The role of the liver in the modulation of glucose and insulin in non alcoholic fatty liver disease and type 2 diabetes

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Short title: **Central role of the liver in NAFLD and T2D**

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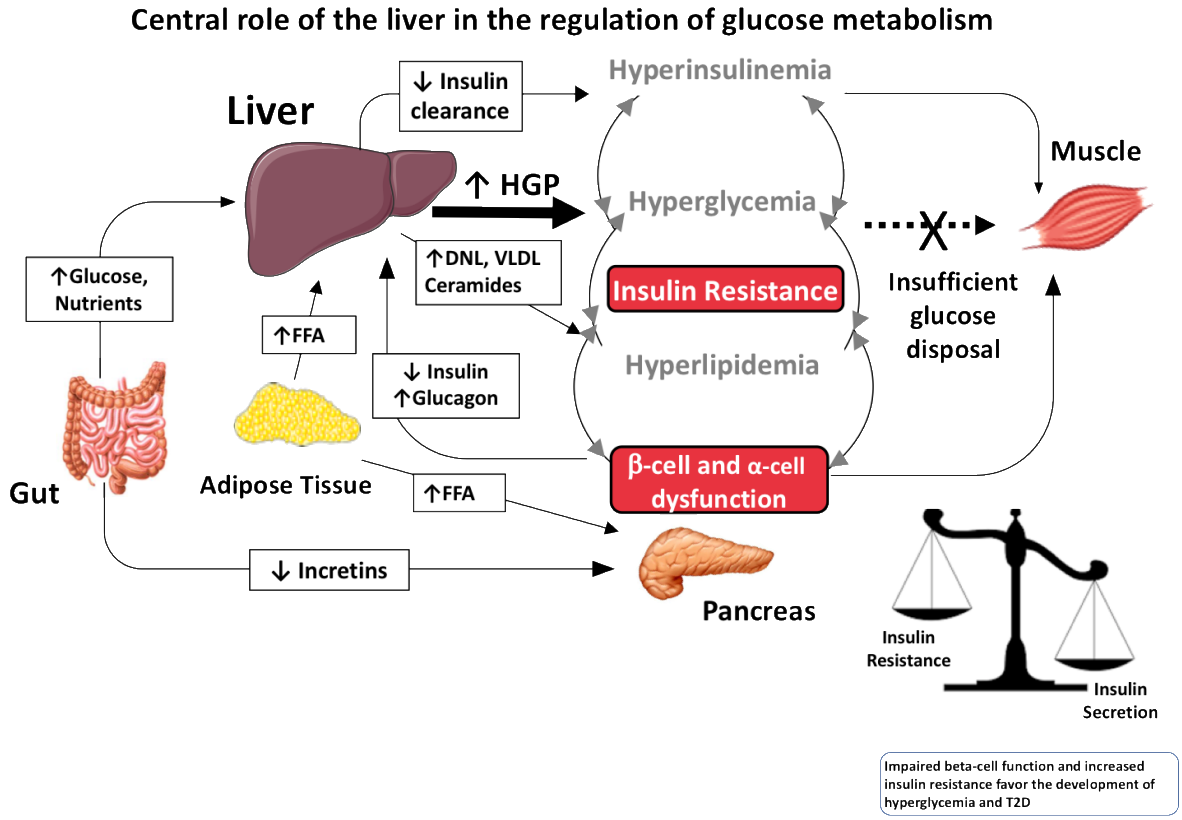
Graphical abstract



Abstract

In this review we have discussed how the liver plays a central role in the regulation of glucose metabolism and in insulin clearance. Both non-alcoholic fatty liver disease (NAFLD) and diabetes (T2D) are characterized by high plasma insulin concentrations, hepatic insulin resistance, high hepatic glucose production (HGP), in particular gluconeogenesis (GNG), that are increased proportionally to fasting hyperglycemia, while postprandial hyperglycemia is due to impaired suppression of HGP by insulin, and reduced hepatic glycogen storage. The liver acts also as a modulator of peripheral insulin since most of insulin secreted by the pancreas is cleared by the liver during the first pass. Hepatokines and hepatic lipids can act in either autocrine or paracrine way and can be responsible of the changes in insulin sensitivity and alterations in glucose metabolism.

