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Dietary carbohydrate and control of hepatic gene expression: mechanistic links from ATP and phosphate ester homeostasis to the carbohydrate-response element-binding protein

Loranne Agius
Institutes of Cellular Medicine and Ageing and Health, Medical School, Newcastle University, Newcastle upon Tyne NE2 4HH, UK

Type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) are associated with elevated hepatic glucose production and fatty acid synthesis (*de novo* lipogenesis (DNL)). High carbohydrate diets also increase hepatic glucose production and lipogenesis. The carbohydrate-response element-binding protein (ChREBP, encoded by *MLXIPL*) is a transcription factor with a major role in the hepatic response to excess dietary carbohydrate. Because its target genes include pyruvate kinase (*PKLR*) and enzymes of lipogenesis, it is regarded as a key regulator for conversion of dietary carbohydrate to lipid for energy storage. An alternative hypothesis for ChREBP function is to maintain hepatic ATP homeostasis by restraining the elevation of phosphate ester intermediates in response to elevated glucose. This is supported by the following evidence: (i) A key stimulus for ChREBP activation and induction of its target genes is elevation of phosphate esters; (ii) target genes of ChREBP include key negative regulators of the hexose phosphate ester pool (*GCKR*, *G6PC*, *SLC37A4*) and triose phosphate pool (*PKLR*); (iii) ChREBP knock-down models have elevated hepatic hexose phosphates and triose phosphates and compromised ATP phosphorylation potential; (iv) gene defects in *G6PC* and *SLC37A4* and common variants of *MLXIPL*, *GCKR* and *PKLR* in man are associated with elevated hepatic uric acid production (a marker of ATP depletion) or raised plasma uric acid levels. It is proposed that compromised hepatic phosphate homeostasis is a contributing factor to the elevated hepatic glucose production and lipogenesis that associate with type 2 diabetes, NAFLD and excess carbohydrate in the diet.

Type 2 diabetes: Non-alcoholic fatty liver disease: ATP: Carbohydrate-response element-binding protein: Glucokinase: *GCKR*: Glucose 6-phosphatase: Uric acid

Type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD) are chronic metabolic diseases that associate with overnutrition. T2D manifests as a defect in blood glucose homeostasis with altered insulin secretion and liver glucose metabolism and insulin resistance[1]. NAFLD comprises a spectrum of changes ranging from raised liver fat (steatosis) to inflammation and fibrosis[2], and associates with insulin resistance and T2D[3]. High-carbohydrate diets and particularly dietary sugars are a risk factor for both T2D and NAFLD[4–6]. The transcription factor carbohydrate-response element-binding protein (ChREBP, encoded by *MLXIPL*), is a major mediator of the hepatic changes induced by high-carbohydrate diets and is conventionally regarded as a key mechanism for promoting conversion of dietary carbohydrate to fat for energy storage[7–11]. This review

Abbreviations: ChREBP, carbohydrate-response element-binding protein; DNL, *de novo* lipogenesis; F1P, fructose 1-phosphate; F2,6P$_2$, fructose 2,6-bisphosphate; F6P, fructose 6-phosphate; Gckr, glucokinase inhibitor protein; G6P, glucose 6-phosphate; G6pc, glucose 6-phosphatase; KHK, ketohexokinase; NAFLD, non-alcoholic fatty liver disease; PE, phosphate esters; Pi, inorganic phosphate; PKLR, pyruvate kinase; T2D, type 2 diabetes.
Corresponding author: L. Agius, email Loranne.agius@ncl.ac.uk

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evaluates the hypothesis that a key function of ChREBP is to preserve liver ATP homeostasis in conditions of compromised intracellular metabolite homeostasis as occurs with overnutrition on high-carbohydrate diets. In health, liver cell ATP is robustly homeostatic despite large diurnal changes in glucose metabolism. The hepatic concentrations of intermediates of glucose metabolism comprising phosphate esters (PE) are dynamic but over a narrow concentration range, that has been described as the stability paradox[12]. Evidence is reviewed that key changes in hepatic gene expression that associate with high-carbohydrate diets or metabolic disease are an adaptive response to maintain intracellular PE and ATP homeostasis.

