

Effect of oral fructose on urate production

B. T. EMMERSON

From the University of Queensland Department of Medicine at the Princess Alexandra Hospital, Woolloongabba, Brisbane, Australia, Q. 4102

The oral or intravenous administration of fructose to children results in dramatic rises in both urate and lactate concentrations in serum and an increase in the excretion of uric acid in the urine, findings interpreted as being caused by rapid nucleic acid degradation due to the increased intracellular lactic acidosis in the liver (Perheentupa and Raivio, 1967). As lactate also reduces renal excretion of urate (Yü, Sirota, Halpern, and Gutman, 1967), these authors were unable to assess the relative importance of hepatic and renal factors in the resultant hyperuricaemia, but the increase in the urinary uric acid suggested either a sudden increase in urate production or a shift of urate from an intracellular pool to the extracellular fluid. Moreover, Fox and Kelley (1972) were unable to demonstrate any consistent decrease in the fractional clearance of urate after fructose infusions. Studies in Cebus monkeys have also demonstrated hyperuricaemia and increases in urinary uric acid excretion after infusions of high concentrations of hexoses, fructose having the greatest effect and glucose a lesser effect (Simkin, 1969). This has not been a uniform finding, however, and no significant changes in serum urate concentrations were demonstrated during the 3 hrs after the intravenous administration of 100 g. fructose to ten young adult males (Curreri and Pruitt, 1970). Because of the conflicting evidence, the lack of information concerning the possible mechanism involved and the potential importance in patients with hyperuricaemia, it was decided to compare the effect of diets containing large quantities of either added fructose or glucose on urate metabolism, as reflected by the miscible urate pool and turnover rate and the simultaneous incorporation of glycine into both urinary and produced uric acid.

Methods

SUBJECTS

The three patients studied were males who were healthy apart from stable chronic neurological disease. Each was studied on three occasions, and each study was separated by at least 4 months, which allowed complete elimination of isotopes used in the previous study. None had suffered from gout; all had normal serum urate concentrations and

only Subject A had suffered from any renal disease, which in his case had consisted of occasional urinary tract infections responding readily to chemotherapy. All understood the implications of the study and were fully agreeable and cooperative. All studies were carried out in hospital under metabolic ward conditions.

DIET

Purine-free diets containing sufficient calories to maintain a stable weight were used throughout. Each diet was begun at least 5 days before isotope administration and was continued for the 7 study days, during which all urine was collected. Fluids were taken regularly during the day and intake was as large as was desired by the patient but at least sufficient to maintain a urine volume in excess of 1 litre per 24 hrs.

Three dietary regimes were used (Table). The first study in each subject had been completed before the planning of the glucose and fructose studies and was undertaken on a low carbohydrate diet. In the high glucose and high fructose studies, hexose calories replaced those from fat in the low carbohydrate diet, and were administered with flavouring to the fluids in eight equal amounts during the waking hours.

STUDIES

All urine was collected in 12- or 24-hr aliquots from 8.30 a.m. each day, preserved with toluene, and stored at room temperature. At 8.30 a.m. on the day set for commencement, and after bladder emptying, the fasting patient was given by mouth a measured amount (about 10 μ Ci.) of glycine-1-¹⁴C (1-2 mCi. per mmole) (Radiochemical Centre, Amersham) in milk. Immediately thereafter, a measured amount of a sterile solution containing between 22.75 and 24.83 mg. uric acid with a ¹⁵N enrichment between 14.94 and 15.75 atoms per cent. excess (prepared from uric acid-1,3-¹⁵N; Merck Sharp and Dohme, Canada, and dispensed as sterile lyophilized lithium urate by the Commonwealth Serum Laboratories, Australia) was administered into a free-flowing intravenous drip in a volume of less than 10 ml. by means of a glass syringe fitted with a Chaney adaptor. Breakfast was given 1 hr later.

The uric acid content of each 12- or 24-hr collection of urine was estimated by the spectrophotometric method using purified Worthington uricase (Liddle, Seegmiller, and Laster, 1959). Crystalline uric acid was then isolated

Table The effect of different carbohydrate content upon parameters of urate production

