Glucose Production from Fructose

Marshall E. Atwell, M.D., and Christine Waterhouse, M.D., Rochester, New York

SUMMARY

Five adult subjects, three male and two female, were given fructose-U-C-14 by single injection under the condition of fructose loading. The radioactivity in glucose was determined in serial arterial samples. On a separate day, glucose-6-C-14 was given to these same subjects under identical conditions. Using these two curves, the fructose converted to glucose was determined. In these subjects an average of 30.94 per cent of the infused fructose appeared as blood glucose.

Hepatic vein catheterization showed a large positive arteriovenous difference for fructose (50 to 80 per cent of infused fructose) and negative arteriovenous differences for glucose and lactate. There was a discrepancy between the calculated fructose conversion to glucose (30 per cent) from the tracer studies and the glucose production estimated from the hepatic arteriovenous differences of glucose (10 per cent). Supporting data for the validity of the former figure are given.

The simultaneous hepatic production of glucose and lactate by the liver is most likely due to the large substrate load of fructose entering the glycolytic scheme at a point which bypasses the rate limiting enzymes for both glycolysis and gluconeogenesis. DIABETES *20*:193-99, April, 1971.

The value of infusion of fructose as a therapeutic measure either in normal people or in diabetic subjects is still controversial, since the quantitative aspects of fructose metabolism remain in doubt. Many studies have been done determining glucose and fructose blood levels at various rates of fructose administration,[1-10] as well as hepatic arteriovenous (AV) differences[4,7,10] of these two substances. From such data as these, several observations seem incontrovertible. Dispersion of large amounts of infused fructose from the vascular system is more rapid than that of glucose.[9] Insulin appears to have little effect on disposal mechanisms. Lactate and uric acid accumulation are ordinarily seen and hepatic protein synthesis may be inhibited.[3,11,12] Quantitation of fructose uptake by the liver as well as glucose production during the administration of fructose, all calculated from splanchnic arteriovenous differences and blood flow,[4,7] have been highly variable, the uptake ranging from 30 to 50 per cent of the fructose infused and the glucose production roughly 10 per cent.

By means of a different technic using tracer studies, we have obtained quantitative data on one aspect of metabolism, namely that of glucose production from fructose. This technic does not require the estimation of hepatic blood flow nor even hepatic arteriovenous differences, a distinct advantage when contemplating the problems and vagaries of hepatic venous sampling.[13] The technic consists of administering labeled fructose to an individual during the process of intravenous fructose administration and measuring the radioactivity which appears in blood glucose. The blood glucose radioactivity curve is then corrected for the rate of disappearance of glucose from the vascular system and the fraction of the fructose which is converted to glucose may then be calculated.

METHODS AND MATERIALS

Five adult subjects were chosen for this study, three of whom were metabolically normal and two of whom had maturity-onset type diabetes controlled by diet alone (table 1). All subjects were admitted to the Clinical Research Center three to five days prior to the study and each was given a diet containing at least 300 gm. carbohydrate daily.

Each study was begun at 8 a.m. after a 12 to 14-

TABLE 1
Patient data

	Age	Sex	Weight (kg.)	Glucose tolerance
F.S.	27	M	50	Normal
F.H.	70	F	65	Abnormal
J.F.	21	M	58	Normal
P.R.	49	M	68	Abnormal
M.O.	46	F	75	Normal

From the Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York 14620.

Dr. Atwell is a former Associate Resident in Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York 14620.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/20/4/193/346141/20-4-193.pdf by guest on 31 March 2023

hour overnight fast and subjects were kept in bed for the duration of the study period which ranged from 2½ to 3 hours. Indwelling arterial needles were placed for the purpose of blood sampling and hepatic venous catheters were localized under fluoroscopic control via a femoral percutaneous approach in three of the five patients (four studies). D-fructose in 20 per cent solution was infused intravenously at the rate of 1 gm./kg./hr. After 30 to 60 minutes, when a steady state was assumed, D-fructose-U-C-14 10 μc. was given by single intravenous injection. The radioactivity in blood glucose was isolated as potassium gluconate.[14] Arterial blood was also collected for the determination of glucose, fructose, lactate and pyruvate. Total reducing substances were determined by AutoAnalyzer, glucose by the glucose oxidase method and the difference was considered to be fructose, even though other reducing substances may have contributed up to 7 per cent of the value.[15] Blood lactate and pyruvate were determined by the enzymatic method described by Rosenberg and Rush.[16] Hepatic venous samples were handled identically. The disappearance of radioactive D-glucose-6-C-14 from the blood after a single injection of 10 μc. was determined on a separate day, under identical conditions. An assumption of identical glucose disappearance on both days that tracers were given is made.

