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Liver Glucokinase and Lipid Metabolism

Anna Vidal-Alabró, Andrés Méndez-Lucas, Jana Semakova,
Alícia G. Gómez-Valadés and Jose C. Perales
Department of Physiological Sciences II,
University of Barcelona, Barcelona,
Spain

1. Introduction

Control of energy metabolism is crucial for optimal functioning of organs and tissues. Amongst all nutrients, glucose is the principal energy source for most cells and, therefore, minimum blood glucose levels must be guaranteed. Alterations in glycaemia can lead to hyperglycaemic states (producing protein glycosylation and toxicity in glucose-sensitive cells) or hypoglycaemic states (that can affect brain function), both harmful. Therefore, mechanisms must exist to keep glycaemia in a narrow physiological range (4-8 mM) independently of the nutritional state. To achieve control of blood glucose levels, our body has a complex, interorgan signaling system using nutrients (glucose, lipids, amino acids), hormones (insulin, glucagon, ghrelin, etc.) and the autonomic nervous system. In response to these signals, organs and tissues (mainly intestine, endocrine pancreas, liver, skeletal muscle, adipose tissue, brain and adrenal glands) adapt their function to energetic requirements.

The liver plays a pivotal role in the maintenance of glucose homeostasis by continuously adapting its metabolism to energetic needs. In the fed state, when blood glucose levels are high and there is insulin, liver takes-up part glucose to replenish glycogen stores. Besides, when glucose stores are full, the liver has the capacity to synthesize lipids *de novo* from glucose for-long term energy storage. Lipids are packaged in very low-density lipoprotein (VLDL) particles and then transported to the adipose tissue. Conversely during starvation, when glycaemia falls and glucagon increases, the liver produces glucose to maintain circulating glucose levels by breaking down glycogen stores or by synthesizing glucose de novo through gluconeogenesis. Gluconeogenesis, as an energy-consuming pathway, is linked to β-oxidation of fatty acids (fuel supplier pathway).

From this introduction on the regulation of glucose homeostasis, one can appreciate the close relation that exists between carbohydrate metabolism and lipid metabolism in the liver. Therefore, alterations in hepatic carbohydrate metabolic pathways may directly affect hepatic and/or blood lipid levels. Particularly, this chapter will focus on evaluating the incidence of glucokinase (GK) –the first enzyme of the glycolytic pathway in the liver- on lipidemia and on hepatic lipid content. But first, an introductory overview of the physiology behind the first-pass metabolism of dietary glucose in the liver will be presented.

2. Liver glucose metabolism

After a meal rich in carbohydrates, high levels of glucose reach the liver via portal vein. Glucose enters passively the hepatocyte through GLUT-2, a facilitated glucose transporter, and then is rapidly phosphorylated by GK at the sixth carbon to obtain glucose-6-phosphate which cannot escape the cell. From a functional perspective, it is important to recognize that both GLUT2 and glucokinase are expressed in cell types in which glucose metabolism has to vary accordingly to extracellular glucose concentration (glucose sensors). The high Km for glucose of both proteins, and the absence of product inhibition by glucose-6-phosphate, ensure that glucose uptake and phosphorylation in these cells are proportional to extracellular glucose concentration throughout the physiological range of glycaemia
The product of GK reaction, glucose-6-phosphate, is the gateway to the major pathways of glucose utilization: glycogen synthesis, glycolysis, oxidation of glucose and pentose phosphate pathway. It should be noted that hepatic glycolysis provides pyruvate principally for lipid synthesis rather than for oxidation. As glucose is the main substrate for fatty acid synthesis, hepatic glycolytic enzymes can be considered an extension of the lipogenic pathway. Glucose, insulin and parasympathetic nervous system orchestrate these glucose metabolic pathways in the fed state, with the aim of maintaining normal levels of blood sugar.

2.1 Glycogen synthesis

Two enzymes, glycogen synthase and glycogen phosphorylase, control glycogen levels. Both enzymes are regulated by phosphorylation and allosteric modulators. Specifically in the fed state, insulin activates glycogen synthase (limiting enzyme for glycogen synthesis) by promoting its dephosphorylation and, at the same time inhibits glycogen phosphorylase (important for glycogen breakdown). Meanwhile, glucose-6-phosphate binding to glycogen synthase favours its dephosphorylation, promoting glycogen synthase activity (Bollen, 1998; Agius, 2008). As a result, glucose coming from bloodstream fills hepatic glycogen stores.

2.2 Lipogenesis *de novo*

Hepatic lipogenesis is induced upon ingestion of excess carbohydrates to convert extra carbohydrates to triglyceride for long-time energy storage. Once inside the hepatocyte, glucose enters glycolytic pathway and provides pyruvate, which enters mitochondrion where it is converted into acetyl-CoA by pyruvate dehydrogenase. On the other side, in the cytoplasm glucose is also oxidized through the pentose phosphate pathway and NADPH is obtained. Acetyl-CoA will serve for fatty acid and also cholesterol synthesis. The initial steps for fatty acid synthesis are the transfer of acetyl-CoA from mitochondria to the cytoplasm and its conversion into malonyl-CoA under the action of the enzyme acetyl-CoA carboxylase. Importantly, malonyl-CoA is a regulatory molecule because it inhibits carnitine palmitoyltransferase-1, a rate limiting enzyme in β-oxidation of fatty acids. Therefore, increasing malonyl-CoA favours lipogenesis. Malonyl-CoA is elongated using NADPH under the action of the enzyme fatty acid synthase. Once obtained, fatty acids can be esterified with glycerol to form diglyceride and triglyceride. Most of the triglyceride is produced for export to the adipose tissue, but in order to be secreted, it must be packaged in very low-density lipoprotein (VLDL) particles together with cholesterol, phospholipids and apolipoprotein B (Figure 1).

