A Potential New Role for Muscle in Blood Glucose Homeostasis*

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The breakdown of tissue glycogen into glucose is critical for blood glucose homeostasis between meals. In the final steps of glycogenolysis, intracellular glucose 6-phosphate (Glc-6-P) is transported into the endoplasmic reticulum where it is hydrolyzed to glucose by glucose-6-phosphatase (Glc-6-Pase). Although the majority of body glycogen is stored in the muscle, the current dogma holds that Glc-6-Pase (now named Glc-6-Pase-α) is expressed only in the liver, kidney, and intestine, implying that muscle glycogen cannot contribute to interprandial blood glucose homeostasis. Recently we reported a second Glc-6-P hydrolase, Glc-6-Pase-β. Glc-6-Pase-β shares kinetic and structural similarities to Glc-6-Pase-α and couples with the Glc-6-P transporter to form an active Glc-6-Pase complex (Shieh, J.-J., Pan, C.-J., Mansfield, B. C., and Chou, J. Y. (2003) J. Biol. Chem. 278, 47098–47103). Here we demonstrate that muscle expresses both Glc-6-Pase-β and Glc-6-P transporter and that they can couple to form an active Glc-6-Pase complex. Our data suggest that muscle may have a previously unrecognized role in interprandial glucose homeostasis.

Blood glucose homeostasis between meals is maintained by endogenous glucose production via glycogenolysis and gluconeogenesis. In the terminal stages of both pathways, glucose 6-phosphate (Glc-6-P) is hydrolyzed to glucose and phosphate by the endoplasmic reticulum (ER)-associated glucose-6-phosphatase (Glc-6-Pase) complex (reviewed in Refs. 1 and 2). This complex is composed of a Glc-6-P transporter (Glc-6-PT) that transports Glc-6-P from the cytoplasm into the lumen of the ER and a Glc-6-Pase catalytic subunit that hydrolyzes the Glc-6-P to glucose and phosphate. Together they contribute, along with other factors, to the maintenance of blood glucose homeostasis. The Glc-6-PT is encoded by a single copy gene (3) that produces two alternatively spliced transcripts, Glc-6-PT and variant Glc-6-PT (vGlc-6-PT), differing by the absence or presence of a 66-bp exon-7 sequence (3–5). Although the Glc-6-PT transcript is expressed in all tissues examined, the vGlc-6-PT transcript is expressed only in the brain, heart, and skeletal muscle (6).

Two distinct Glc-6-Pase genes, Glc-6-Pase-α (G6PC) (7, 8) and Glc-6-Pase-β (G6PC3) (9–11), have been identified. The family prototype, Glc-6-Pase-α, is a 357-amino acid nine-transmembrane domain ER protein (7, 8, 12), which is expressed primarily in the liver, kidney, and intestine (13, 14). The primary function of Glc-6-Pase-α is to couple with the Glc-6-PT to metabolize hepatic and renal Glc-6-P to glucose. The Glc-6-Pase-α/Glc-6-PT complex is crucial for glucose homeostasis, and disruption of either component results in glycogen storage disease type Ia (GSD-Ia, Glc-6-Pase-α deficiency) or type Ib (GSD-Ib, Glc-6-PT deficiency). Both GSD-Ia and -Ib patients manifest the symptoms of failed Glc-6-P hydrolysis, characterized by a loss of blood glucose homeostasis and disorders of glycogen and lipid metabolism (reviewed in Refs. 1 and 2).

Glc-6-Pase-β is a 346-amino acid ubiquitously expressed phosphohydrolase with similar kinetic properties to Glc-6-Pase-α (10). Like Glc-6-Pase-α, Glc-6-Pase-β is an integral membrane protein in the ER, containing nine transmembrane domains (15). Moreover, the active-site structures of Glc-6-Pase-α and β are similar, and both form covalently bound phosphoryl-enzyme intermediates during catalysis (15, 16). Glc-6-Pase-β also couples functionally with the Glc-6-PT to form an active Glc-6-Pase complex that hydrolyzes Glc-6-P to glucose (10). In addition to Glc-6-PT, Glc-6-Pase-α can couple functionally with vGlc-6-PT (6). Although it is assumed that Glc-6-Pase-β can also couple to vGlc-6-PT, this remains to be demonstrated.

