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The Conversion of Glucose and Fructose to Fatty Acids in the Human Liver¹

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Alterations of the type and amount of the dietary carbohydrate source leads to changes in the plasma triglyceride concentration in the rat, in normal man, and in patients with endogenous, so-called carbohydrate-induced, hypertriglyceridemia (1-14). Of special interest has been the fact that isocaloric amounts of fructose (or the fructose containing disaccharide sucrose) may increase the plasma triglyceride concentration in comparison to non-fructose containing sugars (9-12, 14).

Measurements of the concentrations of glycolytic and tricarboxylic acid cycle intermediates, the incorporation of [¹⁴C₆] glucose and [¹⁴C₆] fructose into fatty acids, and the activity of glycolytic and lipogenic enzymes in rat liver indicated that one possible reason for the difference between the effect of glucose and fructose on plasma triglyceride concentration was that fructose was a better precursor of hepatic fatty acids (15). This difference in the conversion of fructose and glucose to fatty acids was related to the fact that the rate of hepatic glycolysis of fructose was greater than that for glucose. Evidence was also obtained that the conversion of glucose to fatty acids could be increased by increasing the rate at which glucose was metabolized in the Embden-Meyerhof pathways (15). These conclusions are consistent with the isotope data of Cahill *et al.* (16) and Ballard and Hanson (17) which also indicate that the rate of lipogenesis in the rat liver is related directly to the rate of glycolysis.

In the perfused rat liver there is a direct relation between the rate of hepatic fatty acid synthesis and the amount of triglyceride (as lipo-

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protein) added to the perfusate (18). In the absence of dietary fat the liver is the primary organ for the synthesis of plasma lipoproteins (19-21). Thus, the rate of fatty acid synthesis in the human liver could be an important determinant of the plasma triglyceride concentration. A differential conversion of sugars to hepatic fatty acids could be one of the mechanisms for the observed differential effects of different sugars on the plasma triglyceride concentration in man. In order to examine this possibility in the human liver we have measured the rates of incorporation of [$^{14}\text{C}_6$] fructose and [$^{14}\text{C}_6$] glucose into fatty acids, glyceride-glycerol and CO_2 and the activities of hepatic enzymes which could be limiting for the glycolysis of glucose and fructose.

METHODS

The enzymes and cofactors were purchased from Sigma Chemical Company or Calbiochem. [$^{14}\text{C}_6$] glucose and [$^{14}\text{C}_6$] fructose were purchased from New England Nuclear Corporation.

Liver biopsy specimens were obtained from patients who were undergoing elective cholecystectomy. None of these patients was jaundiced, febrile, or poorly nourished at the time of surgery. All patients had been eating an *ad libitum* diet until the time of surgery. Some of the patients whose tissue was used for enzyme assays were given 1 liter of 5% glucose in water in the 8 hours preceding surgery. There were no differences in the enzyme activities between patients who were given intravenous glucose and those who were not. Because of this, the enzyme data for all patients has been combined. The biopsies which were used for the liver slice experiments were taken from patients who were given the glucose infusion. The gross appearance of the liver was normal at the time of surgery for all liver specimens for which data is reported in this paper.

The liver biopsies were placed in the appropriate ice-cold buffer immediately after they were obtained. Portions of tissue were frozen and stored at -85° . The assays of glucokinase (ATP:D-glucose 6-phosphotransferase EC 2.7.1.2), hexokinase (ATP:D-hexose 6-phosphotransferase EC 2.7.1.1), aldolases (ketose-1-phosphate aldehyde-lyase EC 4.1.2.7), and (fructose-1,6-diphosphate D-glyceraldehyde-3-phosphate lyase EC 4.1.2.13), and pyruvate kinase (ATP:pyruvate phosphotransferase EC 2.7.1.40) were made on fresh tissue which had been homogenized in a Potter-Elvehjem tube in 20 volumes of 0.1 M Tris, 5 mM EDTA, 5 mM MgCl_2 , pH 7.50. Phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase EC 2.7.1.11) activity was assayed in fresh tissue homogenized in 20 volumes of 20 mM Tris, 1 mM EDTA, 5 mM MgSO_4 , 120 mM KCl, pH 7.40. There were no differences in

fructokinase (ATP:D-fructose-1-phosphotransferase EC 2.7.1.3) activities when the assay was done on fresh or stored tissue. All other enzymes were assayed on the samples of frozen tissue. These activities and that for fructokinase were measured after homogenization in 20 mM Tris, 1 mM EDTA, 5 mM MgSO₄, 120 mM KCl, pH 7.4. All assays were made on the 105,000g cell free supernatant portion.