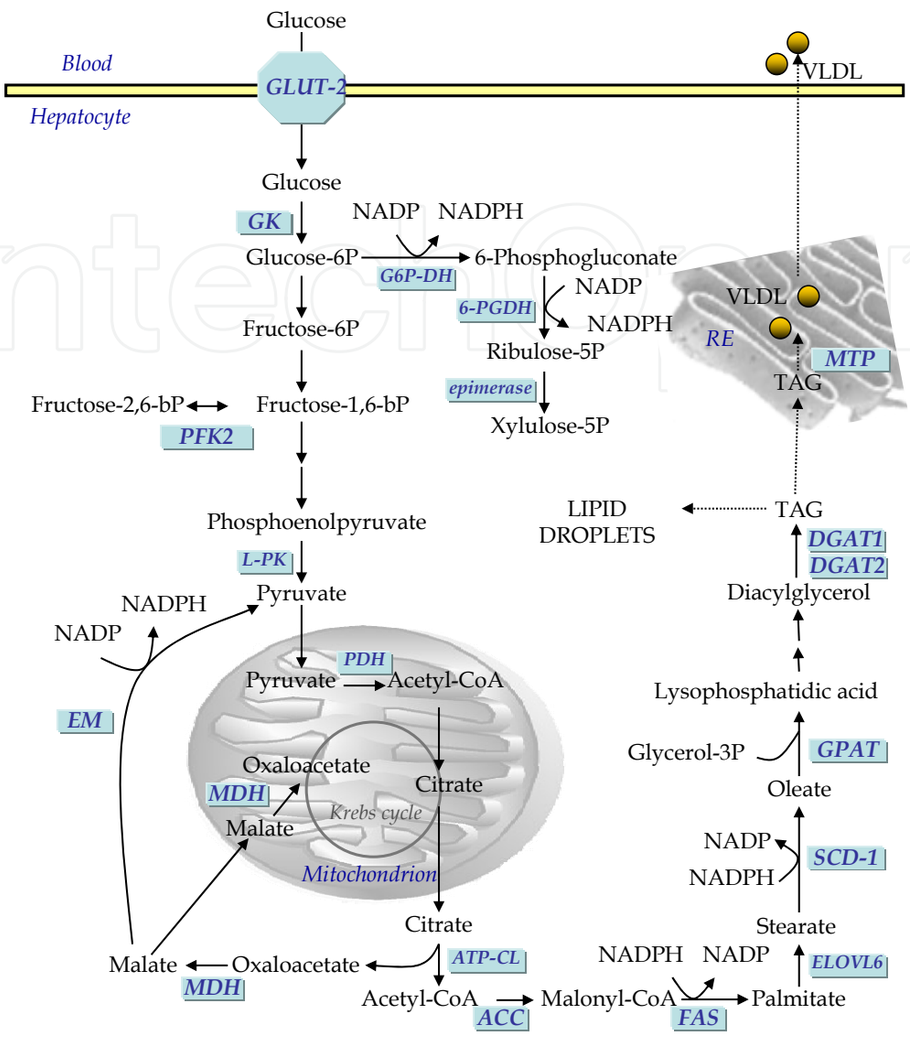

Fig. 1. Scheme of de novo lipogenesis from glucose. Once inside the hepatocyte, glucose is metabolized on one hand through glycolysis to pyruvate (GK means glucokinase; PFK-2, 6, phosphofructo-2-kinase/fructose-2,6-bisphosphatase; L-PK, liver-pyruvate kinase). On the other hand, glucose is oxidized through pentose phosphate pathway to obtain NADPH (G6P-DH means glucose-6-phosphate dehydrogenase; 6-PGDH, 6-phosphogluconate dehydrogenase). Pyruvate enters the mitochondrion to obtain citrate (PDH means, pyruvate dehydrogenase; MDH, malate dehydrogenase and EM, malic enzyme). De novo synthesis of fatty acids starts with citrate (ATP-CL means ATP citrate lyase; ACC, acetyl-CoA carboxylase) and after suffering elongation and desaturation reactions (ELOVL6 means elongase that catalyzes the conversion of palmitate to stearate; SCD-1, stearoyl-coenzyme A desaturase), fatty acids are converted to triglyceride (TAG) (GPAT means glycerol-3-phosphate acyltransferase; DGAT, diacylglycerol acyltransferase). Triglyceride can be stored in the liver but are mostly packaged into VLDL (very low-density lipoprotein) and secreted to bloodstream (MTP means microsomal triglyceride transfer protein). Original artwork.

In mammals, hepatic lipogenesis is controlled by several transcription factors, mainly SREBP-1c (sterol regulatory element binding protein 1c) and ChREBP (carbohydrate-responsive element-binding protein), but also by PPAR-γ (peroxisome proliferator-activated receptor gamma), LXR-α (liver X receptor alpha) and XBP1, all of them regulated by nutritional and hormonal conditions.

SREBP-1c plays a major role in the induction of lipogenic genes by insulin. SREBP-1c is a member of the bZIP transcription factor family that was originally identified as a mediator of sterol signaling (Wang, 1994), and is produced as a precursor form that reside in the endoplasmic reticulum in an inactive state. On one hand insulin stimulates SREBP-1c gene transcription, and on the other hand, induces the maturation of SREBP-1c precursor (Shimomura, 1998). Mature SREBP-1c moves to nucleus and activates transcription of several lipogenic genes with SRE (sterol regulatory elements) sequences in their promoters, for instance fatty acid synthase (FAS), stearoyl-Coenzyme A desaturase 1 (SCD-1), etc. (Figure 2) (Foretz, 1999; Ferre, 2010).

Glucose regulates genes of glycolytic and lipogenic pathways by activating ChREBP (Iizuka, 2008). ChREBP is a transcription factor that binds to ChoRE sequences present in the promoter of ACC (acetyl Coenzyme-A carboxylase), fatty acid synthase (FAS), stearoyl-Coenzyme A desaturase 1 (SCD-1), L-pyruvate kinase (L-PK), etc. (Uyeda, 2006). Under basal conditions, ChREBP is phosphorylated at Ser196 and remains in the cytosol. When glycaemia increase, glucose enters the hepatocyte and is metabolized. Therefore there is an increase in some glucose metabolites such as xylulose-5P, which promotes ChREBP dephosphorylation (Kabashima, 2003). Then, ChREBP rapidly moves to the nucleus and will activate transcription of its target genes (Figure 2).

SREBP-1c and ChREBP are also transcriptionally activated by liver X receptor apha (LXR-α), which could be a glucose sensor although it is controversial (Mitro, 2007; Denechaud, 2008). LXR-α is classically activated by oxysterols and it is important for the transcription of some lipogenic genes, a part form SREBP-1c and ChREBP, since their promoters contain LXRE (LXR response element) sequences (Chen, 2004; Cha, 2007).

XBP1, a transcription factor best known as a key regulator of the unfolded protein response (UPR), has been surprisingly associated with *de novo* fatty acid synthesis in the liver. It seems to be induced by diet carbohydrates and its deletion in mice causes hypocholesterolemia and hypotriglyceridemia, attributed to diminished hepatic lipid production (Lee, 2008). But, there are still some questions about its function to answer: what is its binding site in the promoter regions of these genes? Does it act alone or in partnership with other known transcription factors such as SREBP, ChREBP and LXR?

In summary, hepatic lipogenesis is regulated by several transcription factors that may probably work synergistically (Figure 2). With this complex system, carbons from glucose can be directed to fatty acid synthesis only when there is substrate availability and glycogen depots have been replenished. Altered fatty acid synthesis in the liver can lead to changes in lipid secretion, and consequently to dyslipidemia (Ginsberg, 2006).

2.3 Inhibition of hepatic glucose production

During fasting, liver produces glucose that enters bloodstream in order to maintain glycaemia, ensuring fuel supply for brain and red blood cells. But after a meal, when diet glucose arrives, hepatocytes must switch glucose production to glucose uptake. Insulin and high glucose levels coordinate the inhibition of glycogenolysis and gluconeogenesis (glucose producing pathways).

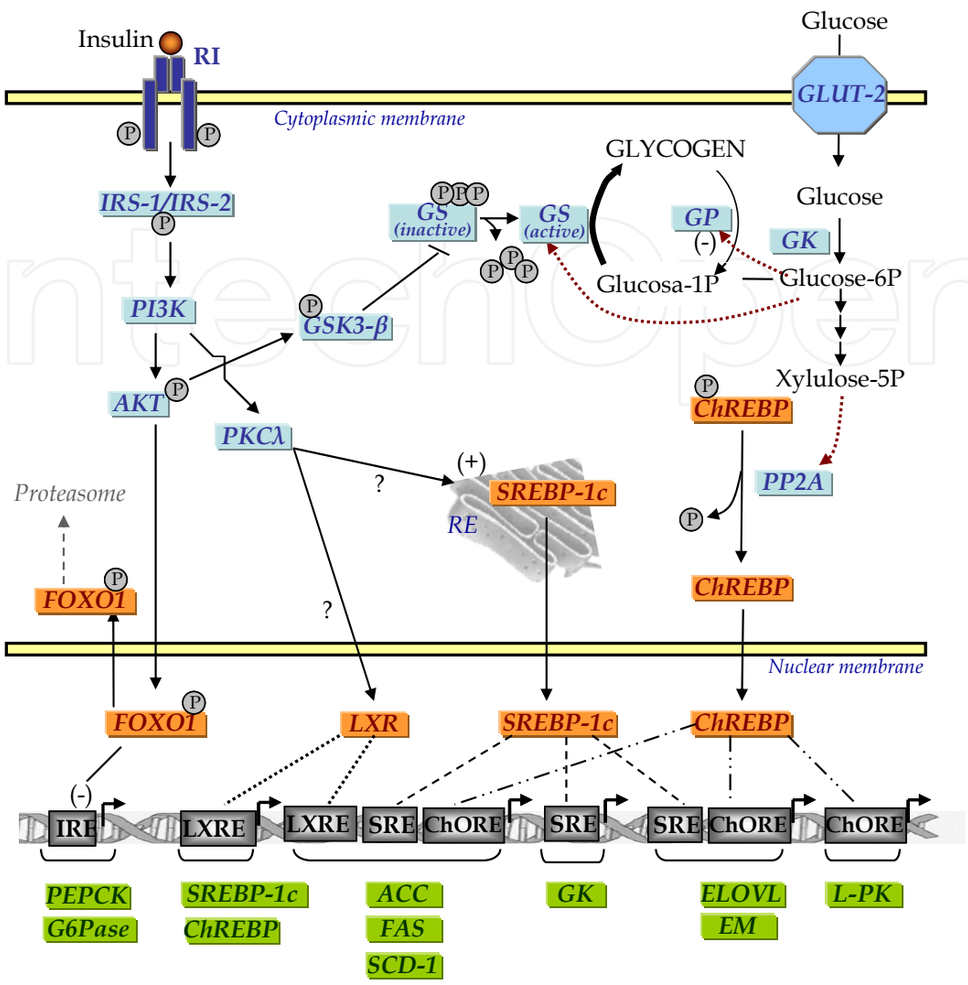

Fig. 2. Main regulatory mechanisms of hepatic metabolism in fed state. Insulin and glucose direct gene transcription to switch from glucose producing pathways to glucose uptake and storage. Briefly, insulin signaling promotes the phosphorylation of FOXO1 that results in its nuclear exclusion and proteasome degradation; consequently, transcription of gluconeogenic genes is inhibited. Besides, insulin stimulates transcription of lipogenic genes through SREBP-1c activation and probably through LXR-α, as well. Finally, insulin signaling causes activation of glycogen synthase function. Glucose also controls allostericaly glycogen synthesis and promote transcription of lipogenic genes via activation of ChREBP. IR means insulin receptor, IRS, insulin receptor substrate; PI3K, phosphoinositide 3-kinse; AKT, Ser/Thr protein kinase; GSK3-β, glycogen synthase kinase 3.beta; FOXO1, forkhead box O1; PCK, protein kinase C; LXR, liver X receptor; SREBP-1c, sterol regulatory element binding protein 1c; ChREBP, carbohydrate response element binding protein; GS, glycogen synthase; GP, glycogen phosphorylase; GK, glucokinase; PP2A, protein phosphatase 2A); IRE, insulin response element; LXRE liver X receptor response element; SRE, sterol regulatory elements; ChORE, carbohydrate-response elements; PEPCK-C, cytosolic phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; ACC, Acyl-CoA carboxylase; FAS, fatty acid synthase; SCD-1, stearoyl-CoA desaturase 1; ELOVL , EM, malic enzyme and L-PK, liver-pyruvate kinase. Original artwork.

