TCPTP attenuates STAT3 and insulin signaling in the liver to regulate gluconeogenesis

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Running title: TCPTP regulates gluconeogenesis

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Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org

Submitted 14 September 2009 and accepted 4 May 2010.

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**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 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 analysed insulin sensitivity and gluconeogenesis in chow versus high fat fed Ptpn2+/− and Ptpn2+/+ mice and insulin and IL-6 signaling and gluconeogenic gene expression in Ptpn2+/− and Ptpn2+/+ hepatocytes.

**Results** – High fat fed Ptpn2+/− mice exhibited lower fasted blood glucose and decreased hepatic glucose output as determined in hyperinsulinaemic euglycaemic 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 b–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.
Type 2 diabetes mellitus has reached epidemic proportions afflicting roughly 170 million people worldwide. Although the underlying genetic causes and the associated pathological symptoms are heterogeneous, 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 glucose transporter 4 (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 due to defective suppression of gluconeogenesis is one of the primary defects contributing to fasting hyperglycaemia in 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 hyperinsulinemia, insulin resistance and glucose intolerance (5-7) and in at least one instance, PEPCK overexpression has been shown to promote weight gain (8). PEPCK catalyses the conversion of oxaloacetate to phosphoenolpyruvate, while G6Pase catalyses 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; whereas 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 IR and several IR substrates (IRS) such as IRS-1. IRS-1 tyrosine phosphorylation allows for the recruitment of PI3K which catalyses the formation of lipid phosphatidylinositol (3,4,5)-triphosphate (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, hypothalamic control of hepatic IL-6 generation and JAK (Janus activated kinase)/STAT3 signalling 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 PTP1B (18-22). PTP1B dephosphorylates the IR PTK in liver and muscle to regulate glucose homeostasis (18; 19; 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 b-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...
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**RESEARCH DESIGN AND METHODS**

*Antibodies and reagents.* JAK PTK inhibitor CMP6 (2-tert-butyl-9-fluoro-3,6-dihydro-7Hbenz[h]-imidaz[4,5-f]isoquin-olone-7-one) was from Calbiochem (San Diego, CA) and dexamethasone and insulin from Sigma-Aldrich (St Louis, MO). Rabbit a-phospho-Akt-S473, a-phospho-STAT3-Y705, a-Akt and a-STAT3 were from Cell Signaling (Beverly, MA); a-actin (sc-1616) was from Santa Cruz Biotechnology (Santa Cruz, CA); rabbit a-phospho-IRb-Y1162/Y1163/a-phospho-IRb-Y972 and a-phospho-JAK1-Y1022/Y1023 from Biosource International (Camarillo, CA); mouse a-IRb (Ab-5) and a-actin were from Thermo Scientific (Fremont, CA) and mouse a-tubulin from Sigma-Aldrich (St Louis, MO). The mouse IL-6 ELISA kit was from eBiosciences (San Diego, CA) and recombinant human and murine IL-6 from PeproTech (Rocky Hill, NJ).

*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 6 generations and genotyped as described previously (32). Mice were fed a standard chow (19% protein, 4.6% fat and 4.8% crude fibre; Specialty Feeds, Australia) or a high fat diet (19% protein, 60% fat and 4.7% crude fibre; Specialty Feeds, Australia) as indicated.

*Metabolic measurements.* Insulin tolerance tests and pyruvate or glucose tolerance tests were performed on 4 and 6 h fasted mice respectively by injecting human insulin (0.75-1.5 mU/g body weight), D-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). Euglycaemic hyperinsulinaemic clamps were performed on overnight fasted and
anaesthetized mice as described previously (34). Fed and fasted blood glucose and corresponding plasma insulin levels were determined as described previously (34).

**Cell culture.** The generation and culture conditions of control HeLa cells and those expressing TCPTP-specific shRNA have been described previously (31). Hepatocytes from 8-12 week old Ptpn2+/– and Ptpn2+/+ mice were isolated by a two step collagenase A (0.05% w/v; Roche Diagnostics, Germany) perfusion as described previously (34). Hepatocytes were cultured in M199 medium (Invitrogen, Carlsbad, CA) containing 10% (v/v) heat inactivated FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, 10 nM dexamethasone, 50 nM insulin and 20 ng/ml EGF (R&D Systems, Minneapolis, MN) for no more than 3 days. Cells were starved in M199 medium alone for 4 h and then stimulated with 10 nM insulin or 1ng/ml IL-6 as indicated.