Despite disruption of the Glc-6-Pase-α complex in GSD-I patients, several studies (17–19) indicate that GSD-Ia patients are still capable of producing glucose. In light of the discovery of Glc-6-Pase-β, this implies that non-gluconeogenic tissues may be contributing to interprandial glucose homeostasis through the activity of a Glc-6-Pase-β/Glc-6-PT or a Glc-6-Pase-β/vGlc-6-PT complex. Because the largest reservoir of glycogen in the body is muscle, we examined whether muscle possesses functional Glc-6-Pase-β and Glc-6-PT activities. Our findings are consistent with our hypothesis that endogenous glucose production in GSD-Ia patients is mediated, at least in part, by the muscle Glc-6-Pase-β complex.

EXPERIMENTAL PROCEDURES

Isolation of Mouse Glc-6-Pase-β cDNA—The entire coding region of mouse Glc-6-Pase-β was isolated by reverse transcriptase-PCR amplification of mouse kidney RNA using oligonucleotide primers derived from nucleotides 232–258 (5′-ATGGAGTTCCAGCGTACGCCGGGCA-TCA-3′, sense) and nucleotides 1253–1272 (5′-TCAAGAGGGGAATG-GCC-3′, antisense) of mouse Glc-6-Pase-β mRNA (GenBank™ accession number XM_126488). The PCR product was cloned into the pSVL vector to generate pSVL-mGlc-6-Pase-β and the pGEM-11Z vector to generate pGEM-11Z-mGlc-6-Pase-β.

Construction of Recombinant Adenoviral Glc-6-Pase-β—Recombinant adenoviruses containing the human vGlc-6-PT (6) were generated by the Cre-lox recombination system (20). The recombinant virus was plaque-purified and amplified to produce viral stocks with titers of 5–10 x 10⁹ plaque-forming units/ml. Recombinant adenoviruses, containing human Glc-6-Pase-α (Ad-Glc-6-Pase-α) and human Glc-6-Pase-β (Ad-Glc-6-Pase-β), have been described (10).

Northern Blot Analysis—Total RNA was isolated by the guanidinium
thiocyanate/CaCl₂ method, fractionated by electrophoresis through 1.2% agarose gels containing 2.2 M formaldehyde, and transferred to a Nytran membrane (Schleicher & Schuell) by electoblotting. The filters were hybridized to a uniformly labeled mouse Glc-6-Pase-β, mouse Glc-6-PT (21), or β-actin riboprobe.

Expression in COS-1 Cells, Glc-6-Pase-α−/−, and Glc-6-PT−/− Mice—COS-1 cells in 150-cm² flasks were grown at a temperature of 37 °C in HEPES-buffered Dulbecco’s modified essential medium supplemented with 4% fetal bovine serum and infected with recombinant virus. The infected cultures were used to isolate microsomes for Glc-6-P uptake analysis after incubation at 37 °C for 24 h as described previously (10).

Mice deficient in Glc-6-Pase-α (22) and Glc-6-PT (23) have been described. All animal studies were conducted under an animal protocol approved by the NICHD, National Institutes of Health Animal Care and Use Committee. To maintain the viability of the Glc-6-Pase-α−/− (GSD-Ia) and Glc-6-PT−/− (GSD-Ib) mice, glucose therapy (23) consisting of an intraperitoneal injection of 25–100 µl of 15% glucose every 12 h was initiated on the first postnatal day. Weaned mice were also given unrestricted access to mouse chow (Zeigler Bros., Inc., Gardiners, PA). Microsomes were isolated from the leg muscle and liver of 6–7-week-old mice essentially as described (8, 24). Each microsomal preparation represents one individual mouse, and at least three independent microsomal preparations were used for each assay.

