Protein Tyrosine Phosphatase 1B Activation Is Developmentally Regulated in Muscle of Neonatal Pigs

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Short title: Muscle PTP1B activity in neonates

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ABSTRACT

The high activity of the insulin signaling pathway contributes to the enhanced feeding-induced stimulation of translation initiation in skeletal muscle of neonatal pigs. Protein tyrosine phosphatase-1B (PTP1B) is a negative regulator of the tyrosine phosphorylation of the insulin receptor (IR) and insulin receptor substrate 1 (IRS-1). The activity of PTP1B is determined mainly by its association with IR and Grb2. We examined the level of PTP1B activity, PTP1B protein abundance, PTP1B tyrosine phosphorylation, and the association of PTP1B with IR and Grb2 in skeletal muscle and liver of fasted and fed, 7- and 26-day-old pigs. PTP1B activity in skeletal muscle was lower (P< 0.05) in 7- compared to 26-day-old pigs but in liver was similar in both age groups. PTP1B abundances were similar in muscle but lower (P< 0.05) in liver of 7- compared to 26-day-old pigs. PTP1B tyrosine phosphorylation in muscle was lower (P< 0.05) in 7- than in 26-day-old pigs. The association of PTP1B with IR and the association of PTP1B with Grb2 were lower (P< 0.05) at 7 than at 26 days of age in muscle but there were no age-effects in liver. Finally, in both age groups, fasting did not have any effect on these parameters. These results indicate that basal PTP1B activation is developmentally regulated in skeletal muscle of neonatal pigs, consistent with the developmental changes in the activation of the insulin signaling pathway reported previously. Reduced PTP1B activation in neonatal muscle likely contributes to the enhanced insulin sensitivity of skeletal muscle in neonatal pigs.

Key Words: Neonate, insulin signaling, insulin sensitivity, protein synthesis.
INTRODUCTION

The enhanced activation of the insulin signaling pathway leading to translation initiation in skeletal muscle of the neonate following food consumption plays an important role in the enhanced responsiveness of muscle protein synthesis to feeding in the neonate (13, 16, 26, 27, 37). We have shown that the feeding-induced activation of the insulin receptor, insulin receptor substrate-1 (IRS-1), and phosphatidylinositol 3-kinase (PI 3-kinase), as well as the abundance of the insulin receptor, are enhanced in skeletal muscle of the neonatal pig and decrease markedly with development. This developmental decline in the feeding-induced activation of early insulin-signaling components parallels the developmental decline in the feeding-induced activation of downstream signaling proteins leading to the stimulation of protein synthesis in skeletal muscle and the developmental decrease in the ability of insulin to stimulate muscle protein synthesis. However, the molecular mechanism that regulates the developmental decline in the insulin sensitivity of skeletal muscle in neonatal pigs has not been elucidated.

When insulin binds to its receptor, it induces autophosphorylation of the receptor on its tyrosine residues, followed by the activation of its tyrosine kinase. The activated insulin receptor binds to its substrates, such as IRS proteins, resulting in the phosphorylation of their tyrosine residues (44). IRS activation triggers the activation of downstream signaling molecules, such as PI 3-kinase, leading to the stimulation of the metabolic effects of insulin, including protein synthesis (32). The accumulated evidence indicates that, in the early steps of the insulin signaling pathway, tyrosine phosphorylation is essential for the activation of insulin signaling (39).

Protein tyrosine phosphatase 1B (PTP1B), a non-transmembrane phosphotyrosine phosphatase, has been shown to attenuate insulin signaling by catalyzing the dephosphorylation of the insulin receptor and IRS-1 (9, 17). Early studies suggest that PTP1B blocks insulin-induced S6
peptide phosphorylation and inhibits insulin-induced maturation of Xenopus oocytes (10, 41). Intracellular delivery of neutralizing antibodies against PTP1B augments insulin receptor and IRS-1 phosphorylation and, conversely, overexpression of PTP1B in transfected cells inhibits insulin receptor and IRS-1 phosphorylation (4, 24). Recent studies show that PTP-1B-deficient mice exhibit hypersensitivity to insulin, suggesting that PTP1B is the major tyrosine phosphatase that regulates IR sensitivity (18, 28). PTP1B is abundantly expressed in insulin sensitive tissues such as muscle and liver, and its activity is an important regulator of the sensitivity of the insulin signaling pathway (2, 30, 33).