**Biochemical analyses.** Tissues were mechanically homogenised in ice cold RIPA lysis buffer (50 mM Heps [pH 7.4], 1% (v/v) Triton X-100, 1% (v/v) sodium deoxycholate, 0.1% (v/v) SDS, 150 mM NaCl, 10% (v/v) glycerol, 1.5 mM MgCl2, 1 mM EGTA, 50 mM sodium fluoride, leupeptin (5 µg/ml), pepstatin A (1 µg/ml), 1 mM benzamadine, 2 mM phenylmethlysulfonyl fluoride, 1 mM sodium vanadate) and clarified by centrifugation (100,000 x g 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).

**Real time PCR.** Liver was dissected and immediately frozen in liquid N2 and RNA extracted using Trizol reagent (Invitrogen, Carlsbad, CA). mRNA was reversed transcribed using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and quantitative real-time PCR performed using the TaqMan™ Universal PCR Master Mix and Gene Expression Assays (Applied Biosystems) for G6pc, Pck1, Fbp1, Srebf1, Fasn and Il6; Gapdh or 18S were used as internal controls. Reactions were performed in quadruplicate and relative quantification achieved using the DDCt method.

**RESULTS**

**Decreased gluconeogenesis and hepatic glucose production in Ptpn2+/– mice.** Ptpn2+/– (BALB/c) mice are healthy and fertile and do not show any overt histopathologies (32). To assess the impact of TCPTP heterozygous deficiency on glucose homeostasis, 8-10 week old Ptpn2+/– versus +/+ littermate male mice were fed a standard chow diet for 20 weeks or a high fat diet (60% fat; 74% energy from fat) for 15 weeks to induce insulin resistance and fasting hyperglycaemia (Fig. 1; Supp Fig. 1 which is available in the online appendix at http://diabetes.diabetesjournals.org). Food intake (high fat diet) and body and tissue weights were determined and insulin sensitivity and glucose homeostasis assessed in insulin tolerance tests (ITTs) and glucose tolerance tests (GTTs) and by monitoring blood glucose and insulin levels. Food intake (high fat diet), body weights and liver and fad pad masses remained unaltered in +/+ versus +/- mice on either diet (Fig. 1a; Supp Fig. 1a). Similarly, no significant differences were noted in ITTs or GTTs (Fig. 1c-d; Supp Fig. 1c-d). However, fasted blood insulin levels were significantly reduced in chow fed mice and this trended with reduced fasted blood glucose (Supp Fig. 1b). More importantly, fasted blood glucose were significantly reduced in high fat fed (HFF) Ptpn2+/– versus Ptpn2+/+ mice (Fig. 1b), approximating those seen in fasted chow fed +/- mice. Therefore, these results indicate that a reduction in TCPTP protein may be sufficient to prevent the fasting hyperglycaemia 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 are not overtly altered in liver, WAT, or muscle in HFF (data not shown) or Ob/Ob obese mice (Supp Fig. 2). The liver is the primary tissue responsible for the control of blood glucose levels in the fasted state generating glucose from non-carbohydrate sources in a process known as gluconeogenesis during periods of fasting, starvation or intense exercise (35). Fasting hyperglycaemia 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 due to 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 HFF 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 (Supp. Fig. 3a). To further characterise the apparent reduced gluconeogenesis in HFF mice, whole body glucose disappearance and production were measured in HFF Ptpn2+/– versus +/+ mice by performing hyperinsulinaemic euglycaemic clamps (Fig. 2c). The rate at which glucose was infused to maintain euglycaemia during the clamps was increased by approximately 30% in Ptpn2+/– mice (Fig. 2c), indicative of enhanced insulin sensitivity. While 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 TCPTP’s potential 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 real time PCR (DDCt) using Gapdh (Fig. 3) or 18S (data not shown) for normalisation. 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 (Supp. Fig. 3b). Given the increased lipogenic gene expression in clamped HFF Ptpn2+/– mice, we monitored for hepatic steatosis by histological 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 ceramides, TAGs and significantly reduced DAGs (Fig. 3f), consistent with the overall enhanced insulin sensitivity evident in hyperinsulinaemic euglycaemic clamps. Taken together these results indicate that hepatic insulin signaling was enhanced, in line with repressed gluconeogenesis and HGP in HFF 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
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decreased gluconeogenic gene expression and HGP in HFF \textit{Ptpn2}+/– mice. We have reported previously that TCPTP can dephosphorylate the IR PTK