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Centre for Cardiopulmonary Biochemistry and Respiratory Medicine, University College London Medical School, London WC1E 6JJ, UK (N A Hernández-Rodríguez BSc, A D Cambrey PhD, R C Chambers PhD, A J Gray PhD, R J McAnulty PhD, Prof G J Laurent PhD); **Respiratory Unit, Morriston Hospital, Swansea** (N K Harrison MD); **Interstitial Lung Disease Unit, National Heart and Lung Institute, London** (A M Southcott MD, R M duBois MD); **Department of Rheumatology, Royal Free Hospital, London** (Prof C M Black MD); **and Thrombosis Research Institute, London** (M F Scully PhD)

Correspondence to: Dr Robin J McAnulty

Endogenous synthesis of galactose in normal men and patients with hereditary galactosaemia

Gerard T Berry, Itzhak Nissim, Zhiping Lin, Alice T Mazur, James B Gibson, Stanton Segal

Despite restricted ingestion of lactose, patients with galactose-1-phosphate uridyltransferase deficiency have raised concentrations of galactose metabolites in blood and urine. Endogenous production of galactose may underlie this phenomenon. Using isotopically labelled galactose in a continuous intravenous infusion, we employed the steady-state flux method to calculate endogenous galactose production rate in three normal men and three patients with classic galactosaemia. We found that galactosaemic patients and normal subjects synthesise gram quantities of galactose per day. The rate of synthesis ranged from 0·53–1·05 mg/kg per h. Endogenous production of galactose may be an important factor in the pathogenesis of the complications of the brain and ovary, and could explain the persistent elevation of galactose metabolites in patients despite dietary restriction of galactose.

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Galactose-1-phosphate uridyltransferase (GALT) deficiency is an autosomal recessive inborn error of metabolism.[1] With unrestricted lactose ingestion, infants usually exhibit poor growth, feeding difficulties, jaundice, and may display cataracts, some degree of encephalopathy, and laboratory evidence of liver disease and renal tubular dysfunction.[1] Dietary lactose restriction essentially either reverses these early manifestations or eliminates their expression in prospectively treated infants. Unfortunately, with time a long-term syndrome emerges characterised by new encephalopathic features of speech defects and learning disabilities, primary ovarian failure in females, and, uncommonly, a severe ataxic condition.[1–4] Since most patients also display persistent increases of erythrocyte galactose-1-phosphate concentrations and urinary galactitol excretion, chronic lack of care with diet or hidden dietary sources such as macromolecular-bound galactose in fruits and vegetables have been considered as potential causes of clinical and laboratory abnormalities.[5] It has been suggested, however, that endogenous galactose production independent of dietary galactose is the mechanism that underlies these phenomena, producing a chronic state of "autointoxication".[6]

In a study on fruits and vegetables as cryptic sources of dietary galactose, we estimated that an adult with galactosaemia produces over 1 g of galactose per day.[7] Using continuous intravenous infusion of isotopically labelled galactose and steady-state flux analysis, we now provide experimental evidence that galactosaemic patients, as well as normal subjects, synthesise de novo gram quantities of galactose on a daily basis.

The subjects in our study were three adult patients with typical features of galactosaemia and three normal adults. The galactosaemic group were a 22-year-old woman with a history of a speech disorder and ovarian failure, a 30-year-old man with a history of a speech disorder and learning problems, and a 31-year-old woman with a history of learning problems who, despite hypergonadotropic hypogonadism, experienced a successful pregnancy. None of the patients had detectable GALT activity in erythrocytes and all were homozygous for the most common GALT gene mutation, Q188R, which in a yeast system expresses an enzyme with no activity.[8] The three control subjects were men ranging in age from 25 to 37 years. The studies were approved by the institutional review board of the Children's Hospital of Philadelphia and each subject provided written consent.

All subjects were studied in the Clinical Research Center of the Children's Hospital of Philadelphia after an overnight fast. The galactosaemic patients had been on their usual lactose-restricted diets. Every patient had raised concentrations of erythrocyte galactose-1-phosphate and urinary galactitol—typical for a patient on a lactose-restricted diet—in specimens obtained just before beginning the study. The normal subjects had been on regular diets not restricted in dairy products, and their baseline metabolite levels were within the normal range. The study commenced with an intravenous priming bolus of 7 mg

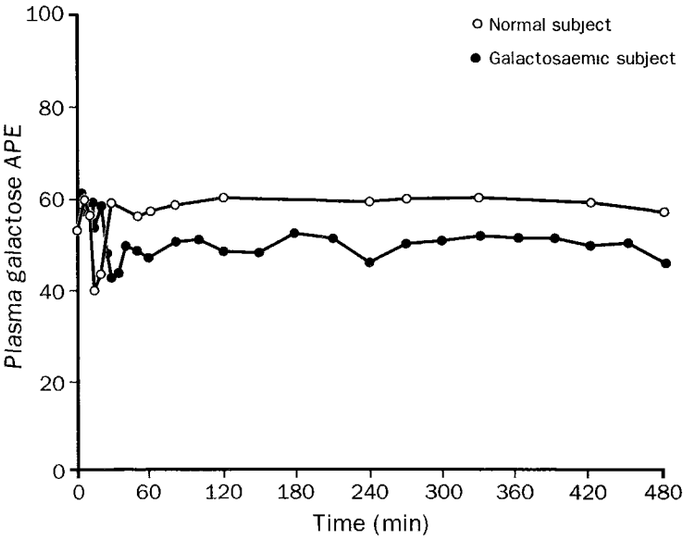

Figure: **Appearance of [1-^{13}C]galactose enrichment in plasma expressed as APE during continuous infusion of [1-^{13}C]galactose in normal man and woman with galactosaemia**

