T-Cell Protein Tyrosine Phosphatase Attenuates STAT3 and Insulin Signaling in the Liver to Regulate Gluconeogenesis

Atsushi Fukushima,1 Kim Loh,1 Sandra Galic,1 Barbara Fam,2 Ben Shields,1 Florian Wiede,1 Michel L. Tremblay,3 Matthew J. Watt,4 Sofianos Andrikopoulos,2 and Tony Tiganis1

OBJECTIVE—Insulin-induced phosphatidylinositol 3-kinase (PI3K)/Akt signaling and interleukin-6 (IL-6)-instigated JAK/STAT3-signaling pathways in the liver inhibit the expression of gluconeogenic genes to decrease hepatic glucose output. The insulin receptor (IR) and JAK1 tyrosine kinases and STAT3 can serve as direct substrates for the T-cell protein tyrosine phosphatase (TCPTP). Homozygous TCPTP-deficiency results in perinatal lethality prohibiting any informative assessment of TCPTP’s role in glucose homeostasis. Here we have used Ptpn2+/- mice to investigate TCPTP’s function in glucose homeostasis.

RESEARCH DESIGN AND METHODS—We analyzed insulin sensitivity and gluconeogenesis in chow versus high-fat–fed (HFF) Ptpn2+/- and Ptpn2+/+ mice and insulin and IL-6 signaling and gluconeogenic gene expression in Ptpn2+/- and Ptpn2+/+ hepatocytes.

RESULTS—HFF Ptpn2+/- mice exhibited lower fasted blood glucose and decreased hepatic glucose output as determined in hyperinsulinemic–euglycemic clamps and by the decreased blood glucose levels in pyruvate tolerance tests. The reduced hepatic glucose output coincided with decreased expression of the gluconeogenic genes G6pc and Pck1 and enhanced hepatic STAT3 phosphorylation and PI3K/Akt signaling in the fasted state. Insulin-induced IR-β-subunit Y1162/Y1163 phosphorylation and PI3K/Akt signaling and IL-6-induced STAT3 phosphorylation were also enhanced in isolated Ptpn2+/- hepatocytes. The increased insulin and IL-6 signaling resulted in enhanced suppression of G6pc and Pck1 mRNA.

CONCLUSIONS—Liver TCPTP antagonises both insulin and STAT3 signaling pathways to regulate gluconeogenic gene expression and hepatic glucose output. Diabetes 59:1906–1914, 2010

From the 1Department of Biochemistry and Molecular Biology, Monash University, Victoria, Australia; the 2Department of Medicine, Heidelberg Repatriation Hospital, The University of Melbourne, Victoria, Australia; the 3Department of Biochemistry, McGill University, Montreal, Quebec, Canada; and the 4Department of Physiology, Monash University, Victoria, Australia.

Corresponding author: Tony Tiganis, Tony.Tiganis@med.monash.edu.au.

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Current address for S.G. is St Vincent’s Institute, Victoria 3065, Australia.

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T-type 2 diabetes has reached epidemic proportions, afflicting roughly 170 million people worldwide. Although the underlying genetic causes and the associated pathologic symptoms are heterogenous, a common feature is high blood glucose due to peripheral insulin resistance. Circulating insulin released from β-cells in the pancreas serves to lower blood glucose by triggering the translocation of the facilitative GLUT4 to the plasma membrane in muscle and adipose tissue (1). Insulin also acts in the liver to promote glycogen synthesis and lipogenesis and to suppress hepatic glucose production (HGP) by inhibiting gluconeogenesis and glycogenolysis (1). Elevated HGP caused by defective suppression of gluconeogenesis is one of the primary defects contributing to fasting hyperglycemia in patients with type 2 diabetes (2–4).