Introduction

It has been estimated that half a billion people are living with diabetes worldwide (90% of these with type 2 diabetes (T2D)) and this number is projected to increase by 25% in the next ten years. Moreover, 374 million people have impaired glucose tolerance (IGT) [1]. Most of these subjects are unaware of their condition, considering that diabetes is undiagnosed in about half (50.1%) of the 463 million people living with diabetes [1].

Both T2D and IGT are characterised by postprandial hyperglycemia while fasting hyperglycemia is present in some non-diabetic subjects, i.e. those with impaired fasting glucose (IFG), but not in all patients with T2D. Moreover, global prevalence of non alcoholic fatty liver disease (NAFLD) among patients with T2D is estimated to be 55.5%, almost double than in the general population, and the prevalence of non-alcoholic steatohepatitis (NASH) is 37.3% [2].

Among the risk factors for the development of T2D there are insulin resistance, family history of diabetes, metabolic syndrome, obesity, pre-diabetic hyperglycemia, i.e. IFG and/or IGT [3]. Beside obesity, preferential abdominal fat accumulation, i.e., hepatic and visceral fat, are major emerging risk factors for T2D and related comorbidities, e.g., NAFLD and cardiovascular disease (CVD) [3-5].

The liver is fundamental in the regulation of plasma glucose levels being an important player during both fasting and postprandial conditions mainly through hepatic glucose production (HGP) and glycogen storage. The liver acts also as a modulator of peripheral insulin since most of the insulin secreted by the pancreas is degraded by the liver during the first pass. In addition, the liver secretes hepatokines and lipids that can also act in either autocrine or paracrine way and they can control insulin sensitivity.

In this review we have discussed how the liver plays a central role in the regulation of glucose metabolism and the development of cardiometabolic diseases specifically non alcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2D).

The Liver and Fasting Glucose Metabolism

The liver is the main organ that produces glucose during fasting, through gluconeogenesis (GNG) and glycogenolysis (**Figure 1**); other organs like the kidney [6] and the intestine [7] might contribute to the production of the glucose through gluconeogenesis but in minimum amount (less than 10%). However, the extrahepatic tissues may increase the contribution to EGP when needed, as in conditions of hypoglycemia and acidosis [8].

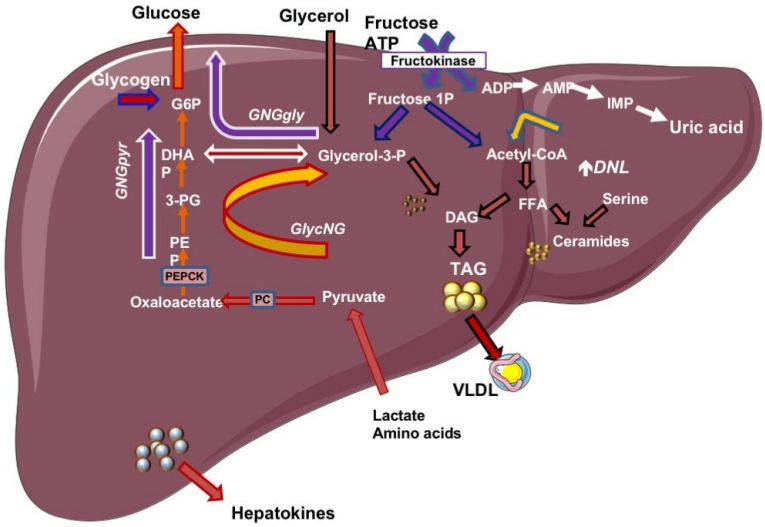


Figure 1: Hepatic glucose production is in part due to glycogenolysis and in part to gluconeogenesis (GNG, from 50-70%). Greater availability of glucogenic substrates (lactate, pyruvate, amino acids and glycerol), type 2 diabetes and obesity contribute to high glucogenic fluxes. The great majority of glycerol that is taken up by the liver is used for GNG, while DAG (diacylglycerols) and TAG (triacylglycerols) are synthesized from glycerol derived either form glyceroneogenesis or glycolysis (in hyperglycemic patients).

Both glucotoxicity and lipotoxicity contribute to the development of hepatic insulin resistance. Lipotoxicity is related to increased de novo lipogenesis (DNL) that synthesizes saturated fatty acids (palmitic acid and for elongation stearic acid), that are used to form TAG and ceramides. Fructose is one of the major substrates used for DNL, and regulation of fructokinase is important for the development of hepatic insulin resistance.

