ARTICLE
Journal of Cellular Biochemistry 113:848–856 (2012)

Journal of Cellular Biochemistry

Nuclear Accumulation of Fructose 1,6-Bisphosphatase is Impaired in Diabetic Rat Liver

Romina Bertinat,[1] Juan P. Pontigo,[1] Moisés Pérez,[1] Ilona I. Concha,[1] Rody San Martín,[1] Joan J. Guinovart,[2] Juan C. Slebe,[1*] and Alejandro J. Yáñez[1*]

[1]*Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Valdivia, Chile*

[2]*Institut de Recerca Biomèdica, Universitat de Barcelona, Barcelona, Spain*

ABSTRACT

Using a streptozotocin-induced type 1 diabetic rat model, we analyzed and separated the effects of hyperglycemia and hyperinsulinemia over the in vivo expression and subcellular localization of hepatic fructose 1,6-bisphosphatase (FBPase) in the multicellular context of the liver. Our data showed that FBPase subcellular localization was modulated by the nutritional state in normal but not in diabetic rats. By contrast, the liver zonation was not affected in any condition. In healthy starved rats, FBPase was localized in the cytoplasm of hepatocytes, whereas in healthy re-fed rats it was concentrated in the nucleus and the cell periphery. Interestingly, despite the hyperglycemia, FBPase was unable to accumulate in the nucleus in hepatocytes from streptozotocin-induced diabetic rats, suggesting that insulin is a critical in vivo modulator. This idea was confirmed by exogenous insulin supplementation to diabetic rats, where insulin was able to induce the rapid accumulation of FBPase within the hepatocyte nucleus. Besides, hepatic FBPase was found phosphorylated only in the cytoplasm, suggesting that the phosphorylation state is involved in the nuclear translocation. In conclusion, insulin and not hyperglycemia plays a crucial role in the nuclear accumulation of FBPase in vivo and may be an important regulatory mechanism that could account for the increased endogenous glucose production of liver of diabetic rodents. J. Cell. Biochem. 113: 848–856, 2012.

KEY WORDS: GLUCONEOGENESIS; FRUCTOSE 1,6-BISPHOSPHATASE; NUCLEAR TRANSLOCATION; DIABETES

Endogenous glucose production maintains normoglycemia during fasting in non-diabetic individuals, but it is also responsible for elevated glucose output in diabetes [Yoshida et al., 2011]. In normal conditions, the main organ involved in the control of glucose homeostasis is the liver, providing glucose by glycogenolysis and gluconeogenesis [Agius, 2007]. Hepatic glycogen is readily depleted during fasting and gluconeogenesis becomes the essential metabolic pathway involved in glucose homeostasis during starvation [Agius, 2007]. Gluconeogenesis is modulated at the level of three enzymes catalyzing irreversible reactions: Glucose 6-phosphatase (G6Pase), fructose 1,6-bisphosphatase (FBPase), and phosphoenolpyruvate carboxykinase (PEPCK). While G6Pase and PEPCK are mainly regulated at the transcriptional level [Barthel and Schmoll, 2003], FBPase is modulated at the level of activity by the negative effectors AMP and Fru 2,6-P_2 [Cárcamo et al., 2000], and by its interaction with fructose 1,6-bisphosphate aldolase [Yáñez et al., 2005]. Due to its strategic position in the gluconeogenic pathway, hepatic FBPase has become an interesting target for development of new anti-diabetic drugs [Erion et al., 2007; Heng et al., 2009; Yoshida et al., 2011].

One important level of regulation for hepatic metabolic pathways is related to the blood supply of the liver and implicates that hepatocytes are functionally different: FBPase [Lawrence et al., 1986], G6Pase, and PEPCK [Rajas et al., 2007] are mainly expressed in periportal hepatocytes where gluoneogenesis is more active. However, a novel mechanism proposed for regulation of metabolism is the coordinated modulation of the phosphorylation and subcellular localization of enzymes from different pathways in

Abbreviations: FBPase, fructose 1,6-bisphosphatase; Fru-1,6-P_2, fructose-1,6-bisphosphate; Fru-2,6-P_2, fructose-2,6-bisphosphate; G6Pase, glucose 6-phosphatase; GK, glucokinase; GS, glycogen synthase; PEPCK, phosphoenolpyruvate carboxykinase; STZ, streptozotocin.

Romina Bertinat was a recipient of a Doctoral Fellowship granted by CONICyT-Chile and Fundación Marcelino Botín-España.

Additional supporting information may be found in the online version of this article.

Grant sponsor: Fondo Nacional de Investigación Científica y Tecnológica (FONDECYT); Grant number: 1090694; Grant sponsor: FONDECYT; Grant number: 1090740; Grant sponsor: DID-UACh; Grant number: 2006-12.

*Correspondence to: Alejandro J. Yáñez and Juan C. Slebe, Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Valdivia, Chile. E-mail: ayanez@uach.cl

Received 11 October 2011; Accepted 12 October 2011 • DOI 10.1002/jcb.23413 • © 2011 Wiley Periodicals, Inc.
Published online 20 October 2011 in Wiley Online Library (wileyonlinelibrary.com).

the single cell. There is substantial in vitro background supporting this idea. Glucokinase (GK) was one of the first examples showing the ability of enzymes from glucose metabolism to interact with a regulatory protein and to translocate into the nucleus in response to the level of intracellular metabolites [De la Iglesia et al., 1999]. The cytoplasmic distribution of liver glycogen synthase (GS) is also modulated by substrate availability, translocating to the actin-rich cell cortex of hepatocytes where glycogen synthesis begins [Ros et al., 2009]. Notably, impaired GK nuclear export is associated with the unresponsiveness of hepatic glucose flux to the rise in plasma glucose and insulin seen in prediabetic ZDF rats [Fujimoto et al., 2004]. In parallel, GS translocation is dependent on glycogen synthesis, indicating that it is distributed throughout the cytoplasm of hepatocytes during diabetes [Ros et al., 2009].

Previously, we have demonstrated that FBPase subcellular distribution is modulated by nutritional stimuli in rat hepatocytes in primary culture [Yáñez et al., 2004], but the effects of diabetes over liver FBPase subcellular distribution have not been reported. It is well known that conventional cultures dissociate tissues in one kind of cell, avoiding inter-cellular processes that occur at the organ level [Khetani and Bhatia, 2008]. Since cellular function is not only modulated by autonomous programs but also by micro-environmental stimuli (neighbor cells, extracellular matrix, soluble factors, and physical forces), regulation of activity and subcellular distribution of enzymes in the multicellular context of the tissue is poorly understood yet. Moreover, differences have been demonstrated when comparing whole organ with in vitro experiments [Jin et al., 2003; Matsuo et al., 2010]. Here, we present evidence that the regulation of FBPase nucleo-cytoplasmic shuttling is impaired during diabetes, whereas the liver zonation was not affected. Besides, FBPase was found phosphorylated only in the cytoplasm of both healthy and diabetic rats, suggesting a link between the phosphorylation state and the insulin-induced nuclear translocation. Exogenous insulin restored FBPase nuclear accumulation in the liver from diabetic rats, demonstrating that insulin and not hyperglycemia does regulate FBPase subcellular localization at the organ level. Since hepatic FBPase is considered a new target for the control of diabetes, the more we know about its regulation, the better we understand the function of specific inhibitors and their effects.

