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Extended [^{13}C]galactose oxidation studies in patients with galactosemia

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Abstract

Since patients with galactose-1-phosphate uridyltransferase (GALT) deficiency have considerable endogenous galactose formation and only limited urinary excretion of galactose metabolites, there must be mechanisms for disposal of the sugar. Otherwise, a steady-state could not be maintained and there would be continuous body accumulation of galactose and alternate pathway products. Previous studies quantitating the amount of galactose handled by oxidation to CO_2 focused on short collection periods of expired air after administering isotopically labeled galactose mainly designed for discerning differences in the capacity to oxidize the sugar in relation to genotype. Assuming that there may be more extensive oxidation than that observed in short-term studies in order to dispose the daily galactose burden, we have examined the amount of [1-^{13}C]galactose oxidized to $^{13}CO_2$ over a 24-h period after either a single bolus or continuous IV administration by 11 patients with classic galactosemia including patients homozygous for the Q188R gene mutation. As much as 58% of the administered galactose was oxidized to $^{13}CO_2$ in 24 h. The pathways involved remain to be determined but a significant amount may be metabolized by non-GALT pathways since a patient homozygous for gene deletion had an oxidative capability. We conclude that classic patients have the ability to slowly oxidize galactose to CO_2 in 24 h in amounts comparable to that which a normal handles in approximately one-fifth the time. This capacity enables the galactosemic to maintain a balance of galactose disposal with the galactose burden imposed by endogenous formation and dietary intake.

Keywords: Galactosemia; Stable isotope; [1-^{13}C]Galactose; Breath test

Introduction

To what extent and by what routes patients with classic galactosemia who have little or no residual galactose-1-uridyltransferase (GALT) activity metabolize galactose has been an important aspect in the comprehension of this enigmatic disorder [1]. Based on the data from man and experimental animals, it appears that the bulk of galactose that would rise in blood following lactose consumption is cleared by the liver, the organ that we consider to be the most important for whole body galactose handling. In the liver, the GALT enzyme plays a pivotal role in the sequence of reactions that allows galactose to be converted to glucose, in which form it may ultimately be exported to extrahepatic tissues for oxidative breakdown to CO_2 and H_2O. A quantitation of the patients' ability to oxidize exogenously administered, isotopically labeled galactose to CO_2 has been the currency for this assessment. Employing intravenously administered ^{14}C-labeled galactose, Segal et al. [2,3] reported that Caucasian galactosemics oxidized up to 8% of the galactose dose in the 5-h period of measurement compared to a normal of 30–35%. There appeared to be no increase in the ability to oxidize galactose with age but African-American patients were found to oxidize galactose to an almost normal extent [3]. Subsequently, Segal and Cuatrecasas [4] examined the oxidation of [^{14}C]galactose labeled in either the C-1 or

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C-2 position over an 8–10-h period, showing that over the longer period of $^{14}CO_2$ collection Caucasian patients oxidized up to 20% of the dose of the [1-^{14}C] galactose, but only about half that amount of [2-^{14}C] galactose. This lead them to postulate that patients with galactosemia could slowly oxidize galactose over time and that much of the oxidation occurred via a directed oxidative pathway [5] involving conversion to galactonate and subsequent preferential release of the labeled C-1.

More recently, the quantitation of galactosemic patients' ability to oxidize galactose has involved the administration of stable isotopically labeled [^{13}C]galactose. In a series of reports [6–8], Berry and associates showed that similar results could be obtained with orally or intravenously administered sugar and that in a 5-h breath collection Caucasian patients oxidized an average of 4% of the dose. When oxidation was correlated with genotype, Q188R homozygous patients could be distinguished by a low level of oxidation at 1–2 h, while patients with other mutations could oxidize greater amounts especially African-American patients with 1 or 2 copies of the S135L mutation [7,9].

With the reports that there is a large daily endogenous production of galactose [10–12] which provides a galactose burden in addition to that imposed by food intake, it is apparent that patients with galactosemia with little or no GALT must have mechanisms for disposal of the sugar. Otherwise, they would continue to accumulate galactose metabolites such as galactose-1-phosphate (Gal-1-P), galactitol and galactonate. This, however, is not the case: most patients manifest their own relatively unique steady-state levels of plasma galactose [13], RBC galactose-1-phosphate [14], galactitol and galactonate [15], and urinary galactitol [16] and galactonate [17] excretion. Estimates are that urinary excretion of metabolites account for about 30% of the daily burden [7,12].

Assuming that there may be a greater amount of oxidation over time than has been measured in 5–8 h expired air collection periods that could account for the apparent galactose metabolic steady-state, we have examined the amount of [^{13}C]galactose oxidized over a 24-h period after galactose administration. We recently demonstrated that an infant with homozygous deletion of the GALT gene had a substantial ability to oxidize [1-^{13}C]galactose to $^{13}CO_2$ if the air samples were collected over a 24-h period [18]. Whereas the fractional elimination of the dose as $^{13}CO_2$ was $<1\%$ in 2 h, by 24 h it was up to 17% providing evidence for function of non-GALT related pathway(s) for galactose disposition. In this report, we extend our observation to 10 additional patients with classic galactosemia and genotypes such as Q188R/Q188R and, indeed, demonstrate their ability to oxidize as much as 58% of exogenously administered galactose in 24 h.

