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Targeting hepatocyte carbohydrate transport to mimic fasting and calorie restriction

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Abstract

The pervasion of three daily meals and snacks is a relatively new introduction to our shared experience, and is coincident with an epidemic rise in obesity and cardiometabolic disorders of overnutrition. The past two decades have yielded convincing evidence regarding the adaptive, protective effects of calorie restriction (CR) and intermittent fasting (IF) against cardiometabolic, neurodegenerative, proteostatic and inflammatory diseases. Yet, durable adherence to intensive lifestyle changes is rarely attainable. New evidence now demonstrates that restricting carbohydrate entry into the hepatocyte by itself mimics several key signaling responses and physiological outcomes of IF and CR. This discovery raises the intriguing proposition that targeting hepatocyte carbohydrate transport to mimic fasting and caloric restriction can abate cardiometabolic and perhaps other fasting-treatable diseases. Here, we review the metabolic and signaling fates of a hepatocyte carbohydrate, identify evidence to target the key mediators within these pathways, and provide rationale and data to highlight carbohydrate transport as a broad, proximal intervention to block the deleterious sequelae of hepatic glucose and fructose metabolism.

Graphical Abstract

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AUTHOR CONTRIBUTIONS

All authors participated in conceptualization, writing and critical review of this work.

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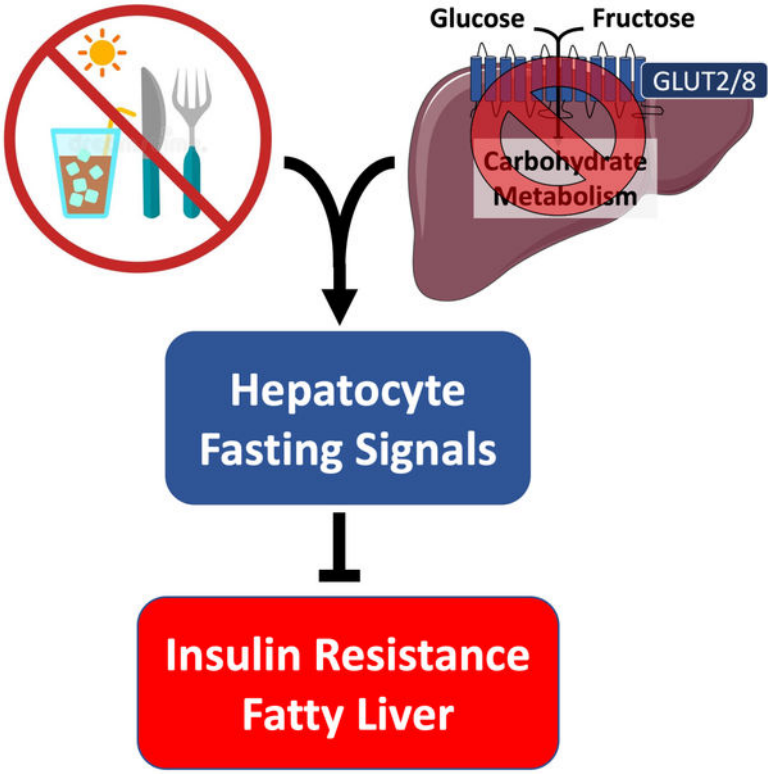

Keywords

Glucokinase; GLUT; ChREBP; Fructose; Obesity; non-alcoholic fatty liver disease; non-alcoholic steatohepatitis; intermittent fasting; caloric restriction; insulin resistance; diabetes; ketogenic diet; cardiovascular disease; heart failure; low-fat diet; glycemic index; trehalose; lactotrehalose; metabolism; NAFLD; mitochondrial pyruvate carrier; sirtuin; FOXO; nicotinamide adenine dinucleotide; fibroblast growth factor 21

INTRODUCTION

Long before three daily meals and interceding snacks became part of modern industrialized life, the Ancient Romans viewed consumption of more than one meal daily to be a form of gluttony, and monastic practices of the Middle Ages forbade food consumption prior to morning Mass (Denise Winterman, *BBC News Magazine*, 2012). The act of “breaking fast”, only became a widespread, shared practice across social classes during the Industrial Revolution, initially as a means to sustain day laborers through long workdays in the mid-1800s.

In contrast with the practice of three meals, caloric restriction and intermittent fasting (CR and IF), have long been known to exert therapeutic effects on healthspan and lifespan in animals from worms to humans (1). In real-world clinical contexts, however, implementing and sustaining such lifestyle changes has proven to be difficult. As a result, we have yet to fully leverage this biology to mitigate a range of fasting-responsive human diseases. Recent

provocative data now demonstrate that restricting carbohydrate entry into the hepatocyte recapitulates several key metabolic effects of CR and IF (2–14). This raises the possibility that fasting mimetic therapies that target hepatocyte carbohydrate metabolism may be viable treatments against metabolic disease, including non-alcoholic fatty liver disease, and type 2 diabetes mellitus (15). The purpose of this review is to briefly examine the metabolic fate of a carbohydrate in the hepatocyte, define downstream hepatocyte carbohydrate signaling, and then finally focus on promising nodes within this axis to leverage against metabolic disease.

Clinical utility of CR, IF, and reduced dietary carbohydrate

IF and CR exert manifold protective metabolic effects in rodents and in humans (16, 17). This includes broadly adaptive effects on hepatic steatosis and inflammation, insulin resistance, and cardiovascular disease (1, 16, 18–29). These effects occurred independently of whether the intervention consisted of chronic daily caloric restriction, or of a fast:fed intermittent fasting of 5:2 days, 16:8hr, or 18:6hr. Furthermore, IF exerts several key effects, even when controlled for caloric intake, and even in the absence of weight loss. For example, 18h intermittent fasting for five weeks reduce insulin resistance, blood pressure, oxidative stress and appetite without lowering weight in pre-diabetic men, when compared with men consuming an isocaloric diet instead over a 12h timespan (23, 29). Together, these observations suggest that IF and CR in humans improve cardiometabolic function.