MATERIALS AND METHODS

ANIMALS

250 g male Sprague-Dawley rats were kept in a conditioned house and fed ad libitum. Two groups of five healthy rats each were starved for 12 h and then one group was fed for 12 h. To induce diabetes, rats were injected with a single intravenous dose of 65 mg/kg streptozotocin (STZ; Merck), and the glycemia was measured periodically since it reached 400 mg/dl in average. At this moment, two groups of five rats each were subjected to the same nutritional conditions as the two healthy groups. For insulin supplementation, six diabetic rats were fasted for 12 h and then three were fed for 12 h, and 2.1 UI of insulin glargine (Lantus, Sanofi Aventis) in 200 μl of saline buffer were added by a single intraperitoneal injection. All experiments were approved by the Institutional Animal Care and Use Committee of Universidad Austral de Chile.

ANTIBODIES

Rabbit antiserum against FBPase and cytosolic PEPCK were prepared in our laboratory. Mouse anti-β actin was from Sigma. Mouse anti-SC35 was from BD Pharmingen. Alexa Fluor 488-conjugated anti-rabbit IgG (Molecular probes, Eugene, OR) was used as secondary antibody for immunofluorescence of FBPase.

NUCLEI PREPARATION

Nuclear and cytoplasmic extracts from rat liver were prepared by using the NE-PER nuclear and cytoplasmic extraction reagents, according to the instructions of the manufacturer (Pierce Biotechnology, Rockford, IL) and previous publication [Anan et al., 2006]. The protein content in the two fractions was estimated using the BCA Protein assay kit (Pierce Biotechnology).

WESTERN BLOT

Liver was homogenized in 20 mM Tris-HCl buffer (pH 7.5) supplemented with 0.1 mM EDTA and 1× protease inhibitor cocktail (Calbiochem, Darmstadt, Germany). Protein concentration was estimated using the BCA Protein assay kit (Pierce Biotechnology). Ten micrograms of total proteins were fractionated in 4–12% SDS–PAGE, transferred to PVDF membranes and probed with anti-FBPase, -PEPCK, or -β actin. Following incubation with an HRP-conjugated IgG secondary antibody (Pierce Biotechnology), reaction was developed using the Pierce ECL Western Blotting Substrate (Pierce Biotechnology).

IMMUNOHISTOCHEMISTRY

Tissue samples were deparaffinized in xylene and rehydrated in graded ethanol. Endogenous peroxidase activity was inhibited with 3% H_2O_2 for 5 min and tissue was blocked with 3% BSA in PBS, and permeabilized with 0.3% Triton X-100 for 30 min. Primary antibody was incubated for 1 h, washed in PBS, and secondary antibody (Universal ICQ LSAB plus kit, DAKO Corporation, Carpenteria, CA) was incubated for 20 min followed by washing in PBS. The reaction was developed with DAB and nuclear counter-staining was carried out with hematoxylin.

IMMUNOFLUORESCENCE

The same as for the immunohistochemical procedure, but Alexa Fluor-conjugated secondary antibodies were used for 45 min. Counter-staining was carried out with propidium iodide (PI). Stained sections were examined with a Fluoview 1000 (Miami, FL) laser scanning confocal microscope at the core facility of UACh, and optical sections of 1 μm were obtained.

ENRICHMENT OF RAT LIVER PHOSPHORYLATED PROTEINS

Briefly, rat liver proteins were extracted by homogenization in lysis buffer containing 0.25% (w/v) CHAPS, protease/phosphatase inhibitors, and benzonase as described in the manufacturer's protocol (Phosphoprotein Purification Kit, Qiagen Inc. Valencia, CA) and centrifuged at 10,000*g* at 4°C for 30 min. The supernatant was diluted to a protein concentration of 0.1 mg/ml in a total of 25 ml lysis buffer and was applied to a lysis buffer-equilibrated PhosphoProtein purification column. Phosphoproteins were eluted with 2 ml of PhosphoProtein Elution Buffer. The flow-through

samples were passed through two additional columns to ensure complete removal of phosphoproteins from the sample [Davis et al., 2006].

ENZYME ACTIVITY

FBPase and PEPCK activities were estimated spectrophotometrically by changes in the absorbance at 340 nm due to reduction or oxidation of NAD, respectively, in a coupled enzyme assay at 30°C in 0.5 ml final volume and 10 μl of dialyzed total liver extract or nuclear and cytoplasmic fractions. For FBPase activity, phosphoglucose isomerase and glucose 6-phosphate dehydrogenase (Sigma) were used as coupled enzymes [Reyes et al., 1987]; for PEPCK activity, malate dehydrogenase (Sigma) was used as the coupled enzyme [Colombo and Lardy, 1981]. The reaction was initiated by adding protein extracts. One unit of FBPase or PEPCK activity was defined as the amount of enzyme that catalyzes the formation of 1 μmol of Fru-6-P or oxalacetate, respectively, per minute under the conditions described.

STATISTICAL ANALYSIS

Results are expressed as means ± S.E., the number of animal in each group is indicated between brackets. Significant differences between means were established with by unpaired Student's *t*-test (significant difference for $P < 0.05$).