Materials and methods

Materials

D-[1-^{13}C]Galactose (APE 99%) was purchased from the Omicron Biochemicals.

Methods

Breath test

During the initial study period of 480 min, the patients who received oral galactose rested in bed or in a chair except for periodic use of a bathroom to void. After that time they were discharged and the breath collections were obtained at home. Patients were permitted to drink only water during the GCRC phase of the study. Upon discharge, they were placed on their regular galactose-restricted diet and the breath samples were collected while they maintained their normal home routine including ambulatory activity. Two baseline breath samples were collected, after which an oral (PO) or an intravenous bolus of 7 mg/kg [1-^{13}C]galactose was administered. Breath samples were collected at frequent intervals for 8 h and at longer intervals thereafter. The sample of air exhaled into a breath-collecting bag at each time point was trapped in a vacuum tube and stored at room temperature until analyzed. Patients receiving a continuous IV infusion of 0.2–1.0 mg [1-^{13}C]galactose/kg/h remained in the GCRC for at least 24 h and breath samples were collected at time 0 and at frequent intervals for 8 h and at longer intervals thereafter.

Measurement of $^{13}CO_2$ in expired air

In each breath sample, the enrichment of ^{13}C in expired CO_2 was measured by automated gas-isotope-ratio mass spectrometry [19]. Results were expressed as the $\Delta\%$ versus the limestone standard, PeeDee Belemnite. To determine the micromoles of $^{13}CO_2$ released into expired air per minute, the CO_2 production rate can be measured directly or calculated using the estimated BMR [20]. The CO_2 production rate was calculated from the Weir equation [21], which relates energy expenditure to CO_2 production and O_2 consumption, assuming a fasting RQ of 0.80. Using the CO_2 production and isotopic enrichment values, the $^{13}CO_2$ production rate was calculated as well as the percentage dose recovered at each time point [6]. After integration of the latter by the trapezoidal method, the CUMPCD recovered at 480–4320 min was determined. In some instances, the enrichment of ^{13}C in CO_2 in samples of expired air was performed by Metabolic Solutions (Merimack, NH, USA).

Data analysis

The difference between the CUMPCD in different groups of subjects was assessed by Student's *t* test, while

the correlation between the CUMPCD and age was assessed by linear regression.

Patient studies

Subjects were studied in the outpatient or inpatient General Clinical Research Center and outpatient clinic of the Children's Hospital of Philadelphia. The protocol was approved by the Institutional Review Board. Informed consent was obtained from a parent or from each adult subject. All of the patients were on a lactose-restricted diet and had not eaten for at least 2 h before administration of [1-^{13}C]galactose. There were 11 patients ranging in age from 2 to 48 years old. One of the subjects was studied on three occasions. The genomic DNA from each of the subjects with reduced erythrocyte GALT activity was analyzed for a GALT gene mutation [22].

Results

Long-term oxidation in bolus studies

The results of the breath test studies in the 10 patients who received a 7 mg [1-^{13}C]galactose/kg bolus are shown in Table 1. Six of these patients received the bolus as an oral dose, while four received the bolus intravenously. By approximately 24 h, the patients had eliminated 17–50% of the dose as CO_2 in expired air. In Figs. 1A and B, the rate of elimination of $^{13}CO_2$ and the CUMPCD versus time are shown for Patient #6 (weight = 59.2 kg) who eliminated 23% of a bolus of 414 mg of [1-^{13}C]galactose in 24 h. In one subject the elimination at 2640 min was 57% and in another at 4320 min was 44%. There was no obvious relationship between the CUMPCD and either the mode of bolus administration or genotype. At approximately 24 h, the mean CUMPCD ± SD was 26 ± 12 ($n = 6$) in the PO group and 34 ± 4 ($n = 4$) in the IV group. At the same time point, the mean CUMPCD ± SD was 32 ± 11 ($n = 7$) in the del/del and Q188R/Q188R patients, and 25 ± 8 ($n = 3$) in the other patients with uncommon genotypes. There was no correlation between the CUMPCD at approximately 24 h and age (Fig. 2). Also, there was no correlation between the CUMPCD and the mg of [1-^{13}C]galactose administered to each individual (Fig. 3).