Expired air was collected in Douglas bags at intervals throughout each tracer study. Three-minute collection periods were done and the CO_2 was extracted in IN NaOH through sintered glass filters. Barium carbonate was precipitated from each sample and weighed. The amount of CO_2 produced per unit time was calculated from this and the radioactivity was determined on weighed aliquots.[17] Fructose loss in the urine was not determined; however, other studies using comparable rates of infusion have found this to be 5 to 10 per cent.[1,4,5,7]

Integration of curves relating the concentration of radioactivity to time was done either by using Simpson's rule for the computer or by planimetry.

RESULTS

Fructose-U-C-14 incorporation into glucose

Figure 1 depicts the appearance and disappearance of labeled glucose in the blood during fructose infusion and after fructose-U-C-14 was given by single injection. Rapid conversion of fructose to glucose is evident by peak counts in glucose in 15 minutes. In all instances the final exponential decay is manifest at 60 to 90 minutes and the integrals under these radioactivity curves are quite comparable. It is clear that these curves are a reflection of both incorporation of the label into glucose and subsequent glucose disappearance from blood.

In figure 2 is shown a typical disappearance curve for glucose-6-C-14 under the condition of fructose infusion. It is apparent that fructose loading produces a similar disappearance of glucose tracer as does glucose

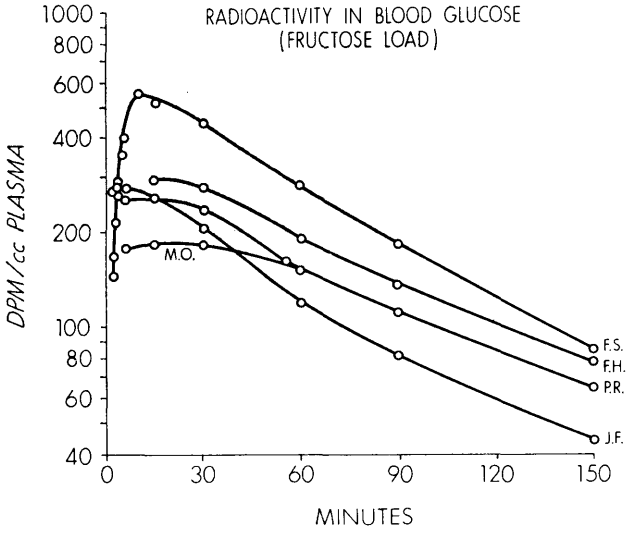

FIG. 1. Appearance of fructose tracer in glucose.

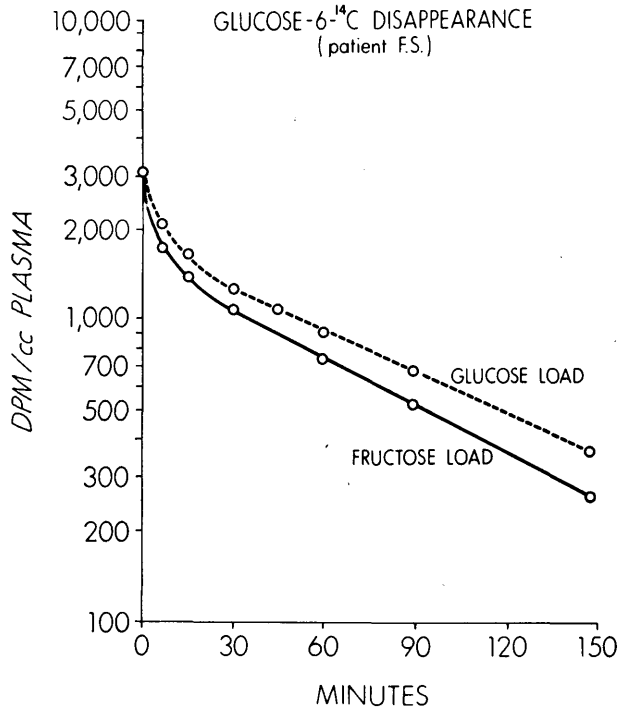

FIG. 2. Glucose tracer disappearance curves.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/20/4/193/346141/20-4-193.pdf by guest on 31 March 2023