RESULTS

EXPRESSION OF FBPase IN RAT LIVER

Two weeks after STZ injection, diabetic and healthy rats were 12 h-fasted and then fed during 12 h, and plasma glucose and insulin levels were determined (Table I). Total liver proteins were prepared and expression of hepatic FBPase was analyzed by Western blot (Fig. 1A). As control, protein levels of cytosolic PEPCK were decreased in response to feeding of healthy but not diabetic rats (Fig. 1A). FBPase expression was not altered between fasting and feeding, or the diabetic state (Fig. 1A). Immunofluorescence analysis of insulin (red) and glucagon (green) in pancreas sections revealed the expected alterations induced by STZ administration (Fig. 1B). Since FBPase is expressed in a zonated fashion through the liver parenchyma, the absence of significant changes in total protein expression may not represent this compartmentalized distribution, which is a higher level of regulation for hepatic metabolism. In order to evaluate the zonal expression of FBPase, fixed liver samples from normal and diabetic rats were assessed by immunohistochemical analysis. As a control, glycogen zonation by means of PAS staining

TABLE I. Glycemia and Insulinemia in Healthy and Diabetic Rats

	Healthy rat	Diabetic rat
Blood glucose (mg/dl)		
12 h fasted	72 ± 4 [5]	354 ± 11 [5]
12 h fasted + 12 h re-fed	137 ± 8 [5]	612 ± 15 [5]
Serum insulin (ng/ml)		
12 h fasted	0.19 ± 0.03 [5]	0.05 ± 0.01 [5]
12 h fasted + 12 h re-fed	0.58 ± 0.08 [5]	0.07 ± 0.01 [5]

Serum was collected from fed and fasted healthy and diabetic rats (12 weeks of age), and glucose and insulin levels were analyzed. The number of animals is indicated between brackets.

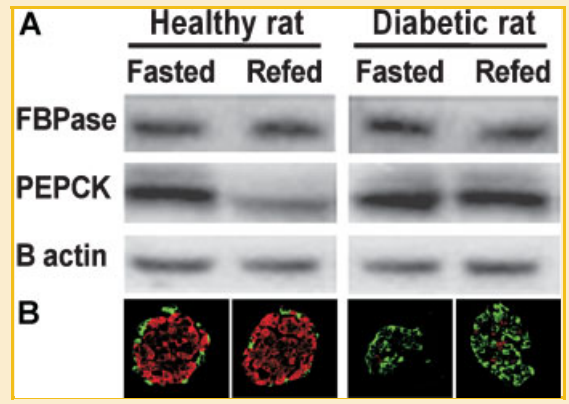

Fig. 1. Protein expression of FBPase in the liver from short-term diabetic rats. After a 12 h fasting, healthy and diabetic rats were re-fed for 12 h. A: Liver proteins were processed for immunoblot against hepatic FBPase, cytosolic PEPCK, and β actin as the control. B: Pancreas sections from the same animals were analyzed for insulin (red) and glucagon (green) expression by immunofluorescence. n = 5. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

was analyzed. Positive cells with a very strong signal were observed throughout the tissue in re-fed healthy rats, but a clear perivenous zonation and weaker signal was detected in re-fed diabetic rats. In the liver from fasted healthy and diabetic rats, no accumulation of glycogen was detected, and the respective controls with amylase treatment before PAS staining were always negative (data not shown). In this context, PEPCK periportal zonation was only and strongly decreased after feeding of healthy rats, whereas FBPase gradient remained unchanged in all conditions (Fig. 2).

FBPase ACTIVITY AND SUBCELLULAR FRACTIONATION

Cytoplasmic (Cy) and nuclear (Nu) fractions from fasted and re-fed healthy and diabetic rats were analyzed for FBPase protein and activity. In the healthy fasted rats, FBPase activity was only detected in the Cy fraction of hepatocytes, whereas after feeding its activity was also detectable in the nuclear fraction, corresponding to ~30% of the cytoplasmic activity (Fig. 3A, black bars). In the diabetic rat, FBPase activity was only detected in the cytoplasmic fraction (Fig. 3A, white bars). Notably, a significant but not proportional difference in the cytoplasmic activity of FBPase between fasted and re-fed healthy rats in spite of 30% activity translocation to the nucleus after re-feeding was observed under these experimental conditions. However, when we examined the ability of the detergent Triton X-100 to solubilize a particulate fraction of FBPase we observed a greater increase in assayable FBPase activity in the cytoplasm from fasted than re-fed rat liver (data not show). Correcting for the activities in the presence of Triton X-100, the sum of nuclear and cytoplasmic FBPase in re-fed liver equals that of cytoplasmic FBPase in fasted liver (data not shown) from healthy rats. Experiments using pure FBPase demonstrated that the action of Triton X-100 does not affect the enzyme activity (data not shown). As control, PEPCK activity was only detected in the cytoplasmic fraction (Fig. 3B), supporting the nuclear-associated activity of FBPase as a fact and not as a consequence of cytoplasmic

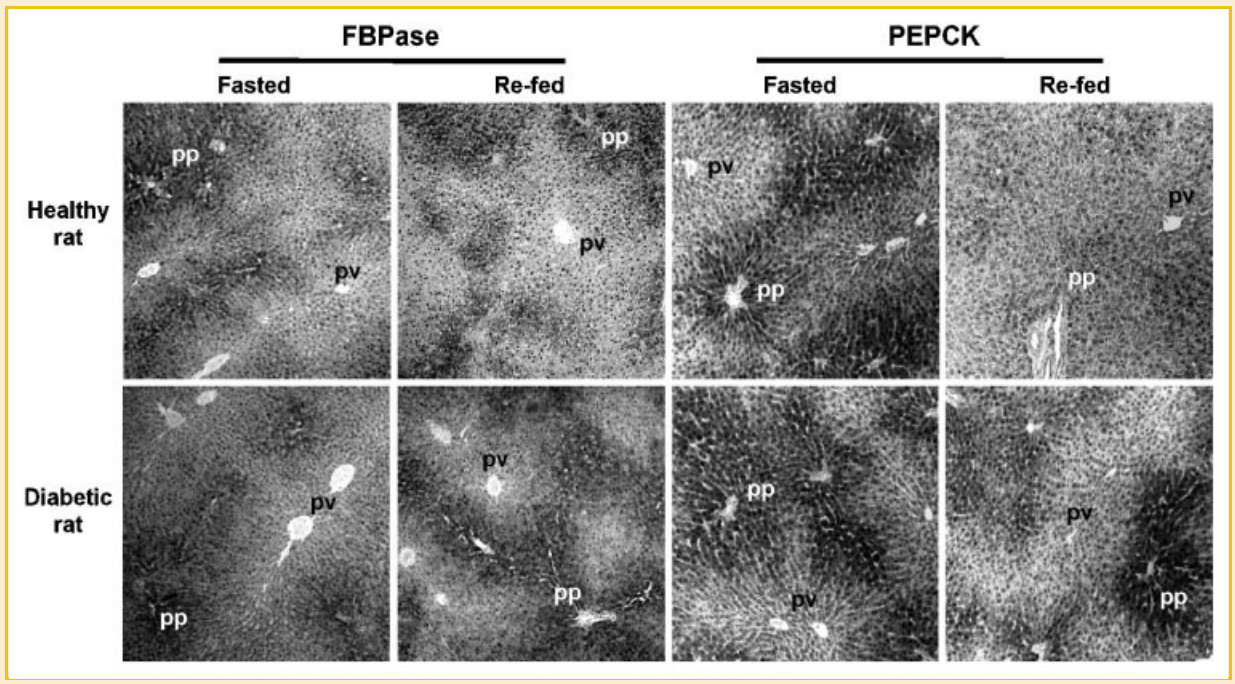

Fig. 2. Zonation of FBPase in the liver from healthy and diabetic rats. After a 12 h fasting, healthy and diabetic rats were re-fed for 12 h and the livers were fixed in Bouin's fluid and embedded in paraffin for histological sectioning. Immuohistochemical analysis was performed with specific antibodies against liver FBPase and cytosolic PEPCK. PP: Periportal area, PV: Perivenous area. n = 5.