Phosphohydrolase and Glc-6-P Uptake Analyses—Phosphohydrolase activity was determined essentially as described previously (10). Glc-6-Pase-β in muscle microsomes was assayed at the optimal temperature of 37 °C, and Glc-6-Pase-α in hepatic microsomes was assayed at 30 °C, although Glc-6-Pase-α activities differ very little between 30 and 37 °C (10). Reaction mixtures (100 μl) containing 50 mM caccodylate buffer, pH 6.5, 250 mM sucrose, and 0.2 mM [U-¹⁴C]Glc-6-P (50 μCi/μmol). The reaction was stopped at the appropriate time by filtering immediately through a nitrocellulose membrane (BA85, Schleicher & Schuell) and washing with an ice-cold solution containing 50 mM Tris-HCl, pH 7.4, and 250 mM sucrose. The radioactivity measured within the microsomes represents both the translocated substrate, [U-¹⁴C]Glc-6-P, and its hydrolytic product, [U-¹⁴C]glucose, produced by the Glc-6-Pase activity localized within the lumen of the ER. Microsomes permeabilized with 0.2% deoxycholate to abolish Glc-6-P uptake were used as negative controls. 2–3 independent experiments were conducted, and at least three Glc-6-P uptake studies were performed for each microsomal preparation.

Statistical analysis using the unpaired t test was performed with the Prism Program (GraphPad Software, San Diego, CA). Data are presented as the mean ± S.E.

Generation of Polyclonal Anti-Glc-6-Pase-β Antibody and Western Blot Analysis—To generate antibodies against Glc-6-Pase-β, nucleotides 445–558 of the coding sequence, encoding amino acids 71–114 of human Glc-6-Pase-β, were cloned into the pET-41a (+) vector (Novagen, Madison, WI) and produced as a glutathione S-transferase fusion protein containing a His₆ tag at the N terminus. The recombinant Glc-6-Pase-β peptide was affinity-purified on a nickel chelate column and used to raise antibodies in New Zealand white rabbits. The antibodies recognize both human and mouse Glc-6-Pase-β efficiently.

For Western blot analysis, mouse microsomal proteins were resolved by electrophoresis through a 12% polyacrylamide-SDS gel and trans-blotted onto polyvinylidene fluoride membranes (Millipore Co., Bedford, MA) at 4 °C. The membranes were incubated overnight with the radiolabeled anti-Glc-6-Pase-β antibody and then with horseradish peroxidase-conjugated goat anti-rabbit IgG (Kirkegaard & Perry Laboratories, Gaithersburg, MD). The immunocomplex was visualized using the SuperSignal West Pico Chemiluminescent substrate from Pierce.

RESULTS

Skeletal Muscle Expresses Glc-6-Pase-β and Glc-6-PT—It has been shown that Glc-6-Pase-α is expressed primarily in the liver, kidney, and intestine (13, 14) and that Glc-6-Pase-β (9) and Glc-6-PT (21) are expressed ubiquitously with high levels in the skeletal muscle. Northern hybridization analysis confirms that the leg muscles of wild-type and GSD-Ia (Glc-6-Pase-α−/−/Glc-6-PT−/−) mice express both Glc-6-Pase-β and Glc-6-PT transcripts (Fig. 1A). As anticipated, muscles of GSD-Ib mice (Glc-6-Pase-α−/−/Glc-6-PT−/−) express only the Glc-6-Pase-β mRNA (Fig. 1A). This was confirmed at the protein level. A polyclonal antibody, raised against amino acids 71–114 of human Glc-6-Pase-β, which also recognizes the in vitro expressed mouse Glc-6-Pase-β (Fig. 1B), detected the presence of the Glc-6-Pase-β protein in the leg muscles of wild-type, Glc-6-Pase-α−/−, and Glc-6-PT−/− mice (Fig. 1B).