TABLE 2

Fructose conversion to glucose

	Fructose loading			
	gm./hr. infused	gm./hr. converted	Per cent converted to glucose	Per cent converted to CO_2
F.S.	50.0	15.9	31.8	43
F.H.	57.2	18.5	32.4	45
J.F.	58.0	18.5	31.9	38
P.R.	68.0	20.4	30.0	54
M.O.	75.0	21.4	28.6	

administration. This implies no impairment of glucose handling even though active fructose metabolism is occurring. This is perhaps to be expected since glucose is metabolized in most body tissues, whereas significant fructose metabolism is confined to the liver.[18]

The percentage of fructose tracer converted to glucose or $\int_0^\infty P_{FG}(t)\,dt / \int_0^\infty P_{GG}(t)\,dt$* is shown in table 2. The mean conversion is 30.94 per cent or 18.94 gm. of fructose to glucose per hour.

Fructose-U-C-14 incorporation into CO_2

To complement the above data, the incorporation of label into CO_2 was determined both from fructose and glucose tracer. Figure 3 represents the appearance of tracer in expired CO_2, plotted as an instantaneous rate in terms of per cent of injected dose per hour. Curve 1 represents mean values of three studies using fructose tracer. Curve 2 is the mean of two studies utilizing glucose tracer during identical fructose loading. This curve is similar to previous data using glucose tracer with glucose loading.[17]

It is apparent that there are significantly more counts in CO_2 at fifteen minutes derived from fructose tracer than from glucose tracer. This is evidence for early direct oxidation of fructose to CO_2 without prior conversion to glucose, and confirms findings of Baker et al.[19] Percentage of fructose conversion to CO_2 was estimated by integrating the area under the CO_2 curve of each individual study, and these are listed in table 2. About 45 per cent of the label eventually appeared in CO_2, but we cannot differentiate direct oxidation of fructose versus that which occurred after conversion to glucose or lactate.

*Where $P_{FG}(t)$ = radioactivity in blood glucose at time t when the tracer given was fructose, and $P_{GG}(t)$ = the radioactivity in blood glucose at time t when the tracer given was glucose, it can be shown that $\int_0^\infty P_{FG}(t)\,dt / \int_0^\infty P_{GG}(t)\,dt$ is equal to the fraction of the fructose which was converted to glucose.[20]

Unlabeled hexoses and metabolic products

Figures 4 and 5 show simultaneous arterial blood levels of fructose and glucose during seven studies with fructose infusion. The variation in fructose levels among subjects may be partially explained by the nonspecific method of measurement of this substance. It is evident that glucose intolerance was not prominent in these subjects compared with diabetic subjects studied by others.[1,2,5,6,8,21] Relatively constant hepatic uptake of fructose is shown in figure 6 in three studies, with early similar findings in a fourth study. Corresponding arteriovenous differences for glucose show hepatic glucose production to be less than fructose uptake, and to diminish with time. Sustained arterial lactate elevations were noted with fructose loading (figure 7), and hepatic arteriovenous difference for lactate, like glucose, appeared to diminish with time.

DISCUSSION

Considerable data have been obtained in the past characterizing the relationships between glucose and fructose metabolism using in vitro, experimental animal and clinical approaches.[22-28] The biochemical pathways involved in hepatic metabolism of these substances have been well defined[29] and are shown in figure 8. Glycolysis

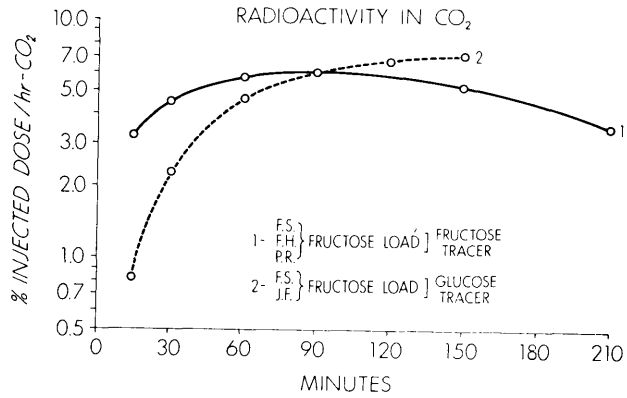

FIG. 3. Appearance of glucose and fructose tracer in carbon dioxide.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/20/4/193/346141/20-4-193.pdf by guest on 31 March 2023