Mechanistic studies of PTP1B’s action suggest that interaction between PTP1B and the insulin receptor is important for catalyzing insulin receptor dephosphorylation (5, 11, 12, 23, 36, 43). Receptor tyrosine kinases appear to undergo internalization and form complexes with PTP1B, thereby removing phosphate groups from tyrosine residues (23). Studies further suggest that the phosphorylated and activated insulin receptor transiently binds and phosphorylates tyrosine residues of PTP1B, thereby increasing its catalytic activity (5, 12). Catalytically active PTP1B can then interact with and dephosphorylate the insulin receptor, thus attenuating insulin action. Finally, PTP1B undergoes auto-dephosphorylation and returns to its inactive basal state (12). There is some evidence that the steady-state phosphotyrosine content of IRS-1 is also regulated by PTP1B (21, 35). In vitro, PTP1B, IRS-1, and Grb2 form a ternary complex, resulting in the dephosphorylation of IRS-1. Therefore, the interaction between Grb2 and PTP1B, with subsequent effects on IRS-1 dephosphorylation, may also play an important role in the regulation of the postreceptor insulin signaling pathway (21).

In the present study, we examined whether PTP1B activation in skeletal muscle of neonatal pigs changes with development by measuring the activity of PTP1B in skeletal muscle of 7- and 26-
day-old pigs in the fasted and fed states. Furthermore, we also studied the mechanisms involved in PTP1B activation by measuring the association of PTP1B with the insulin receptor, the tyrosine phosphorylation of PTP1B, and the association of PTP1B with Grb2 adaptor protein. For comparison, similar measurements were also made in liver, an organ in which insulin does not stimulate protein synthesis (14, 15) and which shows no developmental decline in insulin signaling (37).

EXPERIMENTAL PROCEDURES

Animals. Eight crossbred (Landrace x Yorkshire x Duroc x Hampshire) pregnant sows (Agriculture Headquarters, Texas Department of Criminal Justice, Huntsville, TX) were housed in lactation crates in individual, environmentally controlled rooms two weeks prior to farrowing. Sows were fed a commercial diet (5084, PMI Feeds Inc., Richmond, IN) and provided water ad libitum. After farrowing, piglets remained with the sow and were not given supplemental creep feed. Piglets from 4 liters weighing approximately 2 and 8 kg were studied at 7 (n=6) and 26 day of age (n=6), respectively.

Pigs were either fasted for 18 hours or fed following an 18-h fast by giving two gavage administrations of 30 ml/kg body weight (BW) of porcine mature milk (University of Nebraska, Lincoln, NE) at 60-min intervals. Pigs were killed 30 minutes after the last feed and samples of longissimus dorsi muscle and liver were rinsed in ice cold saline and rapidly frozen. The protocol, which we have previously described (16, 26, 37), was approved by the Animal Care and Use Committee of Baylor College of Medicine and was conducted in accordance with the National Research Council’s Guide for the Care and Use of Laboratory Animals.

Materials. BioMag goat anti-mouse IgG and goat anti-rabbit IgG magnetic beads were obtained from Polysciences, Inc. (Warrington, PA) and the magnetic sample rack was from
Promega (Madison, WI). Reagents for SDS-PAGE were from Bio Rad Laboratories (Richmond, CA). The protein assay kit was purchased from Pierce (Rockford, IL). Anti-phosphotyrosine (PY) antibody and Grb2 antibody were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-PTP1B antibody was purchased from BD Transduction Laboratories (San Diego, CA). The enhanced chemiluminescence Western blotting detection kit (ECL-Plus) was obtained from Amersham (Arlington Heights, IL). Other chemicals and reagents were from Sigma Chemical Co. (St. Louis, MO).