Insulin directly inhibits the transcription of gluconeogenic genes by promoting the phosphorylation of FOXO1 (forkhead box O), a transcription factor necessary for the induction of gluconeogenesis in conjunction with PGC-1α (PPAR-gamma coactivator 1-alpha) (Puigserver, 2003). In addition, SREBP-1c promotes the inhibition of some gluconeogenic genes. Insulin also represses glycogenolysis by phosphorylating glycogen synthase (Bollen, 1998). On the other hand, insulin regulates hepatic glucose production indirectly: a) it suppresses lipolysis in adipose tissue causing a reduction in glycerol (gluconeogenic substrate) availability; b) it inhibits glucagon secretion in the pancreas; and c) it activates hypothalamic pathways important for glucose homeostasis.
Synergistically with insulin, glucose inhibits glycogenolysis allosterically (Bollen, 1998). Glucose inhibition on gluconeogenesis is mediated by glucose metabolites, specifically fructose-2,6-bisphosphate (Wu, 2001) and xylulose-5-phosphate (Massillon, 1998).

3. Glucokinase regulates the fate of glucose carbons in the liver

In order to enter the lipogenic pathway, glucose must be metabolized. The first and rate-limiting step is the phosphorylation of glucose at the 6th carbon to obtain glucose-6-phosphate. This reaction is catalyzed by glucokinase (GK; EC 2.7.1.1), a member of the hexokinase family. However, GK differs from other hexokinases in its particular kinetic properties: affinity for glucose that is within the physiological plasma concentration range ($S_{0.5}$ for glucose of 8 mM), positive cooperativity for glucose although it is a monomeric enzyme, and lack of inhibition by glucose-6-phosphate (Table 1).

	HEXOKINASES 1-3	GLUCOKINASE
Molecular weight	100 KDa	50 KDa
Substrates	Hexoses	Glucose
$S_{0.5}$ for glucose	< 0.5 mM	8 mM
Kinetic	Hyperbolic	Sigmoidal
Product inhibition	Yes	No

Table 1. Hexokinase family kinetic properties

As a result of its kinetic characteristics, intracellular glucose phosphorylation rate inside the hepatocyte correlates with glycaemia. Hence, GK can be considered an intracellular glucose sensor. Consequently, apart from hepatocytes, GK is expressed in glucosensitive cells of the pancreas, hypothalamus, anterior pituitary gland, and entero-endocrine K and L cells of the gut (Schuit, 2001; Zelent, 2006; Vieira, 2007; Iynedjian, 2009), all of them crucial in the control of the whole-body glucose homeostasis.
Liver contains 99.9% of the body GK. Therefore, is not surprising that this enzyme influences intermediary metabolism and energy storage. GK reaction controls the flux of glucose through several metabolic pathways: glycolysis, glucose oxidization, glycogenesis, triglyceride synthesis, phospholipids and cholesterol synthesis, glycogenolysis and gluconeogenesis. For that reason, GK is an enzyme highly regulated in the liver, both at the transcriptional and the post-transcriptional level.

3.1 Regulation of GK activity in the liver

Gck gene has two distinct promoters and one of them directs gene transcription specifically in the liver (Postic, 1995). Hepatic GK expression responds to nutritional changes; it is

activated by insulin and inhibited by glucagon. Insulin induction of Gck gene expression is through the PI_3-kinase/Akt signaling. However, no IRE (insulin response element) has been described in Gck promoter, and it is not clear which transcription factor mediates insulin-directed Gck expression. SREBP-1c is a candidate to mediate insulin-directed expression of Gck, although controversial results exist (Foretz, 1999; Gregory, 2006). Probably, SREBP-1c is not essential for rapid induction of GK transcription, but it can have a role for long-term expression. Other candidates to mediate insulin-dependent expression of Gck gene are the complex HIF-α/HNF-4/p300 (Roth, 2004), and ERR-α -estrogen-related receptor alpha- (Zhu, 2010).

GK can be modulated by covalent modifications such as nitrosylation and phosphorylation. However, the physiological importance of these modifications is still not determined. More importantly, protein interaction affects GK activity and even intracellular distribution. It has been described that GK in the liver can interact with GKRP (glucokinase regulatory protein), BAD (Bcl-xL/Bcl-2-Associated Death Promoter), PFK-2 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase), GKAP (glucokinase-associated protein), etc. (Massa, 2010). From all GK protein partners, GKRP is the best studied and has high physiological relevance in the liver.

3.1.1 Post-transcriptional regulation by GKRP

GKRP regulation of GK affects both the activity and subcellular localization of the enzyme. GKRP is a competitive inhibitor with respect to glucose. Van Shaftingen *et al* proposed a mechanistic model (Van Shaftingen, 2004); GKRP exists in two conformations, one with low affinity for GK and the other with high affinity. Fructose-1-phosphate and fructose-6-phosphate bind to the same binding site in the GKRP protein. When fructose-1-phosphate is bound to GKRP, GKRP adopts a conformation with low affinity for GK, and on the contrary, when the binding of fructose-6-phosphate to GKRP favours its interaction with GK.

But, Kamata *et al* also described that GK can exist in different conformations with different affinity for glucose (Kamata, 2004); in the absence of glucose, the enzyme exists in a super-open conformation thermodynamically stable and with low affinity for substrate. When glucose binds to it, there is a conformational change to an open form and next to a closed conformation that binds ATP. Then the catalytic cycle completes, after reaction products are released, GK can relax to an open or to a super-open conformation, depending on glucose concentrations (considering that the open conformation has higher affinity for glucose). GK conformation is important for GKRP protein interaction, as it can only take place when GK is in the super-open conformation (Anderka, 2008). From these conformational models of GKRP and GK, one can extrapolate the exquisite influence of carbohydrate concentration in regulating GKRP/GK binding and, consequently, GK phosphorylating activity.

GKRP also plays a fundamental role in importing GK to the nucleus, as it can be deduced from animals null for GKRP that present GK permanently in the cytosol (Farrelly, 1999). At low glucose concentrations, GKRP binds to GK and the formation of GKRP/GK complex results in entry and sequestration of both proteins in the nucleus of hepatocytes. However, it is still not resolved how GK is translocated to the nucleus. On the other hand, in metabolic states with high glucose concentrations, accompanied or not by high fructose levels, and sufficient ATP, there is the dissociation of the GKRP/GK complex. GK has a nuclear export signal sequence. Therefore, once dissociated from the complex, GK can be exported to the cytoplasm (Shiota, 1999). Insulin also favours the dissociation of the complex.

The physiological function of GKRP consists of inhibiting GK activity by sequestering it to the nucleus. GKRP binding also serves to stabilize GK protein and protect it from degradation. Thus, thanks to GKRP a big reservoir of GK exists in the nucleus of the hepatocyte at low glucose concentration. After a meal, this reservoir of GK can be rapidly mobilized (translocation is complete within 30 minutes) to the cytosol in order to promote glucose uptake and storage in the liver. This regulation process is much more fast and efficient than the synthesis de novo of GK promoted by insulin. Conversely, when glucose uptake has finished, GK returns to the nucleus in order to save energy because, on one hand, this translocation avoids the futile cycle between glucose and glucose-6-phosphate, and on the other hand, it ends the glucose signal generated by GK activity that activate transcription of glycolytic and lipogenic genes (Figure 3). The consequence of GK translocation to the nucleus in the post-absorptive state is the induction of glycogenolysis and gluconeogenesis.