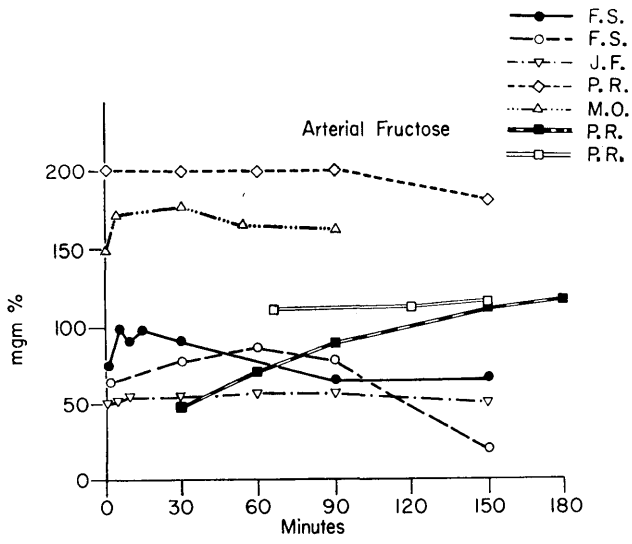

FIG. 4. Arterial fructose levels during fructose infusion. Fructose-U-C-14 was usually given immediately after the blood concentrations were drawn at t = 0. It should be remembered that fructose had been infused for thirty to sixty minutes prior to this.

is promoted and controlled by key enzymes as indicated: (1) glucokinase, (2) phosphofructokinase, and (3) pyruvate kinase. Reactions catalyzed by these enzymes are not reversible and require the enhancement of separate enzymes to reverse the direction of substrate in the process of gluconeogenesis. These enzymes are: (4) pyruvate carboxylase, (5) phosphenol pyruvate carboxykinase, (6) fructose, 1,6 diphosphatase, and (7)

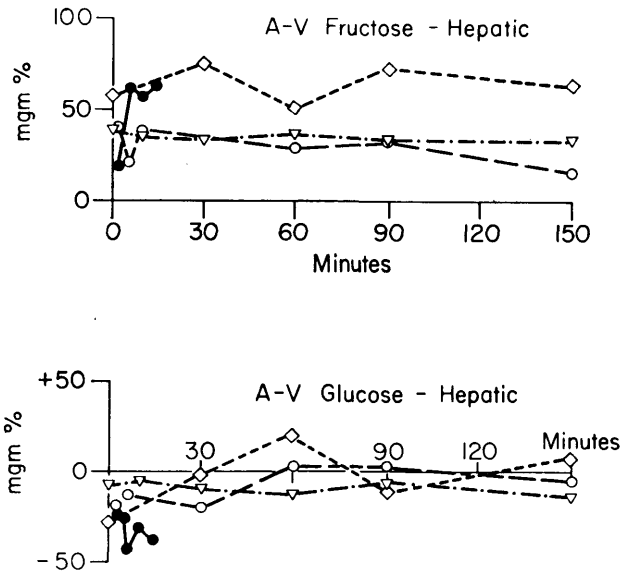

FIG. 6. Hepatic arteriovenous fructose difference (top) and hepatic arteriovenous glucose difference (bottom) during fructose infusion. See caption for figure 4.

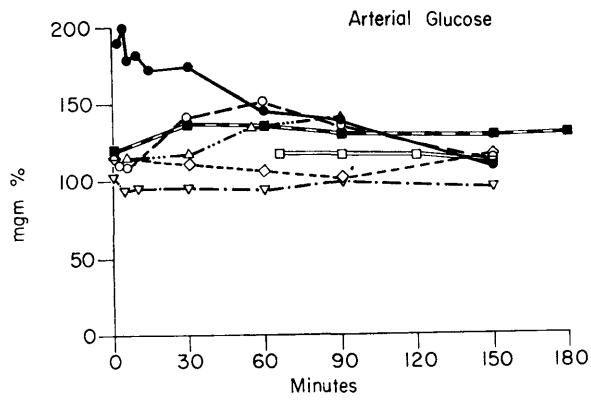

FIG. 5. Arterial glucose levels during fructose infusion. See caption for figure 4.

glucose-6-phosphatase. Under baseline conditions the activity ratios of the enzyme pairs which catalyze the initial steps in glycolysis and gluconeogenesis effect a barrier to each pathway. Phosphofructokinase is rate limiting to orderly glycolysis, and phosphenol pyruvate