contamination. Moreover, PEPCK activity decreased in response to feeding of healthy rats (Fig. 3B, black bars), but it was elevated in both fasted and re-fed diabetic rats (Fig. 3B, white bars). Western blot analysis of 10 μg total proteins from these same fractions confirmed the exclusive cytoplasmic detection of PEPCK, with the associated changes in protein expression (Fig. 3D), and the expected nuclear association of the splicing factor SC35 (data not shown); it also corroborated the ability of FBPase to concentrate

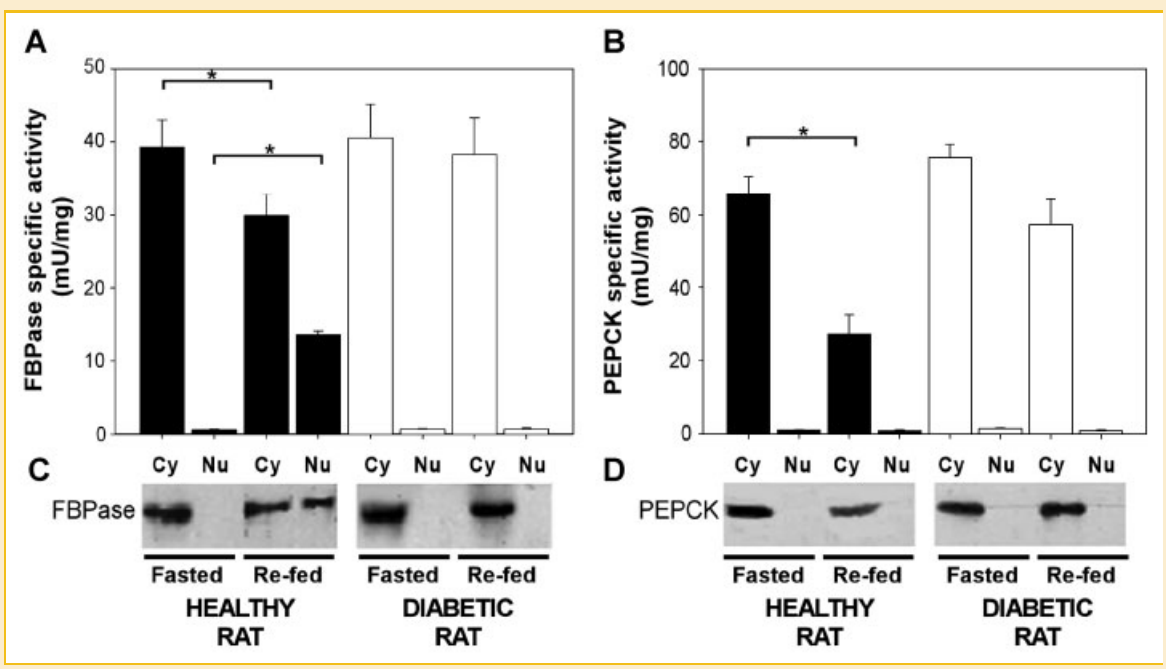

Fig. 3. Fractionation of FBPase activity in the re-fed rat liver. After a 12 h fasting, healthy (black bars) and diabetic (white bars) rats were re-fed for 12 h and liver proteins were processed for nuclear and cytoplasmic fractionation. FBPase (A) and PEPCK (B) activities were estimated and expressed as specific activity (mU/mg total proteins). In parallel, 10 μg of total proteins from each fraction were separated in SDS–PAGE and immunoblotted against FBPase (C) and PEPCK (D). $^{*}P < 0.05$. n = 5.

in the nuclear fraction of hepatocytes only after re-feeding of healthy rats (Fig. 3C).

SUBCELLULAR DISTRIBUTION OF HEPATIC FBPase IN RAT LIVER

Confocal microscopy analysis of hepatic FBPase showed a clear change of its subcellular distribution in response to the fasting and re-feeding condition in the liver from healthy rats (Fig. 4). In the liver of healthy fasted rats, FBPase was almost exclusively and uniformly detected in the cytoplasm of hepatocytes (Fig. 4A–C), while after re-feeding it was prominently localized in the cell periphery and the nuclear compartment (Fig. 4D–F). The opposite behavior was observed when fed rats were fasted, indicating that FBPase nuclear translocation is reversible (data not shown). Strikingly, in the liver from fasted and re-fed diabetic animals, the subcellular distribution of hepatic FBPase remained similar to that of the healthy fasted rat, being mostly localized in the cytoplasm (Fig. 4G–L). As a control, PEPCK was specifically detected in the cytoplasm of hepatocytes from fasted and re-fed healthy and diabetic rats (data not shown).

PHOSPHORYLATION STATE AND SUBCELLULAR DISTRIBUTION OF HEPATIC FBPase

Posphoproteins from rat liver cytoplasmic and nuclear fractions were enriched and analyzed by Western blot against PEPCK and