to suppress insulin signaling (24; 28; 36), whereas others have used overexpression approaches to identify STAT3 as a putative TCPTP substrate (30). Insulin-instigated 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 HFF \textit{Ptpn2}+/– versus +/+ mice by immunoblot analysis. We found that STAT3 Y705 phosphorylation was significantly enhanced in livers from fasted \textit{Ptpn2}+/– mice (Fig. 4a). Importantly, IL-6 in blood or liver were not altered in HFF \textit{Ptpn2}+/– mice (Fig. 4b-c). We also noted that PI3K/Akt signaling as monitored by Akt Ser-473 phosphorylation was elevated in livers from fasted HFF \textit{Ptpn2}+/– mice and this coincided with a trend for elevated IR b-subunit Y1162/Y1163 phosphorylation (Fig. 4a; Supp. Fig. 4a) and IRS-1 tyrosine phosphorylation (Supp. Fig. 4b). There were no significant increases in STAT3 or Akt phosphorylation in muscle or WAT from HFF \textit{Ptpn2}+/– versus +/+ mice (Supp. Fig. 4c). Moreover, neither STAT3 phosphorylation nor PI3K/Akt were elevated in the livers of fasted chow fed \textit{Ptpn2}+/– mice (Supp. Fig. 3c). Interestingly, although hepatic insulin signalling in fasted HFF \textit{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 (Supp. 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 re-fed (4h) and thereon re-fastened (4 h) HFF \textit{Ptpn2}+/– mice. Although we noted no overt difference in PI3K/Akt signaling in +/+ versus \textit{Ptpn2}+/– mice after re-feeding, Akt Ser-473 phosphorylation was significantly elevated in HFF \textit{Ptpn2}+/– mice that were re-fed and subsequently re-fastened (Fig. 4d), consistent with TCPTP heterozygous deficiency prolonging the insulin signal; convincing increases in IR b-subunit Y1162/Y1163 phosphorylation in either +/+ or +/- mice after fasting and re-feeding 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 \textit{Ptpn2}+/– hepatocytes. Our results suggest that the lower fasted blood glucose levels and the decreased gluconeogenic gene expression and HGP in HFF \textit{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 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 \textit{Ptpn2}+/– versus +/+ mice and stimulated them with either insulin or IL-6 (Fig. 5). Basal and insulin-induced IRb Y1162/Y1163 phosphorylation and downstream Akt Ser-473 phosphorylation were enhanced in +/- versus +/+ hepatocytes (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), consistent with TCPTP acting directly on STAT3. Although we have previously established that TCPTP deficiency is associated with elevated IR phosphorylation and signalling in MEFs and HepG2 hepatoma
cells (24; 28; 36), the impact of TCPTP deficiency on IL-6 signalling has not been previously examined. To establish an independent model by which to examine TCPTP’s role in IL-6 signalling 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 TCPTP’s capacity 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 IL6-induced STAT3 signaling on the expression of gluconeogenic genes by quantitative real time 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 suppressed further by insulin (Fig. 6a). IL-6 also suppressed G6pc and Pck1 expression (Fig. 6b) and this could be prevented by pre-treating cells with the JAK PTK inhibitor CMP6 (Fig. 6c). Pre-treating 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 hyperglycaemia 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 hyperglycaemia 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 hyperinsulinaemic euglycaemic clamps 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 signalling in muscle or adipose tissue and we see no overt difference in insulin signalling 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 liver being 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 was 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 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 have reported that TCPTP serves to control the duration rather than the intensity of IR Y1162/Y1163 phosphorylation and downstream PI3K/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 PI3K/Akt signalling remained significantly elevated in HFF $Ptpn2^{+/–}$ mice that were fasted, re-fed 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 hyperinsulinaemia when Fasn and Srebf1 were otherwise induced. Previous studies have established TCPTP’s capacity 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 signalling. 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-1a on gluconeogenesis, whilst 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 as to whether changes in STAT3 acetylation affect its dephosphorylation status by TCPTP.