In parallel, a significant body of data interrogating specific macronutrient withdrawal has come to light. Whereas low-fat foods surged in popularity over the preceding decades amidst an unprecedented rise in obesity in the United States, recent studies have examined the metabolic effects of dietary carbohydrate restriction. Carbohydrate-selective restriction overall appears to improve circulating lipids, glucose and insulin tolerance with equal or greater efficacy as compared with calorie restriction and low-fat intervention. This seems to hold true in varied populations. For example a randomized trial of ketogenic diet (KD) in both obese men and women demonstrated that the low carbohydrate, high-fat, high-protein diet was equally efficacious when compared to a low calorie, low-fat, high-carbohydrate diet with regard to lowering diastolic BP and improving glucose tolerance. In addition, the low-carbohydrate diet produced lower initial weight loss, and increased HDL and lowered TG in ketogenic diet-treated subjects when compared with those on a low-calorie, low-fat diet (30). In 132 severely obese subjects with a high prevalence of diabetes or metabolic syndrome, patients on a carbohydrate-restricted diet lost more weight, and exhibited improved insulin sensitivity and triglycerides when compared with subjects on a low-fat diet (31). In overweight, dyslipidemic subjects, KD was more effective than a calorie-restricted, low-fat diet at reducing endogenous lipogenesis, insulin resistance, body weight, adiposity, and dyslipidemia (32). In obese females without co-morbidities, a very low carbohydrate diet was more efficacious in reducing body weight, raising HDL and ketones without adversely affecting other cardiometabolic risk factors when compared with calorie-restricted low-fat diet (33). Similarly, when compared with calorie restriction and Mediterranean diet interventions, low-carbohydrate diets produced the most weight loss, and had the most favorable effects on lipid profiles in moderately obese human subjects over a 2-year period (34). Moreover, in children with NAFLD, low-glycemic index foods reduced systolic blood pressure, plasma ALT, and insulin resistance indices (HOMA-IR)

after 3- and 6-months treatment (35). In mice, ketogenic diets reduce circulating lipids, intrahepatic lipids, and insulin resistance markers (36), and improve cardiac function during heart failure (37). In balance of these findings, low-carbohydrate diets in some contexts do not outperform other forms of caloric restriction (38). Nevertheless, data from human and mouse models indicate the efficacy of carbohydrate-specific withdrawal, and substantiate further investigation into how carbohydrates regulate metabolic function, and the extent to which targeting carbohydrate metabolism is a therapeutically viable option.

The fate of a carbohydrate in the hepatocyte

The liver sits at the nexus of portal and venous circulations. In this position, the hepatocyte negotiates an organism's present and immediate future systemic energetic status. In light of promising data to suggest that dietary carbohydrate is an important determinant of cardiometabolic risk, significant efforts have focused on carbohydrate metabolism in the liver, and its subsequent host effects.

Carbohydrate entry into the hepatocyte occurs via at least two primary facilitative transporters in the glucose transporter (GLUT) family of solute carrier proteins (Figure 1) (39, 40). These are GLUT2 (encoded by the *Slc2a2* gene) and GLUT8 (encoded by the *Slc2a8* gene), each of which transports glucose and fructose, among other carbohydrates (39, 40). GLUT2 is a high-capacity, low-affinity glucose, fructose, and galactose transporter (39). Its rapid transport kinetics are tuned such that its substrate concentrations quickly equilibrate with the extracellular fed, fasting, or diabetic milieu. GLUT2 is the most highly expressed liver GLUT, and accordingly, its activity comprises the majority of carbohydrate flux into the hepatocyte (39). In contrast GLUT8 is a high-affinity, low-capacity facilitative carrier of glucose, fructose, galactose, and possibly trehalose (39, 41–45). GLUT8 mediates only about 20–25% of hepatocyte glucose transport (44), and it differs from GLUT2 most prominently in that it localizes both to plasma membrane and intracellular organellar membranes (43, 44, 46–48), although its intracellular functions are not fully understood. Together, these two transporters mediate the preponderance of carbohydrate entry into the hepatocyte cytoplasm.

Glucokinase (GCK) and glucokinase regulatory protein (GCKRP) represent the first regulatory step following facilitative diffusion into the hepatocyte (49). GCK (hexokinase IV) phosphorylates glucose to glucose-6-phosphate (G6P), which attenuates glucose excursion to the cell exterior. Indeed, this is a highly regulated step, mediated in large part by GCK regulatory peptide (GCKRP). This enzyme binds and inhibits GCK to attenuate GCK-mediated glucose phosphorylation and subsequent hepatocyte glycolytic metabolism (50, 51). This regulation is particularly critical, because glucose conversion to G6P also might be a rate-limiting step for glycolysis in hepatocytes (49).

Glucose-6-phosphate subsequently has both signaling and catabolic functions (Figure 1) within the hepatocyte (52). In the post-prandial hepatocyte, G6P undergoes glycolysis, and is converted to pyruvate to generate fatty acids via de novo lipogenesis, or it is converted to UDP-glucose, and stored as glycogen (53). First, following G6P conversion to pyruvate through glycolysis, pyruvate is either converted to lactate by lactate dehydrogenase, or transported into the mitochondrial matrix via the mitochondrial pyruvate carrier (MPC)

(54). After entry into the mitochondrion, mitochondrial pyruvate metabolism is important for de novo lipogenesis. Pyruvate can be carboxylated to produce oxaloacetate (anaplerotic metabolism) or oxidized to acetyl-CoA (catabolic metabolism), and both play a role in de novo lipogenesis (54–56). Moreover, pyruvate carboxylation is important for producing new glucose via gluconeogenesis and cholesterol synthesis (54, 56, 57). Second, from a metabolic signaling standpoint, ATP generated by G6P catabolism suppresses AMPK activity, to contribute to hepatic steatosis and obesity (7, 8, 58). In addition, G6P and potentially other metabolites activate a key carbohydrate sensor in hepatocytes, the carbohydrate response element binding protein (ChREBP) (59). Activated ChREBP transcriptionally activates genes of de novo lipogenesis (Figure 1) (60).