Subjects	A (50 kg., S.A. 1.57 m ²)			B (65 kg., S.A. 1.69 m ²)			C (74 kg. S.A., 1.91 m ²)		
	1	2	3	1	2	3	1	2	3
Diet (g./24 hrs)									
Hexose	0	270 glucose	270 fructose	0	290 glucose	290 fructose	0	250 glucose	250 fructose
Carbohydrate	80	115	115	110	140	140	110	180	180
Protein	90	90	90	105	105	105	105	105	105
Fat	150	30	30	160	40	40	160	25	25
Serum urate (mg./100 ml.)‡	4.1 ± 0.5	4.4 ± 0.1	5.1 ± 0.4†	4.5 ± 0.1	4.4 ± 0.7	6.2 ± 0.5*	4.2 ± 0.7	4.8 ± 0.4	5.2 ± 0.3
Urinary urate (mg./24 hrs)‡	333 ± 21	359 ± 25	466 ± 21†	326 ± 9*	363 ± 37	443 ± 47†	286 ± 16†	327 ± 16	340 ± 25
Miscible urate pool (mg.) (95 per cent. range)	865 (707-1,057)	831 (720-959)	1105† (1,002-1,219)	1,098 (827-1,456)	1,034 (808-1,321)	913 (778-1,072)	1,123† (1,031-1,222)	1,019 (984-1,057)	1,110† (1,043-1,181)
Urate turnover (pools/24 hrs)	0.62	0.65	0.63	0.51	0.57	0.71†	0.40†	0.50	0.49
Urate production (mg./24 hrs)	540	537	697	560	590	650	450	514	545
Intravenous urate excreted 7 days (per cent. administered dose)	61.3	61.0	65.9	57.3	60.1	60.2	59.0	59.6	61.9
¹⁴ C-glycine incorporation into urinary urate 7 days (per cent. dose)§	0.32 ± .002	0.31 ± .002	0.73 ± .004	0.23 ± .002	0.26 ± .002	0.33 ± .002	0.14 ± .002	0.09 ± .001	0.17 ± .001
¹⁴ C-glycine incorporation into produced urate 7 days (per cent. dose)§	0.52 ± .003	0.51 ± .003	1.10 ± .006	0.44 ± .003	0.43 ± .003	0.55 ± .003	0.24 ± .003	0.15 ± .001	0.27 ± .001

* Significantly different from glucose study P < 0.05.

† Significantly different from glucose study P < 0.01.

‡ Expressed as mean ± standard deviation.

§ Cumulative incorporation ± radiological counting error (percentage).

from each urine collection by adsorption on to an anion-exchange resin column and elution by a pyridine-formate solution (Johnson and Emmerson, 1972). Repeated recrystallization was undertaken until assay showed a purity of greater than 95 per cent. Crystals were then dried overnight in an oven at 110°C. and stored in a desiccator.

¹⁴C STUDIES

A small quantity of crystals was weighed on an electro-micro-balance, placed in a scintillation vial, dissolved in NCS solubilizer, mixed with a toluene-POP-POPOP scintillator, and counted in a Nuclear-Chicago Mark I liquid scintillation counter at 72 per cent. efficiency. Conversion of c.p.m. to d.p.m. was carried out from the graph of channels ratio obtained with quenched standard solutions and the use of an external standard. From the specific activity of these uric acid crystals and knowledge of the exact dose of ¹⁴C which had been administered (determined by a similar technique), the daily and the cumulative percentage incorporation of ¹⁴C glycine into urinary uric acid could be calculated (Wyngaarden, 1957). Counting errors were expressed at a probability of 0.1, as outlined by Sørensen (1960).

¹⁵N STUDIES

Samples of each crystallization and of the injected lithium ¹⁵N urate solution were digested in concentrated sulphuric acid using a selenium catalyst. Using a stainless steel Bremner-Keeney still with steaming between samples to minimize contamination, and working from samples of low ¹⁵N enrichment up to samples of high ¹⁵N enrichment, the samples were steam distilled into boric acid and titrated against N/10 sulphuric acid. The resulting ammonium sulphate was then analysed for its ¹⁵N enrichment in a mass spectrometer by courtesy of Dr. A. Martin of the Cunningham Laboratory of the Commonwealth Scientific and Industrial Research Organization of Australia.

The ¹⁵N enrichment of the uric acid crystals isolated from the urine forms a single exponential curve against time and the miscible urate pool and the daily turnover of urate were calculated from the best straight line of the natural logarithm of isotope enrichment against time according to the original method of Benedict, Forsham, and Stetten (1949), as followed by Sørensen (1960), and Seegmiller, Grayzel, Laster, and Liddle (1961). Because slightly different amounts of ¹⁵N were administered in

different studies, all results were standardized to a constant administered dose of 22 mg. uric acid containing 15 atoms per cent. excess ^{15}N . The 95 per cent. range of values for the miscible pool and turnover were calculated from the 95 per cent. confidence limits of the best straight line of ^{15}N isotope enrichment against time and the significance of the difference between pools (intercepts) and turnovers (slopes) was calculated by standard statistical procedures comparing regression lines by means of a 't' test.