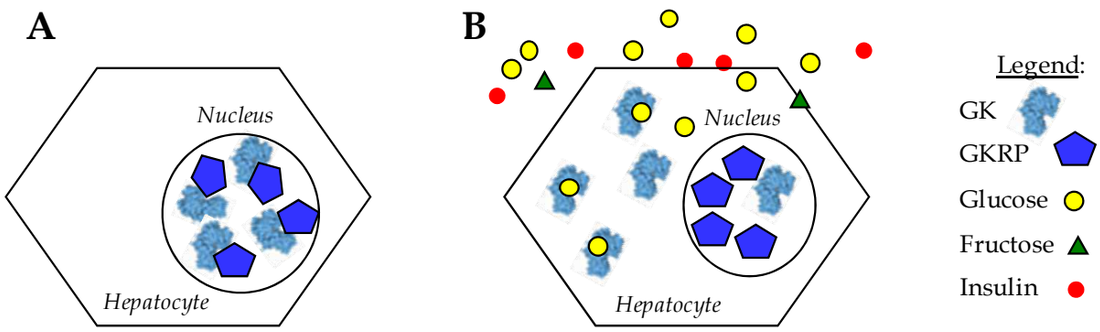

Fig. 3. Subcellular localization of GK regulated by GKRP. (A) During fasting, GK is sequestered in the nucleus where it remains bound to GKRP and inactive. After a meal, nutritional signals (i.e. insulin, glucose and fructose) induce the dissociation of the GK/GKRP complex and free GK translocates to the cytosol. Original artwork.

To summarize, thanks to its kinetic properties and its subtle regulation, GK enables the liver to adapt its metabolism for glucose uptake or glucose production as required, and consequently to regulate energy homeostasis.

3.2 GK modulation in the liver: impact on carbohydrate and lipid metabolism

Numerous natural mutations in GK gene have been associated to disease (Gloyn, 2003 & Osbak, 2009), reinforcing the concept that it is a crucial enzyme in the control of whole-body glucose homeostasis. Mutations that cause decrease or loss of GK activity are associated to maturity onset diabetes of the young-2 (MODY-2) or to permanent neonatal diabetes mellitus (PNDM). In diabetes, as a result of impairment in insulin secretion, the capacity of the liver to uptake glucose is diminished. On the other hand, activating mutations of GK cause persistent hyperinsulinemic hypoglycemia in infancy (PHHI). The phenotype of all these pathologies is mainly dominated by GK function in the pancreatic β-cell, where it regulates glucose-dependent insulin secretion. As insulin controls hepatic GK transcription and influences GKRP regulation, it is difficult to elucidate which are the specific consequences of these mutations on hepatic GK independently of insulin.
Some animal models have been developed to study the specific role of liver GK on metabolism.

3.2.1 Genetic suppression of hepatic GK

A liver specific GK knock-out was obtained using the LoxP-Cre system (Postic, 1999). Transgenic mice showed mild hyperglycemia and hyperinsulinemia in basal conditions, without changes in hepatic glycogen, plasma non-esterified fatty acids, triglycerides or β-hydroxybutyrate. In hyperglycaemic clamp studies, reduced hepatic glucose uptake and glycogen levels were observed in KO animals; however, results on lipid profile were not provided.

3.2.2 Genetic overexpression of GK in the liver

Several liver GK gain-of-function studies, both using transgenic animals and by means of adenovirus gene transfer, have been performed in healthy animals and models of diabetes such as streptozotocin induced type I and type II induced by ingestion of high fat/high carbohydrate diet. Due to heterogeneity, these studies will be examined according to the animal model and analysis conditions.

a. Overexpression of GK in the liver of fed, healthy animals is summarized in Table 2 (Ferre, 1996a, 1996b, 2003; O'Doherty, 1999; Scott, 2003).

Study variables	Ferre 1996a, 1996b Ferre 2003		O'Doherty 1999; Scott 2003	
Animal model	*Mus musculus*		*Rattus norvegicus*	
	Transgenic		Adenoviral gene transfer	
	PEPCK-C promoter		CMV promoter	
GK activity over control	x 2		x 3	x 6.4
Age at analysis	2 months	12 months	5 days post-injection (Rats 200-250 g)	
Glycaemia	decrease	-	no change	Decrease
Blood lactate	-	-	increase	Increase
Blood triglycerides	~ increase	-	~ increase	Increase
NEFA	~ increase	-	no change	increase
Insulin	decrease	increase	no change	decrease
Hepatic glucose-6-P	increase	~ increase	-	-
Hepatic glycogen	increase	decrease	no change	no change
Hepatic triglycerides	no change	increase	-	-
Modulation of enzymes and transcription factors	↑ L-PK ↓PEPCK-C, GLUT-2, TAT	-	↑ L-PK, ACC1, FAS, G6Pase. No change in PEPCK-K	-

Table 2. Hepatic GK overexpression studies in healthy fed animals. Comments: decrease, increase and no change are referred to control group. "~" means no statistically significant; "-", no determined; "CMV", cytomegalovirus; "PEPCK-C", cytosolic phosphoenolpyruvate carboxykinase; "L-PK", liver pyruvate kinase; "TAT", tyrosine aminotransferase; "ACC1", Acetyl-Coenzyme A carboxylase 1; "FAS", fatty acid synthase; and "G6Pase", glucose-6 phosphatase.

In these models, enhancing hepatic glucose uptake by GK overexpression results in a direct reduction of glycaemia. As a consequence of lower blood glucose levels, pancreatic β-cell

secretes less insulin. Therefore, a decrease in insulinemia is a secondary effect of increasing hepatic GK activity. But, O'Doherty et al demonstrate that the influence of GK activity on blood glucose and insulin levels could be dose-dependent, as it occurs only with high doses of their transgene. In the hepatocyte, glucose-6-phosphate derived from GK activity is directed to glycogen synthesis and, consequently, hepatic glycogen levels are increased in the study by Ferre et al. However, glycogen content is not modified by GK overexpression in the study of O'Doherty et al, maybe for intrinsic limitations. In both animal models, increasing GK activity results in glucose signaling that activates transcription of glycolytic and lipogenic genes. Lipogenic proteins together with high availability of citrate and ATP (derived from augmented glucose metabolism) lead to enhanced *de novo* lipogenesis in the liver, and consequently, higher secretion of VLDL to bloodstream that could explain the observed increase in blood triglycerides. Augmented blood fatty acids might be explained by insulin levels; low levels of this hormone result in low inhibition of lipolysis in the adipose tissue, and consequently fatty acids raise in the bloodstream. Importantly, Ferre et al show that long-term GK overexpression drives to hyperinsulinemia and hepatic steatosis.

b. Studies of GK overexpression in the liver of fasted, healthy mice are listed in Table 3 (Hariharan, 1997; O'Doherty, 1999; Desai, 2001; Ferre, 2003 & Scott, 2003)

Study variables	Hariharan 1997	Desai 2001	Ferre 2003	O'Doherty 1999 Scott 2003
Animal model	*M. musculus*	*M. musculus*	*M. musculus*	*R. norvegicus*
	Transgenic	Adenovirus	Transgenic	Adenovirus
Promoter	apoA1-SV40	RSV	PEPCK-C	CMV
GK activity over control	x5	x1.5	x2	x2.1 or x3
Age at analysis	5 weeks	3 weeks post-injection	12 months	4-5 days post-injection
Glycaemia	Decrease	no change	-	no change
Blood lactate	Decrease	no change	-	~ decrease
Blood triglycerides	no change	no change	increase	increase
NEFA	~ increase	no change	-	no change
Insulin	Decrease	decrease	increase	no change
Hepatic glucose-6-P	-	-	~ increase	-
Hepatic glycogen	-	-	no change	increase
Hepatic triglycerides	-	-	increase	-
Modulation of enzymes and transcription factors	-	-	-	↑ L-PK, ACC1 No change: PEPCK-C, PFK-2

Table 3. Hepatic GK overexpression studies in healthy fasted animals. Comments: decrease, increase and no change are referred to control group. "~" means no statistically significant; "-", no determined; "CMV", cytomegalovirus; "RSV", rose sarcoma virus; "apoA1-SV40", apolipoprotein A1 enhancer and simian vacuolating virus 40 promoter; "PEPCK-C", cytosolic phosphoenolpyruvate carboxykinase; "L-PK", liver pyruvate kinase; "ACC1", Acetyl-Coenzyme A carboxylase 1; "PFK-2", 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase.