Glucose-6-phosphatase (G6Pase; encoded by G6pc) and phosphoenolpyruvate carboxykinase (PEPCK; encoded by Pck1) are key enzymes involved in the rate-limiting steps of gluconeogenesis (1). The overexpression of PEPCK or G6Pase in rodent models results in hyperinsulinaemia, insulin resistance, and glucose intolerance (5–7), and in at least one instance, PEPCK overexpression has been shown to promote weight gain (8). PEPCK catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, whereas G6Pase catalyzes the dephosphorylation of glucose 6-phosphate to free glucose, the final step of both gluconeogenesis and glycogenolysis. The expression of these key gluconeogenic enzymes is controlled by signaling pathways that are activated by insulin, glucagon, and IL-6. Although insulin and IL-6 suppress G6pc and Pck1 expression, glucagon stimulates their expression (1,9–11). Insulin exerts its effects via the PI3K/Akt pathway. Insulin binds to its cell surface receptor to stimulate intrinsic protein tyrosine kinase (PTK) activity, resulting in the phosphorylation of the insulin receptor (IR) and several IR substrates (IRS), such as IRS-1. IRS-1 tyrosine phosphorylation allows for the recruitment of PI3K, which catalyzes the formation of lipid phosphatidylinositol-3,4,5-trisphosphate (PIP3) at the plasma membrane (1). Increases in PIP3 activate several Ser/Thr protein kinases, including Akt, which phosphorylates and prevents the translocation of the transcription factor Foxo1a to the nucleus, where it otherwise functions in concert with peroxisome proliferator-activated receptor γ coactivator 1a (PGC1a) to increase the transcription of the gluconeogenic genes G6pc and Pck1 (1,12,13). Several studies have also implicated signal transducer and activator of transcription-3 (STAT3) in the PGC1a-independent suppression of hepatic gluconeogenic gene expression (11,14). In particular, hypo-
thalamic control of hepatic IL-6 generation and JAK (Janus activated kinase)/STAT3 signaling has emerged as an important mechanism for the regulation of HGP (11,15–17).

Several protein tyrosine phosphatases (PTPs) have been implicated in the modulation of glucose homeostasis in vivo, including the prototypic protein tyrosine phosphatase 1B (PTP1B) (18–22). PTP1B dephosphorylates the IR PTK in liver and muscle to regulate glucose homeostasis (19,18,21,22). PTP1B also dephosphorylates and inactivates the JAK2 PTK in the hypothalamus to antagonize leptin-induced JAK2/STAT3 signaling and thus leptin’s effects on body mass and peripheral insulin sensitivity (20,23). PTP1B dephosphorylates the IR β subunit Y1162/Y1163 autophosphorylation site, which is necessary for IR activation, as well as the Y972 site that contributes to IRS-1 recruitment (24). Muscle or liver-specific PTP1B knockout mice exhibit increased insulin-induced IR Y1162/Y1163 phosphorylation and P3K/Akt signaling and concomitant improved glucose tolerance associated with enhanced glucose uptake and decreased HGP respectively (21,22).

The T-cell protein tyrosine phosphatase (TCPTP) (encoded by Ptpn2) is a ubiquitous tyrosine-specific phosphatase (25). The catalytic domains of PTP1B and TCPTP share a high degree of primary (72% identity) and tertiary structure similarity and have similar active sites. In particular, both PTPs share a second “phosphotyrosine-binding pocket” that allows for the selective recognition of tandem tyrosyl phosphorylated substrates (26,27), such as the IR (24,26,28) and JAK PTKs (JAK1–3 and TYK2) (29). Despite their similarity, TCPTP and PTP1B exhibit a high degree of substrate selectivity and cooperativity in a cellular context. PTP1B can dephosphorylate JAK2, but not JAK1/3, whereas TCPTP dephosphorylates JAK1/3, but not JAK2 (23,29). Moreover, using cell-based approaches, we previously identified the IR as a bona fide substrate for TCPTP (24,28). We reported that PTP1B and TCPTP could act in concert to regulate IR-β Y1162/Y1163 and Y972 phosphorylation and P3K/Akt signaling (24). Additional substrates for TCPTP include STAT family members such as STAT3 (25,30,31). Despite TCPTP’s potential to regulate IR and JAK/STAT3 signaling, it remains unclear whether TCPTP regulates glucose homeostasis in vivo. This is due to the morbidity and lethality that is associated with a global deficiency in TCPTP (32); Ptpn2+/− mice develop inflammatory disease and hematopoietic defects and succumb at 2–3 weeks of age due to a bone marrow stromal cell defect (32,33). In this study we have explored the potential of TCPTP to regulate glucose homeostasis in Ptpn2+/− mice that have a normal life expectancy and an unaltered inflammatory response (32,33). Our studies point toward TCPTP acting as an integral negative regulator of gluconeogenesis and fasting blood glucose.

