Intermediary metabolism of fructose\textsuperscript{1-3}

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**ABSTRACT** Most of the metabolic effects of fructose are due to its rapid utilization by the liver and it by-passing the phosphofructokinase regulatory step in glycolysis, leading to far reaching consequences to carbohydrate and lipid metabolism. These consequences include immediate hepatic increases in pyruvate and lactate production, activation of pyruvate dehydrogenase, and a shift in balance from oxidation to esterification of nonesterified fatty acids, resulting in increased secretion of very-low-density-lipoprotein (VLDL). These effects are augmented by long-term absorption of fructose, which causes enzyme adaptations that increase lipogenesis and VLDL secretion, leading to triglyceridemia, decreased glucose tolerance, and hyperuricemia. Acute loading of the liver with fructose causes sequestration of inorganic phosphate in fructose-1-phosphate and diminished ATP synthesis. Consequently, the inhibition by ATP of the enzymes of adenine nucleotide degradation is removed and uric acid formation accelerates with consequent hyperuricemia. These effects are of particular significance to potentially hypertriglyceridemic or hyperuricemic individuals. *Am J Clin Nutr* 1993;58(suppl):754S–765S.

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**Introduction**

Virtually all the unique metabolic properties of fructose are due to two primary factors: its rapid uptake by the liver, and its entry to the pathway of glycolysis or gluconeogenesis at the triose phosphate level after bypassing the phosphofructokinase regulatory step. The metabolic consequence is the provision of increased substrate in all the metabolic pathways leading from triose phosphate (Fig 1).

Current interest in fructose metabolism has arisen because of the increased use as a sweetener in the food industry in the form of high-fructose corn syrup (HFS). Also, whether based on sound science or not, fructose has been promoted as an appetite depressant, as a food for non-insulin-dependent diabetics, for parenteral feeding, and as a food supplement for endurance athletes. Representing half of the sucrose molecule, fructose has been recognized for many years as being largely responsible for the metabolic effects of high-sucrose diets. Concern has arisen because of the realization that fructose, at elevated concentrations, can promote metabolic changes that are actually or potentially deleterious, eg, hyperlipidemia, hyperuricemia, nonenzymic fructosylation of proteins, lactacidemia, and disturbances in copper metabolism. The paper concentrates on the general metabolism of fructose, particularly concerning its impact on carbohydrate, lipid and purine metabolism. Other aspects referred to are the subjects of other reviews in this volume.

Of further consideration is whether augmented insulin secretion is responsible for some of the metabolic effects of fructose intake. Compared with glucose, fructose feeding does not directly cause an increase in plasma insulin (1) whereas sucrose feeding does (2). Thus whenever intake of fructose is accompanied by glucose, the added effect of insulin secretion must be taken into account. Not only is this the case with sucrose itself but some HFSs are equivalent to hydrolyzed sucrose because they are ≈ 50% glucose and 50% fructose (3). Similar considerations apply to supplements for athletes that contain both fructose and glucose.

**General metabolism**

**Utilization of blood fructose and uptake into tissues**

The consequence of the digestion of sucrose and other fructose-containing foods such as honey, fruits, and some vegetables, is absorption and transport of fructose by the intestinal epithelium into the hepatic portal vein. Therefore, all fructose absorbed flows through the liver initially. Because of the presence of an active hepatic enzyme system for metabolizing fructose, fructose readily passes into the liver, accounting for a fractional uptake of 55% and 71%, respectively, of the fructose presented to the liver after tube feeding fed or starved rats (4). In humans it was shown that the liver metabolized at least half of the fructose injected intravenously (5). In the perfused rat liver we found a value of 40% for the fractional extraction of fructose (6). As a consequence of the high rate of extraction of fructose by the liver, correspondingly low concentrations of fructose are found in the systemic blood vessels after meals containing fructose or sucrose are consumed (4, 7, 8). Some 20% of fructose administered intravenously is taken up by the kidney (9). Thus, somewhat less than this amount would be expected to be taken up by this organ after oral feeding where the liver takes up some 50% of the initial influx. A considerably smaller fraction would be available for adipose tissue (10) and

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Hexokinase will catalyse the phosphorylation of most hexose sugars, including fructose. However, when fructose is present with glucose at physiological concentrations its phosphorylation is largely inhibited by glucose (10). Nevertheless, it is probably via this route that fructose, after uptake from the blood, enters the metabolic pathways in adipose tissue and muscle. Of far greater significance is the pathway discovered by Hers (15), which involves three particular enzymes (fructokinase, aldolase B, and triokinase), two of which are specific for fructose metabolism (Fig 1). These enzymes are present in liver and kidney and probably in the small intestine of some species—such as the golden hamster (16), guinea pig (17), and dog (18)—that are able to convert some absorbed fructose into glucose. However, this is not possible in the intestine of either humans (19) or rats (17), which do not contain glucose-6-phosphatase, the enzyme necessary for releasing glucose. Thus in these two species there is no conversion of fructose to glucose during absorption. In this respect the rat is a good model of human fructose metabolism.

