCR or real-time RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence</th>
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<tr>
<td></td>
<td>5'-AGGTCACACTGGTTGAGACTG-3'</td>
<td>412</td>
</tr>
<tr>
<td>LEF1</td>
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<td>124</td>
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<tr>
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<td>5'-GACTGTGCAAGCCGGCGG-3'</td>
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<tr>
<td>TCF7L1</td>
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<tr>
<td></td>
<td>5'-AGTCAGCTGTTCCTGTCGTCTT-3'</td>
<td>243</td>
</tr>
<tr>
<td>TCF7L2</td>
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</tr>
<tr>
<td></td>
<td>5'-GCATTAAAGACCTTTGCGTG-3'</td>
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<tr>
<td>PEPCCK</td>
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</tr>
<tr>
<td></td>
<td>5'-AGGGGTCTATTGTTGAGACTG-3'</td>
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</tr>
<tr>
<td>G-6-Pase</td>
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<tr>
<td></td>
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TCF7, transcription factor 7; TCF7L1 and -2, transcription factor 7-like 1 and 2, respectively; PEPCCK, phosphoenolpyruvate carboxykinase; G-6-Pase, glucose-6-phosphatase.
TCF-responsive promoter element (Fig. 1A, top). As shown in Fig. 1A, positive LacZ staining was observed in hair follicles (positive control) (4) and adult mouse pericentral hepatocytes. Pericentral hepatocytes are typically associated with reduced levels of gluconeogenesis but elevated levels of lipogenesis (16). We then determined by RT-PCR that both the human HepG2 cell line and mouse hepatocytes [Hepa1–6 cell line, C57BL/6 mouse liver tissue (MLT), and mouse primary hepatocytes (MPH)] express TCF7L2 and three other members of the TCF family (Fig. 1B). Western blotting showed that the human HepG2 cell line and mouse hepatic cells express primarily two isoforms of TCF7L2 of ~78 and 58 kDa in size (Fig. 1C).

Feeding stimulates TCF7L2 expression. To determine whether hepatic TCF7L2 expression levels can be modulated by nutritional availability, we compared TCF7L2 expression levels in the livers of fasted or refed C57BL/6 mice. As shown in Fig. 2, A and B, refed animals showed substantially increased hepatic TCF7L2 protein levels (both 78- and 58-kDa isoforms) compared with fasted mice. Increased TCF7L2 expression was associated with elevated expression of cyclin D1, a known downstream target of the Wnt signaling pathway (Fig. 2, A and C).

Insulin stimulates TCF7L2 expression and β-catenin phosphorylation. Because feeding increases plasma insulin levels and insulin is the principle hormone involved in repress-
ing gluconeogenesis in response to feeding, we assessed directly the effect of 4-h insulin treatment on TCF7L2 expression in human HepG2 and mouse Hepa1–6 cell lines in vitro. As anticipated, insulin treatment stimulated the phosphorylation of Akt (Ser473), GSK-3β (Ser9), and ERK1 (Tyr204) (Fig. 3, A and B). This treatment also increased TCF7L2 protein levels in both cell lines, which was associated with elevated cyclin D1 levels (Fig. 3, A and B). In addition, 4-h insulin treatment was shown to increase TCF7L2 mRNA levels in these two cell lines detected by real-time RT-PCR (Fig. 3, C and D). Furthermore, when a TCF7L2-LUC fusion gene plasmid (38) was transfected into either HepG2 or Hepa1–6 cells, 4-h insulin treatment moderately but significantly increased LUC reporter activity (Fig. 3, E and F).

β-Cat is the essential partner of TCF members in stimulating Wnt downstream target gene expression. β-Cat Ser675 phosphorylation in response to PKA activation was demonstrated in other cell lineages to be positively correlated with the transcriptional activity of cat/TCF (12). We have also shown previously the activation of β-cat Ser675 phosphorylation by insulin in gut cells (37, 42). In the current study, we have first of all determined that in the mouse Hepa1–6 cell line both cAMP/PKA activators forskolin and glucagon were able to stimulate CREB phosphorylation, which was associated with β-cat Ser675 phosphorylation (Fig. 4A). As anticipated, these two agents also increased de novo glucose synthesis in this cell line (data not shown). We then assessed the effect of insulin on β-cat Ser675 phosphorylation in the two hepatic cell lines. As shown in Fig. 4, B and C, insulin was able to stimulate β-cat Ser675 phosphorylation. In the Hepa1–6 cell line, this stimulation could be attenuated by inhibition of either phosphoinositide 3-kinase (PI3K) or mitogen-activated extracellular signal-regulated kinase (MEK) but not by the inhibition of protein kinase B (PKB/Akt) (Fig. 4D). MEK inhibition also markedly repressed basal levels of Ser675 β-cat but did not affect insulin-stimulated Akt Ser473 phosphorylation. Inhibition of PI3K or Akt led to the loss of Akt phosphorylation at Ser473 as expected, although phosphorylation of GSK-3β at Ser9 could still be observed likely due to the involvement of multiple kinases (36). Insulin-