The following enzyme assay methods were used: Aldolases (22), fructokinase (23), phosphofructokinase (24), and pyruvate kinase (25). Glucokinase and hexokinase were assayed by the method of Vinuela, Salas, and Sols (26), except that the amount of NADPH formed was divided by 2 to obtain the value for glucose phosphorylated. The NADH (Alcohol:NAD oxidoreductase EC 1.1.1.1) and NADPH (Alcohol:NADP oxidoreductase EC 1.1.1.2) alcohol dehydrogenases (D-glyceraldehyde as substrate) were assayed by the method described by Heinz and Lamprecht (27).

Triokinase (ATP:D-glyceraldehyde 3-phosphotransferase EC 2.7.1.28) was assayed by the method of Hers (28). The amount of fructose-1-P formed was taken as the measure of D-glyceraldehyde phosphorylated to glyceraldehyde-3-P. This probably underestimates the true triokinase activity whereas the measurement of the disappearance of D-glyceraldehyde tends to overestimate this activity. The important point is that the fructose-1-P formed by D-glyceraldehyde is an index of the lower limit of triokinase activity. Protein was determined by the method of Lowry *et al.* (29).

Portions of liver which were used for liver slice incubations were kept in ice-cold, Krebs-Henseleit-HCO₃ buffer (30). The methods for preparation of slices, incubation media, incubation procedures, extraction of the tissue, and the counting of radioactive metabolites used in this laboratory have been described previously (31, 32) except that ¹⁴CO₂ was trapped in hyamine hydroxide. The duration of incubations and the medium substrate concentrations are noted in the tables and in the text.

RESULTS

Fructose and Glucose Glycolytic Enzymes in Human Liver. The data in Table 1 compare the activities of several enzymes for the metabolism of fructose and glucose. The activity of fructokinase is more than 5-fold greater than the total activity for glucose phosphorylation. Although the activities for glucokinase and hexokinase in Table 1 are V_{max} activities, that for fructokinase is approximately $0.7 V_{max}$ because of the ATP concentration used in the assay (23). As a result, the actual ratio of V_{max} activities of fructokinase to glucose phosphorylating activity would be nearly 50% greater than those apparent from the table. Of the total

TABLE 1
COMPARISON OF ENZYME ACTIVITIES FOR THE GLYCOLYSIS OF FRUCTOSE AND
GLUCOSE IN HUMAN LIVER

Fructose enzymes	Activity ^a	Glucose enzymes	Activity
Fructokinase	27.4 ± 3.6 (6) ^b	Glucokinase	3.13 ± 0.31 (6)
		Hexokinase	1.76 ± 0.35 (6)
		Phosphofructokinase	30.5 ± 5.5 (6)
F-1-P Aldolase	50.4 ± 8.8 (4)	F-1, 6-P Aldolase	54.7 ± 7.0 (4)
Triokinase	34.6 (2)	Pyruvate Kinase	221 ± 39.4 (3)
Alcohol dehydrogenase			
NADH	65.3 ± 5.7 (6)		
NADPH	58.2 ± 6.7 (6)		

^a Units of activity equal m μ moles substrate metabolized/min/mg protein. Mean ± SE.

^b Figure in brackets is number of livers measured.

glucose phosphorylating activity 64% was associated with the high K_m glucokinase.

The activity of phosphofructokinase was 30.5 m μ moles/minute/mg protein, greater than glucokinase and hexokinase activities, and essentially equal to the fructokinase activity.

The data in Table 1 indicate that the activities of human hepatic aldolases for fructose-1-P and fructose-1, 6-P are high in comparison to that for the phosphorylation reactions.

The cleavage of 1 mole of fructose-1-P by aldolase yields 1 mole of dihydroxyacetone-P and 1 mole of D-glyceraldehyde (33-35). The data in Table 1 indicate that the activity of triokinase with D-glyceraldehyde as substrate was 34.6 m μ moles/minute/mg protein, which is high in comparison to glucose phosphorylating activity. The activities of the two enzymes which can reduce D-glyceraldehyde to glycerol thereby allowing D-glyceraldehyde to re-enter the glycolytic pathway as glycerol-1-P were also high. The activity of pyruvate kinase, common to the metabolic pathways of fructose and glucose, was considerably greater than the activity for the other glycolytic enzymes assayed (Table 1).