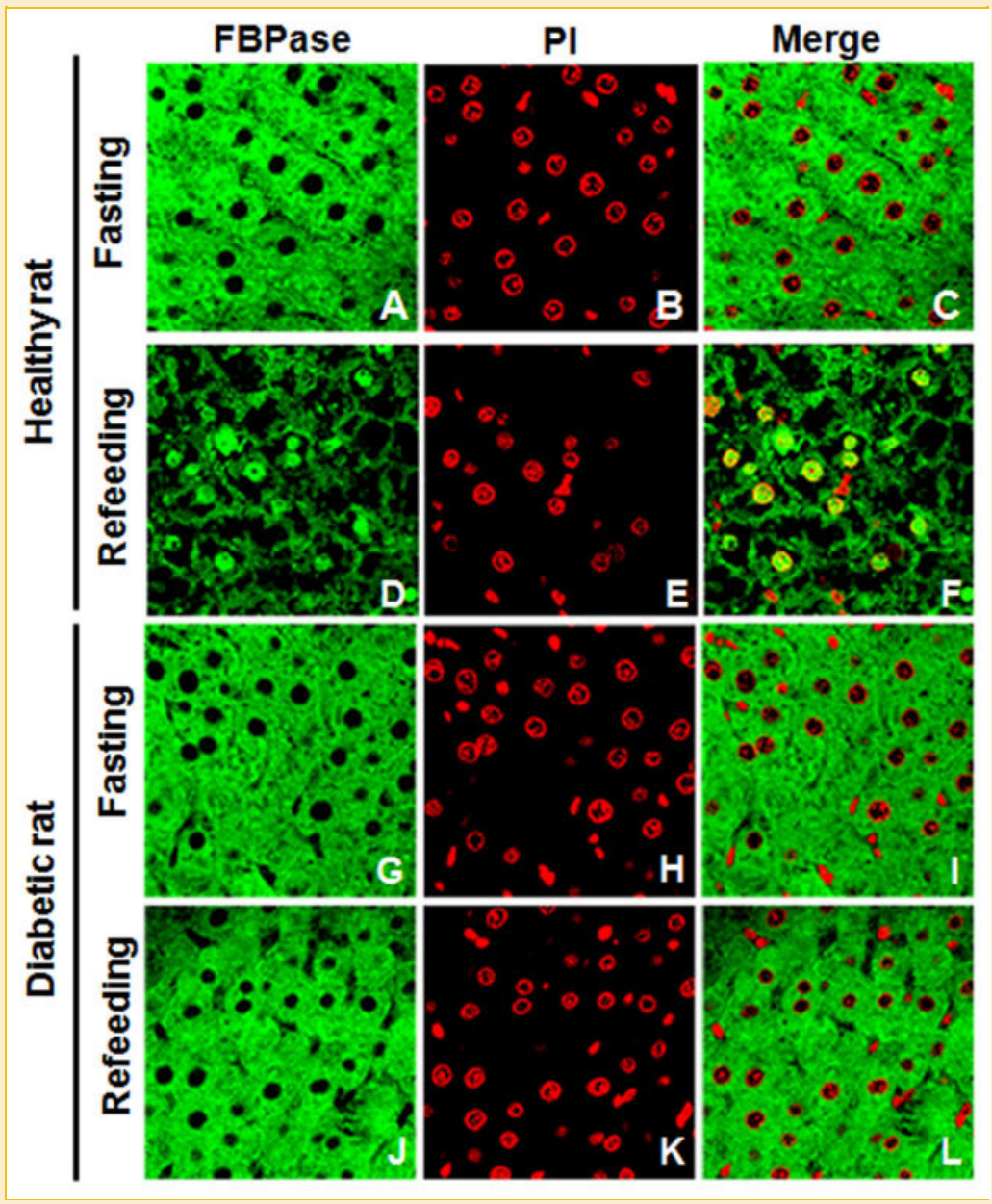

Fig. 4. Subcellular distribution of FBPase in the liver from healthy and diabetic rats. After a 12 h fasting, healthy and diabetic rats were re-fed for 12 h and livers were fixed in Bouin's fluid and embedded in paraffin. Liver sections were probed with anti-FBPase (green) and samples were analyzed by confocal microscopy. Nuclear staining was performed with propidium iodide (red). n = 5. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

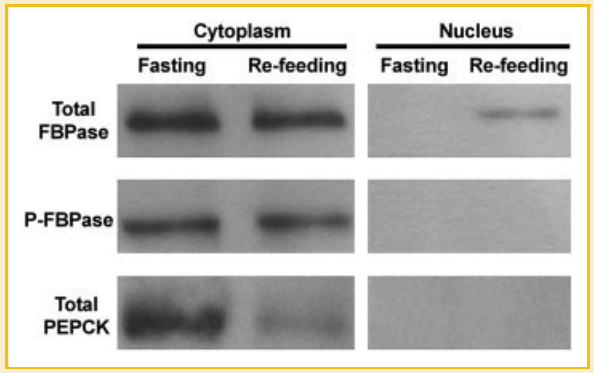

Fig. 5. Phosphorylation state and subcellular distribution of FBPase. After a 12 h fasting, healthy rats were re-fed for 12 h and liver phosphorylated proteins were enriched from nuclear and cytoplasmic fractions. Ten micrograms of proteins were separated by SDS–PAGE and immunoblotted against FBPase and PEPCK. P-FBPase: Phosphorylated FBPase. n = 4.

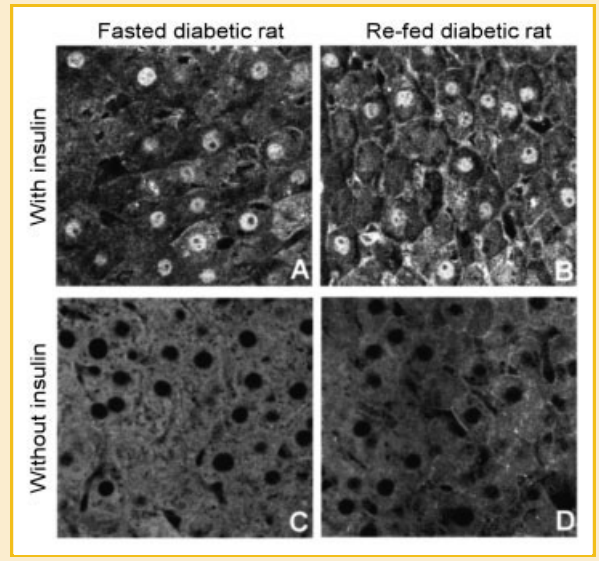

Fig. 6. Subcellular distribution of FBPase in the liver from insulin-supplemented diabetic rats. Diabetic rats were fasted during 12 h and then re-fed for 12 h, and injected with a single intraperitoneal dosis of 2.1 IU insulin (A–B). In parallel, diabetic animals without insulin supplementation were analyzed (C–D). All animals were sacrificed after 4 h and hepatic tissue was fixed in Bouin's fluid and embedded in paraffin. Liver sections were probed with anti-FBPase and analyzed by confocal microscopy. n = 3 for each group.

FBPase. Previous to the phosphoprotein enrichment, FBPase was detected in the cytoplasm of both fasted and re-fed healthy rats, but only in the nucleus of re-fed rats (Fig. 5, FBPase), while PEPCK was only detected in the cytoplasm (Fig. 5, PEPCK). After enrichment, phospho-FBPase was only detected in the cytoplasm of both fasted and re-fed healthy rats (Fig. 5, P-FBPase), whereas PEPCK was not detected (data not shown). In parallel, before phosphoprotein enrichment, cell extracts were incubated with Lambda Protein Phosphatase and then passed through the purification column. No detection of FBPase was observed after this treatment (data not shown).