![Fig. 3. Insulin stimulates TCF7L2 expression in 2 hepatic cell lines. Effect of 100 nM insulin (4 h) on TCF7L2 expression assessed by Western blotting (A and B) and quantitative RT-PCR (C and D) in HepG2 and Hepa1–6 cell lines. E and F: effect of 100 nM insulin (4 h) on TCF7L2 (3.1kb)-luciferase (LUC) reporter expression in HepG2 (E) and Hepa1–6 (F) cell lines. Blots shown are representative of at least 3 independent experiments. In C–F, values in each independent experiment were normalized to the control group (defined as 1-fold) and then presented as means ± SE (n = 3). *P < 0.05 and **P < 0.01 vs. control.](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00249.2012)
stimulated expression of cyclin D1 was attenuated by either Akt or PI3K inhibition (Fig. 4D). This is likely due to the involvement of Akt in regulating protein translation via mTOR activation (37). These observations collectively suggest that in hepatocytes insulin is able to cross-talk with the Wnt signaling pathway by both increasing TCF7L2 expression levels and stimulating β-catenin Ser675 phosphorylation. The activation of β-catenin Ser675 phosphorylation by insulin...
appears to be an Akt-independent phenomenon, as we have observed previously in gut endocrine L cells (42).

**Wnt activation by lithium reduces gluconeogenesis.** We then assessed the contribution of Wnt activation on gluconeogenesis in vivo. As an inhibitor of GSK-3, lithium has been widely utilized to mimic the activation of Wnt via increasing nuclear levels of β-cat (35, 41). We found that it has no appreciable effect on TCF7L2 expression or β-cat Ser675 phosphorylation in hepatocytes in vitro (data not shown). Male C57BL/6 mice at the age of 12 wk were injected ip with lithium chloride (3 mmol/kg body wt). Four hours after injection, mice were euthanized, followed by extraction of liver tissue for RNA as well as protein isolation. In fasted animals, this short-term Wnt activation by lithium injection reduced hepatic expression of PEPCK mRNA significantly (Fig. 5A, left) and generated a trend of reducing the level of G-6-Pase mRNA (Fig. 5A, right), although the effect did not reach statistical significance. As expected, feeding reduced hepatic PEPCK and G-6-Pase mRNA levels (17), although lithium injection in fed animals generated no additive reduction (Fig. 5A). As shown in Fig. 5B, lithium injection also reduced hepatic PEPCK protein level in animals during fasting. Feeding reduced PEPCK protein level, and lithium injection generated no further reduction (data not shown).
We then assessed the effect of lithium in the HepG2 cell line in vitro. Forskolin (which mimics the effect of glucagon on cAMP elevation) treatment significantly stimulated PEPCK and G-6-Pase mRNA levels, whereas insulin or lithium treatment significantly reduced the expression of these two gluconeogenic genes (Fig. 5C). To assess whether the repressive effect of lithium on PEPCK expression occurs at the transcriptional level, we obtained the PEPCK promoter sequence (from −595 to +67 bp) by PCR and constructed a LUC reporter. As anticipated, this fusion gene construct can be positively regulated by PKA activation (data not shown). When this construct was transfected into the HepG2 cell line, lithium treatment was shown to modestly but reproducibly reduce its expression (Fig. 5D). Finally, lithium treatment was found to significantly reduce relative glucose output in mouse primary hepatocytes (Fig. 5E). Although the in vitro glucose production assay utilized here alone cannot eliminate the potential involvement of glycogenolysis, our observation that lithium represses gluconeogenic gene expression (Fig. 5, A–C) indicates that the Wnt pathway activator lithium represses gluconeogenesis.

**Wnt-3a reduces gluconeogenesis.** To verify the repressive effect of Wnt activation on gluconeogenesis in vitro, we directly utilized the canonical Wnt ligand Wnt-3a along with the noncanonical Wnt ligand Wnt-11 as a negative control. Wnt-3a is known to be differentially expressed at critical stages of human liver development in vivo (11) and is commonly used as a canonical Wnt ligand. In addition, we detected the expression of Wnt-3a in human and mouse hepatocytes via RT-PCR (Fig. 6A), consistent with a previous study demonstrating its expression in the mouse liver (20). As shown in Fig. 6B, treatment of HepG2 cells with Wnt-3a, but not with Wnt-11, reduced the levels of PEPCK and G-6-Pase mRNA significantly. Wnt-3a, but not Wnt-11, was also found to reduce glucose output in mouse primary hepatocytes (Fig. 6C). Wnt-3a, however, could not further reduce gluconeogenic gene expression upon insulin treatment (Fig. 6D). These observations suggest that Wnt signaling is a negative regulator of hepatic gluconeogenic gene expression and glucose production.

