CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Hope's Institutional Research Archive

Regulation of growth hormone induced JAK2 and mTOR signalling by hepatic protein tyrosine phosphatase 1B

C. Owen[a], E. K. Lees[a], N. Mody[a] and M. Delibegović[a†].

[a]Institute of Medical Sciences, School of Medical Sciences, University of Aberdeen, Aberdeen, UK.

[†]Corresponding author:

Dr. Mirela Delibegović

Address: Institute of Medical Sciences, School of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK.

Tel: +44 1224 437587

Fax: +44 1224 437465

Email: m.delibegovic@abdn.ac.uk

© 2015. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/

Abstract

Protein tyrosine phosphatase 1B (PTP1B) regulates various signalling pathways including insulin, leptin, IGF-1 and growth hormone (GH) signalling. Transmission of the GH signal depends on JAK2, which is how PTP1B is thought to modulate GH signalling in the liver, based on studies utilising global PTP1B knockout mice (*Ptp1b*$^{-/-}$). Here, we investigated the liver-specific role of PTP1B in GH signalling, using liver-specific *Ptp1b*$^{-/-}$ mice (*alb-crePtp1b*$^{-/-}$), under physiological (chow) or insulin resistant (high-fat diet (HFD)) feeding conditions. Body weight and adiposity were comparable between female *alb-crePtp1b*$^{-/-}$ and *Ptp1b*$^{fl/fl}$ control mice. On chow diet, under 48-hour fasting GH-resistant conditions, GH stimulation *in vivo* led to a robust stimulation of the JAK-STAT signalling pathway. *Alb-crePtp1b*$^{-/-}$ mice exhibited significantly higher GH-induced JAK2 phosphorylation and *SOCS3* gene expression post-GH stimulation. However, STAT3, STAT5 and ERK1/2 phosphorylation and *SOCS2* gene expression were similar between groups. Interestingly, GH-induced mTOR phosphorylation was significantly higher in *alb-crePtp1b*$^{-/-}$ mice 5-min post-GH stimulation compared to controls, revealing this part of the pathway under direct control of PTP1B. Under *ad lib* HFD-fed conditions, GH-induced STAT5 phosphorylation significantly increased in *alb-crePtp1b*$^{-/-}$ mice only, with no alterations in the controls. Overall, our data demonstrate that liver-specific PTP1B deletion leads to significant alterations in GH signalling with increased phosphorylation of JAK2, STAT5 and mTOR phosphorylation and *SOCS3* gene expression.

Keywords: Liver, PTP1B, JAK2, STAT, Growth Hormone.

Introduction

Human growth hormone (hGH) is an important metabolic hormone which has effects on glucose, protein and lipid metabolism [1, 2] Secondary consequences such as increased adiposity and insulin resistance can occur in individuals suffering with abnormal hGH secretion [1, 3]. Thus it is important to understand the regulators of growth hormone signalling *in vivo*.

Initial events in growth hormone signalling include homodimerization of the GH receptor (GHR), recruitment of Janus kinase 2 (JAK2) to the cytoplasmic domain of the receptor, and activation of JAK2 via autophosphorylation [4, 5]. Various signalling proteins are then recruited to high affinity binding sites on tyrosine phosphorylated JAK2 and GHR which leads to the activation of signal transducers and activators of transcription (STATs) 1, 3, and 5b, and several other pathways including Ras-MAP and IRS-1/PI3K/Akt [2, 5].

The mechanisms terminating GHR signalling are less well understood. A study in 2003, identified protein tyrosine phosphatase 1B (PTP1B) as a negative regulator of growth hormone signalling, using livers from globally-deficient PTP1B knockout mice [6]. An absence of PTP1B resulted in GH-dependent hyper-phosphorylation of JAK2 and enhanced activation of STAT3 and STAT5 [6]. However, since this study examined PTP1B deletion in globally-deficient mice, which exhibit decreased body weight under high-fat diet (HFD) feeding conditions, it is unclear whether the effects on GH signalling were specifically caused by PTP1B deletion in the liver.

In order to determine the specific role of liver-PTP1B in GH signalling [6], phosphorylation of the JAK-STAT pathway and downstream signalling targets were assessed in adult, female chow- and HFD-fed liver-specific knockout mice (*alb-crePtp1b*$^{-/-}$), following a hGH stimulation time course under *ad lib*-fed and 48-hour fasted states (to induce GH resistance).