**Preparation of tissue extracts.** Samples for immunoblotting were prepared according to Goodyear et al. (22). Briefly, samples of frozen muscle and liver were pulverized in liquid nitrogen and homogenized in ice-cold buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 10 mM NaPP, 2 mM Na$_3$VO$_4$, 1 mM MgCl$_2$, 1mM CaCl$_2$, 10 mM NaF, 5 mM Na-EDTA, 2 mM phenylmethylsulfonyl fluoride (PMSF), 5 µg/ml leupeptin, 1% NP-40, and 10% glycerol). The homogenate was incubated for 45 min at 4°C with gentle mixing and then centrifuged at 35,000 g for 1 hour at 4°C. The supernatant was collected and an aliquot was assayed for protein concentration using the BCA assay (Pierce, Rockford, IL). Supernatants were used to determine PTP1B content and immunoprecipitation.

**Immunoprecipitation.** To determine the tyrosine phosphorylation of PTP1B and the association of PTP1B with the insulin receptor (IR) and Grb2, protein samples from tissue extract preparations were immunoprecipitated with anti-mouse PY, anti-IR and anti-Grb2 antibody, respectively. The immunoprecipitation procedure was conducted according to Fox et al. (19). Briefly, equal amount of protein samples (500 µg protein in 500 µl buffer) were incubated by gently rocking overnight at 4°C with 20 µl of primary antibody (PY, IR, or Grb2 antibody), 230 µl PBS, and 12.5 µl Triton X-100. The next day, 500 ul of secondary antibody (BioMag goat anti-mouse
IgG or goat anti-rabbit IgG linked to magnetic beads) was added. After 1 hour incubation at 4°C, the samples were washed three times in low-salt buffer (20 mM Tris, 150 mM NaCl, 5 mM disodium EDTA, 0.5% Triton X-100, and 0.1% β-mercaptoethanol, pH 7.4) using a magnetic sample rack, and resuspended in 500 µl of low-salt buffer containing 1% dry skim milk. Each sample was added to 500 µl of resuspended beads and rocked for at least 1 hour at 4°C. The beads were captured using the magnetic rack and washed twice in low-salt buffer and once in high-salt buffer (50 mM Tris, 500 mM NaCl, 5 mM disodium EDTA, 1% Triton X-100, 0.6% sodium deoxycholate, 0.1% SDS, and 0.04% β-mercaptoethanol, pH 7.4). The captured beads were resuspended in 100 µl 1X sample buffer (2% SDS, 100 mM Tris HCl, pH 6.8, 5% β-mercaptoethanol, 12% (vol/vol) glycerol, and 0.02% (wt/vol) Bromphenol blue), boiled for 5 min, and stored at –80°C until electrophoresis.

**Western blot analysis.** To measure PTP1B abundance, equal amounts of samples were subjected to SDS-PAGE (8% wt/vol), as described by Laemmli (with a Mini-PROTEAN II electrophoresis system, Bio-Rad, Richmond, CA). Electrophoretic separation was carried out in 1% SDS, 25 mM Tris, and 200 mM glycine (pH 8.4) at 200 V for 45-60 min at room temperature with a Bio-Rad model 1000/500 power supply. A polyvinylidene difluoride membrane (Immobilon-P, Millipore, Bedford, MA) was activated in 20% methanol for 1 min. The proteins were then transferred to the membrane in 25 mM Tris, 192 mM glycine, and 20% methanol (vol/vol; pH 8.3) at 100 V and 250 mA for 1 h at 4°C. The membrane was incubated for 1 h at room temperature in a Tris-buffered saline-Tween 20 solution (TBS-T) containing 10 mM Tris, 0.5 M NaCl, and 0.5% Tween 20, pH 7.4, with 5% (wt/vol) nonfat dried milk. After the blocking step, the membrane was incubated with PTP1B antibody for 1 h on a rocking platform and washed four times in rinsing solution. Membranes were then incubated with secondary antibody (horseradish peroxidase-
conjugated IgG fraction of goat anti-rabbit IgG or goat anti-mouse IgG) diluted 1:2000 in TBS-T. The membranes were then washed in TBS-T three times for 10 min and developed with an enhanced chemiluminescence detection kit (ECL-Plus, Amersham Pharmacia, Piscataway, NY) before exposure onto Kodak-X-Omat film. The blots were quantified by computerized densitometry (Molecular Dynamics Pharmacia, Piscataway, NY). To measure PTP1B tyrosine phosphorylation, the association of PTP1B with IR and the association of PTP1B with Grb2, equal amounts of respective immunoprecipitant (PY, IR, and Grb2) were subjected to SDS page followed by immunoblotting as above, using PTP1B as the primary antibody.