**TCF7L2 negatively regulates gluconeogenesis.** Norton et al. (26) have shown that knockdown of TCF7L2 in the rat hepatoma cell line H4IIE increased glucose production significantly in the presence or absence of insulin or metformin treatment. Here, we have established the methodology to knockdown TCF7L2 in both human and mouse hepatocytes using siRNA and directly assessed the contribution of TCF7L2 in regulating gluconeogenic gene expression and glucose output. Figure 7A shows the RNA sequence of two TCF7L2 siRNA oligonucleotides used in this study. These two siRNA sequences would collectively recognize and knock down major isoforms of TCF7L2 in both human and mouse. Knockdown of TCF7L2 resulted in a significant elevation of PEPCK and G-6-Pase mRNA levels (Fig. 7B), which was associated with increased glucose output in the mouse Hepa1–6 cell line and mouse
primary hepatocytes (Fig. 7C). However, the repressive effect of insulin on gluconeogenic gene expression was still present after the silencing of TCF7L2 (Fig. 7D), consistent with the report by Norton et al. (26) in the rat H4IIE hepatoma cell line.

DISCUSSION

TCF7L2 has been recognized as the most prominent type 2 diabetes risk gene due to the robust association between its selected SNPs and the risk of type 2 diabetes (9, 10, 31). However, the functional role of TCF7L2 in pancreatic islets remains unclear and controversial (3, 19, 22, 30, 33, 34). Because TCF7L2 is also expressed in organs other than pancreatic islets, including liver, brain, muscle, and fat tissues, which are likewise important in metabolic homeostasis, it is necessary to define the function of TCF7L2 and further study the metabolic function of Wnt signaling in each of those organs. Although it is well known that Wnt signaling is important for the development and zonation of the embryonic liver (8), little effort has been made to explore the potential hepatic role of TCF7L2 and Wnt signaling in mediating glucose homeostasis in adulthood until recently. Liu et al. (20) found that starvation induced the expression of mRNAs encoding different Wnt isoforms. They have also demonstrated with gain- and loss-of-function models the role of /H9252-H9252-cat in hepatic glucose production. This group, however, did not directly assess the contribution of TCF7L2 in gluconeogenesis. Norton et al. (26) demonstrated that TCF7L2 silencing led to increased basal levels of hepatic glucose production in the rat hepatic cell line H4IIE, which is associated with the overexpression of gluconeogenic genes, including PEPCK and G-6-Pase. Utilizing chromatin immunoprecipitaiton (ChIP) combined with massively parallel DNA sequencing (ChIP-Seq),
Norton et al. (26) detected more than 2,000 potential binding events across the genome. They suggested that TCF7L2 may affect fasting and postprandial hyperglycemia in carriers of type 2 diabetes risk SNPs of TCF7L2 (26). This group, however, did not assess whether TCF7L2 expression can be regulated by nutrient availability or a metabolic hormone. In our study, we present direct evidence for the first time that hepatic TCF7L2 expression can be stimulated by feeding in vivo and that insulin is able to stimulate cat/TCF activity by increasing TCF7L2 expression as well as facilitating β-cat Ser675 phosphorylation. We then presented novel evidence of the involvement of both TCF7L2 and the canonical Wnt ligand (Wnt-3a) in repressing gluconeogenesis. Both lithium and Wnt-3a were shown to repress whereas TCF7L2 knockdown was shown to increase gluconeogenic gene expression and glucose output. Taken together, our results led us to suggest that Wnt signaling and TCF7L2 negatively regulate gluconeogenesis and that TCF7L2 and β-cat are downstream effectors of insulin in hepatocytes.

It has been well established that, within the liver lobule, a functional gradient lies along the axis between the portal triad and the central vein (16). On the one hand, periportal hepatocytes, which receive incoming more aerobic blood, are specialized for oxidative functions such as gluconeogenesis and express higher amounts of gluconeogenic genes (16). On the other hand, in pericentral hepatocytes, which typically receive outgoing oxygen-depleted blood, processes that include gluconeogenesis are downregulated. The strong LacZ staining that we have observed in pericentral hepatocytes in the TOPGAL transgenic mice is consistent with a previous report from a different research angle (13). This observation further supports our suggestion that Wnt signaling is a negative regulator of gluconeogenesis. It will be interesting to assess whether the expression of TCF7L2 or Wnt ligands varies across the liver lobule gradient. Since pericentral hepatocytes are also specialized in lipogenesis (16), whether Wnt signaling functions as a positive regulator of lipogenesis deserves a systematic further examination.