In T2D subjects, especially if obese, fasting endogenous glucose production (EGP) is much higher than in non-diabetic subjects due to the unbalance of different regulatory factors, like hepatic insulin resistance, greater availability of glucogenic substrates (lactate, pyruvate, amino acids and glycerol), hormones and hepatokines [4, 5].

In non-diabetic non-obese subjects, the contribution of GNG to EGP is around 50% after an overnight fasting, but increases to 60-70% of EGP in patients with T2D [9] (**Figure 2, Panel A**). It is important to note that in non-diabetic obese subjects the contribution of GNG to EGP is also around 60% but the GNG flux is lower than in T2D [9].

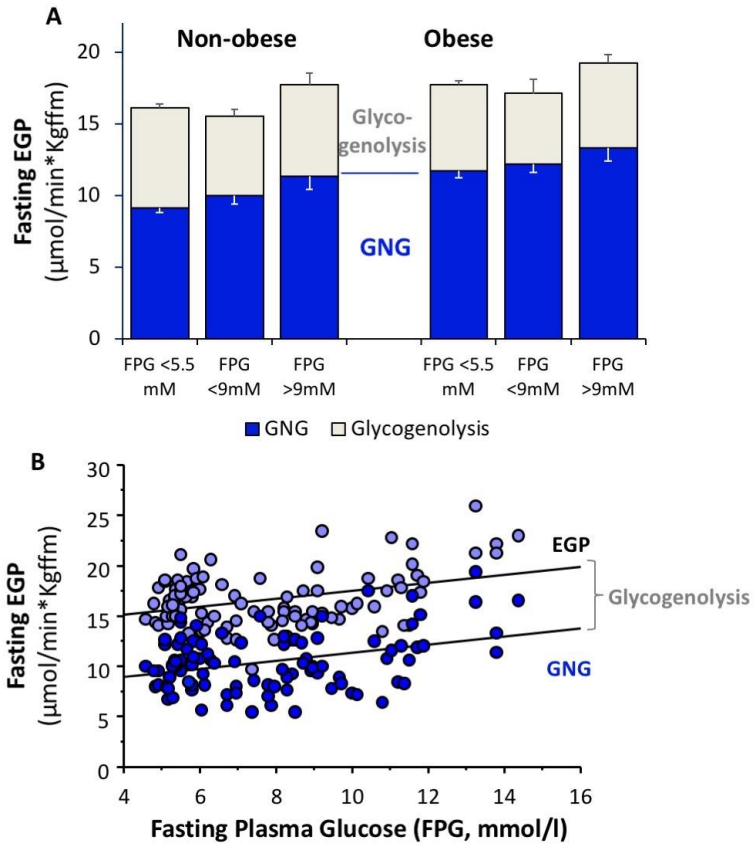


Figure 2 Panel A: Endogenous glucose production (EGP) is mainly driven by gluconeogenesis (GNG, from 50-70%) and only in part by glycogenolysis. Obese subjects have higher GNG compared to non obese, regardless of diabetes. Panel B: GNG and EGP are increased similarly and explain the increase in fasting plasma glucose in T2D patients, while the glycogenolysis fluxes has no relationship with fasting plasma glucose (redrawn from reference [9]).

The increased gluconeogenesis might be explained by several factors, including increased expression of key gluconeogenic genes, increased enzymatic activity, and precursor substrate flux (mainly lactate, pyruvate, amino acids and glycerol) (**Figure 1**). Glucogenic substrates and FFA are increased in T2D and insulin resistant states and drive gluconeogenesis [10-12]. The key enzymes involved in GNG are phosphoenolpyruvate carboxylase (*Pck1*), glucose-6-phosphatase (*G6Pase*) and pyruvate carboxylase (PC). The expression of *Pck1* and G6Pase