In fast state, the influence of hepatic GK overexpression on glycaemia is not clear. Hariharan et al showed a decrease in glycaemia, accompanied by a decrease in insulinemia that could explain a reduction of glycolysis in skeletal muscle, causing the observed decline in serum lactate. Low insulin levels can also explain the increment of blood fatty acids. Interestingly, 20-weeks old mice were smaller than controls and presented reduced body mass index. On the contrary, long-term analysis of transgenic mice developed by Ferre et al showed that increasing GK activity in the liver lead to hepatic steatosis, hyperglycemia, hyperinsulinemia, obesity and insulin resistance. On the other hand, adenoviral gene transfer models for hepatic GK overexpression in fasting revealed induction of lipogenesis and consequently a tendency to increase blood triglycerides, without affecting glycaemia.

c. Studies on hepatic GK overexpression in the context of type 1 diabetes mellitus: This is an autoimmune disease with specific destruction of insulin-producing β-cells in the pancreas, and results in loss of insulin production. As insulin stimulates the transcription of Gck gene in the liver, type 1 diabetic subjects do not have GK protein in their livers and consequently hepatic glucose metabolism is impaired. Gene therapy has been tested to restore liver glucose uptake capacity by increasing hepatic GK protein (Ferre, 1996a; Morral, 2002, 2007). In type 1 diabetic liver, all models present a similar phenotype. When restoring glucose signaling in diabetic hepatocytes via GK, glucose catabolic pathways are induced and, on the contrary, hepatic glucose production is inhibited. Consequently there is a reduction of diabetic hyperglycemia accompanied by incremented hepatic glycogen depots and de novo lipogenesis. Decreasing blood glucose levels forces muscle and adipose tissue to use fatty acids as energetic substrates, and in consequence, serum fatty acids are decreased in type 1 diabetic mice expressing GK in the liver. Lower blood fatty acids, together with increased glucose metabolism in the liver, inhibit hepatic β-oxidation of fatty acids. Therefore, these models suggest that hepatic overexpression of GK in type 1 diabetes leads to normoglycaemia thanks to increments in hepatic glucose uptake and fatty acid oxidation in peripheral tissues.
d. Finally, hepatic GK overexpression in the context of type 2 diabetes: type 2 diabetes is a complex metabolic disorder caused by two physiologic defects: insulin resistance in combination with insulin secretion deficiency. Type 2 diabetes is characterized by glucose metabolism alterations such as failure of insulin to inhibit hepatic gluconeogenesis and impaired skeletal muscle glucose uptake. However, lipid metabolism is also altered. This is often reflected by increased circulating free fatty acids and triglycerides together with increased fat accumulation in non-adipose tissues. Thus, changes in the equilibrium between glucose and fatty acid metabolism in liver and muscle could be responsible for glucose homeostasis alterations. Obesity, hyperinsulinemia, in combination with hyperglycemia, inhibits fatty acid oxidation in many tissues. As a result, lipogenesis is favored over fatty acid oxidation leading to an increase in fat accumulation and a decrease in energy expenditure. A hypothetical strategy for type 2 diabetes therapy is increasing glucokinase activity, with the aim of enhancing glucose uptake in the liver that could contribute to gluconeogenesis inhibition with consequent restoration of glycaemia. If glycaemia is restored, plasma insulin levels could be secondarily lowered and it could be able to elevate energy expenditure and reduce obesity.

However, liver GK activity is increased in mild type 2 diabetes, but diminished in morbid obese diabetic patients. Animal diabetic models linked to obesity, show that GK deficiency in the liver occurs only in the case of obesity, and in severe or long-term forms of the disease. Although hepatic GK expression is different depending on disease stage, some strategies

based on increasing GK activity in the liver have been tested in some models of high fat diet induced type 2 diabetes (Desai, 2001 & Ferre, 2003) , in obesity models (Wu, 2005 & Torres, 2009) and in transgenic mice with hepatic insulin resistance (Okamoto, 2007). All these studies have in common that the increase in hepatic GK activity produces glycaemia normalization. Hepatic GK, through glycolysis and glycogenesis activation, increases blood glucose clearance while it inhibits hepatic glucose production. On the other hand, liver GK activity results in increased malonyl-CoA, a lipogenic substrate and inhibitor of β-oxidation. It is difficult to draw clear conclusions when evaluating consequences of liver GK overexpression on lipid metabolism in type 2 diabetic models. Wu et al report an expected increase in hepatic and serum triglycerides, together with higher serum fatty acids. However, Wu et al report that, although hepatic fatty acid β-oxidation was decreased, muscle increased fatty acid oxidation as a consequence of lower glycaemia and insulinemia. Conversely, Desai et al showed no changes in hepatic and serum lipid levels. Otherwise, Torres et al & Okamoto et al obtained an increase in serum triglycerides with no changes in fatty acid levels. The most striking model is presented by Ferre et al: under high fat diet, liver GK-transgenic mice became insulin resistant faster than controls and showed hepatic steatosis. It contrasts with results obtained in GK gene locus transgenic mice (Shiota, 2001). Besides exhibiting a reduction of the blood glucose concentration, mice with a greater than normal amount of GK also exhibited a dramatic resistance to the development of hyperglycemia and hyperinsulinemia normally brought on by consumption of a high fat diet.

Taken together, all these models have convincingly demonstrated that increasing GK protein in the liver leads to a direct reduction of glycaemia, but sometimes it can be accompanied with the risk of serious alterations in lipid metabolism deriving in hepatic steatosis and/or overt dyslipidemia. This aspect is essential when considering the possibility of using GK overexpression in the liver for diabetes therapy. At this point, it would be important to find out which are the causes of the different phenotypes observed in those animal models of hepatic GK overexpression previously described. There are several possible reasons:

a. Species-specific results: one possibility is that GK overexpression in mouse liver may be more effective stimulating glucose disposal than the same degree of expression in a larger animal such as rat.
b. Side-effects of gene transfer technology: when using adenoviral gene transfer, adenoviruses involve *per se* hepatic metabolic changes. When using transgenic, germ-line manipulated animals overexpress GK throughout life, including intrauterine life, possibly resulting in compensatory changes in insulin secretion, insulin action, or in other metabolic variables that do not occur with acute manipulation of GK via adenovirus technology.
c. Promoter that directs transgene expression can affect two important variables. On one hand, taking into account the metabolic hepatic zonation concept (Jungermann, 1995), the promoter determines which set of hepatocytes express the transgene. It is well known that physiological GK expression predominates in the perivenous area of the liver (Moorman, 1991; Jungerman, 1995 & Jungerman, 2000). However, most studies of hepatic GK gain of function did not use perivenous promoters. For instance, Ferre et al used a PEPCK promoter that directs the transgene to the periportal area of the liver, specialized in gluconeogenesis. In contrast, RSV or CMV promoters are ubiquitous promoters that transfect both perivenous and periportal hepatocytes. On the other hand, promoter directs the regulation of transgene expression by nutrients and hormones. For instance, GK under the PEPCK promoter is expressed under glucagon signaling and is inhibited by glucose

and insulin. Therefore, hepatic GK transgenic mice described by Ferre et al express GK at the periportal area of the liver during fasting, and not in fed state.

d. Transgene dose: Desai et al and O'Doherty et al described different metabolic impact of hepatic GK overexpression depending on the dose of transgene that they used.

In our laboratory we aimed to re-examine the conclusions of these studies and the differentiated effects that GK activity could have on the metabolism, clearly differentiated, of periportal and perivenous hepatocytes. To evaluate the issue, we have developed a hydrodynamic gene transfer technique that served us to pursuit GK overexpression studies exclusively in perivenous liver (Liu, 1999; Zhang, 1999; Gomez-Valades, 2006; Budker, 2006 & Suda, 2007). With the injection of a plasmid for green fluorescent protein (GFP) and immunohistochemistry for PEPCK (periportal marker), we could visualize that hydrodynamic injection generate two separate populations of hepatocytes: green hepatocytes that expressed GFP and red hepatocytes showing PEPCK-C staining (Figure 4). We could conclude that in our conditions the hydrodynamic gene transfer technique delivered the transgene only in the hepatocytes surrounding the central vein of the liver acinus.

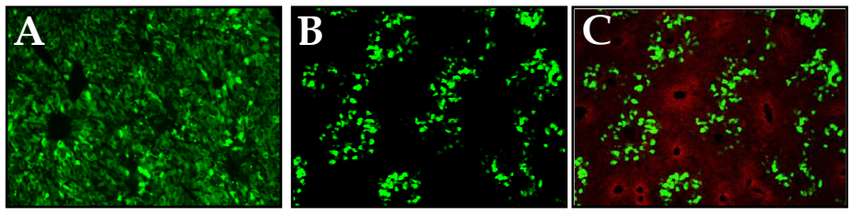

Fig. 4. Visualization of liver transfection achieved with adenoviral and hydrodynamic gene transfer techniques. (A) Healthy mice were injected with $5.5 \cdot 10^9$ IU of an adenovirus that codified for the green fluorescent protein (GFP). Green fluorescence was observed in liver sections (200X), demonstrating a homogeneous presence of the transgene all over the liver acinus. (B) A plasmid for GFP was hydrodynamically injected to healthy mice and, as it can see appreciated in liver sections, resulted in non-homogenous green fluorescence signal. (C) Slices from hydrodynamically-injected mice were immunostained for PEPCK-C (red signal), a periportal marker.