In many other cell lineages, cat/TCF has been shown to serve as an effector for a variety of signaling molecules, including insulin, IGF-I, several peptide hormones that use cAMP as a second messenger, and the lipid metabolite LPA (2, 5, 14, 15, 37). Insulin and Wnt inhibit GSK-3 activity via different mechanisms to carry out distinct downstream signaling events such that stimulation by insulin may not result in free β-cat accumulation, whereas stimulation by Wnt does (6).

We show in this study that in hepatocytes insulin is able to stimulate TCF7L2 transcription and activate β-cat Ser675 phosphorylation, which may account for the observed stimulation of Wnt reporter activity by insulin in other systems (5). In addition, feeding substantially increased hepatic TCF7L2 protein level. These observations, along with the fact that insulin is a strong inhibitor of hepatic gluconeogenesis in response to food intake, revealed a novel physiological application of the cross-talk between insulin and the Wnt signaling pathways. The metabolic hormone insulin, in response to food intake, may utilize the Wnt signaling effector cat/TCF7L2 as a mediator in repressing gluconeogenic gene expression and glucose production. Knockdown of TCF7L2, however, did not diminish the repressive effect of insulin on gluconeogenic gene expression, which was assessed by Norton et al. (26) in a rat cell line and by this study in a human cell line (Fig. 7). A potential explanation is that insulin can also repress gluconeogenesis via attenuating the effect of the transcription factor forkhead box O (FoxO), a known positive regulator of gluconeogenesis (23). This effect is presumably independent of the stimulatory effect of insulin on TCF7L2 expression.

The regulation of TCF7L2 expression by insulin has been recognized in several investigations across various tissues. Whereas it has been demonstrated that insulin upregulated TCF7L2 expression in intestinal cells, other studies have shown that insulin can downregulate the expression of TCF7L2 in adipocytes or pancreatic cells (1, 2, 38, 42). These seemingly controversial occurrences could manifest as a result of tissuespecific applications of the cross-talk between insulin and TCF7L2. In intestinal endocrine L cells, insulin stimulates the expression of the proglucagon gene (gcg) and production of the incretin hormone glucagon-like peptide-1 via TCF7L2 and β-cat (42). However, insulin is known to be a potent inducer of adipocyte differentiation, whereas Wnt signaling inhibits adipogenesis (1, 29, 32). Downregulation of TCF7L2 by insulin in adipocytes may mediate the stimulation of adipogenesis by insulin. Indeed, insulin has been shown to up- and downregulate the expression of specific genes in cell type-specific manners. For example, insulin is known to repress pancreatic gcg expression (27) in contrast to its stimulatory effect on gcg expression in the gut (42). Understanding the detailed molecular mechanisms underlying the cell type-specific regulation of TCF7L2 expression by insulin requires further investigation.

Liu et al. (20) found that β-cat depletion led to reduced hepatic glucose production. However, observations made by

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**Fig. 8.** A schematic presentation of the role of Wnt signaling and TCF7L2 in hepatic gluconeogenesis. Feeding upregulates plasma insulin levels, which leads to β-cat Ser675 phosphorylation and TCF7L2 expression. A: increased levels of TCF7L2 may repress gluconeogenesis via competing with forkhead box O (FoxO) for β-cat. B: in addition, cat/TCF7L2, as the effector of the Wnt signaling pathway, may possess an intrinsic repressive effect on gluconeogenesis. P, Ser675 phosphorylation.
Activity via increasing TCF7L2 expression and elevated insulin in response to feeding can also upregulate Wnt expression. The observations made in our current study suggest that phosphorylation and nuclear exclusion of FoxO1, thereby attenuating gluconeogenic gene expression. The observations made in our current study suggest that elevated insulin in response to feeding can also upregulate Wnt activity via increasing TCF7L2 expression and β-cat Ser675 phosphorylation. Increased levels of TCF7L2 may compete with FoxO1 for β-cat and hence, attenuate gluconeogenesis. However, insulin also increases the levels of active β-cat (Ser675; Fig. 4, B–D). This led us to speculate that cat/TCF7L2 may possess an intrinsic repressive effect on gluconeogenic gene expression. This speculation is further supported by our observation that both Wnt-3a and lithium can repress gluconeogenesis, and that TCF7L2 knockdown upregulates signaling in liver zonation and beyond. Development 126: 4557–4568, 1999.

In conclusion, we have obtained evidence that insulin cross-talks with the Wnt signaling pathway via upregulating TCF7L2 expression and β-cat Ser675 phosphorylation in hepatocytes, that the extracellular canonical Wnt ligand Wnt-3a downregulates gluconeogenesis, and that TCF7L2 knockdown upregulates gluconeogenesis in primary hepatocytes. We speculate that insulin may partially utilize the Wnt signaling pathway effector cat/TCF7L2 in negatively regulating gluconeogenesis. How TCF7L2 and Wnt signaling downregulate hepatic gluconeogenesis via modulating the expression of a network of Wnt downstream target genes deserves further investigation.

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