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Small Amounts of Dietary Fructose Dramatically Increase Hepatic Glucose Uptake Through a Novel Mechanism of Glucokinase Activation

Glucokinase plays a major role in the control of hepatic glucose uptake and storage as glycogen. Small amounts of fructose-1-phosphate, in the presence of relatively high glucose levels, markedly stimulate glucokinase through a novel mechanism of regulation involving dissociation from a regulatory protein and translocation from the nucleus into the cytosol. Using this understanding of glucokinase regulation, a recent study demonstrated that very small amounts of fructose (infused into the duodenum) could increase hepatic glucose uptake and glycogen storage, and reduce peripheral glycemia and insulin levels in the dog. These results suggest that very small amounts of dietary fructose could be beneficial in type 2 diabetes.

Key Words: glycogen storage, fractose, glucokinase, hepatic glucose uptake

Fructose is the sweetest simple sugar and has been promoted over the years as an appetite suppressant, an ideal fuel for endurance exercise, and perhaps most often as a suitable sweetener for type 2 diabetics. Such claims are mainly based on the fact that dietary fructose, when compared with glucose or sucrose, does not elicit a large insulin response. By contrast, fructose has also been cast as a villain having the potential to cause severe problems with lipid, purine, and copper metabolism; in some studies fructose has even been reported to increase insulin secretion in type 2 diabetic subjects. In truth, most claims either lack direct scientific evidence or they are only supported by work in vitro and/or the use of supraphysiologic fructose levels. At moderate dietary fructose levels, therefore, any benefits or problems are still open to question.[1–3] Recent work from Cherrington and colleagues[4] suggests that very small (catalytic) amounts of dietary fructose may be beneficial in lowering postprandial glycemia by increasing hepatic glucose uptake and glycogen storage.

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During the postprandial period (the first 2 to 3 hours after feeding) dietary glucose is distributed within the body according to a set pattern.[5] Approximately one-third is used by the brain, undergoing complete oxidation to carbon dioxide and water. Another one-third will is taken up by skeletal muscle where it is either stored as glycogen or metabolized to lactate or carbon dioxide and water, according to the amount and intensity of muscle contraction. Small amounts are used by oligatory glycolytic tissues (red blood cell, retina, renal medulla) and adipose tissue, but most of the remainder is taken up by the liver primarily to be stored as glycogen. Immediately prior to eating, the liver would show net glucose production, through either glycogenolysis or gluconeogenesis, but upon ingestion of carbohydrate, net hepatic glucose output ceases and the liver quickly switches to net glucose uptake. One of the characteristics of uncontrolled diabetes is that net hepatic glucose output continues after consumption of carbohydrates and this contributes to the hyperglycemia and increased need for insulin. Maintenance of postprandial hepatic glucose production could be the result of impaired hepatic glucose uptake, a failure to shut off the glucose producing pathways, or a combination of both. By applying knowledge of the regulation of hepatic glucose metabolism, Shiota et al.[4] reasoned that they could use small amounts of fructose to stimulate hepatic glucose uptake and thereby decrease hepatic glucose output and lower glycemia.

The control of hepatic glucose uptake and output lies within three related pathways: glycogen metabolism (synthesis and degradation), gluconeogenesis, and glycolysis (essentially a reversal of gluconeogenesis) (Figure 1). These processes share important common steps: transport of glucose into and out of the cell, phosphorylation of glucose to glucose-6-phosphate, and hydrolysis of glucose-6-phosphate to glucose. The liver expresses unique proteins for these three functions; glucose transport is carried out by GLUT 2, a member of the facilitative glucose transporter family, glucose is phosphorylated by glucokinase (hexokinase D or IV), and hydrolysis of glucose-6-phosphate is achieved by glucose-6-phosphatase. The GLUT 2 system moves glucose down a concentration gradient and thus making net flux dependent on the relative intra- and extracellular glucose