Fructose is rapidly phosphorylated by ATP in the liver to form fructose 1-phosphate, catalyzed by the first enzyme of the fructose pathway—fructokinase (20). This enzyme is virtually specific for fructose because it is a ketohexokinase, and fructose is the only ketohexose of physiological significance in the diet. The high activity of fructokinase underlies the ability of the liver to extract so much of the fructose passing through it. Fructose-1-phosphate is split by liver aldolase (aldolase B) into glyceraldehyde and dihydroxyacetone phosphate, a member of the glycolysis sequence of intermediates. Aldolase B also functions in the liver in glycolysis.
Effects on glycolysis, glycogenesis, and gluconeogenesis

As a result of the loading of the initial pathways of fructose metabolism there is a tendency for intermediates of glycolysis to increase in concentration, resulting in an increased flux through the pathway, evidenced by increased lactate formation and raised blood lactate concentrations (36, 37). In the span of glycolytic reactions from glyceraldehyde-3-phosphate to pyruvate and lactate, the rate-controlling step is catalyzed by pyruvate kinase. This enzyme is normally under feed-forward control because of allosteric activation by fructose-1,6-bisphosphate. Although this metabolite may double in concentration when fructose is added to hepatocytes (31), of more significance are the large increases in fructose-1-phosphate concentrations, which extend a similar but more enhanced activation of pyruvate kinase (38), thus facilitating passage of fructose carbon to pyruvate and lactate.

Glycogen synthesis and breakdown is controlled by a complex series of reactions involving covalent modification by protein phosphorylation and dephosphorylation. Briefly, regulation centers around two rate-controlling enzymes—glycogen synthase and glycogen phosphorylase. (See ref 25 for a general review.) The active form of glycogen synthase (synthase a) is the dephosphoenzyme, whereas the inactive synthase b is phosphorylated. On the other hand active glycogen phosphorylase a is the phosphoenzyme, whereas the inactive b form is dephosphorylated. Protein kinases carry out phosphorylations and protein phosphatases carry out dephosphorylations of these enzymes. Both processes are controlled by hormonal and allosteric modifiers. Control of glycogen metabolism in liver differs in some detail from that of glycogen metabolism in muscle and has been reviewed by Hers (39).

A study of the literature reveals a disparity in results on whether fructose promotes liver glycogen deposition, with the balance of studies in vivo in the fed condition indicating that fructose is a better promoter of glycogenesis than is glucose (31). The net deposition of glycogen appears to result from both activation of glycogen synthase (40, 41) and inhibition of glycogen phosphorylase (40, 42). This appears to be brought about by several mechanisms. Phosphorylase a is inhibited by fructose-1-phosphate (42–44), which accumulates after administration of fructose. Also, glucose-6-phosphate increases in concentration and activates glycogen synthase and inhibits phosphorylase (45). We have carried out perfusions with the liver of fed rats, using whole blood containing physiological concentrations of glucose, amino acids, and free fatty acids into which was infused glucose, fructose, or both sugars at physiological rates (46). When either sugar was infused alone, there was a net output of glucose from the liver, with no change in glycogen concentration. However, when glucose and fructose were infused together, there was a marked uptake of glucose and an increase in glycogen. Fructose uptake was the same with or without a concomitant glucose infusion.
Thus, fructose on its own does not seem to be glycogenic but in the presence of extra glucose, as would be the case after a sucrose meal, fructose ‘opens the door’ for hepatic glucose uptake and synthesis of glycogen. This is achieved, presumably, by activation of glycogen synthase and inhibition of phosphorylase, but fructose itself is not able to make use of the facility because an increase in fructose-1,6-bisphosphate inhibits fructose-1,6-bisphosphatase (22). It was significant that lactate concentrations increased only in the presence of a fructose infusion, the uptake of which into the liver could account for the increased lactate production. In the presence of a simultaneous glucose infusion, there was an additional small increment in lactate production. Thus in the fed state, fructose would appear to be predominantly glycolyzed to lactate rather than converted to glycogen or glucose. An oral load of fructose or sucrose, but not glucose, also leads to increases in blood lactate concentration in humans (47, 48).