Materials and methods

Ethics statement. All animal procedures were approved by the University of Aberdeen Ethics Review Committee Board and performed under a project license approved by the Home Office under the Animals (Scientific Procedures) Act 1986 (PPL60/3951).

Animal studies. Female *Ptp1b*$^{fl/fl}$ mice and *alb-crePtp1b*$^{-/-}$ mice expressing *Cre* recombinase under the control of the serum albumin promoter were described previously [7]. DNA extraction and genotyping for the *Ptp1b* floxed allele and the presence of *Cre* by PCR were performed as described previously [8]. Mice studied were age-matched littermates, which were generated on a C57BL/6 background. Mice were housed in groups and maintained at 22-24ºC on a 12-h light/dark cycle with free access to food and water. At weaning (~21 days), mice were placed on standard 3.4% fat chow pellet diet (Rat and Mouse Breeder and Grower, Special Diets Services, DBM, Scotland) or HFD (Adjusted Calories Diet, 55 % fat, Harlan Teklad, USA) and weight was recorded every two weeks. The approximate fatty acid profile of Adjusted Calories Diet (% total fat) was 28% saturated, 30% trans, 28% monounsaturated (cis) and 14% polyunsaturated (cis), as described previously [9]. For signalling studies, chow- or HFD-fed female *alb-crePtp1b*$^{-/-}$ and *Ptp1b*$^{fl/fl}$ mice were injected with saline or 1 µg/g body weight hGH. Mice were either fasted for 48-hours (to induce GH resistance) or used *ad lib* fed. Mice were sacrificed by cervical dislocation at 0, 5, 10, 30 and 90 min. Mice in the 0 min group were injected with saline. Tissues were dissected immediately and frozen in liquid nitrogen.

Body composition using dual-energy X-ray absorptiometry (DXA). The DXA instrument used was the Lunar PIXIMUS-Densitometer (GE Medical Systems, USA). DXA scans and analyses were carried out as instructed by the manufacturer. A quality control phantom mouse was scanned daily before samples were scanned. The phantom and samples were mounted on an adhesive disposable plastic tray placed on the imaging surface. Mice were anesthetised with isoflurane (1.5-2.0% with 2 l/min O_2). Mice were then spread out on the

adhesive trays in the prostrate position with limbs extended away from the body. Mouse tails were not measured. As recommended by the manufacturer, a region of interest (ROI) was created to exclude the head from analyses.

Immunoblotting. Tissue lysates were prepared in RIPA buffer containing fresh sodium orthovanadate and protease inhibitors, as described previously [10]. Proteins were separated by 4-12% SDS-PAGE and transferred to nitrocellulose membranes. Immunoblots were performed using antibodies from Cell Signaling (Cell Signaling by NEB, Hitchin, UK) (unless stated otherwise) against PTP1B (Millipore), SHP2 (Santa Cruz), pJAK2 Y1007/1008 (Invitrogen), JAK2, pSTAT3 Y705, STAT3, pSTAT5 Y694/699, STAT5, pERK1/2 MAPK T202/Y204, p-FRAP/p-mTOR S2448 (Santa Cruz), mTOR, pS6 ribosomal protein S235/236, S6 ribosomal protein, Akt/PKB (Santa Cruz) and IRβ (Santa Cruz). Immunoblots were developed using horseradish peroxidase-conjugated secondary antibodies, visualised using enhanced chemiluminescence, and quantified by densitometry scanning with Image J or Bio1D software (PeqLab, Fareham, UK).

Gene expression analysis. Total RNA was isolated from mouse liver using TRI Reagent (Ambion, Warrington UK), according to the manufacturer's protocol. First strand cDNA was synthesized from 1 µg of total RNA employing the Bioline Bioscript™ Pre-amplification System and oligo$(dT)_{12\text{-}18}$. Two (2) µl of diluted cDNA (1:10) was used to amplify target genes by real-time RT-PCR (10 µl), using GoTaq qPCR Master Mix (Promega, Southampton, UK). The Roche LightCycler® 480 System (Roche Diagnostics, Burgess Hill, UK) was used for analysis. Relative gene expression was calculated using the comparative Ct ($2^{-\Delta\Delta Ct}$) method. A geometric mean of three commonly used reference genes; hypoxanthine-guanine phosphoribosyltransferase (*Hprt*), 18S ribosomal RNA (*18S*) and Glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) were used to normalise data. A geometric mean of the relative copy numbers of mouse PCRs were followed by melting curves (70-95°C).