**PTP1B Activity Assay.** The in vitro PTP1B activity was assayed according to a procedure previously described by Taghibiglou et al. (38). Briefly, samples of frozen skeletal muscle and liver were pulverized in liquid nitrogen and homogenized in ice-cold solubilization buffer (PBS containing 1% NP-40, 1% deoxycholate, 5 mM EDTA, 1 mM EGTA, 2 mM PMSF, and 0.1 mM leupeptin). The lysates were centrifuged for 10 min at 4 °C in a microcentrifuge. The supernatants were collected for immunoprecipitation. Prior to immunoprecipitation, the supernatants were subjected to preclearing with non-immune serum and protein A/G Plus Agarose (Santa Cruz Biotechnology, Inc. Santa Cruz, CA) for 15 min at 4 °C. Equal amount of protein samples (750 µg of total protein) were subjected to immunoprecipitation with anti-PTP1B antibody (BD Transduction Laboratories, San Diego, CA) at 4 °C overnight. PTP1B immunocomplexes were precipitated with protein A/G Plus Agarose at 4 °C for additional 2 hours. Immunoprecipititates were washed in PTP assay buffer (100 mM HEPES, pH 7.6, 2 mM EDTA, 1 mM dithiothreitol (DTT), 150 mM NaCl, and 0.5 mg/mL bovine serum albumin). The pp60c-src C-terminal phosphoregulatory peptide (TSTEPQpYQPGENL, Biomol, Plymouth Meeting, PA) was added to a final concentration of 200 µM in a total reaction volume of 60 µL in PTP1B assay buffer. The sample mixtures were
incubated for 1 hour at 30 °C. After the reaction, 40 µL aliquots were placed into 96-well plate and 100 µL of Biomol Green reagent (Biomol, Plymouth Meeting, PA) was added to each sample. After incubation for 30 min at room temperature, the absorbance was measured at 630 nm.

Statistics. Analysis of variance was used to assess the effect of development and food intake. Probability values of <0.05 were considered statistically significant. Data are presented as mean ± SEM.

RESULTS

To determine whether there was an effect of development on the PTP1B activity in skeletal muscle and liver, equal amounts of protein were subjected to immunoblotting using an anti-PTP1B antibody followed by enzyme activity assay. The activity of PTP1B in skeletal muscle of 7-day-old pigs was significantly (P<0.05) lower than that in 26-day-old pigs (Fig. 1A). PTP1B activity in liver did not differ between 7- and 26-day-old pigs (Fig. 1B). In both tissues, there was no difference in PTP1B activity between fasted and fed groups.

To evaluate the molecular mechanism for the developmental change in PTP1B activation in skeletal muscle of neonatal pigs, we determined PTP1B protein abundance, PTP1B association with IR, PTP1B tyrosine phosphorylation state, and PTP1B association with Grb2. The results showed that there was no difference between 7- and 26-day-old pigs in PTP1B protein abundance in skeletal muscle (Fig. 2A). In the liver, PTP1B protein abundance was significantly lower (P<0.05) in 7- than in 26-day-old pigs (Fig. 2B). Feeding did not alter PTP1B abundance in both tissues.