INSULIN SUPPLEMENTATION AND FBPase SUBCELLULAR DISTRIBUTION IN DIABETIC RAT LIVER

In order to investigate the physiological effect of insulin over FBPase nuclear accumulation, we restored the hormone by a single intraperitoneal injection of insulin glargine (2.1 UI) to fasted and re-fed diabetic rats. Insulin effect was confirmed by analyzing plasma glucose decline each 30 min after injection (data not shown). Rats were sacrificed 4 h after injection and liver samples were analyzed by immunofluorescence and confocal microscopy. As control, livers from fasted and re-fed diabetic rats without insulin administration were analyzed in parallel (Fig. 6).

DISCUSSION

Previously, we have shown that both glucose and insulin were independently able to induce FBPase nuclear translocation in primary culture of rat hepatocytes [Yáñez et al., 2004]. Control of hepatic glucose production occurs through both hormonal and non-hormonal mechanisms, with hyperglycemia being prevented, in part, by the restraint exerted on glucose production by insulin, as well as by glucose per se. Although insulin and glucose levels rise simultaneously, they act to inhibit hepatic glucose production independently [Edgerton et al., 2009]. Our in vivo results demonstrate that FBPase is exclusively localized in the cytoplasm of hepatocytes during fasting, and it translocates to the cell periphery and the nucleus after feeding. In this context, both postprandial insulinemia and hyperglycemia can be responsible for the regulation of the subcellular localization of FPBase. The use of type I diabetic model provided us with a useful tool to analyze and separate the effects of hyperglycemia and hyperinsulinemia during feeding. Despite the severe hyperglycemia, FBPase was unable to translocate to the hepatocyte nucleus during diabetes, highlighting the importance to corroborate in vitro data with physiological experiments.

In contrast with PEPCK and G6Pase, a controversy exists about the degree of transcriptional regulation of FBPase. Changes in protein level and activity of FBPase were not observed in our model. Discrepancies may be the result of different fasting protocols, since more than 96 h of fasting were required to induce FBPase activity in rabbit liver [Pontremoli et al., 1974]. Moreover, the effect of metabolic changes over zonation of FBPase protein has not been analyzed; even the zonation of FBPase mRNA was reported to change during the starvation-refeeding cycle [Eilers et al., 1995]. As a control for the zonal response of the liver, glycogen accumulation was changed as reported by previous studies [Sokolović et al., 2008]. Opposite to the expectation for a gluconeogenic enzyme, periportal zonation of FBPase was unaltered in the fasted-to-fed transition. By contrast, expression and periportal zonation of cytosolic PEPCK was decreased, confirming previous studies [Miethke et al., 1986]. Our data are consistent with a poor short-term transcriptional regulation of hepatic FBPase, and support the idea that other factors, such as oxygen tension but not insulin, are involved in the zonated

expression of gluconeogenic enzymes in the rat liver [Kietzmann et al., 1997].

In addition to segregation of metabolic pathways in different cell types, other regulatory mechanisms are phosphorylation and subcellular localization of enzymes at the single cell level [Ros et al., 2009]. These studies are scarce in the regulation of enzymes from the glucoconeogenic pathway. Contrary to what we previously observed in isolated hepatocytes, hyperglycemia could not stimulate FBPase nuclear translocation at the physiological context of the liver from diabetic rats. This result strongly suggests that insulin is the main physiological stimulus involved in the regulation of FBPase subcellular dynamics. Moreover, our in vivo results clearly showed that regulatory mechanisms are more complex than in cell culture, where oxygen tension and cell–matrix interaction, among other micro-environmental stimuli, are lost after cell isolation [Kietzmann et al., 1997; Khetani and Bhatia, 2008]. Insulin is a potent and direct inhibitor of hepatic glucose production and its primary effect on the liver comes about by virtue of its interaction with its receptor. It can also reduce liver gluconeogenesis by indirect inhibition of glucagon secretion, since glucagon induces PEPCK and G6Pase gene expression [Barthel and Schmoll, 2003]. Glucagon also stimulates PKA-mediated FBPase phosphorylation [Ekdahl and Ekman, 1987]. We could not determine which residues are phosphorylated, because commercial antibodies against phospho-residues did not recognize FBPase, but we detected total phosphorylated FBPase mainly in the cytoplasmic fraction, accordingly with a putative insulin-induced dephosphorylation mechanism responsible for FBPase nuclear translocation [Ekdahl and Ekman, 1987]. Insulin stimulates glucose metabolism and an intermediate metabolite could be participating in FBPase nuclear translocation. GK is the predominant glucose phosphorylating activity in hepatocytes, and transcription of its gene is induced by insulin and dominantly repressed by glucagon, hence hyperglucagonemia and hipoinsulinemia during diabetes reduces GK by more than 90% in the liver [Iynedjian et al., 1988]. Following insulin administration to diabetic animals, the amount and activity of GK increased after 4 h [Iynedjian et al., 1988], approximately the same time required for accumulation of FBPase in the nuclei. Insulin stimulation of FBPase nuclear translocation might result from the indirect effect through GK-induced accumulation of a glucose intermediate, such as xylulose 5-P, which activates protein phosphatase 2A (PP2A) [Nishimura and Uyeda, 1995]. PP2A dephosphorylates the bifunctional enzyme fructose-6-phosphate,2-kinase:fructose-2,6-bisphosphatase, activating its kinase domain [Nishimura and Uyeda, 1995]. It could also be involved in FBPase dephosphorylation. Moreover, dephosphorylation of the bifunctional enzyme produces the accumulation of Fru-2,6-P_2, which once bound to FBPase might make it a better substrate for dephosphorylation and nuclear translocation. A possible explanation is that dephosphorylation exposes a nuclear localization signal (NLS). We found that liver FBPase contains a non-canonical putative NLS [Yáñez et al., 2003], which is totally conserved between FBPases isolated from mammalian liver till date. Since mice and rats are very closely related species, we expected the same behavior of liver FBPase in response to nutritional challenge. Nevertheless, when we assessed the subcellular distribution of mouse liver FBPase, only 30% of nuclear accumulation efficiency per hepatocyte (vs. 60% in rat) was observed after re-feeding (supplementary material, Fig. 1). Despite the high degree of identity between mammalian liver FBPases, rat liver FBPase has a C-terminal 25 residue-long extension, with three unique phosphorylatable serines which provide poor kinetic control in vitro [Ekdahl and Ekman, 1985]. We did not find significant kinetic differences between *wt* and C-terminal truncated rat liver FBPase, indicating that this unique extension does not participate in the enzyme activity (supplementary material, Fig. 2) and rather, it could be involved in nuclear translocation or retention of the enzyme. Unfortunately, FBPase nuclear accumulation was not observed in cell lines, and the use of isolated hepatocytes was restricted by low reproducibility of insulin-induced glycogen synthesis and nuclear translocation of FBPase and GK (data not shown). More studies are needed to fully understand the implication of the differences in nuclear accumulation between mice and rat liver FBPase. In this sense, Andrikopoulos et al. [1993] have shown that insulin-responsive gluconeogenic enzymes are normally regulated in the NZO mouse, a model of NIDDM, but an abnormality in the regulation of FBPase may contribute to the increase hepatic glucose production. Studies of the nucleo-cytoplasmic shuttling of FBPase in these mice will be important to address this question.