Glc-6-Pase-β Couples with vGlc-6-PT to Form an Active Glc-6-Pase Complex—We have shown previously that a functional Glc-6-Pase complex can be formed between Glc-6-Pase-α/Glc-6-PT (3), Glc-6-Pase-α/vGlc-6-PT (6), and Glc-6-Pase-β/Glc-6-PT (10). Because muscle expresses both Glc-6-PT and vGlc-6-PT (6), we examined whether Glc-6-Pase-β exhibits a similar functional coupling to vGlc-6-PT. Microsomes isolated from mock-infected COS-1 cells have a very low level of Glc-6-P uptake activity (data not shown). Microsomal Glc-6-P transport activity was increased significantly in COS-1 cells co-infected with Ad-vGlc-6-PT/Ad-Glc-6-Pase-β or Ad-vGlc-6-PT/Ad-Glc-6-Pase-α (Fig. 2A), which have similar time courses for microsomal Glc-6-P accumulation. The maximal Glc-6-P accumulation mediated by Glc-6-Pase-β/vGlc-6-PT is ~37% that of Glc-6-Pase-α/vGlc-6-PT, consistent with the relative phosphohydrolase activity of Glc-6-Pase-β and -α (10). As expected, COS-1 cells co-infected with Ad-vGlc-6-PT/Ad-Glc-6-Pase-β or Ad-Glc-6-Pase-β/Ad-Glc-6-Pase-α also have similar time courses and relative activities for microsomal Glc-6-P accumulation (Fig. 2B).

Skeletal Muscle Contains an Active Glc-6-Pase-β—Muscle is the primary storage site of glycogen in the body (25). Although muscle is known to express Glc-6-PT and vGlc-6-PT, it lacks Glc-6-Pase-α and was thought to be unable to contribute to blood glucose homeostasis. The discovery of Glc-6-Pase-β and its expression in the muscle led us to reexamine this tenet.

A previous study (10) has shown that Glc-6-Pase-α and Glc-6-Pase-β are very similar. Both are acid-labile and inhibited readily by vanadate, although Glc-6-Pase-β shows a greater sensitivity to the inhibitor than Glc-6-Pase-α does. The activities share a common pH optimum of 6.5 but differ in their temperature profiles. Glc-6-Pase-α has similar activities at both 30 and 37 °C, whereas Glc-6-Pase-β activity at 37 °C is ~1.5-fold higher than that of the activity at 30 °C (10).

Microsomes isolated from the leg muscles of wild-type mice have a detectable Glc-6-P hydrolase activity. Previous reports...
of such activities ascribed 80% of the activity to acid-resistant nonspecific phosphatases (26), but the assays were performed under conditions optimal for Glc-6-Pase-α, namely 30 °C and pH 6.5. Because the optimal condition for Glc-6-Pase-β activity is 37 °C, pH 6.5 (10), we performed assays at 37 °C to optimize for Glc-6-Pase-β. To exclude contributions from the acid-resistant nonspecific phosphatases we performed two measurements, one on disrupted microsomes to measure total phosphatase activity, and the other on disrupted microsomes preincubated at 37 °C, pH 5.0, for 10 min to measure the residual acid-resistant nonspecific phosphatases. The difference between these measurements reflects the acid-sensitive Glc-6-Pase-specific activity. In wild-type mouse muscle the initial Glc-6-P hydrolytic activity of 7.11 ± 0.32 nmol/mg/min is reduced to 1.74 ± 0.19 nmol/mg/min after acid incubation, yielding an acid-labile activity of 5.37 ± 0.32 nmol/mg/min (Table I), which should represent Glc-6-Pase-β. The acid-resistant hydrolytic activity measured here is consistent with previous measurements in other tissue microsomes (data not shown).

To exclude the possibility that a low level of expression of Glc-6-Pase-α occurs in muscle and is more readily measured at the higher temperature we used to assay, we examined the Glc-6-P hydrolytic activity of muscle microsomes from Glc-6-Pase-α−/− mice. The Glc-6-Pase-α−/− muscle had identical activity to the wild-type muscle excluding any contribution from Glc-6-Pase-α in the muscle (Table I).