**RESULTS**

Mice. Mice were maintained on a 12-h light-dark cycle with free access to food and water. Age- and sex-matched mice were used for all experiments. Ptpn2+/− mice on a 129sv x BALB/c mixed background (32) were backcrossed onto BALB/c background for six generations and genotyped as described previously (32). Mice were fed a standard chow diet (10% protein, 4.0% fat, and 4.8% crude fiber; Specialty Feeds, Australia) or a high-fat diet (19% protein, 60% fat, and 4.7% crude fiber; Specialty Feeds, Australia) as indicated.

**Metabolic measurements.** Insulin tolerance tests and pyruvate or glucose tolerance tests were performed on 4- and 6-ha fasted mice, respectively, by injecting human insulin (0.75–1.5 mU/kg body weight), n-glucose (1–2 mg/g body weight), or pyruvate (1–2 mg/g body weight) intraperitoneally and measuring glucose in tail blood as described previously (34). Euglycemic hyperinsulinemic clamps were performed on overnight-fasted and anesthetized mice as described previously (34). Fed and fasted blood glucose and corresponding plasma insulin levels were determined as described previously (34).

**Gene expression.** The generation and culture conditions of control HeLa cells and those expressing TCPTP-specific shRNA have been described previously (31). Hepatocytes from 8- to 12-week-old Ptpn2−/− and Ptpn2+/+ mice were isolated by a two-step collagenase A (0.05% wt/vol; Roche Diagnostics, Germany) perfusion as described previously (34). Hepatocytes were cultured in M199 medium (Invitrogen, Carlsbad, CA) containing 10% (vol/vol) heat-inactivated FBS, 100 units/ml penicillin, 100 μg/ml streptomycin, 10 mmol/l Hepes, 50 mmol/l Glucose (Hyclone, Logan, UT), and 10% N2 for no more than 3 days. Cells were starved in M199 medium alone for 4 h, and then stimulated with 10 mmol/l insulin or 1 ng/ml IL-6, as indicated.

**Biochemical analyses.** Tissues were mechanically homogenized in ice cold RIPA lysis buffer (50 mmol/l Hepes [pH 7.4], 1% (vol/vol) Triton X-100, 1% (vol/vol) sodium deoxycholate, 0.1% (vol/vol) SDS, 150 mmol/l NaCl, 1% (vol/vol) glycerol, 1.5 mmol/l MgCl2, 1 mmol/l EDTA, 50 mmol/l sodium fluoride, 50 mmol/l leupeptin (5 μg/ml), pepstatin A (1 μg/ml), leupeptin (2 mmol/l phenylmethylsulfonyl fluoride, 1 mmol/l sodium vanadate) and clarified by centrifugation (100,000g for 20 min at 4°C). Tissue and cell lysates were resolved by SDS-PAGE and immunoblotted. Lipid analyses were performed as described previously (34).

**RT-PCR.** Liver was dissected and immediately frozen in liquid N2, and RNA extracted using Trizol reagent (Invitrogen, Carlsbad, CA). mRNA was reverse transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and quantitative RT-PCR performed using the TaqMan Universal PCR Master Mix and Gene Expression Assays (Applied Biosystems) for G6pc, Pck1, Prip1, Sreh1, Fasn, and Igf1; Gapdh or 18S was used as internal controls. Reactions were performed in quadruplicate and relative quantification achieved using the DDCt method.