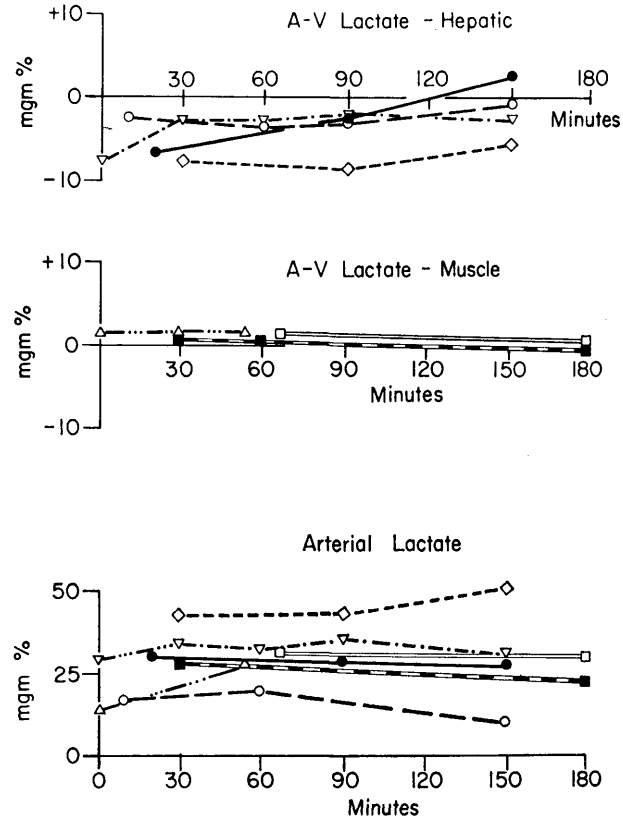

FIG. 7. Arterial lactate levels and hepatic arteriovenous lactate difference during fructose infusion. See caption for figure 4.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/20/4/193/346141/20-4-193.pdf by guest on 31 March 2023

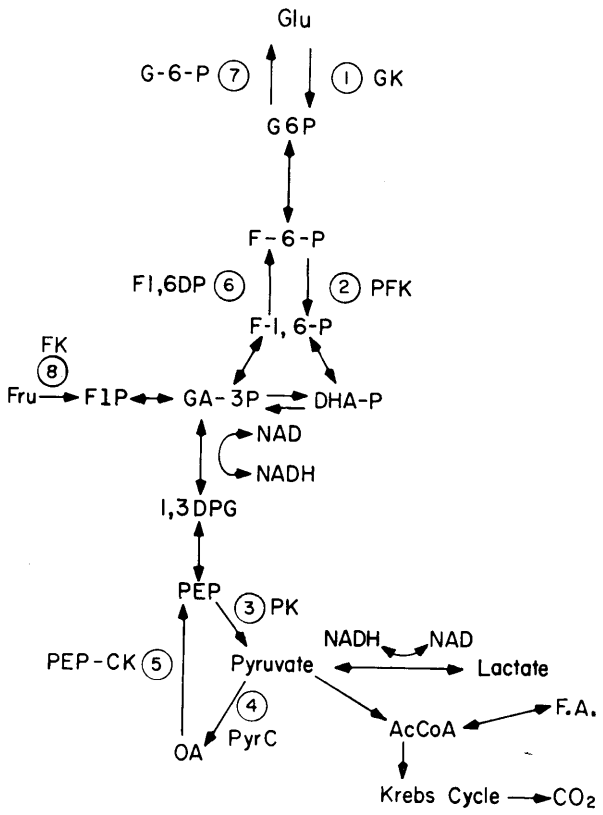

FIG. 8. Glycolysis scheme in the liver.

carboxykinase and pyruvate carboxylase to gluconeogenesis. When large quantities of fructose are introduced into the blood stream, the majority of the infused fructose is taken up by the liver (50 to 80 per cent). Fructokinase, predominantly an hepatic enzyme, catalyzes the phosphorylation of fructose rapidly to fructose-1-phosphate and in the intact animal is little influenced by hormones or substrates. Enzymes are readily available in the normal or diabetic liver which cleave this molecule into two trioses, subsequently phosphorylating them so that they become true intermediates of the glycolytic scheme.[30,31] This substrate overload which results from fructose molecules entering midway in glycolysis tends to activate both the glycolytic and gluconeogenetic pathways which under ordinary circumstances act as unidirectional units. Thus, fructose bypasses the initial blocking steps of both metabolic pathways and we see the paradoxical hepatic production of both lactate and glucose.