Glc-6-Pase-α is expected to be the predominant acid-labile Glc-6-P hydrolyase activity in the liver (13, 14), whereas Glc-6-Pase-β is expected to be a minor component (9). Consistent with this, there was a high level of Glc-6-Pase activity in the wild-type liver but no detectable activity in the Glc-6-Pase-α−/− liver (Table I). In the Glc-6-PT knock-out mouse, the Glc-6-PT−/− muscle had a similar activity to the wild-type and Glc-6-Pase-α−/− muscles, as would be expected for Glc-6-Pase-β. In contrast, the Glc-6-PT−/− liver microsomes had a 3.3-fold higher Glc-6-P hydrolyase activity than the wild-type liver microsomes (Table I). This is not unexpected. The expression of Glc-6-Pase-α in rat hepatocytes is stimulated by extracellular glucose (27) mediated within the cell via Glc-6-P (27, 28). In a Glc-6-Pase−/− liver, cytoplasmic Glc-6-P levels are elevated markedly over wild-type levels, because Glc-6-P produced by glycogenolysis, gluconeogenesis, and glucose phosphorylation can not be transported into the lumen of the ER to be hydrolyzed to glucose (1, 2). Therefore the higher intracellular levels of Glc-6-P in the Glc-6-Pase−/− liver are expected to elevate the Glc-6-Pase-α activity.

To further support the identification of the labile muscle activity as Glc-6-Pase-β, the pH dependence (Fig. 3A) and vanadate sensitivity (Fig. 3B) of the Glc-6-Pase in muscle was compared with the hepatic Glc-6-Pase-α. Muscle Glc-6-Pase had a pH optimum of 6.5 (Fig. 3A), identical to the liver Glc-6-Pase-α, but vanadate inhibited the muscle Glc-6-Pase−/− (vanadate)0.5 = 1.3 mM) more effectively than hepatic Glc-6-Pase-α (vanadate)0.5 = 2.9 mM) (Fig. 3B). These findings were totally consistent with profiles of Glc-6-Pase-β and -α obtained with adenosviral-mediated expression studies (10) and further support the identity of the muscle Glc-6-Pase as Glc-6-Pase-β.

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![Fig. 2. Glc-6-Pase-β couples with vGlc-6-PT to mediate microsomal Glc-6-P uptake. A, microsomal Glc-6-P uptake activity in COS-1 cells co-infected with Ad-vGlc-6-PT/Ad-Glc-6-Pase-β (○) or Ad-vGlc-6-PT/Ad-Glc-6-Pase-α (●). B, microsomal Glc-6-P uptake activity in COS-1 cells co-infected with Ad-Glc-6-PT/Ad-Glc-6-Pase-β (○) or Ad-Glc-6-PT/Ad-Glc-6-Pase-α (●). [U-14C]Glc-6-P uptake analysis was performed as described under “Experimental Procedures.” The radioactivity accumulated in the lumen of the ER consists of both [U-14C]Glc-6-P and [U-14C]glucose. The amount of Ad-Glc-6-Pase-α or Ad-Glc-6-Pase-β was 25 plaque-forming unit/cell and Ad-vGlc-6-PT or Ad-Glc-6-PT was 50 plaque-forming unit/cell. The results are given as mean ± S.E.](image)

**TABLE I**

| Mice | Muscle | Liver |
|------|--------|-------|
| Wild-type | 5.37 ± 0.32 | 217.2 ± 6.0 |
| Glc-6-Pase-α−/− | 5.53 ± 0.37 | Not detectable |
| Glc-6-PT−/− | 5.40 ± 0.18 | 724.3 ± 10.9 |