are under hormonal control, i.e. inhibited/enhanced by insulin and glucagon. A major role in the regulation of hepatic glucose production and gluconeogenesis is exerted by *Foxo* proteins (ie the transcription factor Forkhead box of O family) that regulate liver metabolism by differentially controlling expression of the target genes, such as *G6pc*, *Pck1*, and *Gck* [13]. It has been shown that the activation of *G6pase* and inhibition of glucokinase is dependent on FOXO1 that also regulates PCK1 [13]. FOXO1 is inhibited by insulin, but in presence of insulin resistance this mechanism is reduced leading to excess glucose production despite high insulin levels and explaining hepatic insulin resistance observed even in lean NAFLD [14, 15]. Mitochondrial pyruvate metabolism is essential for the process of gluconeogenesis from pyruvate. The mitochondrial enzyme pyruvate carboxylase (PC) catalyzes the conversion of pyruvate to oxaloacetate, a precursor of malate and citrate. Thus, PC is critical for both hepatic gluconeogenesis and de novo lipogenesis (fatty acid synthesis). In presence of excess energy substrates (mice fed with a diet that induced obesity, DIO), PC drives gluconeogenesis and contributes to the development of hyperglycemia [16]. On the other hand, liver pyruvate carboxylase knockout (LPCKO) mice exhibited improved glucose control in obesity but worsens liver inflammation [16]. A major role is also played by the mitochondrial pyruvate carrier (MPC), a recently discovered protein complex in the inner mitochondrial membrane that mediates the rate of entry of pyruvate into the mitochondria where subsequent oxidative metabolism occurs. After high fat-fructose and cholesterol diet mice with liver-specific deletion of MPC2 were protected from development of NASH on this diet [17].

Fasting glucose levels are increased proportionally to fasting EGP(**Figure 2, Panel B)**, and occurs even in presence of high fasting plasma insulin concentrations, indicating severe hepatic insulin resistance [11, 18]. The high fasting glucose concentrations observed in T2D subjects are strongly related to increased EGP and gluconeogenesis (GNG), (**Figure 2, Panel B**), rather than glycogenolysis [9]. Insulin is able to suppress glycogenolysis, but not gluconeogenesis; fasting hepatic glucose production and glucose concentrations remain within normal ranges until insulin is able to suppress glycogenolysis to compensate for high GNG (hepatic autoregulation). Presence of obesity is associated with a further increase in EGP, gluconeogenesis, hepatic and peripheral insulin resistance (**Figure 2, Panel A**). This condition is further exacerbated in proportion to hepatic (NAFLD) and visceral fat accumulation (abdominal obesity) (**Figure 3)** [5, 19].

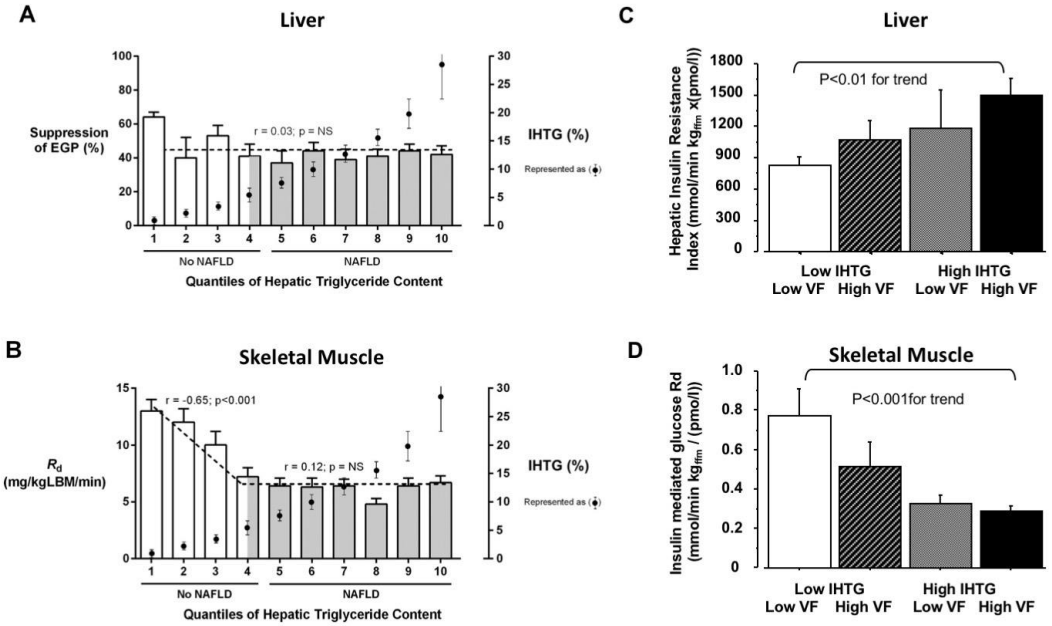


Figure 3 Panel A: suppression of endogenous glucose production (EGP) during euglycemic hyperinsulinemic clamp is impaired in presence of accumulation of hepatic triglycerides even when below 5% (threshold for diagnosis of NAFLD) (data from ref. [19]). Panel B: Glucose disposal during euglycemic hyperinsulinemic clamp is reduced as hepatic fat accumulation increases and similarly low in subjects with (data from ref. [19]). Similarly fasting hepatic insulin resistance (Panel C) is increase with hepatic fat accumulation bit even more in presence of visceral fat accumulation. Also insulin mediated glucose disposal (Panel D) is reduced proportionally to both hepatic and visceral fat accumulation (data from ref. [5])