Our studies were designed to quantitate precisely only one fate of the infused fructose, namely its conversion to glucose which appeared in the circulating blood. It was demonstrated in all our tracer studies that about 30 per cent of the infused fructose was converted to glucose which appeared in the circulating blood. This amounted on the average to about 50 per cent of the hepatic uptake of fructose as measured by splanchnic arteriovenous differences of fructose and estimating hepatic blood flow. This relatively high rate of conversion of infused fructose to blood glucose deserves comment. Other studies[7] have indicated that hepatic glucose production is not significantly increased during fructose infusion over that of the fasting state. Since the latter figure has been quite well established at about 7 gm. an hour, this implies that a maximum of 10 per cent of the usual injected dose (50 to 75 gm./hr.) is converted to glucose. Our hepatic arteriovenous difference data would also suggest such a figure provided no change in hepatic blood flow occurred. Yet it is obvious that the blood sugar does rise with fructose infusion in these experiments, since all our fasting blood glucose levels were less than 93 mg./100 ml. The disappearance of glucose as determined by tracer is also more rapid than in the fasting state, implying an increased production of glucose[16] during fructose infusion over and above that found in the fasting state. Furthermore, two studies of glucose tracer disappearance during fructose infusion allow calculation of the irreversible disposal rate of glucose under this circumstance by means of the formula:

$$\text{Disposal rate} = \text{ID}/\int_0^\infty \frac{\text{radioactivity}}{\text{mass}}$$.[20] In these instances we have good correlation of the disposal and production rates so determined with the fructose conversion to glucose (13.5 versus 15.9 gm. for F.S. and 18 versus 18.5 gm. for J.F.). It seems highly unlikely that glucose is being produced from any other source than fructose in these experiments, and we suggest the agreement between data from the two tracer experiments is evidence for the validity of the 30 per cent conversion of fructose to glucose. How then can we account for the underestimation of glucose production as calculated from arteriovenous differences and blood flow? Arteriovenous differences are generally small, and at high blood glucose levels vary considerably from minute to minute making calculation of uptake over a prolonged period inaccurate.[13] While this may account for some inaccuracy, we are loathe to ascribe all the discrepancy to this. Glucose production from fructose has been documented in the kidney by Krebs[32] and in the intestinal mucosa by Cook.[33] Possibly the contributions by these tissues not measured with hepatic arteriovenous differences but included in our tracer data might help account for the difference. At any rate, our cor-

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/20/4/193/346141/20-4-193.pdf by guest on 31 March 2023

respondingly high rates of gluconeogenesis as determined by two different sets of tracer data make us confident that a significant proportion of the infused fructose is converted to glucose even when glucose levels are well above fasting.

Questions still remain concerning the ultimate fate of fructose not removed by the liver, and also concerning that taken up by the liver but not accounted for by glucose or lactate production, or oxidation. The decrease in production of glucose, lactate and pyruvate with time has been noted by others with constant fructose infusions.[7,8] Though other possible destinations of fructose carbon were not measured in our study, there is evidence that more labeled fructose compared with labeled glucose becomes incorporated into triglyceride[34] not only in vitro[35] but also in clinical studies.[36] Furthermore, sucrose or fructose feeding in animals[37] and man[38] is known to raise cholesterol and triglyceride levels respectively. Obviously fructose carbon could be incorporated either in the glycerol moiety or fatty acid or cholesterol via acetyl Co A. There have been no estimates of fructose retention as glycogen. Thus it appears that fructose conversion to glucose, lactate, pyruvate and CO_2 (direct oxidation—figure 4) diminishes with time, and one must conclude that there is a corresponding increase in either conversion to lipid or storage as glycogen. No data are available to explain these changes in fructose handling with time.

ACKNOWLEDGMENT

This study was supported by Public Health Service Research Grants Number RR-00044 from the Division of Research Facilities and Resources N.I.H. and Number CA-07123 from the National Cancer Institute.

REFERENCES

[1] Miller, M., Drucker, W. R., Owens, J. E., Craig, J. W., and Woodward, H., Jr.: Metabolism of intravenous fructose and glucose in normal and diabetic subjects. J. Clin. Invest. *31*:115, 1952.

[2] Smith, L. H., Jr., Ettinger, R. H., and Seligson, D.: A comparison of the metabolism of fructose and glucose in hepatic disease and diabetes mellitus. J. Clin. Invest. *32*:273, 1953.

[3] Weichselbaum, T. E., Margraf, H. W., and Elman, R.: Metabolism of intravenously infused fructose in man. Metabolism *2*:434, 1953.