![Fig. 3. The pH dependence and vanadate sensitivity of Glc-6-P phosphohydrolase activity. Phosphohydrolase assays were performed using disrupted microsomes isolated from the leg muscle (○) or the liver (●) of wild-type mice. A, pH dependence of Glc-6-P phosphohydrolase activity of deoxycholate-disrupted mouse microsomes. The liver enzyme was assayed at 30 °C, and the muscle enzyme was assayed at 37 °C. B, vanadate inhibition of Glc-6-P phosphohydrolase activity of deoxycholate-disrupted microsomes. The liver enzyme was assayed at pH 6.5 and 37 °C. The results are given as mean ± S.E.](image)
courses of Glc-6-P uptake activity in the intact microsomes of E time course of Glc-6-P uptake activity in intact microsomes isolated from wild-type, Glc-6-Pase-α−/−, and Glc-6-PT−/− mice as described under "Experimental Procedures." The radioactivity accumulated in the lumen of the ER consists of both [U-14C]Glc-6-P and [U-14C]glucose. A, time course of Glc-6-P uptake activity in intact microsomes isolated from the muscle of wild-type (○), Glc-6-Pase-α−/− (△), or Glc-6-PT−/− (▲) mice. B, microsomal Glc-6-P uptake activity. The results are given as mean ± S.E.

Glc-6-Pase to form an efficient Glc-6-Pase complex (3, 6, 22). A comparison of Glc-6-P uptake in wild-type, Glc-6-Pase-α−/−, and Glc-6-PT−/− mouse muscle shows that although the time courses of Glc-6-P uptake activity in the intact microsomes of wild-type and Glc-6-Pase-α−/− mice are identical, the Glc-6-PT−/− mice do show a markedly reduced Glc-6-P accumulation (Fig. 4) consistent with the absence of Glc-6-PT in these mice (23). Glc-6-Pase-α−/− mice show a 95% loss of Glc-6-P uptake activity in the liver (Fig. 4B) consistent with this tight functional coupling (22). However, the muscle Glc-6-P uptake activity is not attenuated in Glc-6-Pase-α−/− mice, which is consistent with a muscle Glc-6-Pase-β coupling with Glc-6-PT.

Knock-out of Glc-6-PT in mice results in an ~97% loss of liver Glc-6-P uptake activity and an ~77% loss of muscle activity (Fig. 4B) confirming the importance of Glc-6-PT in both tissues. Because ~23% of the wild-type Glc-6-P uptake activity does remain in the muscle of Glc-6-PT-deficient mice, another minor Glc-6-P transport protein cannot be excluded in the muscle. This possibility awaits further examination.

The uptake of Glc-6-P in wild-type muscle and liver show a similar time course (Fig. 5A) and pH dependence (Fig. 5B), but in contrast to the 40-fold difference between the muscle and liver Glc-6-P phosphohydrolase activities (Table I), Glc-6-P uptake in muscle is only 4.4-fold lower than that in the liver (Fig. 4D). Overall, the rates of Glc-6-P uptake are ~0.9−3% of the rates of the hydrolysis of either Glc-6-Pase, implying that Glc-6-PT-mediated Glc-6-P uptake is the rate-limiting step of the Glc-6-Pase complex.

**DISCUSSION**

Blood glucose homeostasis between meals depends on the coupled actions of a Glc-6-P transporter and a Glc-6-P phosphohydrolase to metabolize Glc-6-P to glucose in the terminal steps of gluconeogenesis and glycogenolysis (1, 2). There are two distinct Glc-6-P phosphohydrolases, the liver/kidney/intestine Glc-6-Pase-α (7, 8) and the ubiquitous Glc-6-Pase-β (9−11). Both couple with Glc-6-PT or vGlc-6-PT to form a functional Glc-6-Pase complex (3, 6, 10). Deficiencies in the Glc-6-Pase-α complex result in the metabolic disorder GSD-I, which is characterized by hypoglycemia, growth retardation, hepatomegaly, nephromegaly, hyperlipidemia, hyperuricemia, and lactic acidemia (1, 2). Despite the absence of the Glc-6-Pase-α complex, GSD-I patients are capable of endogenous glucose production (17−19). We now show that muscle may be able to contribute to interperiodal blood glucose homeostasis through the activity of Glc-6-Pase-β/Glc-6-PT and Glc-6-Pase-β/vGlc-6-PT.