Subjects with NAFLD have increased insulin resistance, not only at the level of the liver but also at the level of the muscle and adipose tissue [5], and presence of T2D further worsen the degree of insulin resistance in all tissues [11] (**Figure 3).** Early sign of hepatic lipotoxicity (i.e. liver fat below the cut-off for the diagnosis of NAFLD that is 5%) is already associated with impaired suppression of hepatic glucose production and reduced peripheral glucose disposal (**Figure 3 Panel A and B**). The abdominal fat accumulation as visceral fat worsens both hepatic and peripheral insulin resistance (**Figure 3 panel C and D**).

The Liver and Postprandial Insulin Metabolism

During postprandial state the pancreas (β-cells) increases insulin secretion proportionally to changes in blood glucose, to keep glucose concentrations within a tight range . Insulin is released into the portal vein and first is absorbed by the liver. Insulin action is defective in T2D. The first action of insulin is the suppression of hepatic glucose production, stimulation of hepatic glycogen synthesis and storage of glucose. The liver is the organ where most of the ingested glucose is accumulated in the form of glycogen, but it is well established that T2D patients have also an impairment in the hepatic accumulation of glycogen thereby contributing to postprandial hyperglycemia, as reviewed in [18]. Moreover, earlier studies using euglycemic hyperinsulinemic clamp and tracers have shown that patients with T2D have both peripheral and hepatic insulin resistance with decreased peripheral glucose disposal and reduced suppression of hepatic glucose production [18], mainly because the contribution of gluconeogenesis to HGP remains very high [20].

The liver modulates the amount of insulin in the periphery by adjusting hepatic insulin clearance and degradation to allow higher peripheral insulin concentrations to overcome peripheral insulin resistance (**Figure 4**). It has been estimated that up to 60% of insulin secreted is degraded by the liver while the rest is cleared by the muscle and the kidney but insulin clearance is decreased in conditions of insulin resistance (IR) [5, 21, 22]. Moreover, insulin clearance is associated to obesity and hepatic fat accumulation [11, 19]. Thus, peripheral insulin concentrations depend not only by the pancreas (as secretory organ) but also by the liver (insulin modulator).

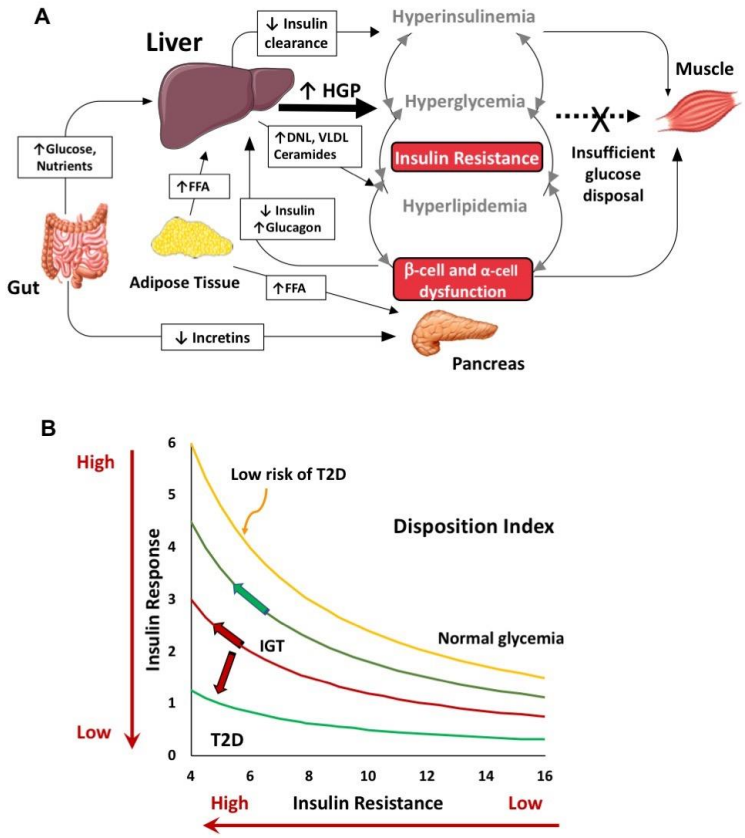


Figure 4: Panel A: the diagram shows the central role of the liver in the regulation of glucose metabolism and glucose tolerance. Panel B shows Disposition index (DI) trajectories in subjects with normal glycemia (NGT, glucose intolerance (IGT) or type 2 diabetes (T2D). A low DI is a sign of increased risk of T2D.