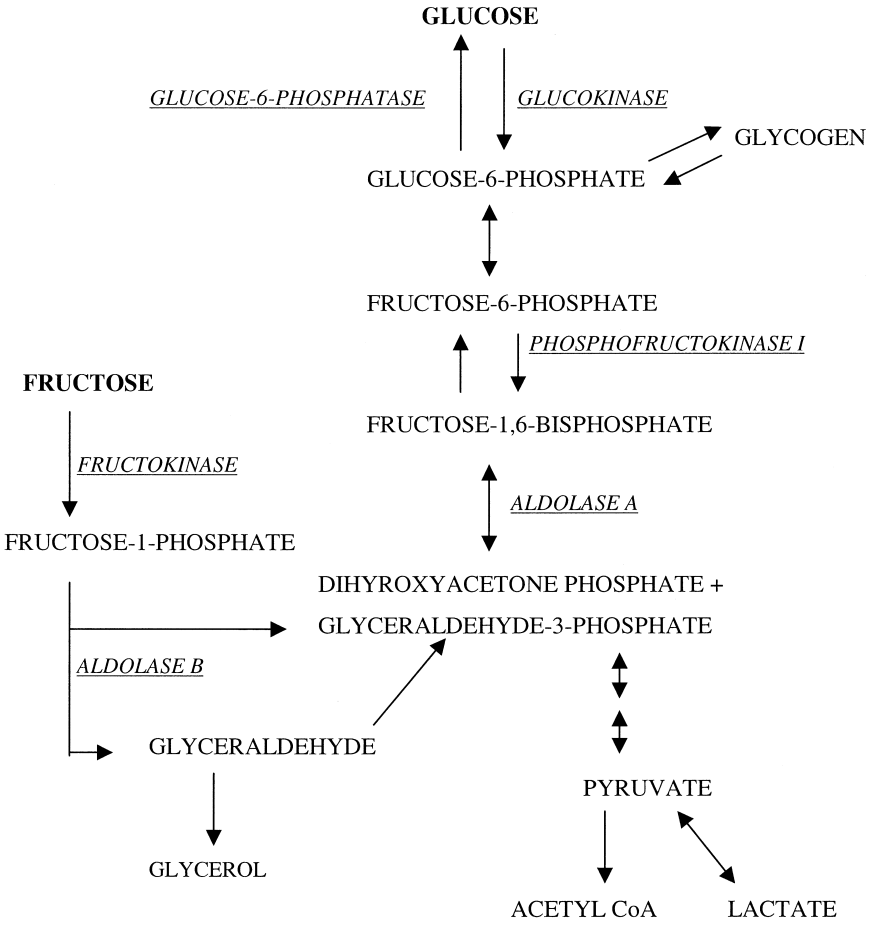

Figure 1. Hepatic glucose and fructose metabolism. Only the key enzymes are shown (underlined) and a number of steps from glyceraldehyde-3-phosphate to pyruvate are omitted for clarity. For detailed explanation see text.

concentrations. Glucose-6-phosphatase is highly regulated but it plays a minor (and indirect) role in the control of glucose uptake and will not be considered further in this article (a thorough review of glucose-6-phosphatase regulation has been recently published[6]). Glucokinase has a number of unique regulatory properties, however, and has been shown to exert considerable control over hepatic glucose uptake and glycogen synthesis.[7,8]

Glucokinase is an isozyme of the mammalian hexokinase family that has a relatively low affinity for glucose, showing sigmoidal kinetics with a $K_{0.5}$ of 7 to 8 mM. Furthermore, by contrast with the high-affinity (Km $<$0.1 mM) hexokinases found in extrahepatic tissues, glucokinase is not subject to inhibition by physiologic concentrations of the product glucose-6-phosphate.[9,10] These properties mean that flux through glucokinase only occurs when glucose in the portal vein is very abundant and that flux continues even as intracellular glucose-6-phosphate levels rise. This allows the liver to take up dietary glucose, store it as glycogen, and thus moderate peripheral glycemia. In keeping with this glucose homeostatic role, glucokinase is not expressed until weaning (change from a high-fat to a high-carbohydrate diet) and the enzyme is subject to long-term regulation, with expression being decreased during diabetes and starvation, and increased upon feeding high-carbohydrate diets. In addition, work over the past decade has demonstrated a novel mechanism for the short-term regulation of glucokinase activity by reversible binding to a regulatory protein and translocation to and from the nuclear compartment. It has long been known that glucose uptake and phosphorylation by liver in vitro is highly stimulated by trace amounts of fructose.[11,12] Although fructose can be phosphorylated in the 6 position, the Km for fructose of most mammalian hexokinases is very high ($>$3 mM for most isozymes and $\sim$400 mM for glucokinase).[9,10] However, the liver possesses a specific fructokinase that will readily phosphorylate fructose (Km $<$0.5 mM) at the 1 position producing fructose-1-phosphate that can be cleaved by aldolase B to yield the triose phosphate intermediates of the glycolytic and gluconeogenic pathways (Figure 1). Fructose metabolism has the potential to perturb hepatic metabolism because, by entering the glycolytic pathway below the phosphofructokinase 1 regulatory step, fructose carbon can be readily metabolized to yield acetyl CoA and subse-