In the starved condition gluconeogenesis and glucose production from fructose is an active process, even exceeding the rates of gluconeogenesis from lactate (23, 49). Unfortunately, many studies on gluconeogenesis from fructose, particularly in vitro, have used unphysiologically high concentrations of fructose (ie ≥ 5 mmol/L) and will not be considered in the present discussion. The glycolysis and gluconeogenic pathways make use of many common intermediates and enzymes but are controlled by several nonequilibrium enzymes unique to each pathway (25). Two pivotal reactions, for which the activities are reciprocal and coordinated, dominate these pathways. These are catalyzed by phosphofructokinase in glycolysis and fructose-1,6-bisphosphatase in gluconeogenesis (Fig 1). Their activities are both induced by hormones and also by allosteric modifiers often acting on the key regulatory molecule—fructose-2,6-bisphosphate—which activates phosphofructokinase and inhibits fructose-1,6-bisphosphatase (50). The concentration of fructose-2,6-bisphosphate is elevated in liver from fed rats but falls during starvation, thus activating fructose-1,6-bisphosphatase and the gluconeogenic pathway and inhibiting phosphofructokinase and glycolysis. Under these conditions, fructose-1,6-bisphosphate does not accumulate even when the flux from fructose metabolites at the triose phosphate level increases. Therefore, there is no inhibition of gluconeogenesis by fructose. In isolated hepatocytes there is some increase in fructose-2,6-bisphosphate under physiological concentrations of fructose, which leads to increased substrate cycling at the locus of phosphofructokinase and fructose-1,6-bisphosphatase (51). However, there is no evidence that this inhibits gluconeogenesis from fructose.

Generally, it has been the case that glucose tolerance has been worse under a long-term sucrose regime compared with a starch-based diet, both in animals (53) and humans (54). The reason for the decreased glucose tolerance does not appear to be an insufficiency in insulin secretion, rather the reverse. Increased plasma insulin concentrations have been reported after sucrose feeding (55-58) and insulin sensitivity, as measured by the hypoglycemic response to administered insulin, is less (59). Thus decreased glucose tolerance is due to increased insulin resistance, brought about most likely by increased nonesterified fatty acid (NEFA) concentrations and utilization (60, 61).

The decreased utilization of glucose, as found in the whole organism when placed on a high-sucrose or -fructose diet, is also exhibited in studies of individual tissues. However, this is usually accompanied by an increased ability to metabolize fructose. Thus, in the liver there is decreased utilization and oxidation of glucose (62, 63). This is no doubt due to depressed glucokinase and increased activity of glucose-6-phosphatase in the liver after fructose-containing diets are consumed (64). As opposed to the immediate effect of fructose in facilitating conversion of glucose to glycogen in the liver (46), long-term feeding of fructose-containing diets reduces the conversion of glucose to liver glycogen (65, 66). However, conversion of fructose to liver glycogen is increased because of enzyme adaptation (64). Consumption of a sucrose diet before consumption of a sucrose load did not elicit any extra increase in blood lactate compared with a starch diet (67).

Muscle tissue also shows decreased ability to metabolize glucose and increased ability to oxidize fatty acids after animals have been fed high-fructose diets (68). Likewise, the utilization of glucose in adipose tissue is impaired with respect to its uptake, oxidation, and conversion to glycogen (62, 69).

**Effects of fructose on lipid metabolism**

**Immediate effects on the initial pathways of lipid metabolism and on lipogenesis**

Fructose has both immediate and long-term effects on lipid metabolism. Short-term or acute effects are those that occur as a result of fructose metabolism by existing enzyme capacity, whereas long-term effects result mainly from enzyme adaptation to diets containing high concentrations of sucrose or fructose. Because of the importance of the liver in fructose uptake from the blood, many of its effects on lipid metabolism are found in this organ. They involve the major pathways of fatty acid oxidation and esterification, and lipogenesis. In this respect, dangers of applying results in experimental animals to humans must be appreciated. For example, it is likely that lipogenesis is a more important pathway in rats than in humans, where lipogenesis may be confined to the liver and where even there the key enzymes of lipogenesis may be low in activity (70).

In the liver, lipid metabolism is affected by fructose at the dihydroxyacetone phosphate and pyruvate locations. Dihydroxyacetone phosphate is in equilibrium with glycerol-3-phosphate, cosubstrate for esterification of long-chain acyl-CoA in the synthesis of triglyceride and phospholipids. Triglyceride is the major precursor and determinant of very-low-density lipoproteins (VLDLs) secreted by the liver and which constitute the bulk of endogenously derived plasma triglyceride (71). Fructose also generates pyruvate, which, besides forming lactate, enters the mitochondrion to form acetyl-CoA as a result of pyruvate de-
citrate lyase. Acetyl-CoA is converted to long-chain fatty acid CoA in the cytosol by the action of the lipogenic enzyme, Acetyl-CoA is converted to long-chain fatty acid via the important cytosolic intermediate malonyl-CoA. By these pathways, fructose provides carbon atoms for both the glycerol and the acyl portions of the acylglycerol molecule.