The mechanisms that regulate hepatic insulin clearance are still unknown. Insulin clearance is not a static process since lower clearance rates are observed in postprandial compared to fasting state, and it is influenced by several factors, like nutrient intake [23] and some hormones [24, 25]. We and other have also shown that insulin clearance was higher during isoglycaemic intravenous glucose infusions (IGIVI) to match the plasma glucose levels observed during the oral challenges [26, 27]. Bariatric surgery, in particular gastric bypass (RYGB), is able to improve IR in liver and muscle [28, 29], insulin secretion and beta cell function [30, 31] but also to increase insulin clearance in liver and extrahepatic tissues [27, 32, 33].

In the liver, insulin is degraded by the insulin-degrading enzyme (IDE); mice with liver-specific deletion of Ide (L-IDE-KO) exhibited higher fasting and non-fasting plasma glucose levels, glucose intolerance and insulin resistance [34]. Contrary to what expected plasma

insulin levels and hepatic insulin clearance were similar to control mice indicating that IDE is not a rate-limiting regulator of plasma insulin levels in vivo. However, L-IDE-KO mice had lower plasma membrane insulin receptor levels in liver, reduction in insulin-stimulated phosphorylation of the insulin receptor, and its downstream signaling molecules, AKT1 and AKT2. moreover, FoxO1 was increased and the gluconeogenic genes Pck1 and G6pc were upregulated explaining the high glucose levels [34].

Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), a surface membrane substrate of the insulin receptor, is also involved in the regulation of hepatic insulin clearance [35]. CEACAM1promotes the internalization of the insulin-insulin receptor complex mediating its endocytosis and targeting to the degradation pathway [36]. Liver-specific CEACAM1deletion is associated with glucose intolerance, reduced hepatic insulin clearance, hyperinsulinemia and IR both in the muscle and in the liver [35].

The Liver and Postprandial Glucose Metabolism

Postprandial glycemia is maintained within the normal ranges by the balance between insulin secretion and insulin action. Thus, postprandial hyperglycemia is due in part to alteration in secretion and action of postprandial hormones, like insulin, glucagon like peptide-1 (GLP-1) and glucagon, in part to insulin resistance [9, 18].

In the pathophysiology of type 2 diabetes insulin secretion is increased following as subjects progress from NGT to IGT, but when beta cell failure causes a decrease in insulin secretion they develop type 2 diabetes [18, 37, 38] (**Figure 4**). Increased IR in several tissues (i.e., liver, muscle and adipose tissue) is a characteristic feature of T2D but it has also been shown that subjects with IGT have already a severe beta cell dysfunction since glucose stimulated insulin response is already decreased by 80% in subjects with IGT [9, 18]. However, until peripheral insulin concentrations are sufficiently high to compensate the defect in peripheral insulin signalling and insulin resistance, glucose tolerance is maintained within normal limits. Thus, not only insulin secretion (i.e., the pancreas), but also insulin clearance, and thus the liver (that as said above clears the majority of secreted insulin), are major players in the maintenance of normal glucose tolerance.

The relationship between peripheral insulin secretion after a stimulus and insulin sensitivity is hyperbolic (and is commonly known as disposition index, DI) (**Figure 4 Panel B**). DI is measured by the product of insulin sensitivity and insulin response to a stimulus (i.e. an oral or intravenous glucose load) and lower values of DI have been found in subjects with IGT and T2D compared to NGT [18, 37, 38]. In subjects with liver disease (either NAFLD or

chronic hepatitis C, CHC) the disposition index is reduced in patients with CHC both NGT and IGT, while only in NAFLD only those that developed IGT have lower disposition index corresponding to a higher risk to develop type 2 diabetes [39].