Subject	Age	Sex	APE (%)	Galactose production rate	
				mg/kg/h	g/day
Control					
1	25	M	59	0·53	1·1
2	27	M	45	0·93	2·1
3	37	M	44	0·97	2·0
Galactosaemic					
1	31	F	49	0·79	1·2
2	22	F	42	1·05	1·3
3	30	M	50	0·76	1·1

Table: **APE of plasma [1-^{13}C]galactose during steady-state conditions and the apparent galactose production rates in control and galactosaemic subjects during a constant intravenous infusion of [1-^{13}C]galactose**

[1-^{13}C]galactose/kg body weight, which was followed by a continuous infusion of [1-^{13}C]galactose at the rate of 0·76 mg/kg body weight per h for 8 h. The pure D-[1-^{13}C]galactose (Omicron Biochemicals Inc, South Bend, IN, USA) was 99% enriched with ^{13}C. Blood samples were obtained at baseline and every 10 to 30 min. In an aliquot of plasma, galactose was derivitised with butylboronate and acetic anhydride and analysed by gas chromatography-mass spectrometry (GC-MS) with a Hewlett-Packard 5890 in the selected ion monitoring mode.[9] The m/z 237 and m/z 238 fragments of derivitised-galactose were used for analysis of the atom percent enrichment (APE) of [1-^{13}C]galactose.

After about 2 h, a constant enrichment of plasma galactose was reached in all subjects. The figure shows representative enrichment curves for a control and galactosaemic subject. By the steady-state equation for turnover—production rate=(rate of infusion of tracer/APE)×100−rate of infusion of tracer—we calculated the apparent rate of de novo synthesis of galactose.[10] In the table, the steady-state APE for plasma galactose and the production rates for each subject are shown. The rates for the three control subjects were 0·53, 0·93, and 0·97 mg/kg per h or 1·1, 2·1, and 2·0 g per day. The production rates in the galactosaemic patients were 0·79, 1·05, and 0·76 mg/kg per h or 1·2, 1·3, and 1·1 g per day. These results may represent a minimal rate of synthesis because some [1-^{13}C]galactose could be recycled. In one of the control subjects the rate per kg was almost 50% lower than the other two normal individuals. This finding raises the possibility of heterogeneity in endogenous galactose synthesis.

This is the first evidence of a quantitative nature for whole-body de novo galactose synthesis in normal adults as well as in patients with hereditary galactosaemia. In retrospect, the results are not unexpected. The daily body production of galactose-containing glycoproteins and membrane-bound complex lipids is not likely to be simply dependent on milk consumption. In normal cell metabolism, UDP-galactose is synthesised from UDP-glucose, which is readily formed from glucose-1-phosphate and UTP. The interconversion of these uridine sugar nucleotides is catalysed by the enzyme UDP-galactose-4-epimerase, which maintains an equilibrium ratio, UDP-glucose to UDP-galactose, of three to one. UDP-galactose is the obligate donor of galactose in the synthesis of complex glycoconjugates. We suggest that the cycle that dictates the galactose production which we observed involves UDP-galactose-dependent galactosylation of complex molecules in the cell Golgi apparatus and the subsequent turnover of these glycoconjugates, which releases free galactose following lysosomal hydrolysis. Lactose production in the mammary gland is also another example of UDP-galactose-dependent galactosylation. Another possibility to explain the production of galactose is the pyrophosphorlytic cleavage of UDP-galactose, followed by the hydrolysis of released galactose-1-phosphate.[6] As with this alternate mechanism, the critical step in the macromolecular cycle is the epimerisation of UDP-glucose to UDP-galactose, which makes the body production of galactose-containing substances important in normal growth and development independent of dietary galactose intake. Since concentrations of galactose-1-phosphate are higher in cord blood than those observed in older patients on restricted diets, it is possible that the de novo production rate is higher in the fetus and infant than in the child and adult.

These findings have profound implications for the patient with galactosaemia. The endogenous production rate of 0·76–1·05 mg/kg per day or 1·1–1·3 g of galactose per day far exceeds the free galactose intake in adult galactosaemic patients on lactose-restricted diets. For example, in two adult patients on restricted diets we determined an intake of galactose that ranged from 4–27 mg per day.[7] A rate of de novo galactose synthesis of this degree or higher may explain the persistently raised concentrations of erythrocyte galactose-1-phosphate and urinary galactitol in patients of all ages on well controlled galactose-restricted diets. In the face of an endogenous galactose production rate of the magnitude we have observed, minute dietary galactose manipulations can have no substantial effect on galactosaemic patients. Endogenous production of galactose must be considered in the pathogenesis of the complications of galactosaemia that are largely dietary independent. New strategies for the treatment of galactosaemia are needed.[4] Realisation of this goal may hinge on our understanding of the different facets of endogenous galactose synthesis in man.

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Department of Pediatrics, University of Pennsylvania School of Medicine, Division of Biochemical Development and Molecular Diseases, Children's Hospital of Philadelphia, 34th Street and Civic Center Boulevard, Philadelphia, PA 19104, USA (Prof G T Berry MD, Prof I Nissim PhD, Z Lin MA, A T Mazur MA, J B Gibson MD, Prof S Segal MD)

Correspondence to: Prof Gerard T Berry