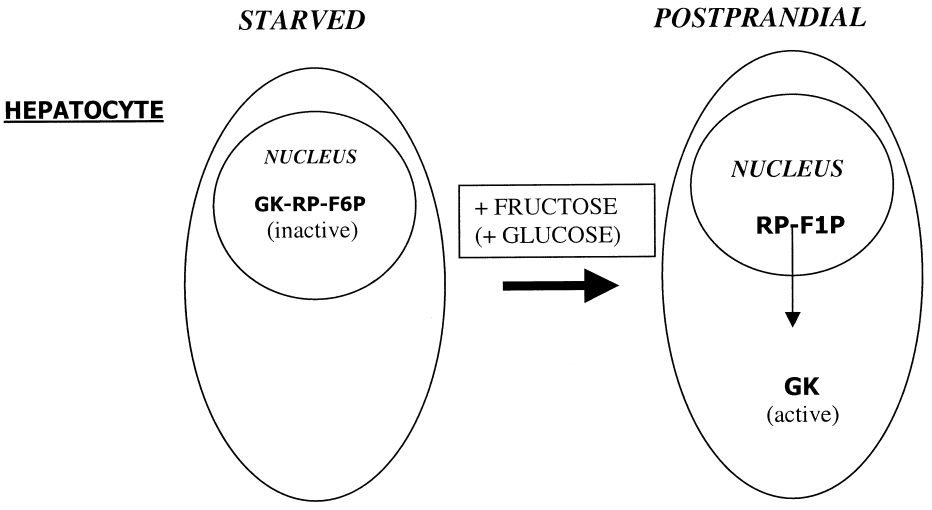

Figure 2. Short-term regulation of glucokinase activity. In postabsorptive liver glucokinase (GK) is inactive and complexed with the regulatory protein (RP) and fructose-6-phosphate (F6P) within the nucleus. In the postprandial state (presence of fructose and glucose) the fructose-6-phosphate is displaced from the regulatory protein by fructose-1-phosphate (F1P), at the same time releasing and activating the glucokinase, which translocates to the cytosol.

quently fatty acids. In addition, aldolase B initially yields glyceraldehyde that may be converted to glycerol, which can be phosphorylated and so promote triglyceride synthesis. However, at traditional levels of dietary intake the rate of fructose absorption in the intestine probably limits the rate of delivery to the liver such that the liver is able to remove most of the incoming fructose without deleterious consequences. Because even in the fed state flux through glycolysis is relatively low in liver (in the fed state the liver probably obtains most of its energy from the partial oxidation of amino acids[5]), the fate of most dietary fructose carbon is likely to be deposition in liver glycogen stores.

Van Schaftingen showed that in liver from starved animals, glucokinase was present as a complex with a regulatory protein plus fructose-6-phosphate, and that in this complex the glucokinase activity was inhibited.[11–13] In liver in vitro and in vivo provision of small amounts of fructose result in displacement of fructose-6-phosphate from the regulatory protein by fructose-1-phosphate. Upon binding fructose-1-phosphate the regulatory protein dissociates from, and no longer inhibits, glucokinase. This model has been refined in recent years and we now know that the glucokinase–regulatory protein–fructose-6-phosphate complex is localized within the nuclear compartment; upon binding fructose-1-phosphate the two proteins dissociate and the glucokinase translocates to the cytosol where it is active (Figure 2).[8] High levels of glucose (10–20 mM in isolated hepatocyte systems) also increase the translocation of glucokinase from the nucleus and Agius and Stubbs[14] suggested that glucose is the primary translocation signal and that fructose-1-phosphate acts to increase the affinity for glucose. Based on this understanding of the mechanism of glucokinase regulation, it has been proposed[15] that small amounts of fructose could significantly increase hepatic glucose uptake in the presence of high glucose levels and thereby lower peripheral glycemia.