Nutrient ingestion plays an important role in glucose metabolism since they stimulate gut secretion of incretin hormones like GLP-1 and GIP that potentiate the pancreatic secretion of insulin and suppress glucagon secretion. The effect of incretins on hepatic glucose and lipid metabolism is still under debate, but recent studies have shown that GLP-1 infusion reduces hepatic glucose production [40] and treatment with GLP-1RA or dual GLP-1/GIP receptor agonists and are effective in improving glucose and lipid metabolism and in reducing hepatic steatosis and markers of liver damage [41-43].

Hepatic lipid accumulation and synthesis and relation to insulin resistance

Hepatic fat is associated to insulin resistance and decrease in hepatic fat following hypocaloric diet. In T2D weight loss is also associated to improvement of both glucose and lipid metabolism and decrease in both hepatic and pancreatic fat [44]; however, return to non-diabetic glucose control after weight loss depends upon the beta cell ability to recover and is associated to shorter diabetes duration.

Hepatic fat accumulation occurs especially in postprandial state when hepatic metabolism is shifted from glucose synthesis to lipid synthesis since insulin promotes fatty acid esterification into diacyl-(DAG) and triacyl-glycerols (TAGs) (**Figure 1**). DAGs and TAGs are synthesized in the liver from the re-esterification of two and three fatty acids bound to a glycerol backbone. respectively Subjects with IR, NAFLD or diabetes have increased accumulation of toxic lipids, like saturated TAGs, ceramides and dihydroceramides (markers of de novo ceramide synthesis) [45], but also higher de novo lipid synthesis (DNL) [46] and glyceroneogenesis [12] not only in postprandial state but also during fasting.

One of the main substrates for de novo lipogenesis (DNL) and synthesis of saturated fatty acids is fructose (**Figure 1**). Hepatic fructose metabolism has gained interest since western diet abuses the utilization of sucrose (that is composed by one molecule of glucose and one of fructose) in soft drinks, cookies, baked good and juice. Fructose can also be metabolized as uric acid through the purine cycle (**Figure 1**). Thus, excessive fructose consumption is a possible cause of dysregulation of glucose and lipid metabolism. Fructose is metabolized mainly in the intestine and in the liver by fructokinase (KHK), a specific enzyme that initiates fructose metabolism through phosphorylation. Excess intake of fructose-containing sugars promotes de novo lipogenesis and hepatic fat accumulation [47]; the blockade of fructose

metabolism (like in whole body KHK-A/C KO mice) is sufficient to prevent hepatic fat accumulation, alterations in liver enzymes and hepatic insulin resistance due to high sucrose intake [48]. Deletion of fructokinase at whole body or organ level (intestine and liver) have different effect on the metabolic handling of excessive intake of fructose and glucose . The selective deletion of KHK in the intestine leads to reduced fructose intestinal clearance that in turn increases hepatic uptake of fructose while hepatic KHK deletion does not prevent the toxic hepatic effect of excess fructose [48]. These data in part explain why after high fructose intake in the presence of a stable energy intake (i.e. lower intake of other dietary sugars) healthy subjects do not show any metabolic alteration nor hepatic fat accumulation [49].

Although there is a strong link between hepatic accumulation of lipids (TAG, DAG, and Sphingolipids including Ceramides) and hepatic insulin resistance [45, 50], the exact mechanisms are not yet known. Hepatic TAG accumulation is associated to both hepatic and peripheral insulin resistance [11, 19, 45, 50-52]. DAGs are precursor of TAGs and have been shown to be positively correlated with HOMA-IR [45, 51] and associated to hepatic insulin resistance [51, 52]. The proposed mechanism for DAG induced HepIR is related with the activation of protein kinase Cε (PKCε), inhibiting insulin action through the phosphorylation of insulin receptor. This will decrease the phosphorylation of insulin receptor and consequently reducing the insulin activation of 1-phosphoinositol 3-kinase (PI 3-kinase) and Akt2. A reduced insulin-Akt activation decrease glycogen synthesis and decreased suppression of gluconeogenesis [52]. However, the role of hepatic PKCε has been recently challenged since only deletion of global but not hepatic PKCε protects mice against diet-induced glucose intolerance and insulin resistance [53]

Sphingolipids are another class of bioactive lipids that have been associated to both hepatic and peripheral IR [45, 54]. The accumulation of ceramides in the liver is increased in subjects with NASH and associated to both HOMA-IR and peripheral IR measured by the euglycemic hyperinsulinemic clamp [45, 50]. The exact mechanism by which ceramides influence Hep-IR are not known but some proposed mechanism relates ceramides and inhibition of Akt. The activation of protein kinase C-ζ (PKCζ) by NADH-ceramide interaction impairs translocation of Akt to the plasma membrane, hampering insulin receptor action on the other hand, ceramide activates protein phosphatase 2A (PP2A) leading to dephosphorylation and inactivation of Akt [54, 55]. The changes in Akt position and function impair FOXO1 phosphorylation and consequently increasing expression of hepatic gluconeogenic, as Pck1, and lipogenic enzymes[13, 56].