Comparison of the Conversion of [¹⁴C₆] Fructose and [¹⁴C₆] Glucose to Fatty Acids, CO₂, and Glyceride-Glycerol in Human Liver Slices. If lipogenesis is related to the glycolytic rate, the data in Table 1 suggest that the conversion of fructose to fatty acids should be greater than that for glucose. This was tested by incubating liver slices with [¹⁴C₆] fructose or [¹⁴C₆] glucose. Comparison of the data in Tables 1 and 2 indicate that the conversion of labeled fructose and glucose to fatty acids correlates directly with the hepatic enzyme activity for the metabolism of fructose and glucose to pyruvate. In four separate liver specimens, the

TABLE 2
COMPARISON OF THE CONVERSION OF 5 mM [$^{14}\text{C}_6$] FRUCTOSE AND 5 mM [$^{14}\text{C}_6$] GLUCOSE TO FATTY ACIDS, CO_2 , AND GLYCERIDE-GLYCEROL IN HUMAN LIVER SLICES

Metabolite:	Fatty acids		CO_2		Glyceride-glycerol	
	Fructose	Glucose	Fructose	Glucose	Fructose	Glucose
Experiment ^a						
1	71.7	2.72	1620	559	600	119
2	127	17.0	3455	505	559	99.4
3	430	55.0	3368	704	563	158
4	197	20.7	6264	1021	—	—

^a Each experiment represents duplicate incubations from one liver. Results are given as μmoles of ^{14}C -substrate recovered as metabolite/gm liver/90 minutes of incubation.

recovery of [$^{14}\text{C}_6$] fructose as fatty acids was greater in comparison to [$^{14}\text{C}_6$] glucose (Table 2). Although there was a wide variation in recovery of the labeled sugars as fatty acids, there was no overlapping of results from fructose and glucose incubations. More [$^{14}\text{C}_6$] fructose than [$^{14}\text{C}_6$] glucose was recovered as CO_2 and glyceride-glycerol. The difference between [$^{14}\text{C}_6$] fructose and [$^{14}\text{C}_6$] glucose recovery was greater for the fatty acids than either CO_2 or the glyceride-glycerol.

The data in Tables 1 and 2 suggest that the pathway from fructose to fatty acids bypasses the glucose-6-P, fructose-6-P, and fructose-1,6-P portion of the glycolytic pathway. Thus a rapid breakdown of glycogen to glucose-6-P could lead to an unequal dilution of the conversion of ^{14}C -glucose and ^{14}C -fructose to fatty acids. In order to investigate this possibility we measured the difference between [$^{14}\text{C}_6$] fructose and [$^{14}\text{C}_6$] glucose recovered in glycogen since fructose and glucose pass through a common glucose-6-P port in the pathway to glycogen. The data in Table 3 indicate that [$^{14}\text{C}_6$] fructose was converted to glycogen to a greater extent than [$^{14}\text{C}_6$] glucose. Thus, it is unlikely that the data in Table 2 can be explained by postulating a differential dilution of [$^{14}\text{C}_6$] fructose as compared to [$^{14}\text{C}_6$] glucose.

The Effect of Medium Glucose Concentration on the Recovery of [$^{14}\text{C}_6$] Glucose as Fatty Acids, CO_2 , and Glyceride-Glycerol. The relationship between the hepatic glycolytic rate and lipogenesis was also investigated by examining the effect of the medium glucose concentration on the recovery of [$^{14}\text{C}_6$] glucose as fatty acids, CO_2 , and glyceride-glycerol. This was done because the data in Table 1 indicated that the major portion of glucose phosphorylating activity is associated with a

TABLE 3
COMPARISON OF THE CONVERSION OF 5 mM [$^{14}\text{C}_6$] FRUCTOSE AND 5 mM [$^{14}\text{C}_6$]
GLUCOSE TO GLYCOGEN IN HUMAN LIVER SLICES

Experiment ^a	Substrate	
	[$^{14}\text{C}_6$] Fructose	[$^{14}\text{C}_6$] Glucose
1	772	73.8
2	1691	109.0
3	653	51.2

^a Each experiment represents the mean of triplicate incubations from one liver. Results are given as μmoles substrate recovered as glycogen/gm liver/90 minutes of incubation.

high K_m glucokinase. With increasing medium glucose concentration there was increased recovery of [$^{14}\text{C}_6$] glucose as fatty acids, CO_2 , and glyceride-glycerol (Table 4). In Exp. 3 there was no difference in [$^{14}\text{C}_6$] glucose recovered as fatty acids at 5 and 10 mM glucose, but the recovery at 30 mM glucose was approximately 3-fold greater. Experiments 2, 3, and 4 in Tables 2 and 4 are taken from the same liver specimens. Comparison of the data for the incorporation into fatty acids of 5 mM [$^{14}\text{C}_6$] fructose with that for 30 mM [$^{14}\text{C}_6$] glucose shows that at a concentration of 30 mM, [$^{14}\text{C}_6$] glucose conversion to fatty acids is beginning to approach that for 5 mM [$^{14}\text{C}_6$] fructose.