Although glucose and fructose both ultimately activate ChREBP, fructose and glucose entering the hepatocyte take initially divergent metabolic pathways. These key differences in initial fructose and catabolism are considered to mediate their distinct physiological sequelae (5, 6, 61–63). Whereas GCK phosphorylates free glucose to G6P, ketohexokinase (KHK) phosphorylates free fructose to fructose-1-phosphate (F1P) upon entry into the hepatocyte. Aldolase B catabolizes F1P to generate dihydroxyacetone phosphate (DHAP) and (after the triose kinase or triose phosphate isomerase reactions) glyceraldehyde-3-phosphate (GA3P). GA3P is the common metabolite in glycolysis at which glucose and fructose metabolism converge.

At least two additional points of divergence exist when comparing fructose metabolism with glucose metabolism. First, KHK is an ATP-dependent enzyme, and AMP generated in the KHK reaction provides substrate for AMP-deaminase, a committed enzymatic step in uric acid synthesis (61). Both decreased intracellular [phosphate] and F1P allosterically activate AMP deaminase to exacerbate uric acid generation, which predisposes the host to cardiometabolic syndrome unless cleared by the kidney or intestine (64–67). A second divergence between fructose and glucose metabolism is that carbon from F1P is used to produce the triacylglycerol backbone, glycerol-3-phosphate (G3P). This occurs via concerted catalysis of aldolase B and glyceraldehyde phosphate dehydrogenase (GAPDH). TAGs are exported systemically in the form of VLDL, which by itself portends cardiometabolic risk (68). Overall, the divergence between fructose and glucose metabolism may explain added risk in response to intracellular fructose (6).

Carbohydrate-Induced Pathways as Targets for Metabolic Intervention

Our current understanding of hepatocyte carbohydrate metabolism and signaling gives us glimpses into therapeutic targets against metabolic disease (Table 1). We have alluded to the hepatic and extrahepatic metabolic effects of hepatocyte GLUT deletion (11, 44, 69, 70), and will discuss this specific therapeutic approach in greater detail in a subsequent section.

Glucokinase may be an attractive target, because it is a proximal enzyme that links glucose influx with glycolysis and downstream metabolism, such as glycogen synthesis and lipogenesis. This is highlighted by data that pharmacological GCK activation as a method to treat type 2 diabetes mellitus produces hypoglycemia, hyperlipidemia and hepatic steatosis (71). Genetic GCK overexpression similarly increased hepatic triglyceride deposition, and induced hyperglycemia, hyperinsulinemia, insulin resistance and glucose intolerance in

mice (72). Conversely, liver-specific GCK-deficient mice have decreased hepatic glycogen content (73, 74), and reduced de novo lipogenic gene expression in liver, including pyruvate kinase and fatty acid synthase (73). However, GCK heterozygous mice develop fasting hyperglycemia (73) and liver-specific GCK-knockout mice also exhibited impaired glucose tolerance. Thus, targeting GCK to treat NAFLD may not be ideal, since many patients with NAFLD are also insulin resistant and/or diabetic, and inhibiting GCK has the potential to exacerbate these conditions.

GCKR encodes the GCK regulatory protein, GCKRP. This protein inhibits glucose trapping and metabolism by binding and inhibiting GCK. Therefore it is unsurprising that the GCKR locus is associated with NAFLD in humans (68, 75, 76). More specifically, polymorphisms that prevent GCKRP-GCK binding (e.g. P466L) result in lower plasma glucose, and increased hepatic steatosis, circulating triglycerides and cholesterol in human subjects (68, 77, 78). Similarly, pharmacologically disrupting the GCKRP and GCK interaction lowered circulating glucose and increased respiratory exchange ratio (RER) in Zucker diabetic rats. The data indicate a switch from fat oxidation to a glucose oxidative predilection (79), although direct measurements of circulating or intrahepatic lipid contents are not reported. Overall, the data point to an important glucose homeostatic function for the interaction between GCK and GCKRP. However, understanding how to optimally target this segment of hepatocyte glucose metabolic pathway is required, due to the delicate, inverse relationship between GCK activity and hepatic steatosis.

In regard to fructose metabolism, recent promising pre-clinical and clinical demonstrations suggest that proximal fructose metabolism blockade improves hepatic and peripheral energy metabolism. KHK regulates the first committed step in fructose metabolism upon entry into the hepatocyte cytoplasm. There are two major KHK isoforms, a minor A-isoform, and a major C isoform. KHK-A and KHK-C deletion together, germline whole-body KHK-C deletion, and liver-specific KHK-C knockdown each block fructose-induced metabolic dysfunction in multiple models (6, 80, 81). These data suggest that targeting dual KHK-A/C isoforms, or single-isoform KHK-C alone is a viable therapeutic strategy against metabolic disease. This is underscored by recent phase 2 clinical trial data, which demonstrate that pharmacologic KHK inhibition reduces hepatic steatosis, as measured by magnetic resonance imaging proton density fat fraction in patients with NAFLD (82). In contrast, patients with aldolase B deficiency have increased intrahepatic lipid content (83), and genetic aldolase B inhibition in mice phenocopies hereditary fructose intolerance in humans. Data by Lanaspa and colleagues recently implicated the product of KHK enzymatic action, fructose-1-phosphate (F1P), as a potential mechanistic link that explains differential outcomes due to aldolase B and KHK targeting (80). They showed that aldolase B deletion exacerbated fructose-induced hepatic TG and uric acid accumulation. In contrast deleting KHK-C reversed these detrimental effects of aldolase B on fructose-induced metabolism, indicating that F1P availability in hepatocytes may be deleterious (80). Thus, aldolase B targeting has not been a major focus for clinical development against metabolic disease (84, 85).