Effects of type 2 diabetes and non-alcoholic fatty liver disease on hepatic glucose metabolism and *de novo* lipogenesis

The liver maintains blood glucose homeostasis by net production of glucose in the post-absorptive state and net uptake of glucose in the postprandial state in response to the rise in blood glucose in the portal vein which delivers the products of carbohydrate digestion from the gut. In the post-absorptive state the liver produces glucose by glycogen degradation and gluconeogenesis. The glucose 6-phosphate (G6P) generated in the cytoplasm from these pathways is transported into the endoplasmic reticulum on the G6P-transporter (encoded by *SLC37A4*) and hydrolysed within the lumen by glucose 6-phosphatase (G6pc) to glucose and inorganic phosphate (Pi)[13]. After a meal when the glucose concentration in the portal vein rises, hepatic glucose metabolism undergoes a large metabolic transition from net production to net uptake. The liver extracts about one-third of the glucose load and of this about 60 % is converted to glycogen; the rest is metabolised by glycolysis and the pyruvate is either transported into mitochondria or exported as lactate[14]. This rapid metabolic transition is mediated by endocrine and neural signals, and by metabolite signals linked to the increase in glucose concentration in the portal vein[14,15].

T2D is characterised by defects in secretion of insulin and glucagon and by elevated hepatic glucose production, lack of suppression of hepatic glucose production by insulin and lower hepatic glucose uptake[1]. Poorly controlled T2D is associated with low flux through glucokinase[1] and low glucokinase activity[16] and mRNA expression[17]. T2D is also associated with raised plasma TAG levels and a greater fractional contribution of hepatic *de novo* lipogenesis (DNL), representing the synthesis of fatty acids from acetyl units[18], to TAG secretion[19].

In NAFLD, glycerolipid accumulates in hepatocytes because of an imbalance between fatty acid uptake (from NEFA and dietary lipids) plus synthesis (via DNL) relative to fatty acid disposal by mitochondrial β-oxidation plus secretion as TAG in VLDL[20–22]. In the healthy fasted state, the major sources of substrate for hepatic TAG secretion are NEFA derived from adipose lipolysis with a smaller contribution of NEFA from dietary chylomicrons that escape uptake by extra hepatic tissues and a negligible contribution of DNL[18]. NAFLD is associated with increased fatty acid supply by adipose tissue lipolysis[22] and an increased fractional contribution of DNL to TAG secretion[20–22] from <5 % in controls to 15–20 % in patients[20,22]. Both the rate of VLDL–TAG secretion derived from DNL and the fractional contribution of DNL to VLDL secretion are elevated in NAFLD and correlate with liver fat content[22]. Other hepatic metabolic abnormalities in NAFLD include impaired suppression of hepatic glucose production by insulin[23]; elevated gluconeogenesis and increased flux through tricarboxylic acid cycle oxidation and anaplerosis, without increased turnover of ketone bodies[24].

A key paradox is that despite a greater fractional contribution of DNL to TAG secretion in NAFLD, the net flux through DNL is very small (<5 %) relative to carbohydrate turnover[25]. Accordingly DNL is not a quantitative route for disposal of carbohydrate. Similar considerations apply in relation to ethanol which increases fractional DNL to >30%; however, the major disposal product is acetate released into the circulation rather than DNL which represents <5 % of ethanol metabolism[25]. However, DNL has a regulatory role through changes in cellular levels of malonyl-CoA (the product of acetyl-carboxylase) which inhibits mitochondrial fatty acid oxidation[26]. This may explain the correlation between raised fractional DNL and raised TAG secretion rate[21]. DNL may represent a spill over of acetyl-units (as citrate efflux from mitochondria) as suggested by the association between intrahepatic lipid and flux through tricarboxylic acid cycle oxidation plus anaplerosis (and increased gluconeogenic flux), in the absence of elevated ketone body production[24].

Effects of dietary carbohydrate on *de novo* lipogenesis and hepatic glucose production

The fractional contribution of hepatic DNL to hepatic TAG secretion is dependent on dietary carbohydrate intake[27]. Healthy subjects consuming diets with lower (25–50 %) or higher (25–50 %) carbohydrate content for 5 d showed a near-linear relation between fractional DNL and dietary carbohydrate[28]. Raised fractional DNL with high carbohydrate was also observed in isoenergetic conditions[29] and more so with dietary sugars than with starch[30]. Fractional DNL correlates with raised VLDL–TAG secretion rates and raised plasma TAG levels[31]. Fructose has a greater effect than glucose on DNL, plasma lipid levels and hepatic steatosis[32–34]. Fructose increases DNL more strongly and more rapidly (within hours) than glucose when administered acutely[18,25]. This rapid effect of fructose is attributed to its high flux of metabolism, whereas the effect of chronic consumption of glucose-containing high-carbohydrate diets is thought to be predominantly transcriptional. Despite the large fractional increase in DNL (from 1 to about 30 %) caused by either acute fructose infusion or chronic carbohydrate surplus[28,35] the net daily flux through DNL in comparison with dietary carbohydrate

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load is small (⩽5 %) in both man and rodents[25,35] indicating that even in healthy subjects on high-carbohydrate diets the role of DNL is regulatory for fuel selection for oxidation[26,28] and not as a quantitative route of disposal of dietary carbohydrate[25].