TABLE 4
EFFECT OF MEDIUM GLUCOSE CONCENTRATION ON THE CONVERSION OF [$^{14}\text{C}_6$]
GLUCOSE TO FATTY ACIDS, CO_2 AND GLYCERIDE-GLYCEROL IN HUMAN LIVER SLICES

Experiment ^a	Glucose concentration (mM)	Fatty acids	CO_2	Glyceride-Glycerol
1	5	3.04	495	66.6
	30	21.4	3240	328
2	5	17.0	505	99.4
	10	30.4	906	168
	30	57.6	1881	354
3	5	55.0	704	158
	10	47.4	1106	240
	30	165.0	2446	583
4	5	20.7	1021	—
	30	151.0	3593	—

^a Each experiment represents duplicate or triplicate incubations from one liver. Results are given as μmoles [$^{14}\text{C}_6$] glucose recovered as metabolite/gm liver/90 minute incubation.

DISCUSSION

It is difficult to obtain human liver tissue so that the dietary and environmental exposure of each patient is constant. Despite this variation of exposure of liver tissue, the relation between fructose and glucose glycolytic enzyme activities, the difference between [$^{14}\text{C}_6$] fructose and [$^{14}\text{C}_6$] glucose conversion to fatty acids and the effect of glucose concentration on [$^{14}\text{C}_6$] glucose recovery as fatty acid was qualitatively the same in all specimens. We cannot exclude the possibility that the patients' disease and the operative intervention did not alter the activity of adaptive enzymes. However, investigation of the activity of jejunal glycolytic enzyme activities in normal, fed males has yielded data identical to that for liver in the present paper (36). Our studies in the human and rat indicate that the glycolytic enzyme activity of jejunal mucosa is an accurate index of activity in the liver (37). Thus, it is unlikely that the differences between fructose and glucose metabolism are due to the pre-operative and operative environment of the liver tissue.

On the basis of the data in the rat we concluded that fatty acid synthesis could be limited by the availability of substrate rather than by control of the activity of fatty acid synthesizing enzymes (15). A similar relationship between fatty acid synthesis and substrate availability (glycolytic rate) seems to be present in the human liver. Although an increased concentration of glycerol-1-P (see Tables 2 and 4) could enhance fatty acid synthesis by diminishing end-product inhibition of acetyl-CoA carboxylase (EC 6.4.1.2) (38), the increased recovery of [^{14}C] as glyceride-glycerol from [$^{14}\text{C}_6$] fructose as compared to [$^{14}\text{C}_6$] glucose or that seen with increasing [$^{14}\text{C}_6$] glucose concentrations could result from a primary increase in fatty acid synthesis. Further, fructose administration does not produce a sustained, large increase in the glycerol-1-P concentration in the rat liver (15, 39-42). It is also difficult to explain the regulation of fatty acid synthesis in terms of the long chain acyl-CoA inhibition of acetyl-CoA carboxylase because: (a) This effect is poorly reversible (43); (b) acyl-CoA non-specifically inhibits a number of enzymes (44-49); (c) the cell protein content is high enough to bind acyl-CoA and thereby block inhibition of acetyl CoA carboxylase (50, 51); (d) there is a poor correlation between the *in vitro* glycerol-1-P stimulation of fatty acid synthesis and changes in the medium acyl-CoA concentration (52); and (e) palmityl-CoA inhibition of the fatty acid synthetase enzyme complex is not site-specific but is due to the detergent properties of acyl-CoA compounds (53). It is therefore unlikely that the differential conversion of fructose and glucose to fatty acids is related

to different effects on the hepatic glycerol-1-P and long chain acyl-CoA concentrations.

Di- and tricarboxylic acids activate acetyl CoA carboxylase (54-56). We cannot exclude that fructose and glucose have differential effects on fatty acid synthesis due to an effect on the concentration of these intermediates. On the other hand, a consideration of the kinetic parameters of some lipogenic enzymes suggests that the relationship between fatty acid synthesis and glycolysis depends on the fact that *in vivo* lipogenesis is limited by the availability of substrate. Comparison of the K_m values for the substrates for citrate cleavage enzyme (EC 4.1.3.6) (57) and acetyl-CoA carboxylase (38) with the tissue concentration of these intermediates (15) (Table 5) shows that the substrate concentrations are