The activity of PDH is a key factor in determining the fate of pyruvate. It is activated by a decreased ratio of [ATP] to [ADP] and by an increase in pyruvate (74); it is inhibited by increased concentrations of NEFA (74, 75). As discussed in reference 76, fructose administration, particularly at high amounts, causes depletion not only of ATP but of all adenine nucleotides; therefore, the ratio of [ATP] to [ADP] may not change appreciably. Oral administration of large amounts of fructose to humans produces hyperuricemia, which is known to follow depletion of ATP and other adenine nucleotides (77, 78). No decrease in hepatic [ATP] was found in fructose-fed rats (79). Adenine nucleotide concentrations and PDH activity did not change when fructose was added to the perfused rat liver at physiological concentrations of 1.3 mmol/L. However, at 11.0 mmol/L both ATP and total adenine nucleotides were decreased and PDH was activated (80). At a fructose concentration of 1.5 mmol/L there was a perceptible increase in PDH activity, which was not accompanied by any change in adenine nucleotide concentrations (13). Of more significance was the fact that at this physiological concentration of fructose, the inhibitory effect of increased oleate (a NEFA) on PDH activity was reversed. Generally, these results support the view that fructose causes increases in PDH activity by increasing pyruvate concentrations, rather than by decreasing the ratio of [ATP] to [ADP] (81). In summary, it would appear that some activation of PDH occurs with high physiological concentrations of fructose, especially in antagonism to the depressant effect of increased concentrations of NEFAs.

When fructose was injected intravenously into rats there was an immediate though transient increase in glycerol-3-phosphate and pyruvate concentrations (82). In contrast, after glucose administration there was no immediate increase. When fructose was administered intraperitoneally there was an increase in hepatic dihydroxyacetone phosphate, glycerol-3-phosphate, pyruvate, and lactate (83). Similar results have been obtained in the perfused liver (27, 84, 85). Acetyl-CoA concentrations also increase (72). It is also likely that malonyl-CoA concentrations rise when fructose is administered because an increase has been recorded in hepatocytes in the presence of lactate and pyruvate (86), both of which increase in the presence of fructose. Thus, fructose causes increased concentrations of its metabolites in both the glycerol-3-phosphate and glycglycotic and acetyl-CoA branches of its metabolism. Although many lipogenic intermediates increase in concentration after administration of fructose, there is little evidence that fructose on its own has an immediate effect on lipogenesis. Thus, fructose stimulated lipogenesis from acetate in chick liver slices, but not when fructose was added alone (87). Using the tritiated water technique we were unable to demonstrate any increase in lipogenesis in perfused livers from fed rats in the presence of physiological concentrations of fructose (13).

It is likely that if fructose were administered with an equal amount of glucose, there might well be increased lipogenesis, particularly if this was accompanied by a rise in insulin concentration.

Immediate effects on fatty acid oxidation and esterification and on lipoprotein formation and utilization

Although fructose does not seem to cause any marked immediate increase in lipogenesis by itself, it does have marked and immediate effects on the fate of NEFAs. These fatty acids arise as a result of lipid mobilization in adipose tissue or from hydrolysis of triglyceride-rich lipoproteins by lipoprotein lipase. We established in the perfused rat liver that NEFAs, which are taken up by the liver to the extent of 30–40% per pass, are either oxidized or esterified (88, 89). An inverse relationship was demonstrated between the quantity of fatty acids oxidized to carbon dioxide and ketone bodies on the one hand, and those esterified in liver acylglycerols and in VLDLs on the other. Regulation of this balance controlled both ketogenesis and VLDL secretion in a reciprocal manner. For a given load of fatty acids taken up by the liver, livers from fed animals oxidized less fatty acids but esterified more than did livers from starved animals, demonstrating that a mechanism exists, which is affected by the nutritional state, for regulating the partition of fatty acids between these two major pathways. We showed (89) that livers from fasted animals maintained a constant fractional rate of esterification irrespective of the NEFA load, demonstrating that glycerol-3-phosphate availability could not have been rate limiting in fatty acid esterification. It has now been firmly established that the regulatory site for controlling the balance between oxidation and esterification lies in the oxidative pathway, where long-chain acyl groups enter the mitochondrion (90). The rate-limiting step in mitochondrial oxidation of long-chain fatty acids is catalyzed by palmitoyltransferase I. Fatty acids failing to enter the mitochondria for oxidation do not accumulate but are esterified immediately. Palmitoyltransferase I is inhibited by malonyl-CoA (90), whose concentration increases in the fed condition when there is active lipogenesis. This can explain the change in balance between esterification and oxidation of fatty acids between livers of fed and starved animals.

To test whether fructose and insulin had direct and immediate effects on VLDL secretion by the liver, we perfused livers from fed rats with whole blood into which [14C]oleate NEFA was infused to maintain a constant physiological concentration (46, 91). Increased concentrations of insulin or an infusion of a physiological quantity of fructose decreased oxidation and increased esterification of fatty acids and secretion of VLDL triglyceride from the liver. When insulin was added together with fructose, the separate effects were additive with a further decrease in oxidation and enhancement of esterification and VLDL secretion. Malonyl-CoA is the product of acetyl-CoA carboxylase activity and it is known that this enzyme is activated by covalent modification by insulin and inhibited by glucagon (92). Therefore, insulin and fructose can independently increase the concentration of malonyl-CoA. Fructose may also enhance esterification by raising the concentration of glycerol-3-phosphate (93, 94) although this seems unlikely if physiological concentrations of fructose do not in fact influence the concentration of this intermediate (82). Also, we have shown that mitochondrial glycerol phosphate acyltransferase activity is enhanced by insulin (95),
and this could also account for a direct effect of insulin in promoting esterification and VLDL secretion.