[4] Mendeloff, A. I., and Weichselbaum, T. E.: Role of the human liver in assimilation of intravenously administered fructose. Metabolism *2*:450, 1953.

[5] Daughaday, W. H., and Weichselbaum, T. E.: Utilization of intravenous fructose in diabetic acidosis and in a pancreatectomized human. Metabolism *2*:459, 1953.

[6] Moorhouse, J. A., and Kark, R. M.: Fructose and diabetes. Amer. J. Med. *23*:46, 1957.

[7] Tygstrup, N., Winkler, K., and Lundquist, F.: The mechanism of the fructose effect on the ethanol metabolism of the human liver. J. Clin. Invest. *44*:817, 1965.

[8] Metz, R., Mako, M., Stevens, T., and Franklin, J.: The metabolism of fructose in diabetes mellitus. J. Lab. Clin. Med. *69*:494, 1967.

[9] Bergstrom, J., and Hultman, E.: Synthesis of muscle glycogen in man after glucose and fructose infusion. Acta Med. Scand. *182*:93, 1967.

[10] Elliott, W. C., Cohen, L. S., Klein, M. D., Lane, F. J., and Gorlin, R.: Effects of rapid fructose infusion in man. J. Appl. Physiol. *23*:865, 1967.

[11] Perheentupa, J., and Raivio, K.: Fructose-induced hyperuricemia. Lancet *2*:528, 1967.

[12] Maenpaa, P. H., Raivio, K. O., and Kekomaki, M. P.: Liver adenine nucleotides: Fructose-induced depletion and its effect on protein synthesis. Science *161*:1253, 1968.

[13] Bondy, P. H.: Spontanous fluctuations in glucose content of the hepatic venous blood in resting normal human beings. J. Clin. Invest. *31*:231, 1952.

[14] Blair, A., and Segal, S.: The isolation of blood glucose as potassium gluconate. J. Lab. Clin. Med. *55*:959, 1960.

[15] Sunderman, F. W., Jr., and Sunderman, F. W.: Measurement of glucose in blood, serum and plasma by means of a glucose oxidase-catalase enzyme system. Amer. J. Clin. Path. *36*:75, 1961.

[16] Rosenberg, J. C., and Rush, B. F.: An enzymatic-spectrophotometric determination of pyruvic and lactic acid in blood. Methodologic aspects. Clin. Chem. *12*:299, 1966.

[17] Waterhouse, C., and Kemperman, J. H.: Changes in oxidative metabolism with glucose ingestion. J. Lab. Clin. Med. *68*:250, 1966.

[18] Wick, A. N., Sherrill, J. W., and Drury, D. R.: The metabolism of fructose by extrahepatic tissues. Diabetes *2*:465, 1953.

[19] Baker, N., Shreeve, W. W., Shipley, R. A., Incefy, G. E., and Miller, M.: C-14 studies in carbohydrate metabolism. I. The oxidation of glucose in normal human subjects. J. Biol. Chem. *211*:575, 1954.

[20] Waterhouse, C., and Keilson, J.: Cori cycle activity in man. J. Clin. Invest. *48*:2359, 1969.

[21] Metz, R.: The effect of glucagon on fructose metabolism in diabetic and nondiabetic human beings. Metabolism *17*: 305, 1968.

[22] Renold, A. E., and Thorn, G. W.: Clinical usefulness of fructose. Amer. J. Med. *19*:163, 1955.

[23] Luke, R. G., Dinwoodie, A. J., Linton, A. L., and Kennedy, A. C.: Fructose and glucose tolerance in uremia. J. Lab. Clin. Med. *64*:731, 1964.

[24] Morris, R. S., Jr., Ueki, I., and Loh, D.: Absence of renal fructose-1-phosphate aldolase activity in hereditary fructose intolerance. Nature *214*:920, 1967.

[25] Zalitis, J., and Oliver, I. T.: Inhibition of glucose phosphate isomerase by metabolic intermediates of fructose. Biochem. J. *102*:753, 1967.

[26] Morris, R. C., Jr.: An experimental renal acidification defect in patients with hereditary fructose intolerance. I. Its resemblance to renal tubular acidosis. J. Clin. Invest. *47*:1389, 1968.

[27] Levin, B., Snodgrass, G. J. A. I., Oberholzer, V. G., Burgess, E. A., and Dobbs, R. H.: Fructosemia. Amer. J.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/20/4/193/346141/20-4-193.pdf by guest on 31 March 2023

Med. 45:826, 1968.