Another effect of high-carbohydrate diets in man is an increase in hepatic glucose production. During chronic carbohydrate under-feeding or over-feeding (for 5 d) hepatic glucose production increased in parallel with DNL with increasing carbohydrate load despite the elevated plasma insulin[28]. Elevation in basal glucose production also occurred with a high-carbohydrate diet in isoenergetic conditions[36]. The parallel elevation in glucose production and DNL with high-carbohydrate diets are similar to the metabolic characteristics of NAFLD[24].

Effects of dietary carbohydrate on hepatic gene expression: role of carbohydrate-response element-binding protein

In animal models high-carbohydrate diets induce liver enzymes of DNL[8,9]. Two transcription factors involved are sterol regulatory element binding protein 1c that is induced by insulin and targets predominantly enzymes of DNL and ChREBP that is induced by high glucose and targets enzymes of DNL but also several additional genes, including pyruvate kinase [7–9]. Because ChREBP is induced by dietary carbohydrate, the generally held explanation for ChREBP function is that it represents a mechanism for efficient conversion of dietary carbohydrate to fat as a 'thrifty gene'[8–11]. The decrease in DNL[37–39] and in steatosis[38,39] in ChREBP knock-down models[37] is consistent with such a hypothesis but it does not exclude the alternative explanation[25] that the role of DNL is regulatory for fuel selection because it is not a quantitative route of carbohydrate disposal[25,28].

ChREBP binds to the promoter of its target genes in complex with Max-like protein X (Mlx), a member of the Myc/Max superfamily of transcription factors[9,40]. Mlx has other partners, including MondoA, the paralogue of ChREBP[40]. Gene microarrays in hepatocytes using a dominant-negative Mlx, identified several candidate target genes of Mlx, including not only enzymes of DNL and L-pyruvate kinase (PKLR), but also two enzymes of gluconeogenesis (G6pc and fructose bisphosphatase-1) which catalyse generation of Pi from PE and the inhibitory protein of glucokinase encoded by the *Gckr* gene[41]. These target genes were also confirmed by chromatin immunoprecipitation-PCR[42,43] or chromatin immunoprecipitation-sequencing[44]. Glucokinase which is the major determinant of hepatic glucose disposal[15], is not a target gene for ChREBP and it is repressed rather than induced by high glucose[42]. The induction by high glucose via ChREBP-Mlx of the two major counter-regulators of glucokinase, the glucokinase inhibitor protein (Gckr) and G6pc which dephosphorylates the product of the glucokinase reaction, argues against a role for ChREBP-Mlx in net conversion of glucose to fat.

To evaluate the hypothesis that the function of ChREBP is to maintain homeostasis of PE and ATP in conditions of high-carbohydrate diets or compromised intracellular metabolite homeostasis, the following issues need to be considered: (1) To what extent are the hepatic concentrations of PE of glucose metabolism elevated postprandially? What is the function of these changes? (2) What are the consequences of disrupted regulation on cell Pi and ATP homeostasis? (3) What mechanisms exist to restrain the elevation in PE within physiological limits? (4) What is the effect of ChREBP deletion on hepatic PE and ATP homeostasis? (5) What are the signals for ChREBP activation?