**RESEARCH DESIGN AND METHODS**

**Antibodies and reagents.** JAK PTK inhibitor CMP6 (2-tert-butyl-6-fluoro-3,6-dihydro-7H-benzo[h]imidazole), IL-6 was from Calbiochem (San Diego, CA), and desamethasone and insulin from Sigma-Aldrich (St Louis, MO). Rabbit α-phospho-Akt-S473, α-phospho-STAT3-Y705, α-Akt and α-STAT3 were from Cell Signaling (Beverly, MA); α-actin (sc-1616) was from Santa Cruz Biotechnology (Santa Cruz, CA); rabbit α-phospho-IRβ-Y1162/Y1163, α-phospho-IRβ-Y972 and α-phospho-Akt-Y1102/1183 from Biosource International (Camarillo, CA); mouse α-IRβ (Ab-5) and α-actin were from Thermo Scientific (Fremont, CA), and mouse α-tubulin from Sigma-Aldrich (St Louis, MO). The mouse IL-6 ELISA kit was from eBioscences (San Diego, CA), and recombinant human and murine IL-6 from PeproTech (Rocky Hill, NJ).
Therefore, these results indicate that a reduction in TCPTP protein may be sufficient to prevent the fasting hyperglycemia that is associated with high-fat feeding-induced insulin resistance.

TCPTP is expressed in liver, white adipose tissue (WAT), and skeletal muscle (Fig. 2A), the key insulin responsive tissues responsible for the control of glucose homeostasis. TCPTP protein levels were not overtly altered in liver, WAT, or muscle in HFF (data not shown) or Ob/Ob obese mice (supplementary Fig. 2). The liver is the primary tissue responsible for the control of blood glucose levels in the fasted state, generating glucose from noncarbohydrate sources in a process known as gluconeogenesis during periods of fasting, starvation, or intense exercise (35). Fasting hyperglycemia in type 2 diabetes is linked to elevated gluconeogenesis and HGP (2–4). One possibility is that the lower fasted blood glucose levels in the HFF Ptpn2+/− mice may be caused by decreased gluconeogenesis. To assess this, we performed pyruvate tolerance tests (PTTs); administration of the gluconeogenic substrate pyruvate increases blood glucose levels by promoting gluconeogenesis in the liver. Administration of pyruvate (1 mg/g body weight) significantly enhanced blood glucose levels in Ptpn2+/+ mice, but this was attenuated in HFF Ptpn2+/− mice (Fig. 2B), indicating reduced gluconeogenesis; no differences were noted in PTTs in chow-fed mice (supplementary Fig. 3A). To further characterize the apparently reduced gluconeogenesis in HFF mice, whole-body glucose disappearance and production were measured in HFF Ptpn2+/− versus +/+ mice by performing hyperinsulinemic euglycemic clamps (Fig. 2C). The rate at which glucose was infused to maintain euglycemia during the clamps was increased by ~30% in Ptpn2+/− mice (Fig. 2C), indicative of enhanced insulin sensitivity. Although glucose disappearance (mainly in muscle and fat) remained unaltered, the ability of insulin to suppress whole-body (mainly hepatic) glucose production was increased in Ptpn2+/− mice (Fig. 2C). Taken together, these results indicate that insulin sensitivity was increased in HFF Ptpn2+/− mice and that this was ascribed to decreased HGP.

**Decreased gluconeogenic and increased lipogenic gene expression in Ptpn2+/− mice.** To further assess the potential of TCPTP to regulate hepatic gluconeogenesis, we examined the expression of the rate-limiting gluconeogenic genes G6pc and Pck1 in livers from fasted HFF Ptpn2+/− mice and from those subjected to clamps by quantitative RT-PCR (ΔΔCt) using Gapdh (Fig. 3) or 18S (data not shown) for normalization. We also measured the expression of genes encoding the lipogenic enzymes SREBP-1c (sterol regulatory element-binding protein 1c; encoded by Srebf1) and Fas (fatty acid synthase; encoded by Fasn) that are normally increased in expression in response to insulin (1). We found that G6pc and Pck1 were reduced in both fasted (Fig. 3A) and clamped HFF Ptpn2+/− mice (Fig. 3B), whereas Fasn and Srebf1 were increased in clamped (Fig. 3D), but not fasted mice (Fig. 3C); hepatic G6pc and Pck1 were not altered in chow-fed Ptpn2+/− versus +/+ mice (supplementary Fig. 3B). Given the increased lipogenic gene expression in clamped HFF Ptpn2+/− mice, we monitored for hepatic steatosis by histologic means and by measuring ceramide, diglyceride (DAG), and triglyceride (TAG) levels in HFF Ptpn2+/− versus Ptpn2+/+ mice. Histologically, steatosis appeared to be decreased in HFF Ptpn2+/− mice (Fig. 3E), and this coincided with a trend for reduced hepatic