Data analysis. Data are expressed as mean ± SEM and *n* represents the number of mice or biological replicates. Statistical analyses were performed using one-way ANOVA with Tukey's multiple comparison post-tests, repeated measures two-way ANOVA with Bonferroni multiple comparisons post-tests or two-tailed Student's t-tests, as appropriate. The critical alpha level (P) was set at 0.05. $P < 0.05$ was considered statistically significant. GraphPad Prism 5 statistical software was used for analyses.

Results

Liver-PTP1B deletion has no effect on body weight or adiposity. Body weight was comparable between HFD-fed *alb-crePtp1b*$^{-/-}$ and HFD-fed *Ptp1b*$^{fl/fl}$ control female mice throughout the study (Figure 1A), as previously reported in male mice [7]. There was ~50% decrease in PTP1B protein levels in whole liver lysates (Figure 1B). Furthermore, body weight, adiposity and bone mineral density were comparable between chow-fed *alb-crePtp1b*$^{-/-}$ and *Ptp1b*$^{fl/fl}$ control mice (Figure 1C, D and E), as assessed by dual-energy X-ray absorptiometry (DXA).

Liver-PTP1B deletion increases JAK2 and mTOR but not STAT phosphorylation in mice on chow diet in the fasted state.

To assess whether liver-PTP1B deletion attenuated growth hormone-mediated JAK-STAT phosphorylation, chow-fed *alb-crePtp1b*$^{-/-}$ and *Ptp1b*$^{fl/fl}$ control mice, fasted for a period of 48-hours to induce GH resistance, were injected intraperitoneally with hGH for 5 or 10 min.

hGH stimulation significantly increased JAK2 phosphorylation at both 5 and 10 min in both groups (Figure 2A and B). However, JAK2 phosphorylation was significantly higher in *alb-crePtp1b*$^{-/-}$ mice compared to *Ptp1b*$^{fl/fl}$ control mice at 10 min (Figure 2A and B).

While hGH stimulation increased STAT5 phosphorylation at both 5 and 10 min post-GH stimulation, there were no differences between the two groups (Figure 2A and C). STAT3 phosphorylation was also comparable between groups at all time points (Figure 2A and D).

hGH stimulation significantly increased mTOR phosphorylation at 5 and 10 min in both groups, with *alb-crePtp1b*$^{-/-}$ mice displaying significantly higher phosphorylation than *Ptp1b*$^{fl/fl}$ control mice at the 5 min time point (Figure 2A, F and G).

ERK1/2 and S6 phosphorylation was comparable between groups at all time points examined (Figure 2A, E).

Liver-PTP1B deletion increases *SOCS3* gene expression at 30 min post-hGH stimulation following a 48-hour fast.

As expected from previous studies, hGH stimulation significantly increased gene expression of *SOCS2* and *SOCS3* at 90 min.

SOCS3 gene expression was significantly increased in *alb-crePtp1b*$^{-/-}$ at 30-min post-GH stimulation (two-tailed t-test; p=0.047), while at 90-min post-GH stimulation this was no longer significant (two-tailed t-test p=0.07).

There were no significant differences in *SOCS2* expression levels between *alb-crePtp1b*$^{-/-}$ mice *Ptp1b*$^{fl/fl}$ control mice (Figure 3A and B).

Liver-PTP1B deletion results in a small but significant effect on STAT5 phosphorylation in response to growth hormone (GH) stimulation.

To assess the role of liver-PTP1B deficiency in growth hormone signalling under diabetic, insulin-resistant conditions, *alb-crePtp1b*$^{-/-}$ and *Ptp1b*$^{fl/fl}$ control mice were fed HFD for 32 weeks and then injected with hGH for 30 min under *ad lib* fed or 48-hour fasted conditions (to induce GH resistance).

Interestingly, under *ad lib* HFD-fed conditions, hGH-stimulation was not able to significantly increase STAT3 phosphorylation in *alb-crePtp1b*$^{-/-}$ or *Ptp1b*$^{fl/fl}$ control mice (Figure 4B, D) but it did robustly increase STAT3 phosphorylation under 48-hour fasted conditions in control mice (Figure 4F and H).

hGH robustly stimulated STAT5 phosphorylation under chow 48-hour-fasted conditions in both groups of mice. However, under HFD conditions, a significant increase in STAT5 was only observed in *alb-crePtp1b*$^{-/-}$ *ad lib* HFD-fed mice (Figure 4C, D, G and H).