Hepatic concentrations of phosphate esters in the fasted-to-fed transition

In the rodent model, the hepatic concentration of G6P, the first intermediate of glucose metabolism (Fig. 1) and also downstream PE intermediates of glycolysis and the pentose phosphate pathway are elevated about 2-fold in the fed state on a starch-containing diet compared with the fasted state[45]. The increase in G6P is sustained during the absorptive state[46]. Larger elevations in PE, particularly of the pentose pathway intermediates, occur on a sucrose-containing diet[45]. Changes in G6P are associated with parallel changes in fructose 6-phosphate (F6P) which is in equilibrium with G6P via phosphoglucoisomerase, and with elevation in fructose 2,6-bisphosphate ($F2,6P_2$) which is a 'regulatory' rather than 'intermediate' metabolite that is a potent activator of phosphofructokinase-1 and thereby glycolysis[47]. $F2,6P_2$ is generated from F6P by the bifunctional enzyme phosphofructokinase-2/fructose biphosphatase-2, which also degrades $F2,6P_2$ to F6P. The postprandial elevation in G6P has a major role in stimulating glycogen storage (Fig. 1) because G6P is an allosteric effector of glycogen phosphorylase and glycogen synthase[15]. The elevation in $F2,6P_2$ mediates stimulation of glycolysis by activation of phosphofructokinase-1, with consequent elevation in its product fructose 1,6-bisphosphate, which is an allosteric activator of pyruvate kinase which converts phosphoenolpyruvate, the last PE intermediate of glycolysis to pyruvate. The greater elevation in PE caused by a sucrose-diet compared with a starch-diet[45] can be explained by the different pathways of glucose (Fig. 1) and fructose (Fig. 2) metabolism in liver as discussed later.

Effects of glucose on hepatocyte phosphate esters: the restraining role of glucokinase inhibitor protein and glucose 6-phosphatase

In hepatocytes, the G6P content is a sigmoidal function of glucose concentration (5–35 mM) with half-maximal ($S_{0·5}$) stimulation at about 25 mM glucose as for glucose phosphorylation[15,48]. This represents a lower affinity than for glucokinase kinetics ($S_{0·5}$ 8 mM) and is explained by the inhibition of glucokinase by the Gckr protein[48]. Gckr sequesters glucokinase in the nucleus of hepatocytes in the fasted state, and enables its translocation to the cytoplasm in response to elevated glucose in the portal vein postprandially (Fig. 1)[15]. Allosteric effectors that enhance dissociation of glucokinase from Gckr

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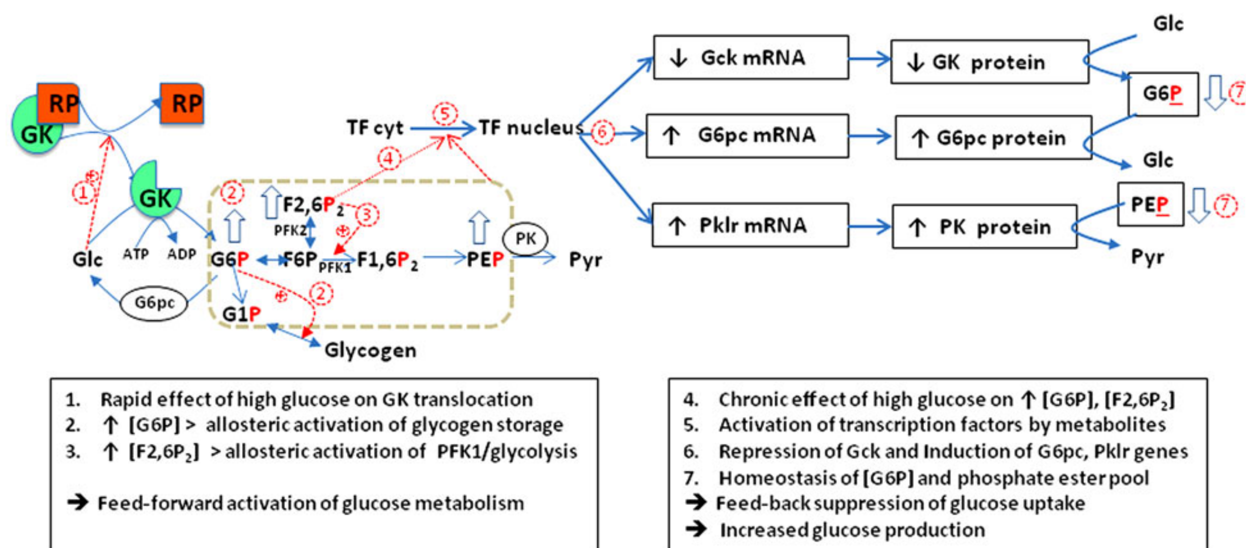

Fig. 1. (Colour online) Glucose stimulates glucose disposal by allosteric mechanisms but it also causes feed-back regulation of gene expression. Elevated glucose concentration in the portal vein causes rapid dissociation of glucokinase (GK) from the Gckr regulatory protein (RP) resulting in a moderate increase in the cellular concentrations of glucose 6-P (G6P) and fructose 2,6-bisphosphate (F2,6P_2) which cause allosteric activation of enzymes promoting glycogen storage and glycolysis. Chronic elevation in G6P and F2,6P_2 causes activation of transcription factors which down regulate the GK gene (Gck) and upregulate the G6pc and Pklr genes. This gene regulation serves to maintain homeostasis of the cell phosphometabolite pool and results in restraint of glucose uptake and elevated glucose production.