PERCENTAGE INCORPORATION OF GLYCINE INTO PRODUCED URATE

The percentage intravenous (^{15}N) urate excreted is calculated from the sum of the products of the daily ^{15}N enrichments of uric acid and the urinary uric acid excretion for 7 days and expressed as a percentage of the administered dose of ^{15}N uric acid. This value may be used to correct the cumulative per cent. incorporation of ^{14}C glycine into urinary uric acid so that one can calculate the 7-day cumulative incorporation of ^{14}C glycine into produced uric acid (Seegmiller and others, 1961).

Results

The results in all studies are summarized in the Table. The studies were planned to compare a high glucose with a high fructose diet, and the low carbohydrate study, which had been performed previously, is included only for comparison.

COMPARISON OF HIGH FRUCTOSE WITH HIGH GLUCOSE DIETS

Serum and urine urate

In all cases, both the serum and the urine urate were greater during fructose administration, the increase being statistically significant in two subjects (A and B). There was no tendency for this effect to decrease with the passage of time during the fructose feeding.

Miscible urate pool and turnover

In two subjects (A and C), the pool size was significantly greater during fructose administration, whereas in the other (B) it was less, although the reduction was not of significant degree. In the two subjects in whom the pool size was greater, the turnover in pools per day did not change significantly but in the third in whom the pool size was less, the turnover rate was significantly greater. Consequently, the product of pool and turnover, which in the steady state can be equated with urate production, was greater in all three subjects. Statistical methods have not been formulated, however, whereby the degree of significance of this increase can be assessed. In Subject A, the increase appears to be significant, but less so in the other two.

Percentage intravenous urate excreted in 7 days

This appears to have changed appreciably only in Subject A, who is the subject who exhibited the

greater increase in both his urate production and his 24-hr urinary urate excretion. This measurement reflects chiefly the percentage of produced urate which is excreted by the kidney, but it must also reflect to some degree the extent of dilution of ^{15}N urate within the urate pool and the rate of production of newly synthesized urate.

Glycine incorporation into urate

All three subjects exhibited increases in percentage glycine incorporation into urate, whether measured into urinary uric acid, or, after correction for extrarenal losses, into the total amount of uric acid produced. In the subject with the greatest change in urate pool and urine urate (A), the increase amounted to a doubling of the glycine incorporation, whereas in another (B) the increase was only a little over 25 per cent.

COMPARISON OF A HIGH GLUCOSE DIET WITH A LOW CARBOHYDRATE DIET

No significant differences between these studies in Subjects A and B existed in regard to serum urate, urate pool and turnover, percentage excretion of intravenous urate or percentage glycine incorporation into urate, although the urine urate was significantly lower in Subject B on the low carbohydrate diet. This is unexplained, except that it may be partly due to the smaller standard deviation and may reflect the slightly lower urate production and the slightly lower percentage excretion of intravenous ^{15}N urate in that study. In Subject C, however, the urine urate is again significantly lower during the low carbohydrate diet and is again associated with a lesser urate production. In each subject, the urinary urate was greater on a high glucose diet than on the low carbohydrate diet and was again greater when the glucose was replaced by fructose. Glycine incorporation into urate, which is not significantly different in Subjects A and B, is intermediate in Subject C between the incorporation obtained during glucose administration and that during fructose administration.

COMPARISON OF A HIGH FRUCTOSE DIET WITH A LOW CARBOHYDRATE DIET

In all subjects, the high fructose study was associated with greater values for serum and urine urate, miscible urate pool and production rate, and greater incorporation of glycine into urinary or produced urate than was found in either the low carbohydrate diet or the high glucose diet.

Discussion

Simultaneous increases in serum and urine urate can result either from (a) an increase in purine synthesis, (b) a contraction of the volume of distribution of urate, or (c) an increase in nucleoprotein destruction.

The present data supports the first explanation and excludes the second. The third possibility is also excluded as the sole explanation, although it might be acceptable if it were the result of a secondary increase in purine synthesis (such as occurs in myeloproliferative disorders). There was, however, no evidence of delay in the time for development of maximum specific activity of uric acid, which is characteristic of this situation. In addition, there was no evidence of any reduction in renal ability to excrete urate.

The metabolism of fructose is completely different from that of glucose. Initially, fructose is rapidly phosphorylated to fructose-1-phosphate by fructokinase in a reaction involving adenosine triphosphate (ATP). The fructose-1-phosphate is then converted by liver aldolase to dihydroxyacetone phosphate and glyceraldehyde. These are then progressively degraded to pyruvate, which is either reduced to lactate or metabolized *via* the citric acid cycle (Herman and Zakim, 1968). Conversion of fructose to glucose occurs only *via* pyruvate after recondensation of trioses and then only when the pyruvate concentration reaches a threshold level (Reynafarje, Oyola, Cheesman, Marticorena and Jimenez, 1969).