Previous studies have identified PTP1B as an important regulator of hepatic insulin receptor signaling and HGP and these effects have been linked to the regulation of IR b-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 TCPTP’s capacity 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 PTP1B regulating additional, insulin-independent pathways pertinent to the control of lipogenesis. In HFF Ptpn2+/– mice, Srebfl and Fasn were not altered under fasted conditions and increased after clamps consistent with TCPTP deficiency enhancing insulin sensitivity. Despite the increased insulin-induced expression of lipogenic genes, steatosis was not evident in HFF Ptpn2+/– 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 focused on PTP1B as a target for development of novel therapeutics for the treatment of both type 2 diabetes and obesity. Anti-sense 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 hyperglycaemia 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 hyperglycaemia in type 2 diabetes and obesity.

Author Contributions: A.F., K.L., S.G., B.F., B.S. and F.W. researched data, M.L.T provided reagents and edited the manuscript, M.J.W. researched data and edited the manuscript, S.A. contributed discussion and edited the manuscript, T.T. directed the research program, researched data and wrote the manuscript.

ACKNOWLEDGMENTS
We thank Christine Yang, Teresa Tiganis, Amy Blair and Jane Honeyman for technical support. This work was supported by the NH&MRC of Australia (to T.T., S.A., M.J.W.). S.A. is a NH&MRC R.D. Wright Fellow and M.J.W and T.T are NH&MRC Senior Research Fellows.
FIGURE LEGENDS

Figure 1. Decreased fasting hyperglycaemia in high fat fed Ptpn2+/– mice. 8-10 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) 4 h and ITTs performed. Results shown are means ± SE; ** p<0.01 by a two-tailed student’s t-test.

Figure 2. Decreased gluconeogenesis and hepatic glucose production in Ptpn2+/– mice. (a) Expression of the 45 kDa (TC45) and 48 kDa (TC48) variants of TCPTP in white adipose tissue (epididymal; WAT), liver, muscle (gastrocnemius) and spleen as well as immortalised Ptpn2−/− and +/+ mouse embryo fibroblasts (MEFs). (b-c) 8-10 week old Ptpn2+/– and +/+ littermate male mice were fed a high fat diet for 15 weeks and (b) pyruvate tolerance tests, or (c) hyperinsulinaemic-euglycaemic clamps. Glucose infusion and disappearance rates were determined and whole body glucose production determined by subtracting the glucose infusion rate from the glucose appearance rate. Results shown are means ± SE; * p<0.05 by a two-tailed student’s t-test.

Figure 3. Altered gluconeogenic and lipogenic gene expression in Ptpn2+/– mice. 8-10 week old Ptpn2+/– and +/+ male mice were fed a high fat diet for 15 weeks. Livers were harvested from (a and c) 4 h fasted mice, or (b and d) at the end of hyperinsulinaemic-euglycaemic clamps and processed for quantitative (ΔΔCt) real time PCR to measure the expression of (a-b) gluconeogenic genes Pck1 and G6pc or (c-d) lipogenic genes Srebf1 and Fasn with Gapdh being used for normalisation; similar results were attained when 18S was used for normalisation. (e) Ptpn2+/– and +/+ male mice were fed a high fat diet for 15 weeks, livers extracted, fixed in formalin, paraffin embedded and processed for histology (hematoxylin and eosin). (f) Mice were fasted for 4 h and livers isolated and triglyceride (TAG), diglyceride (DAG) and ceramide extracted and quantified as described in Methods. Results shown are means ± SE; * p<0.05, ** p<0.01 by a two-tailed student’s t-test.