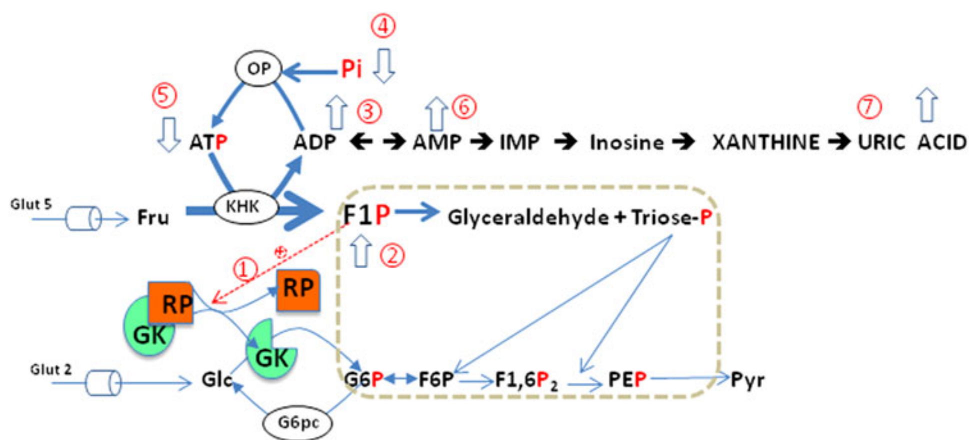

Fig. 2. (Colour online) Fructose is transported and phosphorylated by a distinct mechanism from glucose. Low concentrations of fructose cause moderate elevation in fructose 1-phosphate (F1P), which dissociates glucokinase (GK) from its regulatory protein (RP) leading to stimulation of glucose metabolism (1). Millimolar concentrations of fructose cause marked elevation in F1P (2) because the activity of ketohexokinase (KHK) is higher than that of downstream enzymes. During reconversion of ADP to ATP by oxidative phosphorylation (OP; 3), the cell content of inorganic phosphate (Pi) becomes depleted (4) resulting in depletion of ATP (5) and degradation of AMP (6) to uric acid (7).

(e.g. fructose 1-phosphate (F1P) and Pi) promote translocation of glucokinase to the cytoplasm and elevation in G6P[49–51]. Accordingly the G6P elevation like glucose phosphorylation[50,51] is a function of the glucokinase: Gckr protein ratio and of allosteric ligands of Gckr (e.g. F1P and Pi) which decrease its affinity for glucokinase[49–51]. The activities of enzymes that degrade G6P (e.g. G6pc) also influence the elevation of G6P in response to glucose, as shown by overexpression of G6pc which lowers the G6P elevation by high glucose[52] and by inhibitors of G6P hydrolysis, such as chlorogenic derivatives that target the G6P transporter, which markedly enhance the elevation in G6P[42,53]. The induction by high glucose via Mlx-ChREBP of G6pc and Gckr[41] therefore has a restraining effect on the elevation in G6P[42,43].

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Effects of fructose on hepatic phosphate ester, inorganic phosphate, ATP and uric acid production

The larger elevation in hepatic PE on a diet containing sucrose or fructose[45] can be explained by the high hepatic capacity for phosphorylation of fructose to F1P (by ketohexokinase (KHK)) and the absence of a mechanism for hydrolysis of F1P, the first intermediate (Fig. 2). Fructose transport into hepatocytes is characterised by a high K_m (50–100 mM) and V_{max}[54] and may involve various transporters including Glut5, Glut2 and Glut8[55]. The *KHK* gene encodes two isoforms: a ubiquitous isoform with very low-affinity (KHKa) and the liver isoform (KHKc) which is expressed at high activity in human and rat liver and has a high-affinity (K_m approximately 0·5 mM) for fructose[56–58]. Accordingly, the liver is the major site of fructose clearance[57]. After phosphorylation of fructose to F1P, the latter is metabolised by aldolase-2 to dihydroxyacetone-P and glyceraldehyde, which is phosphorylated by triokinase to glyceraldehyde 3-P. The triose phosphates generated are metabolised by glycolysis and gluconeogenesis. Two key consequences of the high KHKc capacity are: (i) a higher rate of metabolic flux from fructose to pyruvate compared with glucose to pyruvate; and (ii) a much larger elevation of the cellular PE concentrations than occurs with high glucose. This is explained by the high capacity of KHKc relative to the capacity for clearance of triose phosphates by glycolysis and gluconeogenesis[57] and accordingly hepatic F1P can rise to very high levels by micromolar or millimolar fructose reaching the liver in the portal vein[49,58]. A further mechanism for the elevation in PE with fructose is that the elevation in F1P dissociates glucokinase from Gckr (Fig. 2) and elevates G6P derived from glucose by translocating glucokinase to the cytoplasm[48–51]. This mechanism operates also at small fructose loads (20 mg/kg) with modest effects on F1P[59] and is explained by the high affinity of Gckr for F1P[48,49].