These effects of fructose on NEFA metabolism are unique to this sugar. In a direct comparison with glucose, the effect of an infusion providing physiological concentrations of fructose in the perfusate of the perfused liver were compared with a comparable infusion of glucose. Only the fructose infusion boosted \[^{14}C\]NEFA esterification and \[^{14}C\]VLDL secretion (46). When similar amounts of the two sugars were then infused simultaneously to simulate sucrose digestion and absorption, there were no further increases in NEFA esterification or secretion as VLDL. Perfusions were also carried out (73) to test the effect of a fructose concentration above the physiological range (8.9 mmol/L) against a concentration within the physiological range (1.5 mmol/L). At the higher concentration, VLDL production was cut to one-third of the amount found under the physiological concentration. Because lipoprotein production is a complex energy-requiring process, the lower ATP availability associated with high-fructose concentrations (76) was probably the reason for the reduced VLDL output under these conditions.

When a 20% fructose solution was given intravenously, serum triglycerides decreased in female baboons but increased in males, whereas a comparable solution of glucose caused a decrease in both sexes (96). Although the increase in triglycerides is most likely the result of a direct effect on the liver, as described above, the fall in triglyceride concentration is probably due to a decrease in NEFA concentration, which in turn provides less NEFA substrate for the liver and therefore less VLDL production. In humans, fructose causes a decrease in NEFAs with little change in insulin concentrations (97), indicating that any reduced secretion of VLDL is due to decreased NEFA availability and any increased secretion is most likely due to the direct hepatic action of fructose. The net output of VLDL by the liver is therefore a balance between these two opposing effects of fructose.

Fructose is both antiketogenic and ketogenic depending on the circumstances. In vivo, in the starved state, a physiological intake of fructose is antiketogenic. This is nearly always due to its inhibition of NEFA mobilization from adipose tissue (97), which results in reduced uptake of the main ketogenic substrate by the liver. It appears that there is also a direct antiketogenic effect of fructose on the liver, as demonstrated in vivo in the presence of a constant plasma NEFA concentration (98). Similar experiments have been carried out in the perfused liver and isolated hepatocytes in the presence of added NEFA (72, 84, 91, 99, 100). Most of these experiments were carried out in the starved state. It is unclear whether or not malonyl-CoA inhibition of carnitine palmitoyltransferase I can explain these results. It would require the immediate activation of acetyl-CoA carboxylase, which has not been reported under these conditions. Although glycerol-3-phosphate availability does not appear to be rate-limiting on esterification of NEFAs, it is possible that boosting its concentration above the normal concentration as a result of fructose metabolism could cause a greater fractional esterification of NEFAs and less oxidation to ketone bodies (98).

That fructose can also be ketogenic was first shown in perfused livers from starved rats infused with the sugar at 20 mmol/L (72). The phenomenon was also demonstrated with livers from fed animals by using lower but still unphysiological concentrations of fructose (73, 101). To test whether this effect could be obtained with fructose within physiological concentrations we studied ketogenesis in perfused livers from fed animals and showed that ketogenesis progressively increased as the concentration of fructose was raised (13). At 1.5 mmol fructose/L, there was a significant increase compared with control perfusions in the absence of fructose. Some of these studies also used \[^{14}C\]fructose. At 20 mmol/L, all of the carbon atoms found in ketone bodies came from fructose (72) whereas at 8.9 mmol/L, half came from fructose and at the physiological concentration of 1.5 mmol fructose/L, 15% of the carbon atoms originated from fructose (73).

Radiolabeled fructose has also been used to follow the course taken by the sugar in the various metabolic pathways. Initially, there is no dilution of fructose label because there is no endogenous pool of free fructose, but as fructose is converted into intermediates common with glucose and fatty acid metabolism, the label is extensively diluted. Therefore, although it is not possible to quantitate the fate of fructose in a whole animal or tissue without knowledge of the specific radioactivity of intermediary pools, some idea may be obtained of the fate of administered fructose. Thus, when the metabolism of starved rats given either \[^{14}C\]glucose or \[^{14}C\]fructose by gastric intubation was compared, it was shown that although \approx 2.5 times as much newly synthesized triglyceride came from total body glucose as from administered fructose, twice as much of the administered fructose formed triglyceride compared with a similar quantity of administered glucose (102). These data reflect the fact that fructose is taken up predominantly by the liver whereas glucose is utilized mainly by the extrahepatic tissues. In liver slices, radioabeled fructose is converted to lactate, pyruvate, carbon dioxide, and triglyceride more rapidly than is glucose (103). Similar results have been reported for incorporation into plasma lipids in guinea pigs (104) and baboons (105). After administration of \[^{14}C\]fructose, more radioactivity is found in glyceride glycerol than in the fatty acid portions of hepatic triglyceride (102, 103), due in part to greater dilution and exchange of label in the pathway from fructose to long-chain fatty acids than in that to glycerc-3-phosphate (Fig 1). When \[^{14}C\]fructose was infused to maintain a concentration of 1.5 mmol/L in the perfusate of livers prepared from fed rats, 12% was oxidized to carbon dioxide and 2.4% was converted to ketone bodies; 4.1% was in liver lipids, 1.6% was incorporated into VLDLs, and only 0.4% was in total cholesterol (13). As the fructose load was increased, proportionally less of the labeled fructose entered lipid products except for ketone bodies, which remained the same. Thus, ketone bodies and lactate act as overflows of excess carbon as fructose saturates the metabolic pathways. Hence, ketone bodies fulfill a similar role in the liver with respect to carbohydrate as they do when fatty acids are present in excess.