Our results represent the first attempt to overexpress pGK in perivenous hepatocytes. The first approach was the hydrodynamic injection of a plasmid with the Gck gene to healthy mice (Vidal-Alabró; publication pending). Forty-eight hours post-injection, increased GK in perivenous hepatocytes had clear effects on glucose homeostasis (Figure 5A). There was a reduction of glycaemia and insulinemia in the fed state, probably as a direct consequence of increased hepatic glucose uptake. Therefore perivenous GK gain of function reproduced results of periportal GK (Ferre, 1996), and liver-homogeneous GK overexpression (O'Doherty, 1999; Desai, 2001 & Scott, 2003). However, 16 hours-fasted mice did not show differences in blood glucose and insulin levels (data not shown), as Desai et al and O'Doherty et al had obtained with adenoviral GK transfer. Fifty days post-injection, perivenous GK overexpressing-mice presented blood glucose levels similar to control animals but accompanied by hyperinsulinism (Figure 5B). Long-term augmented GK activity in perivenous liver resulted in hepatic insulin resistance, since mice presented a phenotype very similar to liver-specific insulin receptor knock-out mice named LIRKO (Michael, 2000). Briefly, hyperinsulinism was probably due to reduced hepatic insulin clearance. Since peripheral tissues were still insulin-sensitive, hyperinsulinism inhibited lipolysis and induced lipogenesis in adipose tissue. Adipose tissue function together with

reduced hepatic lipogenesis *de novo* could explain the observed decrease in circulating triglycerides and free fatty acids. Although having increased GK activity in the liver, neither glycogen synthesis nor glycolysis was stimulated in those mice. Besides, gluconeogenesis was not inhibited in fed state. Therefore, considering the bibliography, our perivenous model resembled transgenic mice that expressed GK transgene under PEPCK-C promoter at periportal hepatocytes (Ferre, 2003). However, periportal GK overexpressing model showed whole-body insulin resistance linked to obesity and hepatic steatosis. It must be considered that their analysis was in 12 months old mice. If the study was extended to 12 months, we would be able to tell if hepatic insulin resistance observed in our mice model leads to general insulin resistance or, on the contrary, confirm its resemblance to LIRKO animals.

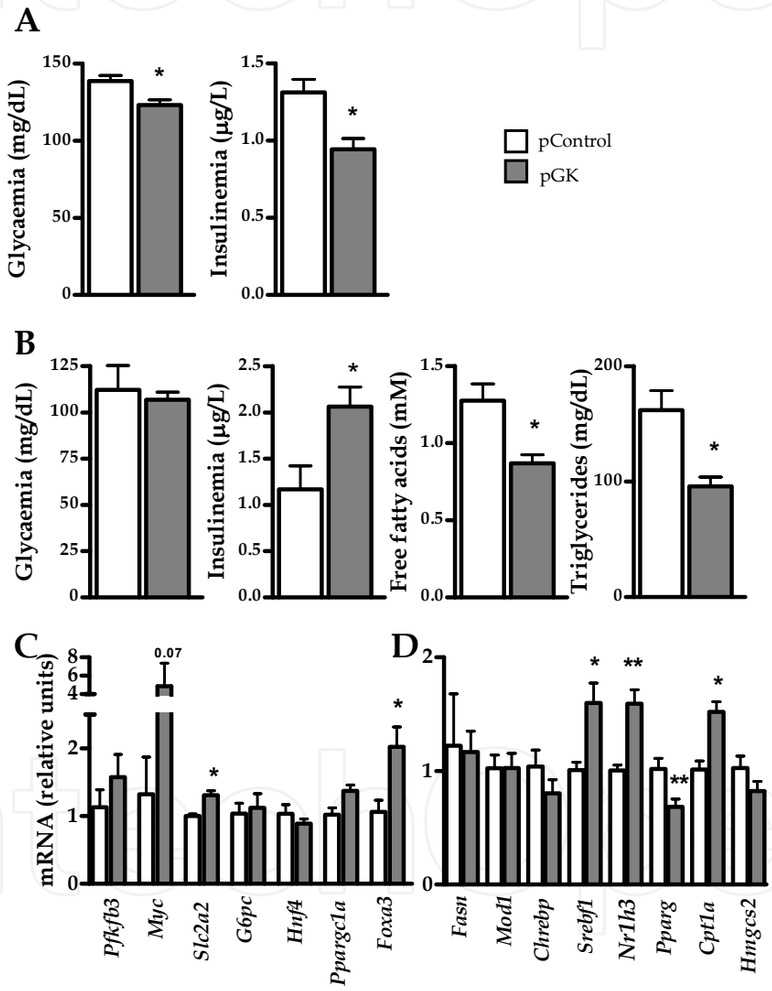

Fig. 5. Analysis of GK-overexpressing healthy mice. (A) Shows glycaemia and insulinemia, 48 hours post-injection of the plasmid that contained the GK gene. Columns represent media ± standard error. (B) 50 days post-injection results on serum nutrients (glucose, free fatty acids and triglycerides) together with insulin levels are represented. (C) After 50 days post-injection, expression of glycolytic and gluconeogenic genes from liver were analyzed by Real-Time PCR. Calculations were done following ΔΔCt algorithm (Applied Biosystems), using β-microglobulin gene expression as a housekeeping gene. (D) The same for lipogenic and lipolysis genes. * $p<0.05$ and ** $p< 0.01$ vs control, determined by t-Student.

In the context of type 1 diabetes induced with streptozotocin, perivenous liver GK gain of function restored hepatic glucose uptake and reduced gluconeogenesis. Therefore, typical increases in hepatic glucose depots (glycogen, triglyceride) occurred and resulted in a reduction of diabetic glycaemia, albeit small. But, perivenous GK expressing mice showed a significant increase in triglyceride and free fatty acid serum concentration, and hepatic lipids (Figure 6) (Vidal-Alabró; publication pending). Therefore our work in type 1 diabetes model reproduces those of periportal GK overexpression (Ferre, 1996a) and those of liver homogeneous GK overexpression (Morral, 2002, 2007) in terms of glycaemia. However, our results on lipid metabolism are more deleterious, probably because perivenous hepatocytes have higher lipogenic potential than periportal hepatocytes (Jungermann, 1995).

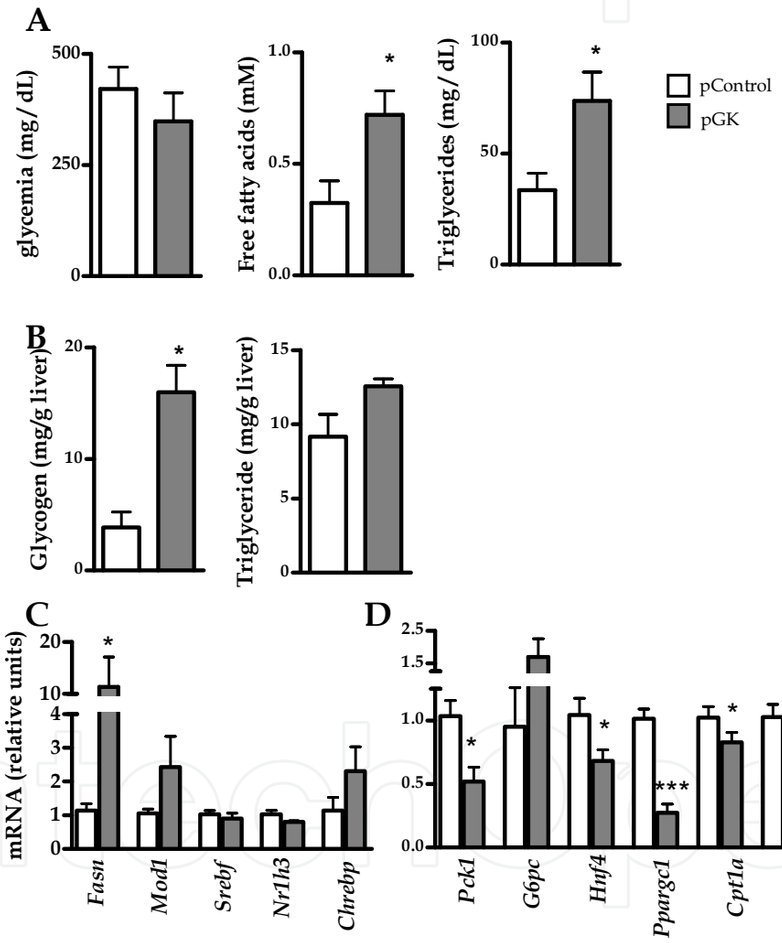

Fig. 6. Analysis of perivenous GK overexpression in type 1 diabetic mice. (A) Shows glycaemia, serum triglycerides and free fatty acids, 48 hours post-injection of the plasmid that contained the GK gene. Columns represent media ± standard error. (B) Hepatic glucose storage was evaluated by measuring glycogen and triglyceride levels. (C) Expression of lipogenic genes in the liver was analyzed by Real-Time PCR. Calculations were done following ΔΔCt algorithm (Applied Biosystems), using β-microglobulin gene expression as a housekeeping gene. (D) The same for gluconeogenic and lipolysis genes. * $p<0.05$, ** $p< 0.01$ and *** $p<0.001$ vs control, determined by t-Student.