Discussion

PTP1B has previously been shown to attenuate growth hormone (GH)-mediated JAK-STAT signalling in hGH-treated fibroblasts and in livers from male globally-deficient PTP1B knockout mice [6]. In order to determine liver-PTP1B specificity of the previously published data [6], JAK-STAT signalling was assessed in female chow- and HFD-fed liver-specific knockout mice following a hGH stimulation time course under *ad lib* fed and 48-hour fasted states (to induce GH-resistance).

As with other mouse models of PTP1B-specific deletion [7, 10, 11] body weight, body fat and bone mineral density of the female liver-specific PTP1B knockout mice did not differ from control mice. This is the first examination of the body weight phenotype in liver-specific PTP1B knockout animals in females and reveals that there is no sexual dimorphism in body weight regulation in these animals.

In our studies, hGH led to a robust stimulation of the JAK-STAT signalling pathway, as expected. Interestingly however, HFD-feeding resulted in an inability of hGH-stimulation to induce phosphorylation of STAT3 under fed conditions. In agreement with previous studies, we observed higher JAK2 phosphorylation with liver-PTP1B deletion after 10 min of hGH stimulation under chow feeding conditions. PTP1B was previously shown to be recruited to

JAK2 in 293-LA cells in response to GH [6]. While we did not observe effects on STAT3 phosphorylation in liver-PTP1B knockout mice, hGH did robustly stimulate and significantly increase STAT5 phosphorylation under both fed and fasted conditions, in *alb-crePtp1b*$^{-/-}$ mice. STAT5a and STAT5b have been suggested to be direct PTP1B substrates [12].

A previous study observed that the abundance of *SOCS2* mRNA, but not *SOCS3* mRNA, was increased 90 min after hGH stimulation in global *Ptp1b*$^{-/-}$ mouse livers relative to controls. In the present study, hGH led to an increase in both *SOCS2* and *SOCS3* gene expression in both groups of mice after 90 min of hGH stimulation. However, only *SOCS3* expression was significantly higher after 30 min of hGH stimulation in the livers of *alb-crePtp1b*$^{-/-}$ mice compared to *Ptp1b*$^{fl/fl}$ mice, while *SOCS2* remained unchanged. Expression of *SOCS3*, an early response GH-target gene, has been found to closely mimic the effects of GH on STAT5 phosphorylation in mice [14]. The previously observed effects of GH on *SOCS2* and *SOCS3* gene expression in livers from global *Ptp1b*$^{-/-}$ mice are somewhat inconsistent with our findings. It may be that a degree of tissue cross-talk is responsible for some of the signalling changes previously observed in the global *Ptp1b*$^{-/-}$ mice. Furthermore, age-related differences could have contributed to the contrary results. The previous study used young 9–10 week old mice, whereas our mice were 6-12 months old and were therefore fully developed.

Recently, novel GH-mediated sites were identified in 3T3-F442A preadipocytes by quantitative phosphoproteomics. 228 phosphosites in 141 proteins that exhibited rapid (5 or 15 min) GH-dependant significant increases or decreases in phosphorylation were identified [13]. Several of the GH-stimulated phosphorylation sites were known (e.g. T203/Y205 in murine ERK1 and T183/Y185 in murine ERK2; Y694 and Y699 in STAT5a and STAT5b, respectively; S939 in tuberous sclerosis protein 2). The remaining 126 GH-stimulated sites that were identified were not previously associated with GH. A number of these newly identified sites were amongst insulin and mTOR signalling pathways. In the current study, mTOR

phosphorylation, but not S6 or ERK1/2 phosphorylation, was significantly increased following hGH stimulation at 5 and 10 min in both groups of mice. hGH-stimulated mTOR phosphorylation was significantly higher with liver-PTP1B deletion compared to controls, revealing this part of the pathway under direct control of PTP1B.

Overall, liver-specific PTP1B deletion leads **to** significant changes in GH signalling and phosphorylation of JAK2, STAT5 and mTOR, revealing a direct role for hepatic-PTP1B in regulation of these signalling processes.

Acknowledgements

We would like to thank Benjamin Neel (Campbell Family Cancer Research Institute, Ontario Cancer Institute, University of Toronto), Barbara Kahn (Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Centre, Boston) and Dr. Kendra Bence (Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania) for *Ptp1b* floxed mice. Carl Owen and Emma Katherine Lees are recipients of BBSRC doctoral-training studentships. Mirela Delibegovic and Nimesh Mody are funded by British Heart Foundation, EFSD/Lilly European Diabetes Programme Grant and Tenovus Scotland.