To determine whether the lower PTP1B activity in muscle of 7-day-old pigs was due to a lower association of PTP1B with IR, equal amounts of protein extracts from skeletal muscle and liver were subjected to immunoprecipitation with anti-IR β subunit antibody. After SDS-PAGE and electrophoretransfer, PVDF membranes were incubated with anti-PTP1B antibody. Figure 3 shows that
the association PTP1B with IR in muscle was significantly lower in 7-day-old compared to 26-day-old pigs. In contrast, there was no different between the age groups in the association of PTP1B with IR in liver. In both tissues, the association of PTP1B with IR was not difference between fasted and fed groups.

Recent studies indicate that PTP1B undergoes tyrosine phosphorylation following insulin stimulation and that this is positively correlated with enzyme activity (8, 12). To determine the effect of development on PTP1B tyrosine phosphorylation, equal amounts of skeletal muscle and liver extracts were immunoprecipitated with anti-PY antibody followed by immunoblotting with anti-PTP1B antibody. Figure 4 shows that PTP1B tyrosine phosphorylation in muscle was significantly lower in 7- than in 26-day-old pigs. PTP1B tyrosine phosphorylation levels in muscle of fasted and fed pigs were similar. The PTP1B tyrosine phosphorylation in liver was undetectable (data not shown).

Because recent studies (21, 35) indicate that the binding of Grb2 adaptor protein with PTP1B is important in forming the PTP1B-Grb2-IRS-1 ternary complex, followed by dephosphorylation of IRS-1, we evaluated the effect of development on the association of PTP1B with the Grb2 adaptor protein. Equal amounts of skeletal muscle and liver extracts were immunoprecipitated with anti-Grb2 antibody, followed by immunoblotting with an anti-PTP1B antibody. Figure 5 shows that Grb2 association with PTP1B in muscle was significantly lower in 7-compared to 26-day-old pigs. In liver, there was no difference between 7- and 26-day-old pigs in the association of Grb2 with PTP1B. In both tissues, there was no difference in the association of Grb2 with PTP1B between fasted and fed pigs.
DISCUSSION

Recent studies from our laboratory have shown an enhanced activation of the insulin signaling pathway leading to translation initiation in skeletal muscle of the neonatal pig following food consumption (16, 26, 37). This feeding-induced activation of the insulin receptor, IRS-I, PI3-kinase, and protein kinase B decreases with development in parallel with the developmental decline in the feeding-induced activation of translation initiation factors that regulate the binding of mRNA to 43S ribosomal complex. These developmental changes also parallel the developmental changes in the ability of feeding and insulin infusion to stimulate protein synthesis and are specific to skeletal muscle (13, 15). Because of the profound developmental decline in an initial step in the insulin signaling cascade, i.e., the tyrosine phosphorylation of the insulin receptor (37), it was crucial that we ascertain the mechanism involved in this developmental decline in insulin receptor activation. We hypothesized that the activation of PTP1B, a major protein tyrosine phosphatase that regulates the phosphorylation of the insulin receptor and IRS-I, increases with development in muscle of neonatal pigs.

Several studies have demonstrated that a reduction in PTP1B activity correlates with an enhanced insulin sensitivity (8). For example, enhanced insulin sensitivity following weight loss is closely correlated with a reduction in the abundance of PTP1B (1). In the current study, we found that PTP1B activity was 30% lower in skeletal muscle of 7- compared to 26-day-old pigs, which is consistent with the previously reported increased tyrosine phosphorylation of the insulin receptor and the high insulin sensitivity of protein synthesis in skeletal muscle of 7- compared to 26-day-old pigs (14, 37, 46). Importantly, no developmental change in PTP1B activation in liver was detected, which is consistent with the previously reported lack of developmental change in insulin sensitivity in the liver (14, 37). There were no differences in PTP1B activity between fasted and fed pigs in
the current study although studies in intact cells have shown that insulin increases PTP1B activity (25, 35). Since the PTP1B activation is transient (23), we may have missed the peak level of insulin-stimulated PTP1B activity by measuring PTP1B activity 90 minutes after feeding. It has been demonstrated recently that basal PTP1B activity in obese Zucker rats is higher than that in lean Zucker rats and that PTP1B activity is positively correlated with insulin resistance (34). The results of the current study suggest that the basal level of PTP1B activity increases from 7 to 26 days in piglets and that this is associated with a developmental decline in insulin sensitivity.