In work reported in 1998, Shiota et al.[16] infused small amounts of fructose into the portal vein of conscious dogs during a hyperinsulemic hyperglycemic clamp. They clearly demonstrated that this resulted in increased glucose uptake and glycogen storage by the liver. In their recently reported study[4] they have extended this work to investigate the effects of intraduodenally delivered fructose and glucose. Using their well characterized chronically catheterized (catheters in an artery and the portal and hepatic veins) conscious dog model they infused glucose (44.4 μmol $\cdot$ kg^{-1} $\cdot$ minute^{-1}) either with or without fructose (2.22 μmol $\cdot$ kg^{-1} $\cdot$ minute^{-1}) into the duodenum of 42-hour starved animals for 240 minutes. Baseline fructose levels were approximately 0.01 mM in both the artery and portal vein as compared with 5.4 $\pm$ 0.2 and 5.6 $\pm$ 0.2 mM, respectively, for glucose (Table 1). Under basal conditions the liver was showing a net glucose output of 9.3 $\pm$ 0.7 to 9.9 $\pm$ 1.7 μmol $\cdot$ kg^{-1} $\cdot$ minute^{-1} in the two groups of animals. Infusion of glucose alone rapidly raised portal vein and arterial glucose to new steady-state levels of 11.5 $\pm$ 0.6 and 9.5 $\pm$ 0.5 mM, respectively, and although net hepatic glucose output had ceased by the first sampling point (15 minutes), it took an additional 15 minutes to see net glucose uptake. Infusion of fructose raised the steady-state portal and arterial fructose levels to 0.100 $\pm$ 0.014 and 0.038 $\pm$ 0.005 mM. This means that 73% of the infused fructose entered the portal vein, and 84% of that was taken up by the liver (although it should be noted that this was only 1.36 μmol $\cdot$ kg^{-1} $\cdot$ minute^{-1}, $<$5% of the total hexose uptake by the liver). However, in the presence of fructose the liver rapidly showed net

Table 1. Liver Glucose and Fructose Metabolism*

	Infusion	
	Glucose Alone	**Glucose + Fructose**
Basal glucose		
Arterial (mM)	5.4 ± 0.2	6.0 ± 0.1
Portal vein (mM)	5.6 ± 0.2	5.9 ± 0.1
Net (μmol · kg^{-1} · min^{-1})	+9.3 ± 0.7	+9.9 ± 1.7
Glucose metabolism		
Arterial (mM)†	9.5 ± 0.5	7.3 ± 0.2
Portal vein (mM)	11.5 ± 0.2	9.8 ± 0.3
Net (μmol · kg^{-1} · min^{-1})	−17.3 ± 5.6	−28.3 ± 4.6
Fructose metabolism		
Arterial (mM)	~0.010	0.038 ± 0.005
Portal vein (mM)	~0.010	0.100 ± 0.014
Net (μmol · kg^{-1} · min^{-1})	zero	−1.36
Glucose equivalents metabolized in 240 minutes‡		
Net glucose uptake	4.06	6.22
Net amino acid uptake	0.76	0.79
Net glycerol uptake	0.15	0.08
Glucose equivalents produced in 240 minutes		
Net lactate release	0.69	1.64
Glycogen synthesis	2.44	3.68
Glycogen synthesis		
Direct pathway§	1.12	2.5
Indirect pathway	1.24	1.18

* Results are means ± SEM (or just means) for nine dogs in each group and are either taken directly or calculated from the results of Shiota et al.[4]

† Arterial and portal vein concentrations and net hepatic uptake (−) or release (+) represent steady-state metabolism, except for the values for glucose in the glucose plus fructose group, which are 240-minute values.

‡ Glucose equivalents and glycogen synthesis are expressed as C6 units removed or produced per kg during the 240-minute experiment.