Also dihydroceramides (the precursors of ceramides) have been linked to insulin resistance and their concentration is increased in subjects with NASH [45, 50]. Dihydroceramides are converted into ceramides by the enzyme dihydroceramide desaturase 1 (DES1) that inserts a conserved double bond into the backbone of dihydroceramides and other predominant sphingolipids. However, when the gene *Degs1* that encodes DES1 was blocked, mice fed with obesogenic diet and leptin-deficient ob/ob mice showed lower levels of ceramides in serum or tissue and the toxic effects of ceramides, i.e. increased lipid uptake and storage and dysfunctional glucose utilization, were no longer present [54]. These mice showed lower body weight, reduced steatosis and improved glucose tolerance and insulin sensitivity compared to wild type mice [54]. Thus, a high dihydroceramide to ceramide ratio that indicates a reduction in DES1 activity might be associated to a more favorable metabolic profile. Not only ceramide synthesis but also degradation (by ceramidases) might play a role in the development of hepatic insulin resistance and increased risk to develop type 2 diabetes. Hepatic overexpression of acid ceramidase was associated to reduced hepatic fat accumulation, increased VLDL secretion but also improved total body glucose homeostasis and insulin sensitivity under HFD feeding [55].

Hepatokines and insulin resistance

The liver secretes several proteins (hepatokines) that can influence metabolic processes through autocrine, paracrine and endocrine signaling and are also linked to insulin resistance [57]. Hepatokines have a crucial interplay with hepatic metabolism to maintain glucose homeostasis, in particular Fetuin A, Fetuin B and Fibroblast Growth Factor 21 (FGF-21).

Fetuin A was the first hepatokine proposed to regulate metabolic homeostasis through inter-organ crosstalk and is an independent risk factor for the development of type 2 diabetes [58]. Serum levels of fetuin-A are increased in obesity and type 2 diabetes [59] and positively correlated with hepatic fat content, insulin resistance and glucose intolerance [60]. This hepatokine directly inhibits not only the phosphorylation IRS-1 and of the insulin signaling cascade but also the translocation of the glucose transporter GLUT4 in insulin target tissues thus impairing insulin sensitivity [57]. The liver secretes also Fetuin B that like Fetuin A correlated positively with the glucose area under the curve during the OGTT but not with insulin resistance [60]. Thus, regulation of glucose homeostasis by Fetuin-A and -B occurs through different mechanisms, the first through alteration in insulin signaling, the second possibly through glucose effectiveness [60].

Fibroblast Growth Factor 21 (FGF-21) is distinguished from the rest of growth factors by its endocrine function, participating in the regulation of glucose and lipid metabolism. FGF-21 is secreted mainly by the liver and is able to reduce glucose concentrations by increasing glucose uptake in the muscle and adipose tissue independently of insulin [57, 61, 62]. FGF-21 is inversely correlated with body mass index (BMI), hepatic fat accumulation and fasting insulin, and is decreased in T2D compared to healthy subjects [61]. FGF21 acts as an insulin sensitizer under physiologic conditions both during prolonged fasting as well as postprandially during overfeeding [62]. Pharmacologically, FGF-21 is a potent insulin sensitizer that can decrease acutely plasma glucose within 1h by 40–50% and its effect can last to about 6h. One of the mechanisms through which FGF21 improves hepatic glucose metabolism is the stimulation of adiponectin production, which in turn reduces hepatic ceramide levels while the effects on TAGs and DAGs synthesis are mild [63].

Summary and conclusions

The liver plays a central role in the regulation of glucose metabolism and the development of metabolic diseases including non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2D). Diabetes can alter the functions of almost any organ in the body, including the liver. However, at the moment liver is not recognized among the organs at risk. Changes in hepatic function, including hepatic glucose and lipid metabolism, should be closely monitored for the prevention and treatment of metabolic diseases.

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Author contribution S.G. Review of the literature, Manuscript draft, Review & Editing
A.G. Conceptualization, Writing - Review & Editing, Supervision and Funding acquisition

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