In man the fructose concentrations in the portal vein can reach 2·5 mM[60]. This is expected to raise hepatic F1P to millimolar concentrations[49]. Studies testing the effects of fructose on hepatic Pi and ATP levels have generally used intravenous fructose loads ranging from 0·5 to 1·5 g/kg body weight given as a bolus load or infusion[58]. MRI for real-time monitoring of hepatic PE, Pi and ATP showed that fructose infusion causes acute elevation of PE and depletion of Pi (about 50 %) and ATP (by 24–29 %)[58], with similar effects in human and rodent liver[58]. During F1P accumulation the ADP generated is used together with Pi for oxidative phosphorylation leading to depletion of hepatic Pi and thereby attenuation of oxidative phosphorylation and depletion of ATP (Fig. 2) The depletion of Pi and ATP results in degradation of AMP to inosine monophosphate via AMP-deaminase and the further metabolism of inosine monophosphate to uric acid[61,62].

In man and in higher primates, uric acid produced from degradation of AMP and purines is not further metabolised to allantoin because of lack of a functional uric acid oxidase gene[63]. Accordingly plasma uric acid levels are higher in man than in most other mammals that convert uric acid to allantoin. Uric acid production by isolated hepatocytes is a very sensitive index of compromised cell ATP homeostasis in response to low concentrations of fructose[62]. However, changes in blood uric acid concentration in man after fructose consumption are not a sensitive index of elevated hepatic uric acid production, because the basal rate of production of uric acid by human liver (3–5 nmol/min per g liver)[64] is low relative to the plasma pool size (1·5 mmol). Therefore, a 2- to 3-fold increase in uric acid production, as occurs at 2 mM fructose[62], may not be detectable in the peripheral circulation. However, evidence for a several-fold increase in uric acid production in man after fructose challenge (0·5 g/kg per h) was evident by catheterisation of the hepatic vein[64] or by ^{14}C-adenine labelling and measurement of ^{14}C-excretory products in the urine[65].

Hepatic ATP homeostasis: robust in response to glucose but not fructose

Robustness of liver function is contingent on maintenance of ATP homeostasis because ATP is required for anabolic pathways such as protein synthesis, urea synthesis, gluconeogenesis and other ATP-dependent processes[66]. Pathways of hepatic glucose and fructose metabolism begin with ATP-requiring steps, generating G6P or F1P, respectively. ATP homeostasis is maintained if the ATP-production rate by oxidative phosphorylation matches ATP consumption. Oxidative phosphorylation is dependent on the *trans*-mitochondrial proton gradient generated by the electron transport chain and on the concentrations of the substrates ADP and Pi. It is therefore dependent on cell Pi homeostasis. The hepatocellular total phosphate content (PE + Pi) is about 50 μmol/g of which about 10 % is Pi, representing cytoplasmic and mitochondrial pools which are substrates for glycogenolysis/glycolysis and oxidative phosphorylation, respectively[67]. Pi transport capacity from the cytoplasm into mitochondria exceeds the rate of ATP synthesis[66]. However, Pi transport across the cell membrane (influx and efflux) is slow relative to oxidative phosphorylation capacity[67]. In steady state homeostasis of ATP and Pi is preserved because ATP consumption is balanced by ATP production and Pi incorporation is balanced by Pi regeneration.