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FIG. 1. Decreased fasting hyperglycemia in HFF Ptpn2+/− mice. Eight to 10-week-old Ptpn2+/− and +/+ littermate male mice were fed a high-fat diet (60% fat) for 15 weeks and (A) body weights, daily food intake, and the indicated tissue weights determined. (B) Fed and fasted (6 h) blood glucose and fasted plasma insulin levels were measured. Mice were fasted for (C) 6 h and GTTs performed, or (D) for 4 h and ITTs performed. Results shown are means ± SE; **P < 0.01 by a two-tailed Student t test.
ceramides, TAGs, and significantly reduced DAGs (Fig. 3F), consistent with the overall enhanced insulin sensitivity evident in hyperinsulinemic euglycemic clamps. Taken together, these results indicate that hepatic insulin signal-
ing was enhanced, in line with repressed gluconeogenesis and HGP in HFP Ptpn2+/− mice.

Enhanced hepatic STAT3 phosphorylation and PI3K/Akt signaling Ptpn2+/− mice. Next we examined the molecular basis for the decreased fasting blood glucose levels and decreased gluconeogenic gene expression and HGP in HFP Ptpn2+/− mice. We reported previously that TCPTP can dephosphorylate the IR PTK to suppress PI3K/Akt signaling and IL-6-induced STAT3 pathways suppress gluconeogenic gene expression and HGP (1,10,11). Accordingly, we assessed the activation of these pathways in the livers of 4-h fasted HFP Ptpn2+/− versus +/+ mice by immunoblot analysis. We found that STAT3 Y705 phosphorylation
was significantly enhanced in livers from fasted *Ptpn2*+/− mice (Fig. 4A). Importantly, IL-6 in blood or liver was not altered in HFF *Ptpn2*+/− mice (Fig. 4B and C). We also noted that PI3K/Akt signaling, as monitored by Akt Ser-473 phosphorylation, was elevated in livers from fasted HFF *Ptpn2*+/− mice, and this coincided with a trend for elevated IR-β subunit Y1162/Y1163 phosphorylation (Fig. 4A; supplementary Fig. 4A) and IRS-1 tyrosine phosphorylation (supplementary Fig. 4B). There were no significant increases in STAT3 or Akt phosphorylation in muscle or WAT from HFF *Ptpn2*+/− versus +/- mice (supplementary Fig. 4C). Moreover, neither STAT3 phosphorylation nor PI3K/Akt were elevated in the livers of fasted Chow-fed *Ptpn2*+/− mice (supplementary Fig. 3C). Interestingly, although hepatic insulin signaling in fasted HFF *Ptpn2*+/− mice appeared to be elevated, we found no significant difference in IR and IRS-1/2 phosphorylation or PI3K/Akt signaling in response to bolus insulin (2 mU/g, 10 min) administration (supplementary Fig. 4A–B), indicating that TCPTP heterozygous deficiency does not alter the acute response to insulin. To further assess the impact of TCPTP heterozygous deficiency on insulin signaling, we monitored for hepatic Akt Ser-473 phosphorylation in overnight-fasted (8 h) and refed (4 h), and thereon refasted (4 h) HFF *Ptpn2*+/− mice. Although we noted no overt difference in PI3K/Akt signaling in +/- versus *Ptpn2*+/− mice after refeeding, Akt Ser-473 phosphorylation was significantly enhanced in HFF *Ptpn2*+/− mice that were refed and subsequently refasted (Fig. 4D), consistent with TCPTP heterozygous deficiency prolonging the insulin signal; convincing increases in IR-β subunit Y1162/Y1163 phosphorylation in either +/- or +/- mice after fasting and refeeding could not be detected with the reagents at hand (data not shown). Nevertheless, these results are consistent with TCPTP-deficiency enhancing insulin signaling.