§ The direct pathway of glycogen synthesis means that the glucose-6-phosphate incorporated into glycogen arose from the direct phosphorylation of glucose via glucokinase, whereas the indirect pathway indicates that the glucose-6-phosphate arose through the gluconeogenic pathway from three carbon intermediates such as lactate, glycerol, and/or amino acids.

glucose uptake (even at the 15-minute sampling time) and at 30 minutes the portal vein and arterial glucose levels were 10.8 ± 0.4 and 8.8 ± 0.3 mM, considerably less than those obtained with glucose alone (12.2 ± 0.2 and 11.2 ± 0.4 mM, respectively) at this time point. Although there was no change in the portal vein–arterial glucose concentration difference or the rate of glucose absorption from the intestinal tract, the rate of hepatic glucose uptake was much higher in the presence of fructose. Sixty minutes after the beginning of the sugar infusion, net hepatic glucose uptake was 17.3 ± 5.6 and 28.3 ± 4.6 μmol · kg^{-1} · minute^{-1} in the absence and presence of fructose, respectively.

Glucose uptake by the liver after a meal is usually accompanied by some lactate release and in this experiment the presence of fructose did increase hepatic lactate production but represented only 14 to 22% of the glucose equivalents taken up by the liver (Table 1). Most of the glucose taken up was used for glycogen synthesis, with glycogen levels rising from and estimated 139 ± 6 μmol glucose equivalents per gram liver, to 249 ± 21 and 321 ± 32 μmol glucose equivalents per gram liver for glucose and glucose plus fructose, respectively. In addition, in those animals receiving only glucose, 49% of the glycogen was synthesized by the direct pathway; this increased to 68% in the presence of fructose, again indicating that glucose phosphorylation had been stimulated. The contribution (as glucose equivalents during the 240 minutes) of the indirect pathway was not changed (Table 1). Other parameters of hepatic glucose metabolism such as the uptake of glycerol and alanine were essentially unchanged by the presence of fructose. However, peripheral insulin levels were markedly lower in the animals receiving fructose. Basal insulin levels were 42 ± 6 pM and rose to 258 ± 6 pM at 30 minutes before declining to a steady state of 198 ± 66 pM in the presence of glucose alone, but 48 ± 6 pM, 126 ± 30 pM, and 96 ± 24 pM over the same time period in those animals receiving fructose. The de-

crease in insulin was presumably due to the lower glycemia, which in turn was due to the stimulation of hepatic glucose uptake.

This study leaves a few unanswered questions, such as what is the fate of the fructose and additional glucose taken up (these are only partially accounted for by glycogen deposition, Table 1). However, this study does indicate that a very small amount of fructose delivered into the duodenum can significantly increase hepatic glucose uptake and thereby moderate peripheral glucose and insulin levels, even in healthy control animals. As this review was going to press, a complementary study was published in which Wolf and colleagues demonstrated that small amounts of fructose, fed together with other carbohydrates in a meal, considerably attenuated postprandial glycemia in the Zucker fatty *fa/fa* rat.[17] In real life, fructose is likely to be ingested as part of a mixed meal (including a large load of amino acids that will also be metabolized by the liver[5]) and the rate of hepatic fructose metabolism is therefore not likely to be constant. Despite this reservation, the results of the infusion study by Shiota et al.[4] and the carbohydrate meal experiments of Wolf et al.[17] clearly support the idea that fructose could be a useful dietary component for diabetic subjects. By contrast with earlier proposals that this sugar would simply replace glucose or sucrose at levels up to 20% of calories,[1–3,17] the current work suggests that only very small amounts of fructose would be required, thereby avoiding any potential problems of excessive fructose consumption.

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Three Vitamins Are Involved in Regulation of the Circadian Rhythm

Photoreceptors in the eyes of mammals synchronize the innate circadian rhythm to the solar light-dark cycle. They differ from the photoreceptors serving vision in rods and cones of the retina and are located in the ganglion cells of the retina. They consist of melanopsin, a protein homologous to rhodopsin, with retinal as the light-sensitive prosthetic group; and cryptochromes 1 and 2, proteins combined with methenyltetrahydrofolate and flavin adenine dinucleotide, which function as blue light–sensitive photoreceptors.

Key Words: circadian rhythm, melanopsin, cryptochrome retinol

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