Hepatic glucose disposal is a good example of metabolic robustness because high glucose concentrations (15–25 mM) as occur in the portal vein or high rates of glycogenolysis do not compromise ATP homeostasis, because G6P and downstream metabolites are modestly elevated[45]. However, fructose metabolism is an example of fragility because 2 mM fructose causes excessive accumulation of F1P[49] with consequent depletion of Pi and ATP and degradation of AMP to uric acid[62]. Two key components of the robustness of glucose metabolism are the glucose 6-phosphohydrolase system within the endoplasmic reticulum (comprising the G6P transporter and G6pc) that dephosphorylates G6P with a high V_{max} and K_m[13], and the glucokinase inhibitor, Gckr. Patients with glycogen storage disease type 1, who have gene defects in G6pc or the G6P transporter, respond to glucagon challenge by acute depletion of Pi and ATP and elevated uric acid production[68], because Pi

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incorporation into PE during activation of glycogen phosphorylase is not balanced by Pi regeneration by G6pc. The induction of G6pc and the G6P transporter (*SLC37A4*) by high glucose can be rationalised as an adaptive mechanism to maintain Pi and ATP homeostasis[41,42]. The induction of Gckr (and the decrease in glucokinase:Gckr ratio) by glucose can also be rationalised as an adaptive response for ATP homeostasis, as shown by hepatocyte studies where an experimentally induced elevation in the glucokinase:Gckr ratio leads to compromised ATP homeostasis and increased uric acid production in response to high glucose challenge[42].

Carbohydrate-response element-binding protein and liver ATP homeostasis: evidence from carbohydrate-response element-binding protein-deletion models and human gene variants

If the function of ChREBP is to maintain hepatic ATP homeostasis by controlling gene expression of enzymes or regulatory proteins that influence PE concentrations (e.g. G6pc, fructose biphosphatase-1, Gckr, Pklr), then ChREBP deletion models would be expected to be associated with raised PE levels, compromised ATP homeostasis and elevated uric acid production. Consistent with this hypothesis, ChREBP deletion models have 2- to 3-fold higher levels of hepatic G6P, dihydroxyacetone-P, 3-phosphoglycerate and phoshoenolpyruvate on either chow or starch diets[37] with somewhat greater differences (about 5-fold) in the hyperphagic ob/ob mouse[38,39]. A study by Burgess and colleagues[69] that determined the hepatic phosphorylation potential, representing the ratio of free concentrations of [ATP]/[ADP]×[Pi] in ChREBP$^{-/-}$ mice showed a 50 % lower phosphorylation potential associated with a 24 % decrease in total ATP and 4-fold elevation of free AMP. The ChREBP$^{-/-}$ mice were viable on chow or starch diets but not on sucrose or fructose diets[37,69]. Two possible explanations for the mortality on fructose are either increased expression of fructose transporters and/or KHKc leading to greater F1P elevation and more severe ATP depletion; or a defect in ATP recovery. The first possibility seems unlikely because *Khk* mRNA levels were lower in the ChREBP$^{-/-}$ model[37]. In support of the second possibility, gene microarrays in cells with ChREBP knockdown identified dihydrofolate reductase which is involved in purine biosynthesis in the down-regulated genes[70], and genes involved in purine metabolism were also identified as ChREBP targets by chromatin immunoprecipitation-sequencing[44]. A role for ChREBP in ATP homeostasis is also indicated by raised plasma uric acid levels in rats with partial ChREBP knock-down on a high-fructose diet[71].

Plasma levels of uric acid in man are elevated in association with insulin resistance[72] and NAFLD[73] and increased hepatic production of uric acid is inferred in these conditions[72]. Genome-wide studies in man have identified twenty-eight loci in association with raised uric acid levels[74]. They include variants for *MLXIPL* and two of the ChREBP target genes, *PKLR* and *GCKR*. The association of these genes with raised uric acid, a marker of ATP depletion[62] is consistent with a role for ChREBP, Pklr and GCKR in ATP homeostasis.

Mechanism for glucose-induced activation of carbohydrate-response element-binding protein: glucose 6-phosphate or multiple phosphate esters?