References

[1] J.J. Kopchick, L.L. Bellush, K.T. Coschigano. Transgenic models of growth hormone action, Annu. Rev. Nutr. 19 (1999) 437–461.

[2] D. Le Roith, C. Bondy, S. Yakar, J. Liu, A. Butler. The somatomedin hypothesis: 2001, Endocr. Rev. 22 (2001) 53–74.

[3] P. Sönksen, F. Salomon, R. Cuneo. Metabolic effects of hypopituitarism and Acromegaly1, Hormone Research in Paediatrics 36 (1991) 27–31.

[4] L.S. Argetsinger, G.S. Campbell, X. Yang, B.A. Witthuhn, O. Silvennoinen, J.N. Ihle, C. Carter-Su. Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase, Cell 74 (1993) 237–244.

[5] J. Herrington, C. Carter-Su. Signaling pathways activated by the growth hormone receptor, Trends. Endocrinol. Metab. 12 (2001) 252–257.

[6] F. Gu, N. Dube, J.W. Kim, A. Cheng, M.d.J. Ibarra-Sanchez, M.L. Tremblay, Y.R. Boisclair. Protein tyrosine phosphatase 1B attenuates growth hormone-mediated JAK2-STAT signaling, Mol. Cell. Biol. 23 (2003) 3753–3762.

[7] M. Delibegovic, D. Zimmer, C. Kauffman, K. Rak, E. Hong, Y. Cho, J.K. Kim, B.B. Kahn, B.G. Neel, K.K. Bence. Liver-specific deletion of protein-tyrosine phosphatase 1B (PTP1B) improves metabolic syndrome and attenuates diet-induced endoplasmic reticulum stress, Diabetes 58 (2009) 590–599.

[8] K. Bence, M. Delibegovic, B. Xue, C. Gorgun, G. Hotamisligil, B. Neel, B. Kahn. Neuronal PTP1B regulates body weight, adiposity and leptin action, Nat. Med. 12 (2006) 917–924.

[9] K. Almind, C.R. Kahn. Genetic determinants of energy expenditure and insulin resistance in diet-induced obesity in mice, Diabetes 53 (2004) 3274–3285.

[10] M. Delibegovic, K.K. Bence, N. Mody, E.G. Hong, H.J. Ko, J.K. Kim, B.B. Kahn, B.G. Neel. Improved glucose homeostasis in mice with muscle-specific deletion of protein-tyrosine phosphatase 1B, Mol. Cell. Biol. 27 (2007) 7727–7734.

[11] C. Owen, A. Czopek, A. Agouni, L. Grant, R. Judson, E.K. Lees, G.D. Mcilroy, O. Göransson, A. Welch, K.K. Bence. Adipocyte-specific protein tyrosine phosphatase 1B deletion increases lipogenesis, adipocyte cell size and is a minor regulator of glucose homeostasis, PLOS One 7 (2012) e32700.

[12] N. Aoki, T. Matsuda. A cytosolic protein-tyrosine phosphatase PTP1B specifically dephosphorylates and deactivates prolactin-activated STAT5a and STAT5b, J. Biol. Chem. 275 (2000) 39718–39726.

[13] B.N. Ray, H.K. Kweon, L.S. Argetsinger, D.C. Fingar, P.C. Andrews, C. Carter-Su. Research resource: Identification of novel growth hormone-regulated phosphorylation sites by quantitative phosphoproteomics, Mol. Endocrinol. 26 (2012) 1056–1073.

[14] C.S. Martinez , V.G. Piazza , L.D. Ratner , M.N. Matos , L. González , S.B. Rulli , J.G. Miquet , A.I. Sotelo . Growth hormone STAT5-mediated signaling and its modulation in mice liver during the growth period. Growth Horm IGF Res. 2013;23:19–28.

Figure Legends

Figure 1. **PTP1B deletion has no effect on body weight, adiposity or bone mineral density.** *A*: Body weight of *Ptp1b*$^{fl/fl}$ mice (n = 9) and *alb-crePtp1b*$^{-/-}$ mice (n = 14) on HFD. *B*: PTP1B deletion immunoblot and quantification of *Ptp1b*$^{fl/fl}$ mice (n = 4) and *alb-crePtp1b*$^{-/-}$ mice (n = 4) on HFD. *C*: Body fat vs. body weight of *Ptp1b*$^{fl/fl}$ mice (n = 7) and *alb-crePtp1b*$^{-/-}$ mice (n = 10) on a chow diet. *D*: Body fat percentage of *Ptp1b*$^{fl/fl}$ mice (n = 7) and *alb-crePtp1b*$^{-/-}$ mice (n = 10) on a chow diet. *E*: Bone mineral density of *Ptp1b*$^{fl/fl}$ mice (n = 7) and *alb-crePtp1b*$^{-/-}$ mice (n = 10) on a chow diet. Groups are indicated in the figures, with *Ptp1b*$^{fl/fl}$ mice in open circles/white bars and *alb-crePtp1b*$^{-/-}$ mice in closed circles/black bars. Data are represented as mean ± SEM; data were analysed using repeated measures two-way ANOVA with Bonferroni multiple comparisons post-tests or two-tailed Student's *t*-test ($^*P < 0.05$).