Anaerobic metabolism of both glucose and fructose generates pyruvate, which can enter the mitochondrion via a carrier-mediated mechanism. The mitochondrial pyruvate carrier

(MPC) is composed of two proteins, MPC1 and MPC2, which form a heterodimer in the inner mitochondrial membrane. Liver-specific MPC1 or MPC2 deletion or knockdown improved basal hyperglycemia and glucose tolerance in high-fat diet-fed or genetically obese db/db mice (86). It is likely that these effects were mediated, at least in part, by blocking pyruvate entry into the gluconeogenic pathway, since that requires pyruvate carboxylation in the mitochondrial matrix (86). Acute pharmacological MPC blockade by the novel PPARγ-sparing thiazolidinedione, MSDC-0602, also attenuated diet-induced insulin resistance, glucose tolerance (87) and hepatic steatosis, inflammation, and fibrosis in mice (56, 88–90). In patients with NASH with or without insulin resistance, MSDC-0602 lowered glycated hemoglobin, circulating insulin, and serum transaminases, but failed to affect NASH histology (91). Moreover, although such data are not yet published in liver, MPC inhibition by MSDC-0160 in neurons induces autophagic flux and compensatory branched chain amino acid catabolism (92). Together, the data suggest that pyruvate transport is the mitochondrial extension of glycolytic flux, and inhibiting substrate catabolism at any of these catabolic steps induces compensatory changes that benefit host metabolism. In addition, the broad, adaptive effects of acute inhibition, and corroborating genetic data highlight an important role for the hepatic MPC in peripheral and hepatic glucose and lipid homeostasis, and hepatic inflammation.

Restricting hepatocyte carbohydrate entry mimics the broader effects of fasting

The above data demonstrate tremendous advances in understanding hepatocyte glucose and fructose intermediary metabolism and signaling. To add to this, preventing hepatocyte carbohydrate entry prior to commitment into glycolysis, fructolysis and downstream signaling merits its own consideration as a viable target to prevent and treat metabolic disease. Our group and others recently examined some of the molecular intermediaries that convey the effects of hepatocyte carbohydrate restriction. In this section, we briefly review the rationale to translate this particular therapeutic approach, define intermediaries and mechanisms that are activated upon blocking carbohydrate entry, and delineate future considerations for study in hepatocyte glucose fasting.

Rationale to target hepatocyte carbohydrate transport—A primary goal of fasting physiology is to provide glucose and ketones to the brain (17). To that end, the hepatocyte switches to anabolism to generate glucose via glycogenolysis and gluconeogenesis, and to generate ketones from fatty acids. It then follows that the absence or paucity of glucose entering the hepatocyte activates homeostatic pathways that signal fasted status to the periphery. These peripheral carbon sources for the latter processes are fatty acids from peripheral adipose stores, and alanine derived from skeletal muscle (17). Aoki, Cahill and colleagues provided some of the early human data to suggest that glucose determines the systemic (and hepatocyte) fasting response in classical experiments (93). In this experiment, Glucose feeding was sufficient to maintain circulating insulin, and suppress ketogenesis and peripheral lipolysis even in the context of profound caloric restriction. Even after three weeks of starvation, 150 g / day glucose (~70% calorie restriction) by itself raised serum insulin, and suppressed hepatic ketogenesis and urea nitrogen excretion. The data underscore that, even during starvation and severe caloric restriction, minimal amounts of glucose are sufficient to abrogate the fasting response. A substantial body of data

have accumulated to demonstrate that both endocrine and cell-autonomous hepatocyte regulation direct ketogenesis, gluconeogenesis, glycogenolysis, and the peripheral lipolysis and glucose-alanine cycling that fuel these processes. Our focus here will remain on cell-autonomous substrate regulation of hepatocyte glucose transport, because this process is quite directly amenable to small-molecule therapeutics.

Cellular Consequences of Hepatocyte Carbohydrate Restriction—Blocking carbohydrate entry activates autophagy primarily by AMPK-dependent (7, 94, 95) and potentially AMPK-independent pathways (Figure 2) (96). This activates homeostatic processes to correct for this perturbation. First, the hepatocyte activates autophagic flux to recycle damaged or misfolded proteins as a means to free substrate to meet subsequent energy demands (7, 8, 13, 14, 97, 98) in part by direct AMPK stimulatory phosphorylation of Beclin 1 (99) and *unc52*-like kinase 1 (ULK1) (94, 95, 100). Secondly, glucose deprivation activates ER and oxidative stress-responsive pathways, and this includes GRP78 (101, 102), NRF2/KEAP1, and AMPK alpha 2 (103) pathways (104). Distally, glucose deprivation activates several key transcriptional regulators, including TFEB (105–107), PGC1α (108–110), and PPARα (111). These factors activate lysosomal biogenesis and fat oxidative transcriptional programs to coordinate the metabolic switch from glucose to autophagy-derived macromolecules and fat. In addition, glucose-stimulated ATP production regulates the redox state through NAD^+/NADH levels to activate the deacetylase sirtuin family member, SIRT1, which deacetylates and activates PGC1α and the stress-responsive factor, FOXO1 (112). Finally, we identified non-canonical fasting mediators of hepatocyte glucose-specific restriction (10, 113), including the lipoxygenase, *Aloxe3* and the arginine ureahydrolase, *Arg2*. Both genes are induced by macronutrient withdrawal, fasting, and trehalose and LT treatment in vivo and in vitro, and mediate aspects of the hepatocyte fasting response (10, 113).