The results of the current study also show that PTP1B protein abundance in skeletal muscle did not change with development. In the liver, however, PTP1B protein abundance was lower in 7- compared to 26-day-old pigs, even though there were no differences in PTP1B activity in liver of both groups. PTP1B protein abundance is generally positively correlated with PTP1B activity (40, 42). However, a study using hepatoma cell culture indicated that treatment with TNF-α does not change PTP1B protein abundance but reduced PTP-1B activity by 66% (3). Furthermore, Bleyle et al. (7) suggested that the level of PTP1B abundance is not the primary determinant in its modulation of insulin receptor kinase activity. Thus, the binding of PTP1B to the insulin receptor may be more important than PTP1B abundance in assessing the enzyme activity. Our results also indicate that there are tissue specific factor(s) that regulate the activation of PTP1B.

A direct interaction between the activated insulin receptor and PTP1B that leads to an increase in PTP1B tyrosine phosphorylation and PTP1B activation in intact cells has been reported (12). Here we found that the lower activity of PTP1B in muscle of 7- compared to 26-day-old pigs was paralleled by a reduced interaction between PTP1B and the insulin receptor and a lower tyrosine phosphorylation of PTP1B in muscle of 7- compared to 26-day-old pigs. In contrast, there was no developmental change in the interaction between PTP1B and the insulin receptor in liver.
We also found that in both tissues there were no differences in the PTP1B-IR complexes between fasted and fed pigs. Recently, Mur et al (35) demonstrated in cell culture that insulin stimulates the interaction of PTP1B with the IR. The lack of feeding-induced formation of PTP1B-IR complexes in the current study (fasted vs. fed pigs) is probably due to the time point that we selected for sacrificing the pigs (1.5 hours after feeding). Indeed, Haj et al (23) found that activated receptor tyrosine kinases become associated with PTP1B by 30 minutes after growth factor stimulation. PTP1B rapidly removes the phosphate groups from the tyrosine residues of the receptors and the receptors are either sent back to the plasma membrane or to degradative lysosomes (23).

PTP1B forms a protein complex through the proline-rich sequence of its C terminus with SH3 domain-containing adaptor proteins, including Grb2 (31). Recent evidence suggests that Grb2, a well known docking protein for IRS-1, mediates the association of IRS-1 with PTP1B, leading to IRS-1 dephosphorylation (21, 35). The formation of a ternary protein complex consisting of PTP1B, Grb2, and IRS-1 has been suggested to play a crucial role in the dephosphorylation of IRS-1 by PTP1B. The results of this study are consistent with the hypothesis and show that the association between PTP1B and Grb2 in skeletal muscle was lower in muscle of 7- compared to 26-day-old-pigs, with no developmental change in liver. Although we were unable to detect the formation of a ternary protein complex consisting of PTP1B, Grb2, and IRS-1 in vivo, in vitro studies that detected ternary complex formation used inactivated PTP1B that binds IRS-1 strongly (21). In our studies, PTP1B catalytic activity may still be active, therefore transient formation of a ternary complex could not be detected. Nevertheless, several studies have shown that Grb2 associates with SH2 domain-containing PTPases in a variety of cell types (29, 40, 45), suggesting that Grb2 has an important cellular role in the regulation of PTPase activity. The results of the current study support this hypothesis.
The available data indicate that there are a number of other PTPase, including PTP1-α, LAR, and SHP2 (9, 20). Therefore, the contribution of these phosphatases in the regulation of insulin sensitivity in the neonate cannot be ruled out. Moreover, there are other tissue-specific functions of PTP1B beside the regulator of insulin signaling. Recently, PTP1B has been identified as the major PTP that dephosphorylates and activates c-Src in several human breast cancer-cell lines (6). PTP1B is also capable of antagonizing signaling by EGF receptor by directly dephosphorylating the epidermal growth factor (EGF) receptor tyrosine kinase (31). Nevertheless, in this study we provide evidence indicating the involvement of PTP1B activation in the increased insulin sensitivity in skeletal muscle of neonatal pigs.