Figure 2. **Effects of liver-PTP1B deletion on growth-hormone induced phosphorylation of JAK2, STAT5, STAT3, S6 and mTOR.** *B*: pJAK2, pSTAT5, pSTAT3, pS6 and p-mTOR immunoblots. Total proteins were used as loading controls. *B*: pJAK2 immunoblot quantification. *C*: pSTAT5 immunoblot quantification. *D*: pSTAT3 immunoblot quantification. *E*: pS6 immunoblot quantification. *F*: p-mTOR immunoblot quantification. *G*: p-mTOR immunoblot quantification to total mTOR. Groups and hGH stimulation time course are indicated in the figures. *Ptp1b*$^{fl/fl}$ mice 0 min (n = 2), 5 min (n = 3), 10 min (n = 3) and *alb-*

crePtp1b$^{-/-}$ mice 0 min (*n* = 2), 5 min (*n* = 3), 10 min (*n* = 4). *Ptp1b*$^{fl/fl}$ mice are indicated by white bars and *alb-crePtp1b*$^{-/-}$ mice by black bars. Data are represented as mean ± SEM; data were analysed using one-way ANOVA with Tukey's multiple comparison post tests or two-tailed Student's *t*-test (*$P < 0.05$).

Figure 3. **Effects of liver-PTP1B deletion on *SOCS2* and *SOCS3* gene expression after 48-hour fasting.** *A*: *SOCS2* fasted gene expression in mice on HFD. *B*: *SOCS3* fasted gene expression in mice on HFD. *Hprt* was used as a reference gene. Groups and hGH stimulation time course are indicated in the figures. *Ptp1b*$^{fl/fl}$ mice 0 min (*n* = 2), 30 min (*n* = 3), 90 min (*n* = 4) and *alb-crePtp1b*$^{-/-}$ mice 0 min (*n* = 3), 30 min (*n* = 5), 90 min (*n* = 6). *Ptp1b*$^{fl/fl}$ mice are indicated by white bars and *alb-crePtp1b*$^{-/-}$ mice by black bars. Data are represented as mean ± SEM; data were analysed using one-way ANOVA with Tukey's multiple comparison post tests or two-tailed Student's *t*-test (*$P < 0.05$).

Figure 4. **Effects of Liver-PTP1B deletion on JAK/STAT phosphorylation under *ad lib* fed and 48-hour fasted diabetic conditions.** *A*: pJAK2 fed immunoblot quantification. *B*: pSTAT3 fed immunoblot quantification. *C*: pSTAT5 fed immunoblot quantification. *D*: pJAK2, pSTAT3 and pSTAT5 fed immunoblot. Total proteins were used as loading controls. Groups and hGH stimulation time course are indicated in the figures. *Ptp1b*$^{fl/fl}$ mice 0 min (*n* = 3), 30 min (*n* = 3) and *alb-crePtp1b*$^{-/-}$ mice 0 min (*n* = 4), 30 min (*n* = 4). *E*: pJAK2 fasted immunoblot quantification. *F*: pSTAT3 fasted immunoblot quantification. *G*: pSTAT5 fasted immunoblot quantification. *H*: pJAK2, pSTAT3 and pSTAT5 fasted immunoblot. Total proteins were used as loading controls. Groups and hGH stimulation time course are indicated in the figures. *Ptp1b*$^{fl/fl}$ mice 0 min (*n* = 2), 30 min (*n* = 3) and *alb-crePtp1b*$^{-/-}$ mice 0 min (*n* = 3), 30 min (*n* = 5). *Ptp1b*$^{fl/fl}$ mice are indicated by white bars and *alb-crePtp1b*$^{-/-}$ mice by black

bars. Data are represented as mean ± SEM; data were analysed using one-way ANOVA with Tukey's multiple comparison post tests or two-tailed Student's *t*-test (*$P < 0.05$).