All in all, our review of the literature together with our own results on the subject will convey that pGK-overexpression in the liver, independent of zonation, will result in changes in glycaemia but with the risk of non-desirable lipid alterations and insulin resistance. However, several undetermined factors influence the results obtained in GK overexpression studies, reinforcing the concept that hepatic GK is a key regulator of whole-body homeostasis, so that little changes in its activity and/or in its regulation affect glucose and lipid metabolism.

4. GKRP modulates the impact of GK activity on glucose and lipid homeostasis

GKRP is the best-known regulator of the hepatic GK at the post-transcriptional level. Therefore, impairments in GKRP should affect GK and consequently glucose metabolism, since GK plays a central role in glucose homeostasis. Nevertheless, mutations in the GKRP gene (Gckr) that caused disease or alterations in glucose metabolism have never been described until now. Recently, several whole-genome analysis have associated polymorphisms in the Gckr gene with fast hypoglycemia and increased serum triglyceride in humans, even though these subjects have reduced risk to type 2 diabetes (Køster, 2005; Sparsø, 2007; Vaxillaire, 2008; Orho-Melander, 2008 & Beer, 2009). The mechanism underlying this phenotype seems to be a reduction in GK inhibition by the variant regulatory protein (Beer, 2009). But, before exploring this issue it should be convenient to consider some aspects of GKRP biology.

Although GKRP research has been focused in the liver, there are evidences that the GKRP protein is also present in hypothalamic neurons (Schuit, 2001; Alvarez, 2002 & Roncero, 2009). GK/GKRP system in the hypothalamus could play a role in glucose-sensing important for the regulation of energy homeostasis by balancing energy intake, expenditure and storage. On the other hand, there is some controversy in the literature as to whether GKRP also regulates GK in pancreatic β-cells. The vast majority of studies state that GKRP is not expressed in rodent β-cells. However, it has been demonstrated that human islets express GKRP at very low levels (Beer, 2009). This issue should be revisited because of the recent publication of several genome-wide association studies that associate GK, GCKR, G6PC2, MTNR1B with type 2 diabetes risk linked to β-cell function (Reiling, 2009 & Bonetti, 2011). Whether, β-cell GK function is affected directly by a hypothetic pancreatic GKRP, or indirectly by liver GKRP impaired activity, still needs clarification. Another question that remains to be resolved is whether GKRP is also expressed and functional in other GK expressing cells, for instance, in the gut and in the pituitary gland.

Consequently, when considering studies of genome-wide association, mutant GKRP protein might affect GK activity in the brain, in the liver and perhaps in the β-cell. Therefore it is difficult to explain the phenotype only taking into account the hepatic GK/GKRP system. The same occurs with the characterization of GKRP-deficient mice (Farrelly, 2002 & Grimsby, 2000). GKRP knock-out mice models, whether heterozygous or homozygous, had normal weight. Interestingly focusing in liver analysis, those mice displayed reduced production of hepatic GK protein while having the same levels of GK mRNA than control animals, and GK protein was localized exclusively in the cytoplasm. That showed the importance of hepatic GKRP in stabilizing and protecting the intracellular GK pool. These animal models exhibited impaired postprandial glycemic control, with lower hepatic glycogen content and lack of inhibition of PEPCK-C gene expression, albeit with no

noteworthy loss in insulin secretion or changes in fasting blood glucose concentrations. Moreover, when challenged with a high-sucrose/high-fat diet the knock-out and normal mice gained body weight at a similar rate but the knock-out mice were hyperglycaemic and hyperinsulinemic. Importantly, no changes in plasma triglycerides and non-esterified fatty acids were observed in basal conditions as well as with a high-sucrose/high-fat diet. In summary, absence of GKRP results in decreased hepatic GK protein content, affecting glucose metabolism without disturbing lipid parameters.

On the other hand, GKRP gain of function in the liver has also been assessed. In vitro studies with HepG2 cells simultaneously transduced with an adenoviral vector expressing GKRP and another adenoviral vector for GK had significantly elevated GK protein and activity levels compared with cells transduced with the GK adenovirus alone (Slosberg, 2001). These data suggest that GKRP serves to stabilize and protect a pool of GK protein (i.e., extend half-life), and is consistent with data obtained in GKRP knock-out studies. But in vivo studies revealed a more complicated situation. Adenoviral-mediated hepatic overproduction of GKRP in mice with high-fat diet-induced diabetes resulted in 23% decrease in GK enzymatic activity. Although reduction of GK activity is commonly associated to diabetes, hepatic GKRP-expressing mice had improved fasting and glucose-induced glycaemia with a concomitant increase in insulin sensitivity and TAG levels, and a decrease in leptin levels. A possible explanation for discrepancies between in vivo and in vitro results on GK levels when overexpressing GKRP is that GK expression in vivo is influenced by insulin and other physiological regulators. To understand how decreased GK activity improved type 2 diabetes phenotype in this model, a possibility is that GK activity may be applied in a more efficient manner toward metabolizing blood glucose. The subcellular compartmentalization by scaffolding proteins of enzymes or signaling proteins into clusters is often used as a means of increasing system efficiencies.

Coming back to genome-wide studies that associate Gckr with fast hypoglycemia and high triglycerides, Beer et al reported that P446L-GKRP has reduced regulation by physiological concentrations of fructose-6-phosphate, resulting indirectly in increased GK activity (Beer, 2009). They predicted that this increased GK activity in the liver enhanced glycolytic flux, promoting hepatic glucose metabolism and elevating concentrations of malonyl-CoA, a substrate for *de novo* lipogenesis, providing a mutational mechanism for the reported association of this variant with raised triglycerides and lower glucose levels. However, their predictions are conflictive with in vivo studies by Slosberg et al (Slosberg, 2001), since GKRP gain of function reduced hepatic GK activity and also resulted in a decrease of blood glucose levels accompanied by an increase of blood triglycerides. Therefore, any other undetermined factor/s must exist to really understand the complex physiology of the GK/GKRP system. Another possibility is that brain P446L-GKRP and β-cell P446L-GKRP (if existent) may exert determinant influences on phenotype.

Another study that may bring light to this issue, relates to defects in glucokinase translocation identified in Zucker diabetic fatty (ZDF) (Fujimoto, 2004 & Shin, 2007). Although having normal GK protein content, GK was predominantly localized in the nucleus regardless of plasma glucose and insulin levels. Nevertheless, sorbitol restored GK translocation. Clearly, there must be two distinct mechanisms bringing about the dissociation of GK from GKRP. How they are related and what differentiate them are questions currently under investigation. Since this defect was discovered in early stage of diabetes, it could cause of the progression to diabetes seen in the adult ZDF rat. Consistently, a MODY-2 mutation in the Gck gene has been reported to increase the physical

interaction of GK and GKRP (García-Herrero, 2007). But, again these data are in conflict with other studies that reported some new GK mutations causing MODY-2 that reduced GK inhibition by GKRP (Veiga-da-Cunha, 1996; Gloyn, 2005 & Sagen, 2006). Once more, it is difficult to draw conclusions, but the importance of proper GK/GKRP function on metabolism and disease is reinforced, as subtle changes in its activity and/or regulation lead to contrary phenotypes.

Several naturally occurring activating mutations have been described that are localized at the same region where synthetic GK activators bind (Kamata, 2004; Heredia, 2006 & Matschinsky, 2009). Both activating mutations and synthetic activators stabilize the open conformation of the GK protein, resulting in higher affinity for glucose and a reduction of the interaction between GK and GKRP, since the super-open conformation of the enzyme (inactive) is not possible. In humans, activation of GK by naturally occurring mutations is associated to persistent hyperinsulinemic hypoglycemia of the infancy (PHHI), syndrome with a heterogeneous phenotype even in the same family but generally with a normal lipid profile. On the other hand, GK activation through administration of GK activation drugs has been tested for their potential in the therapy of type 2 diabetes, considering principally their capacity to increase glucose-stimulated insulin release at the β-cell (Grimsby, 2003; Brocklehurst, 2004; Efanov, 2005; Leighton, 2005; Coope, 2006 & Matschinsky, 2009). Whole-body effects of glucokinase activator drugs demonstrated a dose-dependent reduction of glycaemia, associated with increased insulin secretion in the pancreas and net glucose uptake in the liver. Besides, the administration of a GK activator prevented the development of diabetes in a diet-induced obesity animal model (Grimsby, 2003). Surprisingly, most in vivo studies with GK activators drugs do not show the lipid profile (Grimsby, 2003; Brocklehurst, 2004; Efanov, 2005; Leighton, 2005 & Coope, 2006), except one where treatment of ob/ob mice with GK activator PSN-GK1 did not produce any significant change blood lipids (Fyfe, 2007).