Carbohydrate withdrawal therefore exerts important molecular changes within the hepatocyte. This suggests that methods to target hepatocyte cytosolic carbohydrate excursion via GLUT2 and GLUT8 have translational value. In the liver-specific GLUT2 knockout model (LG2KO), liver fluorodeoxyglucose uptake was blunted when compared with uptake in WT mice (69), but increased skeletal muscle glucose uptake renders basal glycemia in these mice unchanged when compared with wild-type mice. Moreover, although the role of hepatocyte GLUT2 in obese or diabetic models has not been reported, LG2KO mice had decreased fasting cholesterol biosynthesis and were protected from fasting-induced steatosis. This phenotype was attributed to lower glucose substrate entry to fuel lipid droplet deposition. These adaptations were observed despite paradoxically higher expression of ChREBP and its target lipogenic and glycolytic genes, and despite progressive pancreatic β-cell dysfunction over time in LG2KO mice versus WT mice (69). Accordingly, GLUT8 deletion attenuated radiolabeled hepatocyte fructose uptake, fructose-induced ChREBP activation, de novo lipogenic gene expression, and triacylglyerol synthesis when compared with wild-type cultured primary murine hepatocytes (44). In addition, germline whole-body GLUT8-deficient mice were protected from high-fat and high-fructose diet-induced hepatic steatosis, dyslipidemia and glucose intolerance without any obvious chronic maladaptive

metabolic sequelae reported, in comparison with WT littermates. These effects were mediated in part by enhanced fasting-induced hepatic PPARα activity (11, 44, 70, 114).

Pharmacological studies of restricted hepatocyte carbohydrate entry largely recapitulate the metabolic effects of hepatocyte GLUT deficiency. In particular the disaccharide glucose mimetics trehalose and lactotrehalose (LT) are useful probes to define the effects of hepatocyte GLUT blockade (7, 8, 13, 14, 98, 115–118). Both disaccharides block enterohepatic glucose transport (GLUT) (7, 8, 13, 14, 98), thus offering a window into acute effects of enterohepatic GLUT blockade. Trehalose and LT reduced fructose-induced hepatic steatosis and induced hepatocyte fasting response signals, PGC1α, TFEB and FGF21. Extrahepatic cardiometabolic effects of these compounds are broad, including peripheral insulin sensitization (119), activating peripheral thermogenesis, reduced adipocyte hypertrophy (120, 121), in addition to reduced atherosclerotic plaque lesion area (27, 122) and pathological cardiac remodeling in response to injury (123, 124), that mimicked ketogenic dietary effects on heart failure (37). Treating liver-specific GLUT8-deficient mice with oral trehalose did not increase the efficacy of trehalose on diet-induced hepatic steatosis, which implies some mechanistic overlap between GLUT8 deletion and trehalose action (98). Restricting carbohydrate entry into the hepatocyte thus exerts hepatic and extrahepatic therapeutic adaptative effects. The complex interactions between hepatocyte glucose transport and extrahepatic tissues therefore justify much deeper exploration into both the potential limitations and the physiologic and signaling adaptations of hepatocyte GLUT-specific targeting.

By corollary, detailing the intracellular consequences of hepatocyte carbohydrate restriction may illuminate novel therapies against both hepatic and extrahepatic metabolic disease. For example, hepatocyte glucose restriction induces ALOXE3, a lipoxygenase that metabolizes arachidonic acid intermediaries to enhance peripheral insulin sensitivity, thermogenesis and reduce hepatic steatosis in genetic and diet-induced obese models (113). In addition, Arg2 is upregulated during fasting, and we postulate that the purpose of this is to handle the excess liver nitrogen load to fuel gluconeogenesis (17). Forced Arg2 expression in hepatocytes improved insulin resistance, heat generation and hepatic insulin sensitivity in genetic and diet-induced obese models. These actions depended upon an inverse relationship with the hepatocyte regulator of G-protein signaling (RGS) protein, RGS16, although more detailed mechanisms of this axis remain to be fully explored. Elucidating new pathways that hepatocyte glucose restriction activates will continue to offer novel leverage points for new metabolic therapies.

FUTURE DIRECTIONS AND CONCLUSIONS

The metabolic efficacy of IF and CR are widely recognized across basic scientific, medical, and popular domains (1, 16, 22), but the mechanisms and full breadth of utility for therapeutic hepatocyte glucose transport restriction are only beginning to be elucidated. In addition to unbiased screening approaches, we can look to the paradigm process of fasting itself to point us to mechanisms under carbohydrate control. On this basis, hepatocyte glucose transport restriction and its effects on other core fasting-regulated processes remain prime opportunities for future investigation (Figure 2). This includes, in

particular, hepatocyte glucose-specific withdrawal and its effects on NAD^+ metabolism and circadian rhythm (1, 22, 125–127). Lastly, it should be recognized first that the observations regarding effects hepatocyte glucose restriction might not merely represent a subset of the generalized fasting response. Rather, there are likely to be pathways unique to glucose-specific restriction that are key leverage points for therapy. Secondly, glucose restriction might share pathways with other adaptive processes, such as cold thermogenesis (128–131). Notably, if hepatocyte glucose restriction incites a distinct adaptive response, this opens the exciting possibility that this pathway can be utilized additively or synergistically with other distinct leverage points in fasting-like signaling. Certainly, in the face of the ongoing epidemics of obesity, diabetes and NAFLD, the future certainly has never looked sweeter.