[28] Darragh, J. H., Womersley, R. A., and Meroney, W. H.: Fructose in the treatment of diabetic ketosis. J. Clin. Invest. 32:1214, 1953.

[29] Dickens, F., Whelan, W. J., and Randle, P. J.: Carbohydrate metabolism and its disorders. London and New York, Academic Press, 1968.

[30] Herman, R. H., and Zakim, D.: Fructose metabolism. I. The fructose metabolic pathway. Amer. J. Clin. Nutr. 21:245, 1968.

[31] Zakim, D., and Herman, R. H.: Fructose metabolism. II. Regulatory control to the triose level. Amer. J. Clin. Nutr. 21:315, 1968.

[32] Krebs, H. A., and Lund, P.: Formation of glucose from hexoses, pentoses, polyols and related substances in kidney cortex. Biochem. J. 98:210, 1966.

[33] Cook, G. C.: Absorption products of D (—) fructose in man. Clin. Sci. 37:675, 1969.

[34] Glucose versus fructose (Annotations). Lancet 2:1178, 1968.

[35] Zakim, D., Pardini, R. S., Herman, R. H., and Sauberlich, H. E.: Mechanism for the differential effects of high carbohydrate diets on lipogenesis in rat liver. Biochim. Biophys. Acta 144:242, 1967.

[36] MacDonald, I.: Ingested glucose and fructose in serum lipids in healthy men and after myocardial infarction. Amer. J. Clin. Nutr. 21:1366, 1968.

[37] Staub, H. W., and Thiessen, R., Jr.: Dietary carbohydrate and serum cholesterol in rats. J. Nutr. 95:633, 1968.

[38] MacDonald, I., and Turner, L. J.: Serum-fructose levels after sucrose or its constituent monosaccharides. Lancet 1:841, 1968.

Biotin and Glucokinase in the Diabetic Rat

The initial step in metabolic utilization of glucose by the cell is its phosphorylation, mediated by the enzyme glucokinase. The activity of this enzyme is, therefore, the major controlling factor in hepatic uptake of glucose. During the past few years investigations have clearly shown that hormonal and nutritional states of an animal strongly influence the activity of glucokinase. Diabetic rats, for example, have low glucokinase activity, which can be increased to normal by administration of insulin. However, injections of inhibitors of protein synthesis (actinomycin D, DL-ethionine or *p*-fluorophenylalanine) inhibit the response of glucokinase to insulin, indicating that the hormone induced synthesis of the enzyme (M. Salas, E. Vinuela, and A. Sols, J. Biol. Chem. 238:3535, 1963). Fasting or starvation depresses glucokinase activity, a condition readily reversed by feeding glucose (C. Sharma, R. Manjeshwas, and S. Weinhouse, Ibid. 238:3840, 1963).

In a recent paper, K. Dakshinamurti and C. Cheah-Tan (Canad. J. Biochem. 46:75, 1968) reported that hepatic glucokinase activity was depressed in rats with a biotin deficiency fed either high or low carbohydrate diets, and that either insulin or biotin injections restored the enzyme to normal levels. Neither insulin nor biotin had any effect on enzyme activity when added to an in vitro system, indicating they did not directly influence the activity. In a later investigation, the same authors (Dakshinamurti and Cheah-Tan, Arch. Biochem. Biophys. 127:17, 1968) reported that biotin administered to starved rats (not biotin deficient) also increased liver glucokinase activity almost to control values. Therefore, the glucokinase stimulating activity of biotin was not restricted to biotin deficient rats. However, the enzyme response was most noticeable when glucokinase activity was depressed.

Since the glucokinase response to biotin was so similar to that induced by insulin or by feeding glucose, Dakshinamurti, L. Tarrago-Litvak, and H. C. Hong (Canad. J. Biochem. 48:493, 1970) investigated this relationship in the diabetic rat. Fasted male rats were made diabetic by injecting them with alloxan. Those animals excreting at least 0.5 g. glucose per day two days after the treatment were selected as diabetic and used for the various experiments. Liver glucokinase was assayed spectrophotometrically by coupling glucose-6-phosphate formed with the reduction of NADP by glucose-6-phosphate: NADP oxidoreductase and 6-phosphogluconate:NADP oxidoreductase. Activity of the enzyme was expressed as specific activity (units $\times 10^3$ per milligram protein).

An initial experiment compared diabetic rats injected

(*Continued on page 213*)

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/20/4/193/346141/20-4-193.pdf by guest on 31 March 2023