The mechanism by which high glucose activates ChREBP has not been resolved. ChREBP is transcribed as either a full-length ChREBP-α (864 residues), which is cytoplasmic at low glucose and activated by high glucose which causes translocation to the nucleus, recruitment to target genes and transcriptional activation, or as a shorter ChREBP-β isoform (lacking the first 117-residues at the N-terminus), that is constitutively active at low glucose[75]. Several aspects of the post-transcriptional regulation of ChREBP-α have been explored, including covalent modification by phosphorylation[8,76–78], O-GlcNAc-modification[79,80] and acetylation[81]; mutational studies for mapping of the N-terminal glucose sensory domain and 14-3-3 binding sites[82–84], and identification of putative metabolites that mediate the response to high glucose. The proposed metabolites include: G6P[85,86], xylulose 5-P[8], the regulatory metabolite F2,6P$_2$[43,87], ketone bodies[88] and the product of the hexosamine pathway UDP-N-acetylglucosamine[79,80] which is the substrate for O-GlcNAc-modification of proteins. The induction of ChREBP target genes by high glucose is abolished by glucokinase inhibitors and markedly enhanced by inhibitors of G6P hydrolysis[42,32,53]. These inhibitors abolish and enhance, respectively, the elevation in PE pool in hepatocytes comprising G6P and downstream metabolites[42,32,53]. This rules out an effect of glucose and points to mechanism(s) consequent to PE elevation. Current evidence suggests that a combination of mechanisms is most likely involved in ChREBP-α activation by glucose. For example mutation of residues that are substrates for phosphorylation by cyclic AMP dependent protein kinase and AMP-dependent protein kinase (S196, S626, T666; S566) and for dephosphorylation by glucose[8], did not generate a glucose-insensitive ChREBP indicating involvement of additional mechanisms[77]. Covalent modification of ChREBP by O-GlcNAc which is dependent on UDP-N-acetylglucosamine generated by the hexosamine pathway caused ChREBP protein stabilisation and transcriptional activation[77,78]. However, increased flux through the hexosamine pathway at low glucose does not activate ChREBP target genes[43]. A role for G6P as the signalling metabolite for ChREBP activation[85,86] was proposed based on two sets of evidence: (i) a glucose-like effect of 2-deoxyglucose which is phosphorylated to its 6-PE but not further metabolised; (ii) attenuation of the high glucose effect by overexpression of enzymes that metabolise G6P (e.g. G6pc, glucose 6-phosphate dehydrogenase, phosphofructokinase-1, phosphofructokinase-2). However, 2-deoxyglucose does not activate ChREBP target genes in hepatocytes despite accumulation of its 6-PE[43]. Attenuation of the glucose response by overexpression of enzymes that degrade G6P does not rule out hexosamine pathway involvement, because G6P is in equilibrium with F6P, the substrate for the hexosamine pathway. A role for F2,6P$_2$ was proposed based on inhibition of recruitment of

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ChREBP-Mlx when this metabolite was selectively depleted at high glucose with a kinase-deficient bisphosphatase-active variant of phosphofructokinase-2/fructose bisphosphatase-2. However, additive activation of ChREBP target genes by glucose and xylitol which do not have additive effects on F2,6P_2 indicates involvement of additional metabolites or mechanisms[87].

On the premise that G6P activates MondoA[40] and ChREBP[85,86], McFerrin and Atchley[89] conducted a bioinformatic analysis for the putative 'G6P binding motif' within the N-terminal conserved 'glucose sensing module' based on G6P-binding sites of glucokinase and other enzymes, and identified a 'low-specificity' motif (Sx[ST]xx[ST]) similar to that found in glucosephosphate isomerase and glutamine:fructose 6-phosphate amidotransferase[89]. Based on the 'low-specificity' of this motif and that F6P rather than G6P is the common substrate of these two enzymes, this outcome favours F6P or multiple PE.

Conclusion

ChREBP is induced and activated by high-carbohydrate diets[7,8] and more so by a fructose-containing diet[90]. The mechanism by which ChREBP-α is activated remains unresolved. Current evidence suggests a complex mechanism involving phosphorylation and O-GlcNAc modification of multiple residues and multiple PE including F2,6P_2. The target genes of ChREBP include enzymes of DNL and several proteins which have a lowering effect on PE intermediates of glucose metabolism such as G6P (GCKR, G6PC) and phosphoenolpyruvate (Pklr). These target genes support the hypothesis that a key function of ChREBP is to maintain homeostasis of PE and thereby ATP in conditions of compromised cell PE homeostasis. This hypothesis is further supported by: (i) compromised ATP phosphorylation potential and raised hepatic PE in ChREBP knock-down mouse models; (ii) raised plasma uric acid levels in association with common human variants in the ChREBP gene (*MLXIPL*) and two of its target genes (*GCKR* and *PKLR*)[74]. The hepatic changes that associate with T2D and NAFLD share several similarities to the changes induced by high-carbohydrate diets. It is proposed that these changes in metabolic disease may likewise be, at least in part, an adaptive response to preserve ATP homeostasis. Recognition of this mechanism is crucial for the design of therapeutic strategies for metabolic disease with sustained efficacy[91].

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Conflicts of Interest

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Authorship

The author was responsible for all aspects of preparation of this paper.

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