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Abbreviations:

AldoB	aldolase B
ALOXE3	arachidonate lipoxygenase 3
AMPK	AMP-activated protein kinase
Arg2	arginase 2
ChREBP	carbohydrate response element binding protein
CR	caloric restriction
FOXO	forkhead box transcriptiion factor O
GCK	glucokinase
GCKRP	glucokinase regulatory protein
GLUT	glucose transporter
IF	intermittent fasting
KHK	ketohexokinase
MPC	mitochondrial pyruvate carrier
mTOR	mechanistic target of rapamycin
NAD^+	nicotinamide adenine dinucleotide
NAFLD	non-alcoholic fatty liver disease
PGC1α	peroxisome proliferator antigen receptor gamma coactivator 1α
PPAR	peroxisome proliferator antigen receptor

RGS	regulator of G-protein signaling 16
SIRT1	sirtuin1
TFEB	transcription factor EB

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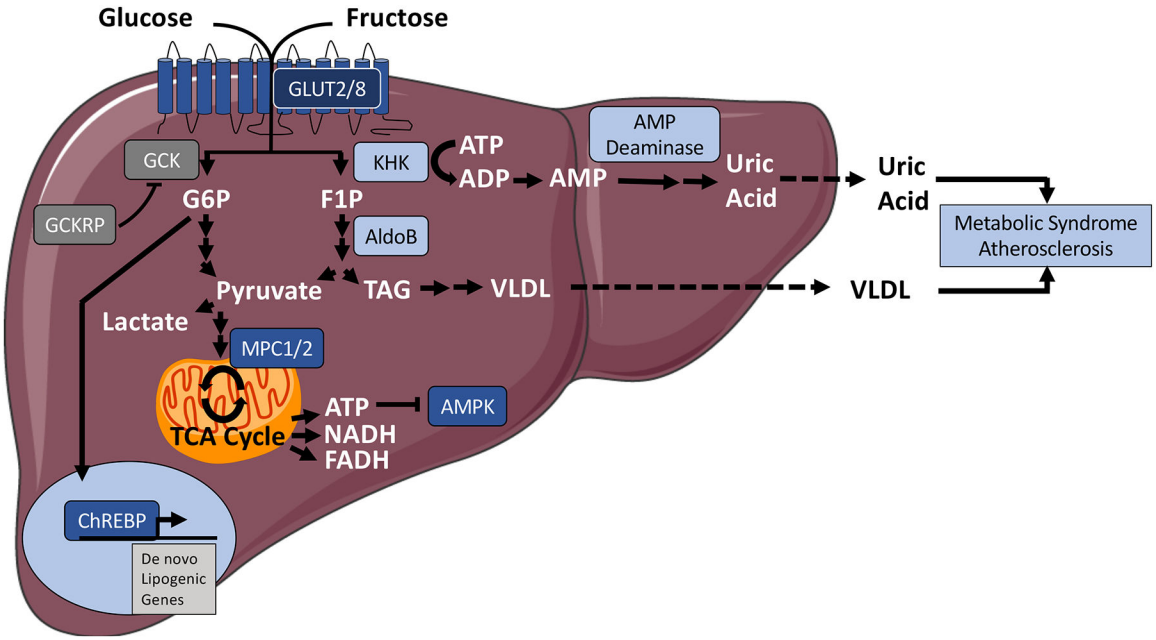

Figure 1.

Post-prandial glucose and fructose fates inside the hepatocyte. In the fed state, glucose and fructose are transported into the hepatocyte via the glucose transporter, and then catabolized to glucose-6-phosphate or fructose-1-phosphate, by glucokinase and ketohexokinase respectively. *Left*, Glucose-6-phosphate is catabolized via glycolysis to pyruvate. Glucose-6-phosphate also induces de novo lipogenic gene transcription by activating the transcription factor, Carbohydrate response element binding protein. Glucokinase regulatory protein regulates glucose catabolism overall by suppressing glucokinase activity. *Right*, The ketohexokinase reaction generates fructose-1-phosphate, from which aldolase B initiates fructolytic conversion toward pyruvate, triacylglycerol, and ultimately VLDL synthesis. Pyruvate from glycolysis and fructolysis is catabolized to lactate, or it is transported via the mitochondrial pyruvate carrier to undergo oxidative metabolism in the mitochondrion. ATP production inhibits a key fasting regulator kinase, AMP-activated protein kinase. The ketohexokinase reaction also generates ADP, which is further catabolized to AMP. This provides substrate, which is shunted toward uric acid production via AMP deaminase. Dark grey enzymes are selective to the glucose-metabolic pathway. Light blue-colored enzymes represent fructose-selective catabolic pathways. Dark blue-colored enzymatic pathways represent common points of intervention in carbohydrate metabolism. Abbreviations: AldoB, aldolase B; AMPK, AMP-activated protein kinase; ChREBP, carbohydrate response element binding protein; CR, caloric restriction; FOXO, forkhead box transcriptiion factor O; G6P, glucose-6-phosphate; F1P, fructose-1-phosphate; GCK, glucokinase; GCKRP, glucokinase regulatory protein; GLUT, glucose transporter; KHK, ketohexokinase; MPC, mitochondrial pyruvate carrier; PPAR, peroxisome proliferator antigen receptor; TAG, triacylglycerol; TCA cycle, tricarboxylic acid cycle; VLDL, very low density lipoprotein.