**Enhanced insulin and IL-6 signaling and decreased gluconeogenic gene expression in *Ptpn2*+/− hepatocytes.** Our results suggest that the lower fasted blood glucose levels and the decreased gluconeogenic gene expression and HGP in HFF *Ptpn2*+/− mice might result from elevated basal PI3K/Akt and STAT3 signaling. Although the liver is comprised primarily of hepatocytes, we cannot formally exclude the possibility that the elevated STAT3 phosphorylation may be attributed to altered hepatic cellularity. To determine whether the enhanced STAT3 phosphorylation was intrinsic to hepatocytes and to further assess TCPTP’s potential to regulate hepatic IR activation and signaling, we isolated hepatocytes from *Ptpn2*+/− versus +/- mice, and stimulated them either with insulin or IL-6 (Fig. 5). Basal and insulin-induced IR-β Y1161/Y1163 phosphorylation and downstream Akt Ser-473 phosphorylation were significantly enhanced in HFF *Ptpn2*+/− mice (Fig. 5A). Furthermore, IL-6-induced STAT3 phosphorylation was enhanced, but the activation of the upstream JAK1 (Y1022/Y1023) PTK was not altered (Fig. 5B). Basal and insulin-induced IR-β Y1162/Y1163 phosphorylation and downstream Akt Ser-473 phosphorylation were significantly increased in HFF *Ptpn2*+/− mice (Fig. 5), consistent with TCPTP acting directly on STAT3. Although we have previously established that TCPTP deficiency is associated with elevated IR phosphorylation and signaling in mouse embryo fibroblasts (MEFs) and HepG2 hepatoma cells (24,28,36), the impact of TCPTP deficiency on IL-6 signaling has not been previously examined. To establish an independent model by which to examine the role of TCPTP in IL-6 signaling, we stably knocked down TCPTP by RNA interference in HeLa cells (31). Knockdown of TCPTP resulted in enhanced IL-6–induced STAT3 phosphorylation (Fig. 5C). Taken together, these results affirm the capacity of TCPTP to negatively regulate STAT3 signaling, including that mediated by IL-6, which in hepatocytes contributes to the suppression of gluconeogenesis.

Next we assessed the impact of elevated insulin-instigated IR phosphorylation and PI3K/Akt signaling and...
IL-6–induced STAT3 signaling on the expression of gluconeogenic genes by quantitative RT-PCR. We found that the elevated basal IR/PI3K/Akt signaling in serum-starved hepatocytes coincided with decreased G6pc and Pck1 expression that could be further suppressed by insulin (Fig. 6A). IL-6 also suppressed G6pc and Pck1 expression (Fig. 6B), and this could be prevented by pretreating cells with the JAK PTK inhibitor CMP6 (Fig. 6C). Pretreating serum-starved hepatocytes with CMP6 did not revert the already reduced G6pc and Pck1 expression to that seen in +/+ cells (data not shown), indicating that the decreased basal gluconeogenic gene expression was independent of the JAK/STAT pathway and most likely attributable to elevated basal IR signaling. These results are consistent with TCPTP heterozygous deficiency promoting both IR and STAT3 signaling in hepatocytes to suppress gluconeogenic gene expression.

**DISCUSSION**

An increased rate of hepatic gluconeogenesis is primarily responsible for the enhanced HGP and fasting hyperglycemia.
mia that is characteristic of patients with type 2 diabetes (2–4). The regulation of gluconeogenesis is dependent largely on the control of PEPCK and G6Pase expression. Although the absolute levels of HGP are only moderately increased in the diabetic state, PEPCK, G6Pase, and HGP are inadequately suppressed by glucose and insulin (2–4). In this study, we have identified TCPTP as a novel regulator of G6pc and Pck1 expression and HGP. Our studies indicate that a heterozygous deficiency in TCPTP in the liver may be sufficient to lower G6pc and Pck1 expression and consequently lower HGP and ameliorate the fasting hyperglycemia that is associated with high-fat feeding and the development of insulin resistance.