Even though there is an abundance of experimental evidence indicating that PTP1B acts as a negative regulator of insulin signaling, direct interaction of PTP1B with the insulin receptor, which is crucial for dephosphorylation of the activated insulin receptor, has only been documented in cultured cell systems or in vitro studies (5, 12, 21, 35, 36, 43). For example, using brown adipocyte culture, the direct interaction between wild-type PTP1B and the insulin receptor was demonstrated in insulin-stimulated cells (35). Our current study, for the first time, showed evidence of the direct interaction between PTP1B and the insulin receptor in the intact animal. Furthermore, to the best of our knowledge, developmental changes in the activation of PTP1B, which may play a significant role in regulating the enhanced insulin sensitivity in the neonate, has not been previously studied.

It is surprising that we did not observe feeding-induced changes in PTP1B activity, PTP1B-IR interaction, PTP1B-Grb2 interaction, and tyrosine phosphorylation of PTP1B. However, the results of this study showed that the basal level of the activation of PTP1B was lower in 7-compared to 26-day-old-pigs and this was associated with higher insulin sensitivity in skeletal
muscle. We suggest that this study is comparable to a rodent study which showed that basal PTP1B activity in obese Zucker rats is significantly higher than that in lean Zucker rats and that PTP1B activity is positively correlated with insulin resistance (34).

**Perspectives.** In the present study, we showed that the activity of PTP1B was lower in skeletal muscle of 7- compared to 26-day-old pigs. This lower activity was associated with a reduced association of PTP1B with the insulin receptor, and Grb2, and decreased PTP1B tyrosine phosphorylation. Furthermore, these developmental changes appear to be tissue-specific because we did not find differences between age groups in PTP1B activation in liver. This developmental increase in skeletal muscle in the activity of PTP1B, which functions to dephosphorylate the activated insulin receptor, was consistent with our previously reported developmental decrease in insulin receptor phosphorylation (37). These changes were also consistent with a developmental decrease in the activation of downstream insulin signaling proteins, i.e., IRS-1, PI 3-kinase, and protein kinase B (26, 37), translation initiation factors (16, 26), and protein synthesis (13) in skeletal muscle and the ability of insulin to stimulate skeletal muscle protein synthesis (46).

Together, these results suggest that the increased insulin sensitivity in skeletal muscle of 7- compared to 26-day-old pigs is due, at least in part, to reduced PTP1B activation. The enhanced activation of the insulin signaling pathway leading to translation initiation in skeletal muscle of the neonate likely plays an important role in determining the high rates of protein synthesis in skeletal muscle and the more efficient use of dietary amino acids for growth in the neonate.
ACKNOWLEDGMENT

We thank D. Burrin for helpful discussion, H. Nguyen and W. Liu for laboratory assistance, J. Cunningham and F. Biggs for care of animals, L. Loddeke for editorial review, and J. Croom for secretarial assistance. This work is a publication of the United States Department of Agriculture/Agricultural Research Service (USDA/ARS) Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, TX. This project has been funded in part by National Institute of Arthritis and Musculoskeletal and Skin Diseases Institute Grant R01-AR-44474 (T. A. Davis) and the USDA/ARS under Cooperative Agreement no. 58-6250-6-001 (T. A. Davis). The contents of this publication do not necessarily reflect the views or policies of the US Department of Agriculture, nor does mention of trade names, commercial products or organization imply endorsement by the US Government.
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FIGURE LEGENDS

Figure 1. PTP1B activity in skeletal muscle and liver of fasted and fed 7- and 26-day-old pigs. Muscle and liver were homogenized as described in EXPERIMENTAL PROCEDURES. Equal amounts of protein were immunoprecipitated with anti-PTP1B antibody followed by PTP1B enzyme activity. A: muscle PTP1B activity. B: liver PTP1B activity. Results are means ± SE in nmol PO₄/mg protein (n=6). The PTP1B activity in muscle, but not liver, was lower in 7- compared to 26-day-old pigs (P<0.05).