With all this puzzling background, we intended to study the expression of an activated mutant form of GK with the aim to decipher the metabolic consequences in the liver of having a GK not regulated by GKRP, with theoretical antidiabetic properties. Particularly we proposed the overexpression of glucokinase A456V (identified in patients of persistent hyperinsulinemic hypoglycemia of the infant), with a $S_{0.5}$ for glucose of 3 mM instead of 8 mM for the wild-type enzyme (Christesen, 2002), and without GKRP regulation (Heredia, 2006). We postulated that GK-A456V overexpression (also as a model for the liver-specific consequences of activating drugs on GK) could increase glucose uptake compared with the wild-type enzyme at equal levels of expression, whilst the metabolic fate of glucose might be different from that of wild-type GK due to its different capacity of interaction with other regulating proteins (GKRP and maybe PFK-2).

By means of hydrodynamic gene transfer of an expression plasmid for GK-A456V in healthy mice, we have been able to demonstrate that the perivenous overexpression of GK-A456V results in a sustained improvement in blood glucose, insulinemia and glucose tolerance, in the absence of dyslipidemia or hepatic lipidosis nor long-term insulin resistance (Vidal-Alabró; publication pending). Importantly, GK-A456V protein levels were similar to GK-control group, suggesting GK-A456V stability although not being directly regulated by GKRP. Its mechanism of action could be explained by its lower $S_{0.5}$ for glucose, so that glucose uptake is stimulated in later phases after ingestion (post absorptive phase) and during early fasting. It is tempting to speculate that glucose taken-up in perivenous liver, both in postprandial and post-absorptive periods, could be directed towards the glycolytic

and oxidative metabolism and not through the pentose phosphate pathway that would favor lipid biosynthesis. This hypothesis is reinforced by results published by Wu et al (Wu, 2005) in which adenovirus expression of wild-type GK in the liver activate the pentose phosphate pathway, in marked contrast to the overexpression of the kinase domain from PFK-2 that stimulates flux through the glycolytic pathway. Surprisingly, GK-A456V transfected animals showed a marked increase in glucose-6-phosphatase. GK overexpression in perivenous hepatocytes does not significantly affect Glc6Pase expression, suggesting that zonation is an important experimental variable not sufficiently addressed to date in the field.
Transfecting GK-A456V in type 1 diabetic mice induced with streptozotocin, also caused an important reduction of diabetic hyperglycemia without dyslipidemia, in contrast with GK overexpression. Again, an induction of glucose-6-phosphatase transcription was observed in the liver GK-A456V -expressing animals (Figure 7) (Vidal-Alabró; publication pending).

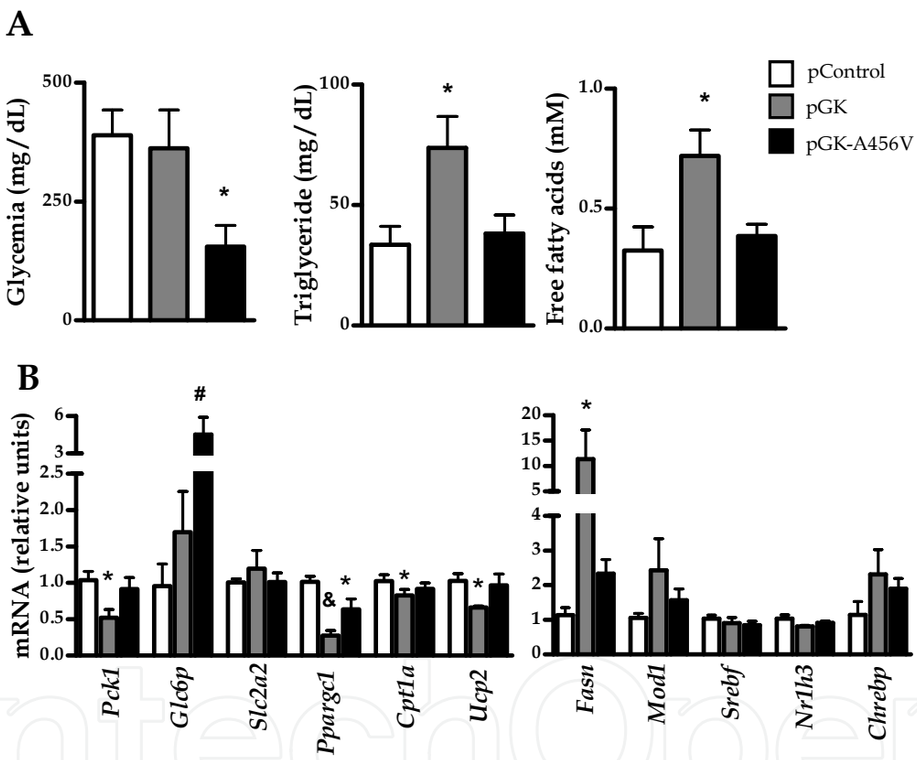

Fig. 7. Study of GK-A456V expression in the liver of type 1 diabetic mice. (A) Shows glycaemia, serum triglyceride and free fatty acid levels, 48 h post-injection of the plasmid for the GK-A456V gene. Columns represent media ± standard error. (B) Expression of gluconeogenic and lipolysis genes from liver was analyzed by Real-Time PCR. Calculations were done following ΔΔCt algorithm (Applied Biosystems), using β-microglobulin gene expression as a housekeeping gene. (C) The same for lipogenic genes. * $p<0.05$, # $p<0.05$ vs control and pGK, and & $p<0.001$ vs control and $p<0.05$ vs pGK-A456V, as determined by One-way ANOVA.

Our results lead us to consider the physiology of glucose-6-phosphatase in the context of glucose and lipid metabolism. Glucose-6-phosphatase dephosphorylates glucose-6-phosphate in the endoplasmic reticulum to obtain glucose, as the last step in the

gluconeogenic pathway. Its transcription is regulated by insulin, so that it is repressed in fed state and induced during fasting. However, glucose induces transcription of this enzyme although the physiological significance of this induction is still not resolved (Nordlie, 2010). Finally glucose-6-phosphatase deficiency causes severe hyperlipidemia and hepatic steatosis (Bandsma, 2002, 2008), therefore giving rise that this enzyme may also participate or influence the GK/GKRP system in the regulation of hepatic glucose fate. To support this hypothesis, Reiling and colleagues described combined effects of single-nucleotide polymorphisms in GK, GKRP and glucose-6-phosphatase on fasting plasma glucose and type 2 diabetes (Reiling, 2009). Therefore, it is a field that needs further exploration.

5. Conclusion

Subtle changes in GK activity or in GKRP function have consequences in glucose and lipid metabolism. However, further studies must be done to completely understand the mechanism underlying GK/GKRP biology. Our results on increasing GK protein in the liver of both healthy and insulin-deficient mice (lacking endogenous GK) resulted in dyslipidemia. On the other hand, our analysis of the metabolic consequences of GK-GKRP deregulation by overexpressing a GK activating mutant (GKA456V) in the liver of both healthy and type 1 diabetic mice demonstrates an impact on glycaemia in the absence of dyslipidemia or hepatic lipid deposition. These data provide novel insights into the capacity of the complex GK-GKRP to influence the fate of metabolized glucose in the liver, providing a framework for further research on GK activating drugs in the liver.

We conclude that GKRP regulation impairment and GK-A456V altered kinetics greatly influence liver metabolism, in line with results in humans carrying a mutant GKRP (Køster, 2005; Sparsø, 2007; Vaxillaire, 2008 & Orho-Melander, 2008). Besides, it suggests that activating GK exclusively in the liver could be a feasible strategy to funnel excess glucose from the diet out of circulation, widening the scope for GK synthetic activators research.

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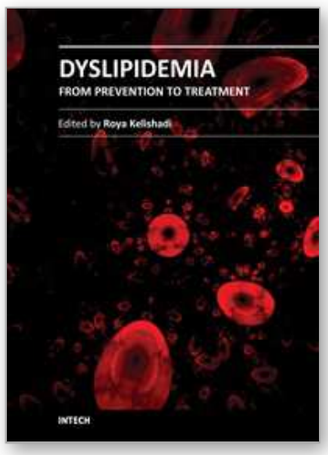

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Dyslipidemia has a complex pathophysiology consisting of various genetic, lifestyle, and environmental factors. It has many adverse health impacts, notably in the development of chronic non-communicable diseases. Significant ethnic differences exist due to the prevalence and types of lipid disorders. While elevated serum total- and LDL-cholesterol are the main concern in Western populations, in other countries hypertriglyceridemia and low HDL-cholesterol are more prevalent. The latter types of lipid disorders are considered as components of the metabolic syndrome. The escalating trend of obesity, as well as changes in lifestyle and environmental factors will make dyslipidemia a global medical and public health threat, not only for adults but for the pediatric age group as well. Several experimental and clinical studies are still being conducted regarding the underlying mechanisms and treatment of dyslipidemia. The current book is providing a general overview of dyslipidemia from diverse aspects of pathophysiology, ethnic differences, prevention, health hazards, and treatment.

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