Most studies of the effect of fructose on serum or urine urates have been limited to a few hours duration. Those of Perheentupa and Raivio (1967) on children showed rapid rises in blood lactate and urate which they attributed to the degradation of purine nucleotides in hepatic cells. Intravenous fructose loading in rats has also been shown to induce a rapid depletion of total adenine nucleotides in the liver, together with severe inhibition of incorporation of amino acid into hepatic protein (Mäenpää, Raivio, and Kekomäki, 1968). The subsequent findings of Woods, Eggleston, and Krebs (1970) have also demonstrated rapid phosphorylation of parenterally-administered fructose, associated with a sharp fall in ATP and AMP and a great increase in inosine monophosphate (IMP), the first purine nucleotide produced in the *de novo* biosynthetic pathway. These authors attributed any increase in uric acid production to two processes:

(1) The extensive dephosphorylation of ATP by the high concentration of fructose, which results in such rapid production of AMP that it is either deaminated to IMP or dephosphorylated to adenosine, both of which are ultimately degraded to the purine base hypoxanthine and thence to uric acid.

(2) The depletion of ATP removes the inhibitory effect which ATP normally exerts upon the degradation of AMP, thereby promoting further its breakdown.

These explanations depend basically upon the existence of depleted levels of ATP, and one would expect such a finding to be less applicable in the present studies where the fructose was administered orally over a period of 12 days than during acute fructose loading. Although orally administered fructose has

been shown to be well absorbed as such from the alimentary tract (Cook, 1969), random sampling during the present study revealed blood levels of fructose of the order of only 10 mg. per 100 ml. One would have expected, therefore, that fructose concentrations of this order would not have exceeded the phosphorylating ability of the liver and might not have resulted in depletion of ATP.

This study has also demonstrated increases in glycine incorporation into urate during prolonged oral fructose administration. As this implies increased *de novo* purine biosynthesis, it suggests that this may also be a factor in the increased urate production which is additional to any increase due to the interconversion of purine nucleotides. However, the possibility must be considered that the increased *de novo* biosynthesis may merely be secondary to decreased utilization of purine bases due to their degradation to uric acid, and that the mechanism might be akin to that which occurs in hypoxanthine-guanine phosphoribosyltransferase deficiency due to lack of re-utilization enzyme activity. Moreover, Heuckenkamp and Zöllner (1971) have shown that the effect of acute fructose infusions on urate metabolism in man is dose related and that brief infusions of less than 0.5 g. per kg. per hour do not induce hyperuricaemia, although they were associated with an increase in uric acid production, as evidenced by increased renal excretion of urate (Sahebajami and Scalettar, 1971). Thus mechanisms of hyperuricaemia after prolonged oral fructose feeding may thus not be precisely the same as those which operate after parenteral infusions of large quantities of fructose.

It is also tempting to suggest that the different hexoses affect either the availability of intermediates or the activity of the enzymes in the early steps of the *de novo* purine biosynthetic pathway and two possible mechanisms for this might be considered. The first would be that the changes in carbohydrate metabolism induced by the high levels of fructose-1-phosphate lead ultimately to increased concentrations of 5-phosphoribosyl-1-pyrophosphate, a phenomenon which has been demonstrated in erythrocytes *in vitro* by Fox and Kelley (1972), although the reverse was found after acute fructose loading *in vivo*. The second possibility would be that the reduced levels of AMP may lead to reduced feedback inhibition of the enzyme involved in the first and rate-limiting step of the purine biosynthetic pathway, glutamine phosphoribosyl pyrophosphate amidotransferase, resulting directly in increased purine biosynthesis (Caskey, Ashton, and Wyngaarden, 1964). This explanation again requires the demonstration that lowered levels of adenine nucleotides, such as were demonstrated during the acute fructose studies of Mäenpää and others (1968), are also present during prolonged oral administration. Whatever the precise mechanism, however, the study clearly shows that dietary factors

have a role in the control of uric acid production and emphasizes their importance in purine synthesis in patients with hyperuricaemia and gout.

Summary

The effect on urate metabolism of a diet high in fructose was compared with that of a diet high in glucose. In three subjects with normal urate metabolism, the high fructose diet induced increases in serum and urine urate and in the daily production of urate. This was accompanied by increases in the incorporation of glycine into urate, indicating greater *de novo* purine biosynthesis during the high fructose

diet. This suggests that the increase in urate production is not derived solely from degradation of adenine nucleotides but that increased *de novo* purine biosynthesis also makes a contribution. This study also demonstrates that variations in dietary compositions may have effects upon urate production which could be of significance for persons with borderline hyperuricaemia.

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