TABLE 5
COMPARISON OF TISSUE SUBSTRATE CONCENTRATIONS TO K_m VALUES FOR THE
CONVERSION OF CITRATE TO MALONYL-CoA

	Reaction	
	Citrate Acetyl-CoA	Acetyl-CoA Malonyl-CoA
K_m for substrate	5.8×10^{-4} M (57) ^a	1.9×10^{-5} M (38)
Tissue substrate concentration	4×10^{-4} M (15)	4.3×10^{-5} M (15)

^a Figure in parenthesis is literature reference to the data.

close to the K_m values for the respective enzymes. Since most of the cell citrate (58) and acetyl-CoA are mitochondrial (59), the supernatant concentrations of citrate and acetyl-CoA are probably less than the K_m values of citrate cleavage enzyme and acetyl-CoA carboxylase for these metabolites. The flux of substrate through these two reactions will therefore be dependent on substrate concentration. An increased rate of pyruvate production, could elevate the hepatic cell supernatant concentration of acetyl-CoA and citrate, thereby causing an increased flux of substrate from acetyl-CoA in the mitochondria to malonyl CoA in the cytoplasm. Although K_m values measured *in vitro* with purified enzymes may have little *in vivo* physiologic meaning, the relationship of the K_m values to the substrate concentration in the early parts of the lipogenic pathway is consistent with the hypothesis that the *in vivo* rate of hepatic lipogenesis is substrate limited.

The data in Table 1 indicate why fructose is metabolized by the human liver at a greater rate than glucose. Although the activity of phosphofructokinase is several-fold greater than that for glucose phosphorylation, the actual *in vivo* activity of phosphofructokinase could

limit the glycolysis of glucose in liver because of the allosteric properties of this enzyme. The fact that the glycolytic rate increased with increasing rates of glucose phosphorylation does not help to decide whether glucose phosphorylation or fructose-6-P phosphorylation is rate limiting for the conversion of glucose to pyruvate in the liver (43). The differential rates of fructose and glucose glycolysis in human liver may not be related solely to the greater activity of fructokinase in comparison to glucokinase and hexokinase (15). Qualitatively similar relationships between enzymes for the glycolysis of fructose and glucose have been reported by Heinz, Lamprecht, and Kirsch (60).

It may be inferred that because of their effects on hepatic fatty acid synthesis, diets containing fructose (or sucrose) or large amounts of glucose would tend to increase the plasma triglyceride concentration in man. Although the differential conversion of fructose and glucose to fatty acids and the effect of glucose concentration on hepatic fatty acid synthesis are qualitatively not related to adaptive changes in hepatic enzyme activity, glucokinase is an adaptive enzyme in human liver (61); and the hepatic activity of lipogenic enzymes can be altered by dietary and hormonal manipulations in the rat (62-64). Thus, the long term response of hepatic lipogenesis to carbohydrate ingestion will depend in part on adaptive changes in the activity of glycolytic and lipogenic enzymes.

The observed effects of different carbohydrate diets on the plasma lipid concentrations in man and animals is consistent with the predictions derived from a consideration of the differential conversion of fructose and glucose to fatty acids and of the effect of glucose concentration on hepatic fatty acid synthesis. However, on the basis of present knowledge these data must be interpreted with caution. There is no direct measure of the absolute rate of hepatic fatty acid synthesis in the human or any large mammal. In order for the differential conversion of different types of dietary carbohydrate to fatty acids in the liver to be of physiologic importance, it would have to be shown that human liver can make fatty acids at a rate consistent with the magnitude of the dietary-induced changes in the plasma triglyceride fatty acid content. An effect on hepatic fatty acid synthesis is only one of several possible mechanisms by which different sugars may have differential effects on the plasma triglyceride metabolism.

SUMMARY

The capacity of the human liver for the glycolysis of fructose is greater than that for glucose as indicated by the greater activity of fructose glycolytic enzymes. In human liver slices [$^{14}\text{C}_6$] fructose was metabolized

to fatty acids, CO₂, and glyceride-glycerol at a greater rate than [¹⁴C₆] glucose. The conversion of [¹⁴C₆] glucose to fatty acids was dependent on the medium glucose concentration. These results, in the human liver, are similar to those in the rat.

The rate of fatty acid synthesis in human liver is related directly to the rate of glycolysis. This dependence of fatty acid synthesis on the glycolytic rate seems to result from the fact that hepatic fatty acid synthesis is limited by the availability of substrate.

The increased lipogenic potential associated with fructose as compared to glucose or with very high glucose concentrations could explain, in part, the differential effects of different carbohydrates on the plasma triglyceride concentration in man.

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