**Long-term effects of a high fructose intake**

Enzyme adaptation is also responsible for many of the long-term effects of a high-fructose diet on lipid metabolism, as well as for the long-term effects on carbohydrate metabolism. In the liver, fructokinase activity increased in rats on a fructose-rich diet (106). Fructose or sucrose feeding also increased the activity of glycerol-3-phosphate dehydrogenase, which is necessary for the conversion of fructose to glycerol-3-phosphate via the glycolytic intermediate dihydroxyacetone phosphor (107, 108). After 50 d on a fructose diet, but not on a sucrose diet, pyruvate kinase in rats increased substantially (109). Similarly fructose diets increased liver PDH activity in rats (110). In the pathway of lipogenesis and triglyceride formation, ATP-citrate lyase (108, 111, 112), acetyl-CoA carboxylase (111–113), fatty acid synthase

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(111–114), and phosphatidate phosphohydrolase (115) are reported to be increased in activity in animals on fructose-containing diets. Also, the enzymes responsible for generation of reducing power for lipogenesis—glucose-6-phosphate dehydrogenase (107–109, 111, 112, 116), 6-phosphogluconate dehydrogenase (107, 108, 111, 112), and NADP malate dehydrogenase (‘malic enzyme’) (107, 111, 112, 116)—are all increased in activity. Many of these adaptive changes in liver enzyme activity are not unique for fructose because glucose feeding will also increase their activities (107), illustrating that this is a more general effect of soluble carbohydrates. Of considerable interest is that sucrose or fructose feeding reduces the activities of hexokinase, pyruvate kinase, PDH, ATP-citrate lyase, acetyl-CoA carboxylase, fatty acid synthase, glucose-6-phosphate dehydrogenase, and NADP malate dehydrogenase in adipose tissue, whereas feeding glucose or starch enhances their activities (52). It would appear from a review of these enzyme activities that lipogenesis is elevated in the livers but depressed in adipose tissue of sucrose- or fructose-fed animals. In keeping with this conclusion the concentrations of the following intermediates in the liver are elevated: pyruvate, malate, acetyl-CoA (117, 118), acetocacetyl-CoA, and long-chain acyl-CoA (117). However, glycerol-3-phosphate concentrations apparently do not increase (119).

Biosynthesis of long-chain fatty acids (lipogenesis) has been shown to increase in liver slices from rats fed either fructose (120) or sucrose (121, 122), as measured directly by using labeled acetate or tritiated water. Increased incorporation of labeled fructose has also been shown (118, 123). In the whole animal, incorporation of tritiated water into liver fatty acids was elevated in rats fed fructose compared with those fed glucose (124). Also, [14C]fructose was incorporated more rapidly into fatty acids and acylglycerol glycerol in plasma and liver in rats on a fructose diet than in rats on a glucose diet (125).

In isolated livers perfused with whole blood, we studied lipogenesis in rats that had been fed the standard laboratory diet or a sucrose-supplemented diet (13, 126). Half the perfusions were initiated with fructose to maintain a physiological perfusate concentration of 1.4 mmol/L. Incorporation of 3H2O into liver acylglycerol fatty acids increased significantly only in the group of perfusates in which fructose was infused into sucrose-fed rats. Thus, lipogenesis is increased on sucrose diets but only when fructose specifically acts as the substrate. This finding is similar to those found in liver slices from rats on a glucose diet, which enhanced selectively the conversion of [14C]glucose to fatty acids, but not [14C]fructose, and where slices from rats on a fructose diet selectively incorporated [14C]fructose into fatty acids but not [14C]glucose. They demonstrate the highly specific and selective nature of the lipogenic adaptations to different sugars in the diet, clearly based on the enzyme adaptations previously discussed.

Plasma triglycerides increase in both humans and rats when diets are enriched with carbohydrate, especially sucrose and fructose (127). This has been, and still is, of considerable interest in view of the fact that raised concentrations of plasma triglyceride (129). However, long-term fructose or sucrose feeding results in hyperinsulinemia (55–58) and increases postheparin lipolytic activity, indicating an enhancement of lipoprotein lipase activity (52). Therefore, raised plasma concentrations of triglyceride in fructose- or sucrose-fed animals, including humans, reflects an increased rate of VLDL secretion by the liver together with an increased rate of VLDL catabolism.