The Glc-6-Pase found in muscle in this study and the in vitro expressed Glc-6-Pase-β (10) have identical kinetic characteristics. Both are acid-labile, readily inhibited by vanadate, with a (vanadate)0.5 of 1.3−1.4 mM, and both share an optimal assay condition of pH 6.5 and 37 °C differing from that of Glc-6-Pase-α at pH 6.5 and 30 °C. Muscle Glc-6-Pase has ~2.5% of the activity of the liver Glc-6-Pase-α, and the muscle Glc-6-Pase/Glc-6-PT or Glc-6-Pase/vGlc-6-PT complex has ~25% of the Glc-6-P transport activity of the liver Glc-6-Pase-α/Glc-6-PT or Glc-6-Pase-α/vGlc-6-PT complex. All of these findings are consistent with previous adenosivral expression studies of Glc-6-Pase-β (10) and, along with the RNA and protein expression profiles, support the conclusion that the acid-labile Glc-6-P hydrolytic activity in muscle is Glc-6-Pase-β.

Muscle consists of 40−45% of the wet body weight of a normal human (25, 29−31) and stores about 300 g of glycogen/70-kg male, making it the largest overall reservoir of glycogen in the body. The finding that there is a specific Glc-6-P hydrolytic activity in muscle that can couple to Glc-6-PT suggests that muscle not only has the ability to store glucose as glycogen but also has the ability to cycle the glucose back and contribute to blood glucose homeostasis, a role previously considered possible only in the liver, kidney, and small intestine. How much of this reservoir is cycled into blood glucose is difficult to judge. Cytoplasmic Glc-6-P has multiple fates that include hydrolysis to glucose, glycogenesis, energy production via glycolysis, entry into the pentose phosphate pathway, and lipid synthesis. Even if blood glucose is the primary destination of the cycled muscle Glc-6-P, the impact on blood glucose homeostasis is still difficult to estimate. It has been shown previously that Glc-6-PT-mediated Glc-6-P uptake is the rate-limiting step of the Glc-6-Pase complex in vitro (14). However, because the rates of hydrolysis have to be measured within disrupted microsomes, while Glc-6-P uptake has to be measured within intact microsomes, it is not yet possible to show what the true rate-limiting step of the Glc-6-Pase/Glc-6-PT complex is in vivo and therefore to show how much blood glucose the muscle Glc-6-Pase system is capable of producing. Currently the best indication of the extent of the endogenous non-hepatic blood glucose production comes from studies of GSD-Ia patients, who lack a functional Glc-6-Pase-α. These studies show that the endogenous glucose production rate improves with age, ranging from 50% normal in young GSD-Ia patients, to 67−100% normal in adult GSD-Ia patients (17−19). Because the muscle mass is only ~20% of the body weight of a newborn but improves through adolescence (36% muscle mass) to adulthood (40−45%) (31), it is reasonable to suggest that the improvement in the susceptibility of patients to hypoglycemia is attributable to endogenous glucose.
production by the muscle Glc-6-Phospho-1/2Glc-6-PT or Glc-6-Phos-1/2vGlc-6-PT complex. Whether muscle may also play a role during fasting, or other hypoglycemic situations, remains to be addressed.

Prior to the discovery of Glc-6-Phospho-1/2, there had been speculation as to the role of Glc-6-PT, which unlike Glc-6-Phospho-1/2 showed an ubiquitous tissue expression profile. With the recognition of Glc-6-Phospho-1/2 and its universal expression profile, new questions arise concerning the physiological significance of Glc-6-Phospho-1/2, whether glucose recycling can actually occur to some extent in all tissues, the importance of this to glucose homeostasis, and what the implications of this are for type II diabetes. A deeper understanding of these issues awaits the analysis of the Glc-6-Phospho-1/2 knockout and overexpression phenotypes.

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