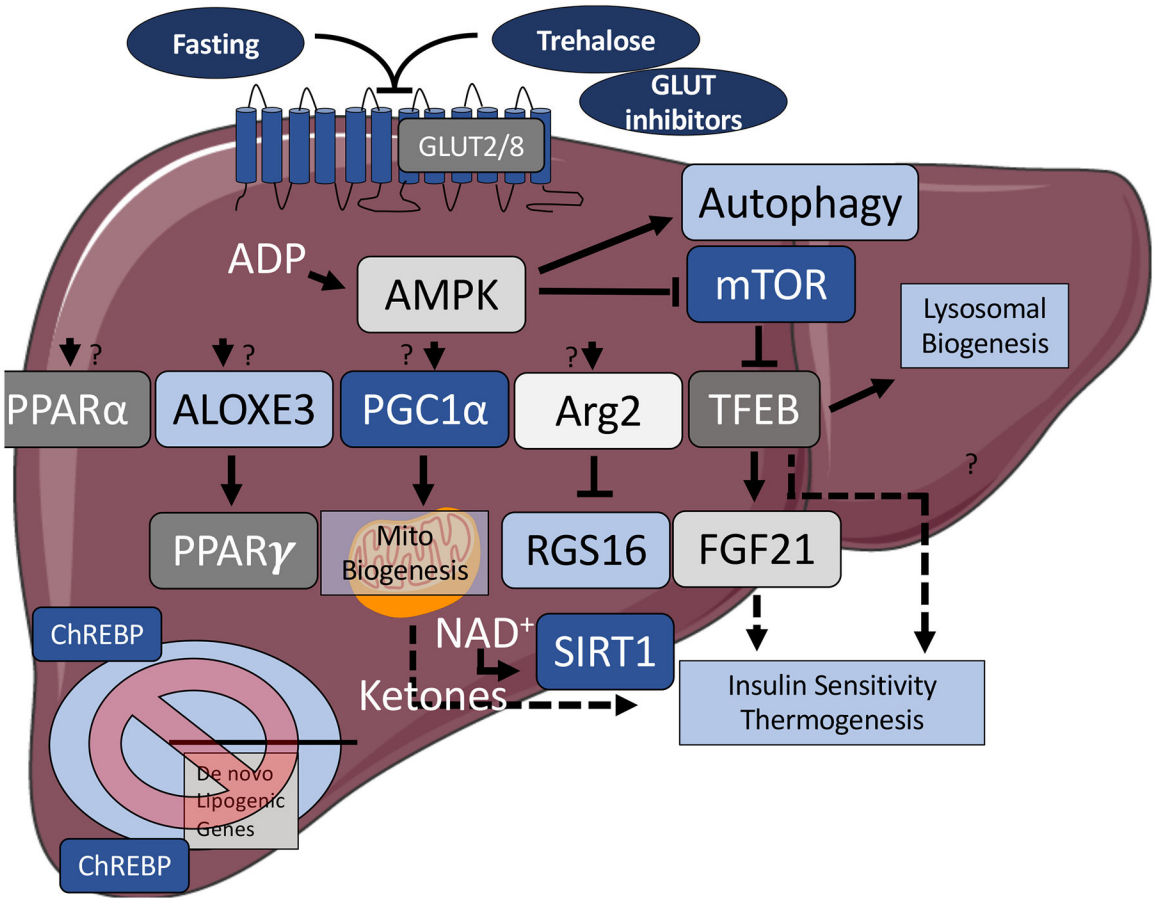

Figure 2.

Some of the key signaling pathways that are activated upon hepatocyte glucose withdrawal, or GLUT blockade. Fasting, and treatment with glucose transporter inhibitors reduce hepatocyte ATP production, and activate key fasting-mimetic signaling pathways, which are metabolically protective to the host. These pathways include induction of autophagy to recycle aging or damaged organelles and proteins to be used as fuel, inhibition of mechanistic target of rapamycin (mTOR) and carbohydrate response element binding protein, and activation of AMP-activated protein kinase. These serve as key proximal fasted-state intracellular sensors. Downstream fasting-like signals upon suppressed carbohydrate entry include peroxisome proliferator antigen receptors α and γ, arachidonate lipoxygenase 3, peroxisome proliferator antigen receptor gamma coactivator 1α;, arginase 2 and sirtuin 1, transcription factor EB, and release of fibroblast growth factor 21 and ketones into the peripheral circulation. The full mechanisms by carbohydrate withdrawal activates these signals, and the full consequences of their activation, however, remain subjects of continued investigation. Dashed arrows: secreted factor. Abbreviations: ALOXE3, arachidonate lipoxygenase 3; AMPK, AMP-activated protein kinase; Arg2, arginase 2; ChREBP, carbohydrate response element binding protein; FGF21, fibroblast growth factor 21; GLUT, glucose transporter; mTOR, mechanistic target of rapamycin; NAD^+, nicotinamide adenine dinucleotide; PGC1α, peroxisome proliferator antigen receptor gamma coactivator 1α;

PPARα, peroxisome proliferator antigen receptor; RGS16, regulator of G-protein signaling 16; SIRT1, sirtuin1; TFEB, transcription factor EB.

TABLE 1.

Summary of outcomes upon targeting glucose intermediary metabolism. Outcomes described (if reported in the cited study) are: glucose homeostasis, lipid homeostasis, and other important readouts.