Figure 4. Increased hepatic STAT3 and PI3K/Akt signaling in fasted Ptpn2+/– mice. 8-10 week old Ptpn2+/– and +/+ male mice were fed a high fat diet for 15 weeks. (a) Livers were harvested from 4 h fasted mice and processed for immunoblot analysis with antibodies to the phosphorylated (Ser-473) and activated Akt (p-Akt), phosphorylated (Y705) STAT3 (p-STAT3) and phosphorylated (Y1162/Y1163) IR b-subunit (p-IR) or the corresponding proteins. Representative blots and quantified results (arbitrary units: AU) are shown (means ± SE); * p<0.05, ** p<0.01 by a two-tailed student’s t-test. (b) Plasma IL-6 levels were determined using an ELISA kit (eBiosciences, San Diego, CA) according to the manufacturers instructions. (c) Livers were harvested from 4 h fasted mice and processed for quantitative (ΔΔCt) real time PCR to measure the expression of Il6. Results shown are means ± SE. (d) Mice were fasted overnight for 8 and at the beginning of the light cycle re-fed for 4 h, or re-fed and re-fasted for 4 h. Livers were harvested from fasted, re-fed and fasted/re-fed and re-fasteds mice and processed for immunoblot analysis as indicated. Representative blots and quantified results are shown (means ± SE); *** p<0.005 by a two-tailed student’s t-test.
TCPTP regulates gluconeogenesis

Figure 5. Increased insulin and IL-6 signaling in Ptpn2+/– hepatocytes. (a-b) Ptpn2+/– versus +/+ hepatocytes were serum starved for 4 h and stimulated with 10 nM insulin or 1 ng/ml IL-6 as indicated and processed for immunoblot analysis. (c) Control HeLa cells or those expressing TCPTP-specific shRNAs were serum starved for 4 h, stimulated with 10 ng/ml IL-6 for 10 min, medium replenished and cells collected at the indicated times for immunoblot analysis. Results shown are representative of three independent experiments.

Figure 6. Increased insulin- and IL-6-induced Pck1 and G6pc suppression in Ptpn2+/– hepatocytes. Ptpn2+/– versus +/+ hepatocytes were serum starved for 4 h ± 2 mM CMP6 (Calbiochem) for the last 1 h and stimulated with (a) 10 nM insulin or (b-c) 1 ng/ml IL-6 for the indicated times and processed for quantitative (ΔΔCt) real time PCR to measure the expression of Pck1 and G6pc. In c, lysates from control and IL-6 (1 h) ± CMP6 treated hepatocytes were also processed for immunoblot analysis. Results shown are means ± SE of three independent experiments performed in quadruplicate.

REFERENCES


TCPTP regulates gluconeogenesis


36. Meng TC, Buckley DA, Galic S, Tiganis T, Tonks NK: Regulation of Insulin Signaling through Reversible Oxidation of the Protein-tyrosine Phosphatases TC45 and PTP1B. *J Biol Chem* 279:37716-37725, 2004


Figure 1

A

B

C

D

TCPTP regulates gluconeogenesis
Figure 2

A

TCPTP regulates gluconeogenesis

B

Pyruvate tolerance test (1 mg/g)

C

Glucose Infusion Rate (umol/min/kg)

Whole Body Glucose Production (umol/min/kg)

Rate of Glucose Disappearance (umol/min/kg)
TCPTP regulates gluconeogenesis

**Figure 4**

A

![Western blots showing p-STAT3 and STAT3 expression](Image)

Liver homogenates from individual mice fasted for 4 h

B

![Graph showing plasma IL-6 levels](Image)

P = 0.18

C

![Graph showing ΔCt values](Image)

P = 0.02

D

![Western blots showing p-Akt and Akt expression](Image)

Liver homogenates from individual mice
Figure 5

A

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<tr>
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| Actin

Hepatocytes

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Hepatocytes

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HeLa cells

TCPTP regulates gluconeogenesis
TCPTP regulates gluconeogenesis

Figure 6

A

![Bar graph showing Pck1 and G6pc expression levels with insulin treatment.](image)

B

![Bar graph showing Pck1 and G6pc expression levels with IL-6 treatment.](image)

C

![Bar graph showing Pck1 expression levels with IL-6 and CMP6 treatments.](image)

(n=3)