Fructose feeding enhances the release of NEFAs from adipose tissue (60), increasing its plasma concentration (60, 61), because of a decreased rate of re-esterification of NEFAs within adipose tissue (69). Esterification of NEFAs in adipose tissue depends on glucose utilization, which is depressed under conditions of fructose feeding. NEFAs are the major precursors of VLDL triglyceride in the liver (71). Therefore, they will augment VLDL formation in animals on high-fructose or -sucrose diets. Mindful of the fact that NEFAs are always present in plasma, we studied the effects on VLDL secretion of a physiological infusion of fructose, and of sucrose supplementation of the diet, in isolated perfused livers infused with [1-13C]oleate NEFA (126). VLDL triglyceride production increased in both the livers infused with fructose alone and in those derived from sucrose-fed rats. When livers from sucrose-fed animals were infused with fructose, the rate of secretion more than doubled. Because shifts in balance between oxidation and esterification of NEFAs in the direction of esterification have been shown to increase secretion of VLDL triglyceride (89), we also measured these indexes. Oxidation of [14C]oleate to 14CO2 was highest and esterification was lowest in control perfusions when livers from normal animals were used. Fructose infusion or sucrose feeding shifted the balance in favor of esterification and increased output of [14C]VLDL. The combination of fructose infusion plus sucrose feeding showed the lowest rate of oxidation and the highest rate of esterification and output of [14C]VLDL. The marked extra increase in VLDL output in the combined group was probably due to increased lipogenesis from fructose in this group (13, 126).

The potential adverse effects of fructose on lipid metabolism in humans has been reviewed (130, 131). It was concluded from a review of a large number of studies that normal individuals consuming diets containing average quantities of fructose have normal fasting triglyceride concentrations. However, this is only a measure of triglyceride clearance and does not give information on prandial and postprandial concentrations of triglycerides, which might be raised on these diets. The condition was exacerbated in individuals having defects in carbohydrate metabolism leading to hypertriglyceridemia, even with low intakes of fructose. Similar conclusions apply to plasma cholesterol, which may increase if higher than normal amounts of VLDLs are present. In addition, the combination of saturated fat and fructose in the diet would appear to favor elevated cholesterol concentrations.

**Effects of fructose on purine metabolism**

**The hyperuricemic effect of fructose**

It was first reported (132) that when fructose was administered intravenously to both normal children and those with hereditary fructose intolerance, there was an increase in serum and urinary uric acid. The hyperuricemic effect appears to be dose dependent and the fructose infusion needs to be > 0.5 g kg body wt−1 h−1 to cause detectible hyperuricemia. Also, the effect is fructose specific because comparable infusions of either glucose or galac-
Mechanism underlying the hyperuricemic action of fructose

To understand the mechanism of the hyperuricemic effect of fructose a knowledge of purine nucleotide metabolism and its regulation is necessary. Adenylate kinase maintains equilibrium between the adenine nucleotides ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP) (Fig 2). Thus, all three adenine nucleotides are able to be degraded and formed via AMP. However, as with many other common metabolites, the pathway of degradation of adenine nucleotides is not the simple reverse of the pathway of biosynthesis. In the degradative pathway, inosine monophosphate (IMP) is formed from AMP by the action of AMP deaminase. Phosphate is then removed by 5'-nucleotidase to form inosine. Alternately, inosine may be formed from AMP via adenosine but experiments in isolated hepatocytes (31) have indicated that this is not as important a route for AMP degradation as that via IMP. In addition, the reaction catalyzed by AMP deaminase appears to be the rate-limiting step in the breakdown of hepatic adenine nucleotides (134-136), and Pi at normal intracellular concentrations is an important inhibitor of this enzyme. Inosine is broken down to uric acid via hypoxanthine and xanthine. In humans and other primates this is the end product of purine metabolism and is excreted as such, but other mammals possess the enzyme uricase, which allows further oxidation to the much more soluble allantoin. A consequence of the absence of uricase in humans is the occurrence of gout, which is associated with hyperuricemia.

IMP is also the first formed purine nucleotide in the pathway of de novo synthesis from ribose 5-phosphate. This pathway involves no less than 11 separate steps (137). Of these, only the first two need to be mentioned because they catalyze the rate-controlling reactions, both of which are held in check by a feedback regulation because of high concentrations of purine nucleotides. In addition, 5-phosphoribosyl-1-pyrophosphate (PRPP), the product of the first reaction in the pathway is an allosteric activator of the second reaction in the pathway catalyzed by PRPP glutamyl amidotransferase (Fig 2).