Study Ref.	Target	Targeting Method	Disease Model	Outcome Measures (targeted relative to untargeted control)
69	GLUT2	Liver-specific KO	Fast (24h)-refeed versus fast alone	• Decreased hepatic glucose uptake. no change in hepatic glucose output. Progressive glucose intolerance and impaired glucose-stimulated insulin secretion. • Reduced cholesterol biosynthesis-related hepatic gene expression. Increased hepatic VLDL secretion, decreased fasting-induced steatosis. • Normal thermogenesis in LG2KO mice.
7	GLUTs (non-specific)	Trehalose	60% fructose diet (10 days)	• Reduced plasma and hepatic TG and cholesterol, reduced hepatic de novo lipogenic gene expression. • Increased hepatic autophagy.
9	GLUTs (non-specific)	Lactotrehalose	60% fructose diet (10 days)	• Reduced hepatic steatosis, de novo lipogenic gene expression markers. • Increased thermogenesis, increased plasma FGF21.
11	GLUT8	GLUT8 Antisense oligonucleotide	Ad libitum fed vs. 12–24h fasting	• Increased relative heat generation in ad libitum-fed and fasted mice treated with GLUT8 ASO.
11	GLUT8	Whole-body KO	Ad libitum fed vs. 12–24h fasting	• Increased heat generation in ad libitum-fed- and fasted GLUT8 KO mice. PPARα-dependent increases in serum β−hydroxybutyrate and FGF21 in fasted GLUT8 KO mice.
44	GLUT8	Whole-body KO	60% fructose diet (24wk)	• Reduced hepatic steatosis in GLUT8 KO mice. Decreased fructose-induced de novo lipogenic gene expression. Decreased TAG synthesis and fat oxidation in isolated hepatocytes.
70	GLUT8	Whole-body KO	60% fructose diet (24wk)	• Improved glucose tolerance and insulin sensitivity in HFrD-fed GLUT8 KO. • Decreased plasma TG. • Increased heat generation in chow- and HFrD-fed GLUT8KO mice.
72	GCK	Hepatocyte-specific overexpression	Chow vs. 42% High-fat diet (12wk)	• Mild hyperglycemia, glucose intolerance. Decreased liver glycogen in chow-fed GCK transgenic mice. Exacerbated HFD-induced hyperglycemia and hyperinsulinemia, steatosis and insulin resistance. • Increased liver TG accumulation in chow-fed transgenic mice. Exacerbated high-fat diet-induced hepatic steatosis in transgenic mice.
74	GCK	Hepatocyte-specific KO	No perturbation	• Impaired glucose tolerance. Deceased hepatic glycogen content in GCK LKO mice.
73	GCK	Hepatocyte-specific haploinsufficiency		• Decreased hepatic glycogen content in GCK heterozygous mice. • Lower PEPCK mRNA in GCK heterozygous mice.

Study Ref.	Target	Targeting Method	Disease Model	Outcome Measures (targeted relative to untargeted control)
79	GCKR-GCK interaction	Small molecule inhibitors: AMG-1694 AMG-3969	ob/ob, db/db, or 60% fat diet (12wk)	• Lower blood glucose in AMG-3969-treated obese mice vs. congenic controls. Switch to glucose oxidative metabolism in AMG-3969 and AMG-2694-treated mice by indirect calorimetry.
6	KHK	Liver-specific KHK siRNA	60% high-fat diet + 30% glucose water or fructose water (10wk)	• Improved glucose tolerance after KHK knockdown in HFD+H_2O, HFD+glucose and HFD+fructose mice. • Decreased hepatic steatosis in KHK knockdown mice on HFD+H_2O, HFD+glucose, and HFD+fructose.
80	KHK-A/C KHK-A	Whole-body KHK deletion Whole-body KHK-A deletion	AldoB KO ± Fructose water AldoB KO ± Fructose water	• KHK deletion corrected fructose-induced hypoglycemia. • KHK deletion reduced fructose-induced hepatic inflammation, fibrosis. • KHK deletion, but not KHK-A deletion, reduced acute fructose-induced hyperuricemia and transaminase elevation.
81	KHK-A/C KHK-A	Whole-body KHK-A/C deletion Whole-body KHK-A deletion	15–30% fructose water (25wk)	• KHK-A/C deletion blocked fructose-induced hyperglycemia, hyperinsulinemia. KHK-A-specific deletion exacerbated these outcomes. • KHK-A/C deletion blocked fructose-induced hepatic steatosis, de novo lipogenic gene expression, weight gain, and epididymal fat accumulation. KHK-A-specific deletion exacerbated these outcomes. • KHK-A/C deletion reversed fructose-induced suppression of plasma β-hydroxybutyrate.
86	MPC1	Germline MPC1 LKO	60% high-fat diet (10–22wk)	• No change in basal glucose and insulin tolerance in chow-fed conditions. Improved glucose tolerance without changes in insulin tolerance in germline HFD-fed MPC1 LKO mice. • Nocturnal switch toward greater fat oxidation by indirect calorimetry in MPC1 LKO mice.
86	MPC1	Acute AAV8-Cre-mediated MPC1 deletion		• Improved glucose and insulin tolerance in HFD-fed acute liver MPC1 KO mice. Lower fasting glucose and insulin in acute liver MPC1 KO mice
87	MPC1/2	MSDC-0602	ob/ob or 60% high-fat diet (10–12wk)	• Lower fasting insulin, improved fasting glucose and glucose and insulin tolerance in MSDC-0602-treated ob/ob mice. Higher (e.g. improved) glucose infusion rate and higher peripheral glucose uptake in MSDC-0602-treated HFD-fed mice. • Decreased plasma TG, cholesterol, and NEFA in MSDC-0602-treated ob/ob mice. Decreased liver TG, TG synthesis and de novo lipogenic gene expression in HFD-fed mice treated with MSDC-0602. • Increased plasma adiponectin in MSDC-0602-treated ob/ob mice.
90	MPC1/2	MSDC-0602	HTF-C (16wk)	• Reduced diet-induced weight gain, transaminase elevations in MSDC-0602-treated mice. • Reduced liver TG, NAS and fibrosis scores in MSDC-0602-treated mice. • Reduced stellate cell activation marker gene expression in MSDC-0602-treated, HTF-C-fed mice. Reduced exosome-mediated stellate cell activation

Study Ref.	Target	Targeting Method	Disease Model	Outcome Measures (targeted relative to untargeted control)
				gene markers in stellate cells incubated with MSDC-0602-treated cell-derived exosomes.
90	MPC2	MPC2 LKO	HTF-C (16wk)	• No reduction in liver TG in MPC2 LKO mice fed HTF-C. Lower diet-induced plasma tranasminases, fibrosis score, fibrosis gene activation • Lower stellate cell activation marker gene expression. Reduced exosome-mediated stellate cell activation marker genes in cell treated with MPC2 LKO-derived exosomes.