The function of nuclear FBPase is still unknown. Measurements of FBPase activity in nuclear fractions do not certainly demonstrate that it is active, because we disrupted nuclei for the experiment. However, even if FBPase retains its tetrameric active structure, there is no evidence about substrate availability in the nucleus. Assuming that substrate channeling impairs the occurrence of Fru-1,6-P_2 in the nucleus, it is possible that the enzyme is sequestered and/or it is exerting a different function. Since FBPase senses AMP, Fru-2,6-P_2, and Fru-1,6-P_2, it could be transmitting the metabolic and energetic status from the cytoplasm through the nucleus. This is the case for LDH and GAPDH, whose nuclear presence makes a link between the redox state and the cell cycle [Dai et al., 2008]. Notably, norepinephrine induced nuclear translocation of muscle FBPase through PKA activation in HL-1 cardiomyocytes [Gizak et al., 2009], which seems to be in contradiction with the present proposal for liver FBPase. However, muscle FBPase is the product of other gene, participates in glycogen synthesis but not gluconeogenesis, and its regulation is quite different to that of hepatic FBPase [Gizak et al., 2009]. Besides, FBPase has been also localized in the nucleus of neurons and astrocytes [Velásquez et al., 2010], indicating that this compartmentalization is important for all three FBPase isoenzymes in mammals, but may be not for the same purpose [Gizak et al., 2009]. In this sense, the nucleo-cytoplasmic shuttling of FBPase in hepatocytes is opposite to that of GK, which is isolated in the nuclear compartment as a rapid mechanism of inactivation during fasting [Chu et al., 2004]. This reciprocal distribution is in relation with the opposite metabolic pathways where each enzyme participates. Toyoda et al. [2000] and Fujimoto et al. [2004] have reported the deficient nuclear export of GK in different models of diabetic rats. Thus, reciprocal alterations in GK and FBPase nuclear-cytoplasmic ratios in the diabetic liver could be involved in the reduction of glucose uptake and the elevation of endogenous glucose production, respectively.

In conclusion, no difference in FBPase protein expression and liver zonation was observed in this study, but nuclear accumulation accounted for removal of enzyme activity from the cytoplasm of hepatocytes after feeding. This mechanism is not functional during diabetes where, despite hyperglycemia, low circulating levels of insulin impair FBPase nuclear accumulation. Together with PEPCK and G6Pase overexpression, these changes in FBPase subcellular distribution could explain the elevated gluconeogenic activity observed during fasting and diabetes. A specific FBPase inhibitor, CS-719, which has shown good results in decreasing plasma glucose with the suppression of hepatic endogenous glucose production and gluconeogenesis in type 2 diabetic rodents may re-establish the FBPase subcellular distribution [Yoshida et al., 2011]. However, it is still unknown if CS-719 regulates the subcellular localization of FBPase.

REFERENCES

Agius L. 2007. New hepatic targets for glycaemic control in diabetes. Best Pract Res Clin Endocrinol Metab 21:587–605.

Anan A, Baskin-Bey ES, Isomoto H, Mott JL, Bronk SF, Albrecht JH, Gores GJ. 2006. Proteasome inhibition attenuates hepatic injury in the bile duct-ligated mouse. Am J Physiol Gastrointest Liver Physiol 291: G709–G716.

Andrikopoulos S, Rosella G, Gaskin E, Thorburn A, Kaczmarczyk S, Zajac JD, Proietto J. 1993. Impaired regulation of hepatic fructose-1,6-bisphosphatase in the New Zealand obese mouse model of NIDDM. Diabetes 42:1731–1736.

Barthel A, Schmoll D. 2003. Novel concepts in insulin regulation of hepatic gluconeogenesis. Am J Physiol Endocrinol Metab 285:E685–E692.

Cárcamo JG, Yañez AJ, Ludwig HC, León O, Pinto RO, Reyes AM, Slebe JC. 2000. The C1-C2 interface residue lysine 50 of pig kidney fructose-1,6-bisphosphatase has a crucial role in the cooperative signal transmission of the amp inhibition. Eur J Biochem 267:2242–2251.

Chu CA, Fujimoto Y, Igawa K, Grimsby J, Grippo JF, Magnuson MA, Cherrington AD, Shiota M. 2004. Rapid translocation of hepatic glucokinase in response to intraduodenal glucose infusion and changes in plasma glucose and insulin in conscious rats. Am J Physiol Gastrointest Liver Physiol 286:G627–G634.

Colombo G, Lardy HA. 1981. Phosphoenolpyruvate carboxykinase (guanosine 5'-triphosphate) from rat liver cytosol. divalent cation involvement in the decarboxylation reactions. Biochemistry 20:2758–2767.

Dai R, Yu F, Goh S, Chng H, Tan Y, Fu J, Zheng L, Luo Y. 2008. Histone 2B (H2B) expression is confined to a proper NAD+/NADH redox status. J Biol Chem 283:26894–26901.

Davis MA, Hinerfeld D, Joseph S, Hui Y, Huang NH, Leszyk J, Rutherford-Bethard J, Tam SW. 2006. Proteomic analysis of rat liver phosphoproteins after treatment with protein kinase inhibitor H89 (N-(2-[p-Bromocinnamylamino-]ethyl)-5-isoquinolinesulfonamide). J Pharmacol Exp Ther 318:589–595.

De la Iglesia N, Veiga-da-Cunha M, Van Schaftingen E, Guinovart JJ, Ferrer JC. 1999. Glucokinase regulatory protein is essential for the proper subcellular localisation of liver glucokinase. FEBS Lett 456:332–338.

Edgerton DS, Ramnanan CJ, Grueter CA, Johnson KMS, Lautz M, Neal DW, Williams PE, Cherrington AD. 2009. Effects of insulin on the metabolic control of hepatic gluconeogenesis in vivo. Diabetes 58:2766–2775.

Eilers F, Modaressi S, Jungermann K. 1995. Predominant periportal expression of the fructose 1,6-bisphosphatase gene in rat liver: Dynamics during the daily feeding rhythm and starvation-refeeding cycle. Histochem Cell Biol 103:293–300.