Mechanism underlying the hyperuricemic action of fructose

The essential finding that led to the explanation as to why fructose administration caused increased uric acid formation was the observation that it was accompanied by a sharp fall not only in hepatic ATP concentration but also in total adenine nucleotides (76). The reason for this became apparent when it was shown that fructose-1-phosphate accumulated and Pi concentrations fell (27). Thus, the sequence of events after fructose administration involves rapid phosphorylation of the sugar to fructose-1-phosphate because of the high activity of fructokinase. This leads to depletion of ATP due to inhibition of oxidative phosphorylation of ADP because of a shortage of Pi sequestered in fructose-1-phosphate. The lowering of ATP concentration is also assisted by the activity of triokinase in utilizing ATP in the phosphorylation of glyceraldehyde to glyceraldehyde-3-phosphate. The depletion of Pi and ATP leads to the removal of the allosteric inhibition on the enzymes that degrade AMP, respectively, AMP deaminase and 5'-nucleotidase. There is a rise in inosine concentration that leads to increased formation of uric acid with depletion of the total adenine nucleotide pool.

The depletion of adenine nucleotides also leads to stimulation of the pathway of purine nucleotide synthesis as a feedback reaction (Fig 2). The lowering of the concentration of adenine nucleotides removes the allosteric inhibition of the first two steps in the pathway catalyzed by PRPP synthetase and PRPP glutamyl amidotransferase. The production of PRPP acts as a further activator of PRPP glutamyl amidotransferase, leading to IMP synthesis. If 5'-nucleotidase is still active, IMP will be degraded to uric acid rather than converted to AMP. In this way the initial production of uric acid from the adenine nucleotide pool is augmented by de novo synthesis.
The reduction in concentration of ATP and other high energy phosphates has other profound effects on metabolism and other functions dependent on a continual supply of these vital sources of free energy, eg, inhibition of protein (76) and nucleic acid synthesis (138).

Summary and conclusions

Many investigations both in vivo and in vitro have been carried out by using unphysiological concentrations of fructose. Deductions made from such observations can be misleading, particularly when applied to humans consuming normal quantities of sucrose and fructose. This aspect has been taken into account in compiling this review.

Specific enzymes in the liver—fructokinase, aldolase B, and triokinase—allow fructose ready access to the triose phosphate pool and all pathways leading from it, after bypassing the phosphofructokinase regulatory step in glycolysis. In the fed state, this allows a greater saturation of the glycolysis pathway with consequent lactate production, activation of PDH, and dominance of the oxidative pathways leading to carbon dioxide formation and ketone body production when under fructose load. Glucose production, glycogenesis, and lipogenesis are not enhanced but there is an immediate shift in the balance between oxidation and esterification of NEFAs in favor of esterification. This leads to augmented output of VLDLs from the liver. In the liver of starved animals, the enzymes of gluconeogenesis are active and fructose will form much more glucose under these conditions.

As a result of enzyme adaptation to the long-term feeding of fructose or sucrose diets, the pattern of fructose metabolism is changed. Enhanced activity of fructose-1,6-bisphosphatase, glycogen synthase, and glucose-6-phosphatase allow more fructose to form glycogen and glucose. Enhanced activity of lipogenic enzymes in the liver, but not in adipose tissue, stimulates long-chain fatty acid synthesis, which augments the immediate hepatic actions of fructose in promoting VLDL triglyceride output. This leads to hypertriglyceridemia. Fructose metabolism in adipose tissue also causes impaired glucose utilization and impaired esterification of fatty acids, which promotes release of NEFAs with consequent raised plasma NEFA concentrations and increased VLDL production. The increased triglyceride and NEFA concentrations impair utilization of glucose in muscle, decreasing glucose tolerance and increasing insulin resistance with consequent hyperinsulinemia. This, in turn, will stimulate the already increased VLDL production by the liver. Because VLDLs contain ≈20% cholesterol, there is a corresponding rise in plasma cholesterol, with little change in LDL cholesterol.

Acute loading of the liver with fructose is also a cause of hyperuricemia. This is due to utilization of ATP in the phosphorylation of fructose and sequestration of Pi in fructose-1-phosphate, preventing oxidative regeneration of ATP from ADP. Because the enzymes of adenine nucleotide degradation are inhibited by ATP and Pi, removal of the inhibition leads to destruction of the total adenine nucleotide pool and generation of uric acid.

Both the hyperlipidemic and hyperuricemic effects of fructose can be demonstrated in humans. Although these effects seem minimal in healthy individuals on normal diets, individuals on very-high-fructose diets and individuals who are actually or potentially hyperlipidemic or hyperuricemic who take in average quantities of fructose may be at increased risk.

It will be apparent that although there have been noteworthy contributions on fructose metabolism in humans, most investigations have been carried out in laboratory animals, particularly rats. This is inevitable when precise information about intracellular processes is required, involving invasive and critical procedures. It is clear that systematic investigations in humans are needed to ascertain the precise amounts, both of fructose consumption and of its concentration in the blood, at which deleterious effects such as hyperlipidemia and hyperuricemia occur. Nuclear magnetic resonance spectroscopy is clearly of value as a noninvasive technique in monitoring intracellular changes in the concentration of key metabolites.

References

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