Figure 2. PTP1B protein abundance in skeletal muscle and liver of fasted and fed 7- and 26-day-old pigs. Muscle and liver were homogenized as described in EXPERIMENTAL PROCEDURES. Equal amounts of protein were subjected to SDS-PAGE followed by immunoblot analysis with anti-PTP1B. A: Muscle PTP1B protein abundance as determined by immunoblot analysis. B: Liver PTP1B protein abundance as determined by immunoblot analysis. Results are means ± SE arbitrary densitometric units (n=6). The PTP1B protein abundance in liver, but not muscle, was lower in 7- compared to 26-day-old pigs (P<0.05).

Figure 3. PTP1B association with insulin receptor in skeletal muscle and liver of fasted and fed 7- and 26-day-old pigs. Muscle and liver were homogenized as described in EXPERIMENTAL PROCEDURES. Equal amounts of protein were immunoprecipitated with anti-IR antibody followed by immunoblotting with PTP1B antibody. A: muscle PTP1B association with IR. B: liver PTP1B association with IR. Results are means ± SE in arbitrary densitometric units (n=6). The PTP1B association with IR in muscle, but not liver, was lower in 7- compared to 26-day-old pigs (P<0.05).

Figure 4. PTP1B tyrosine phosphorylation in skeletal muscle of fasted and fed 7- and 26-day-old pigs. Muscle was homogenized as described in EXPERIMENTAL PROCEDURES. Equal
amounts of protein were immunoprecipitated with anti-PY antibody followed by immunoblotting with PTP1B antibody. Representative immunoblot of PTP1B tyrosine phosphorylation. Results are means ± SE arbitrary densitometric units (n=6). The PTP1B tyrosine phosphorylation in muscle was lower in 7- compared to 26-day-old pigs (P<0.05).

Figure 5. PTP1B association with Grb2 in skeletal muscle and liver of fasted and fed 7- and 26-day-old pigs. Muscle and liver were homogenized as described in EXPERIMENTAL PROCEDURES. Equal amounts of protein were immunoprecipitated with anti-Grb2 antibody followed by immunoblotting with PTP1B antibody. A: muscle PTP1B association with Grb2. B: liver PTP1B association with Grb2. Results are means ± SE in arbitrary densitrometric units (n=6). The PTP1B association with Grb2 in muscle, but not liver, was lower in 7- compared to 26-day-old pigs (P<0.05).
Figure 1

PTP1B Activity (nMol PO\textsubscript{4}/mg protein)

A

Muscle

- Fast
- Fed

7 day 26 day

* *

B

Liver

- Fast
- Fed

7 day 26 day
Figure 2

PTP1B Protein Abundance (Arbitrary Units)

A

Muscle

B

Liver
Figure 3

- A: Comparison of PTP1B:IR Association in muscle (7 day vs. 26 day) between fast and fed conditions.
  - Significant difference indicated by asterisk (*).

- B: Comparison of PTP1B:IR Association in liver (7 day vs. 26 day) between fast and fed conditions.

**Muscle**

- 7 day: 400 units
- 26 day: 900 units

**Liver**

- 7 day: 600 units
- 26 day: 600 units
Figure 4

PTP1B Tyrosine Phosphorylation (Arbitrary Units)

- Fast
- Fed

Muscle

- 7 day
- 26 day

* Indicates statistical significance.
Figure 5

**A**

Muscle

PTP1B:Grb2 Association (Arbitrary Units)

- 7 day
- 26 day

Fast
Fed

**B**

Liver

PTP1B:Grb2 Association (Arbitrary Units)

- 7 day
- 26 day

Fast
Fed

* indicates significant difference.