Ekdahl KN, Ekman P. 1985. Fructose-1,6-bisphosphatase from rat liver. a comparison of the kinetics of the unphosphorylated enzyme and the enzyme phosphorylated by cyclic amp-dependent protein kinase. J Biol Chem 260:14173–14179.

Ekdahl KN, Ekman P. 1987. Effects of epinephrine, glucagon and insulin on the activity and degree of phosphorylation of fructose-1,6-bisphosphatase in cultured hepatocytes. Biochim Biophys Acta 929:318–326.

Erion MD, Dang Q, Reddy MR, Kasibhatla SR, Huang J, Lipscomb WN, van Poelje PD. 2007. Structure-guided design of amp mimics that inhibit fructose-1,6-bisphosphatase with high affinity and specificity. J Am Chem Soc 129:15480–15490.

Fujimoto Y, Donahue EP, Shiota M. 2004. Defect in glucokinase translocation in zucker diabetic fatty rats. Am J Physiol Endocrinol Metab 287:E414–E423.

Gizak A, Zarzycki M, Rakus D. 2009. Nuclear targeting of FBPase in HL-1cells is controlled by beta-1 adrenergic receptor-activated Gs protein signaling cascade. Biochim Biophys Acta 1793:871–877.

Heng S, Gryncel KR, Kantrowitz ER. 2009. A library of novel allosteric inhibitors against fructose 1,6-bisphosphatase. Bioorg Med Chem 17:3916–3922.

Iynedjian PB, Gjinovci A, Renold AE. 1988. Stimulation by insulin of glucokinase gene transcription in liver of diabetic rats. J Biol Chem 263: 740–744.

Jin ES, Uyeda K, Kawaguchi T, Burgess SC, Malloy CR, Sherry AD. 2003. Increased hepatic fructose 2,6-bisphosphate after an oral glucose load does not affect gluconeogenesis. J Biol Chem 278:28427–28433.

Khetani SR, Bhatia SN. 2008. Microscale culture of human liver cells for drug development. Nat Biotechnol 26:120–126.

Kietzmann T, Roth U, Freimann S, Jungermann J. 1997. Arterial oxygen partial pressures reduce the insulin-dependent induction of the perivenously located glucokinase in rat hepatocyte cultures: Mimicry of arterial oxygen pressures by H_2O_2. Biochem J 321:17–20.

Lawrence GM, Jepson MA, Trayer IP, Walker DG. 1986. The compartmentation of glycolytic and gluconeogenic enzymes in rat kidney and liver and its significance to renal and hepatic metabolism. Histochem J 18:45–53.

Matsuo K, Delibegovic M, Matsuo I, Nagata N, Liu S, Bettaieb A, Xi Y, Araki K, Yang W, Kahn BB, Neel BG, Haj FG. 2010. Altered glucose homeostasis in mice with liver-specific deletion of *src* homology phosphatase 2. J Biol Chem 285:39750–39758.

Miethke H, Wittig B, Nath A, Jungermann K. 1986. Gluconeogenic-glycolytic capacities and metabolic zonation in liver of rats with streptozotocin, non-ketotic as compared to alloxan, ketotic diabetes. Histochemistry 85: 483–489.

Nishimura M, Uyeda K. 1995. Purification and characterization of a novel xylulose 5-phosphate-activated protein phosphatase catalyzing dephosphorylation of fructose-6-phosphate,2-kinase:Fructose-2,6-bisphosphatase. J Biol Chem 270:26341–26346.

Pontremoli S, Melloni E, Salamino F, De Flora A, Horecker BL. 1974. Changes in activity and molecular properties of fructose 1, 6-bisphosphatase during fasting and refeeding. Proc Natl Acad Sci USA 71:1776–1779.

Rajas F, Jourdan-Pineau H, Stefanutti A, Mrad EA, Iynedjian PB, Mithieux G. 2007. Immunocytochemical localization of glucose 6-phosphatase and cytosolic phosphoenolpyruvate carboxykinase in gluconeogenic tissues reveals unsuspected metabolic zonation. Histochem Cell Biol 127: 555–565.

Reyes A, Burgos ME, Hubert E, Slebe JC. 1987. Selective thiol group modification renders fructose-1,6-bisphosphatase insensitive to fructose 2,6-bisphosphate inhibition. J Biol Chem 262:8451–8454.

Ros S, García-Rocha M, Domínguez J, Ferrer JC, Guinovart JJ. 2009. Control of liver glycogen synthase activity and intracellular distribution by phosphorylation. J Biol Chem 284:6370–6378.

Sokolović M, Sokolović A, Wehkamp D, Ver Loren van Themaat E, de Waart DR, Gilhuijs-Pederson LA, Nikolsky Y, van Kampen AHC, Hakvoort TBM, Lamers WH. 2008. The transcriptomic signature of fasting murine liver. BMC Genomics 9:528.

Toyoda Y, Ito Y, Tanigawa K, Miwa I. 2000. Impairment of glucokinase translocation in cultured hepatocytes from OLETF and GK rats, animal models of type 2 diabetes. Arch Histol Cytol 63:243–248.

Velásquez ZD, Pérez M, Morán MA, Yáñez AJ, Avila J, Slebe JC, Gómez-Ramos P. 2010. Ultrastructural localization of fructose-1,6-bisphosphatase in mouse brain. Microsc Res Tech [Epub ahead of print].

Yáñez AJ, Bertinat R, Concha II, Slebe JC. 2003. Nuclear localization of liver FBPase isoenzyme in kidney and liver. FEBS Lett 550:35–40.

Yáñez AJ, Garcia-Rocha M, Bertinat R, Droppelmann C, Concha II, Guinovart JJ, Slebe JC. 2004. Subcellular localization of liver FBPase is modulated by metabolic conditions. FEBS Lett 577:154–158.

Yáñez AJ, Ludwig HC, Bertinat R, Spichiger C, Gatica R, Berlien G, Leon O, Brito M, Concha II, Slebe JC. 2005. Different involvement for aldolase isoenzymes in kidney glucose metabolism: Aldolase B but not aldolase A colocalizes and forms a complex with FBPase. J Cell Physiol 202:743–753.

Yoshida T, Okuno A, Takahashi K, Ogawa J, Hagisawa Y, Kanda S, Fujiwara T. 2011. Contributions of hepatic gluconeogenesis suppression and compensative glycogenolysis on the glucose-lowering effect of CS-917, a fructose 1,6-bisphosphatase inhibitor, in non-obese type 2 diabetes Goto-Kakizaki rats. J Pharmacol Sci 115:329–335.