TCPTP’s primary metabolic function may be in the regulation of glucose production since whole-body glucose production and gluconeogenesis, as assessed in hyperinsulinemic euglycemic clamp and pyruvate tolerance tests, respectively, were reduced in fasted-HFF Ptpn2+/− mice, whereas glucose disappearance, a measure of glucose uptake by muscle, remained unaltered. Furthermore, we found no difference in IR signaling in muscle or adipose tissue, and we see no overt difference in insulin signaling in adipocytes differentiated from Ptpn2+/− versus +/+ mouse embryo fibroblasts (Deng and Tiganis, unpublished observations). The liver is the primary tissue responsible for whole-body glucose production, with the kidney playing a smaller role (37). Although we cannot formally exclude the possibility that TCPTP may have a role in the kidney, several lines of evidence support the theory that the liver is an important site of action for TCPTP in the control of blood glucose. First, the STAT3 and PI3K/Akt signaling pathways that suppress gluconeogenesis were enhanced in the livers of fasted Ptpn2+/− mice. Second, this coincided with decreased hepatic gluconeogenic gene expression. And third, insulin and IL-6–induced signaling were increased and downstream gluconeogenic gene expression decreased in Ptpn2+/− hepatocytes. Although our analyses of 1) IR phosphorylation and PI3K/Akt signaling in fasted livers, 2) hepatic lipogenic gene expression in clamped mice, and 3) insulin signaling in isolated hepatocytes all indicate that TCPTP has the capacity to regulate insulin sensitivity, surprisingly, we found that insulin-induced IR phosphorylation and downstream PI3K/Akt signaling in response to bolus insulin administration were not overtly altered in Ptpn2+/− livers. Previously we reported that TCPTP serves to control the duration, rather than the intensity, of IR Y1162/Y1163 phosphorylation and downstream PTP1B/Akt signaling, so that TCPTP-deficient fibroblasts exhibit prolonged, but not enhanced, insulin signaling (24). Therefore, one possibility is that TCPTP heterozygosity may result in prolonged insulin signaling in vivo. This would be evident in the livers of fasted mice, or after clamping, but not after the short periods of acute stimulation used to assess IR activation and signaling. Consistent with this possibility, we found that PTP1B/Akt signaling remained significantly elevated in HFF Ptpn2+/− mice that were fasted, refeed, and fasted once more.

Recent studies have shown that IRS-1 and IRS-2 can differentially contribute to the regulation of hepatic metabolism, with IRS-1 being more closely linked to glucose metabolism, and IRS-2 to lipid metabolism in the fasted state (38,39). In our studies, hepatic IRS-1, but not IRS-2 tyrosine phosphorylation, trended higher in fasted-HFF Ptpn2+/− mice in tune with the increased Akt phosphorylation and the trend for elevated IR Y1162/Y1163 phosphorylation. Although we cannot formally exclude any possible increase in basal IRS-1 tyrosine phosphorylation contributing to the selective suppression of gluconeogenesis in the fasted state, we suggest that G6pc and Pck1 may be primarily suppressed by the hyperphosphorylated STAT3, since further repression of G6pc and Pck1 expression was not evident under conditions of hyperinsulinemia when Fasn and Srebf1 were otherwise induced. Previous studies have established the capacity of TCPTP to dephosphorylate STAT3 (25,30), whereas our studies demonstrate that TCPTP deficiency specifically enhances IL-6–induced STAT3 signaling in hepatocytes and HeLa cells. Several lines of evidence support the contribution of STAT3 to the control of gluconeogenesis. Liver-specific STAT3 knockout mice exhibit insulin resistance and elevated blood glucose levels that are associated with increased hepatic expression of G6pc and Pck1, whereas STAT3 overexpression in lean or obese mice decreases gluconeogenic gene expression and lowers blood glucose levels (11,40). STAT3 is tyrosyl (Y705) phosphorylated and activated by JAK PTKs downstream of all cytokines that act via the gp130 receptor, including IL-6. It is known that insulin signaling in AgRP neurons in the hypothalamus promotes IL-6 release from Kupffer cells in the liver that activates STAT3 in hepatocytes and thus suppresses gluconeogenesis and HGP (11,15–17). In our studies, we found that hepatic IL-6 levels in HFF Ptpn2+/− mice were not altered. In addition, food intake and body weight, which are also suppressed by central insulin action (41,42), were not altered in HFF Ptpn2+/− mice. Thus, the impact of TCPTP heterozygous deficiency on HGP is most likely attributable to the regulation of STAT3 phosphorylation in the liver, rather than the central control of insulin signaling. Recently, STAT3 in hepatocytes has also been shown to be controlled by sirtuin-1–mediated deacetylation (43). Sirtuin-1 is a NAD+ dependent deacetylase that is activated in response to fasting and caloric restriction (44). In the liver, sirtuin-1 activates the stimulatory effects of Foxo1 and PGC-1α on gluconeogenesis, while repressing the inhibitory effects of STAT3 (45,46). In particular, STAT3 deacetylation by sirtuin-1 coincides with STAT3 dephosphorylation (43). Previous studies have shown that STAT1 dephosphorylation by TCPTP can be regulated by STAT1 acetylation (47). It remains unknown whether changes in STAT3 acetylation affect its dephosphorylation status by TCPTP.

Previous studies have identified PTP1B as an important regulator of hepatic IR signaling and HGP, and these effects have been linked to the regulation of IR-β subunit Y1162/Y1163 phosphorylation (22). Interestingly, although liver-specific PTP1B knockout mice exhibited decreased gluconeogenic gene expression and HGP, fasted blood glucose levels were not overtly altered in liver-specific PTP1B heterozygous mice (22), as seen in TCPTP heterozygous mice. Thus, despite the high degree of similarity between the catalytic domains of PTP1B and TCPTP, it appears that the two PTPs may differentially contribute to the regulation of gluconeogenesis. We surmise that this may be attributable, at least in part, to the capacity of TCPTP to also regulate IL-6 signaling. Furthermore, despite the enhanced IR activation, liver-specific PTP1B knockout mice had diminished SREBP and Fas expression in the fed state, and decreased hepatic and serum triglyceride and cholesterol levels (22), consistent with the theory that PTP1B regulates additional, insulin-independent pathways pertinent to the control of lipogenesis. In
expression of lipogenic genes, steatosis was not evident in HFF Ptpm2+/− mice. Srebf1 and Fasn were not altered under fasted conditions and increased after clamps, consistent with the idea that TCPTP deficiency enhances insulin sensitivity. Despite the increased insulin-induced expression of lipogenic genes, steatosis was not evident in HFF Ptpm2+/− mice, but rather decreased, which is consistent with the low hepatic lipid levels observed in insulin-sensitive phenotypes.

PTP1B’s role in IR and leptin signaling has led to considerable attention being placed on PTP1B as a target for development of novel therapies for the treatment of both type 2 diabetes and obesity. Antisense oligonucleotides targeting PTP1B are in clinical trials, whereas drugs that inhibit PTP1B activity are in preclinical development (48–50). The lethality that is associated with TCPTP-deficiency (32) has meant that specific attention has been placed on generating PTP1B inhibitors that do not inhibit TCPTP. However, our studies suggest that the partial inhibition of TCPTP in the liver may be beneficial and contribute to the suppression of fasting hyperglycemia that is associated with high-fat-diet–induced insulin resistance, by enhancing not only IR-dependent, but also IR-independent STAT3-mediated pathways that may be particularly pertinent under conditions of severe insulin resistance. Therefore, we conclude that partial inhibition of TCPTP in the liver, either alone, or in the context of PTP1B inhibition, might be effective for the suppression of gluconeogenesis and the attenuation of fasting hyperglycemia in type 2 diabetes and obesity.

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