Long-term oxidation in continuous intravenous infusion studies

The results of the breath test studies in three patients, two of whom also had a single bolus study are shown in the bottom half of Table 1. One patient had two continuous intravenous studies. In Figs. 1C and D, the rate of elimination of $^{13}CO_2$ and the CUMPCD verses time are shown for Patient #6, the 14-year-old male, who again

Table 1
Long-term oxidation of [1-^{13}C]galactose in patients with galactosemia

Patient	Age	Sex	Wt (kg)	Route[a]	Time (min)	Genotype	CUMPCD (%)
Single bolus							
1	2	F	10	PO	1450	deletion/deletion	17
2	10	M	34	PO	1440	Q188R/Q188R	50
3	10	M	33.6	PO	1440	Q188R/V151A	18
4	11	F	31.5	PO	1440	Q188R/Q188R	27
5	12	M	28	IV	1440	Q188R/M142K	34
6	14	M	59.2	PO	1440	Q188R/Q188R	23
7	30	M	61.4	IV	1440	Q188R/Q188R	29
8	31	F	61.5	IV	1440	Q188R/Q188R	36
9	35	F	109.5	PO	1440	Q188R/Q344K	22
					4320		44
10	48	F	73.8	IV	1380	Q188R/Q188R	39
					2640		57
Continuous infusion							
6	14	M	59.2	IV[b]	1440	Q188R/Q188R	23
7	33	M	62	IV[c]	1440	Q188R/Q188R	22
					1980		42
7	35	M	69.8	IV[d]	1440	Q188R/Q188R	30
11	21	M	109	IV[e]	480	Q188R/Q188R	20
					960		38
					1440		58
					2850		99

[a] Intravenous (IV) or oral (PO) bolus of 7 mg [1-^{13}C]galactose/kg/body weight.
[b] Continuous 24 h IV infusion of 1.0 mg [1-^{13}C]galactose/kg/h following a bolus of 7 mg/kg.
[c] Continuous 24 h IV infusion of 0.76 mg [1-^{13}C]galactose/kg/h following a bolus of 7 mg/kg.
[d] Continuous 24 h IV infusion of 0.76 mg [1-^{13}C]galactose/kg/h with 5% glucose.
[e] Continuous IV infusion of 0.2 mg [1-^{13}C]galactose/kg/h for 8 h, then 0.4 mg/kg/h for 8 h.

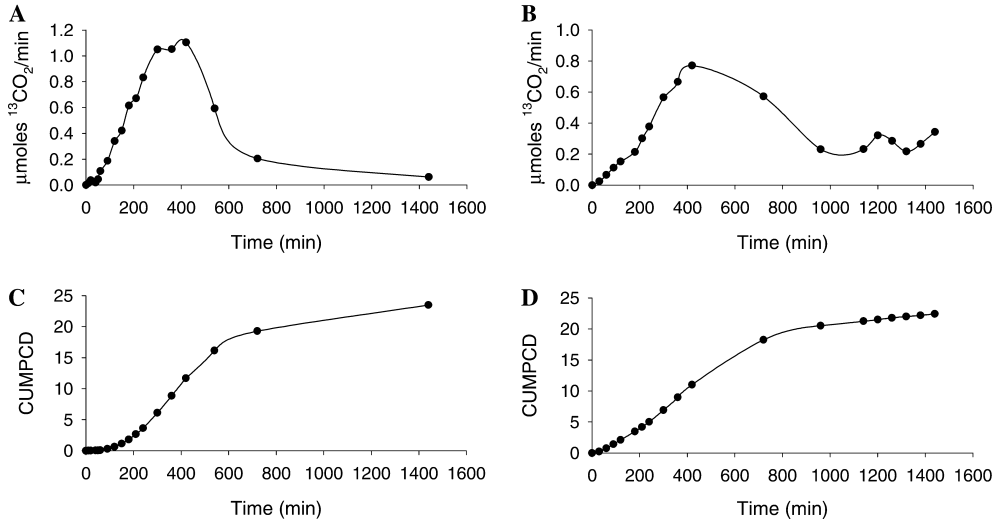

Fig. 1. Breath test in a 14-year-old male (Patient #6) with an oral bolus of 7 mg [1-^{13}C]galactose/kg body weight (A and C) and with a continuous intravenous infusion of [1-^{13}C]galactose (1 mg/kg/h) following a bolus of 7 mg/kg (B and D). Results are shown as µmol $^{13}CO_2$/min versus time in (A) and (B) and the cumulative percent of the dose (CUMPCD) versus time in (C) and (D).

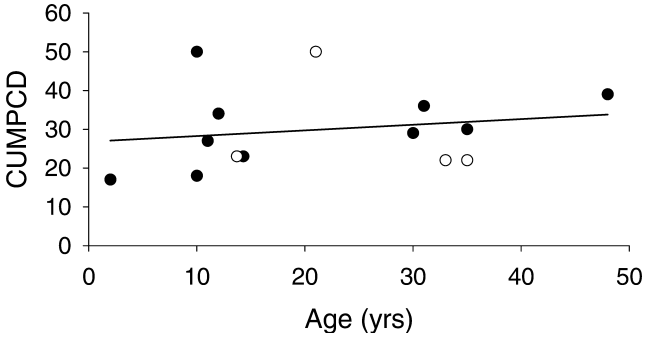

Fig. 2. Relationship between the age of the patient and the CUMPCD at 24 h. The patients who received a continuous intravenous infusion are shown as open circles. The rest of the subjects received an oral or an intravenous bolus alone and are noted as solid black circles.

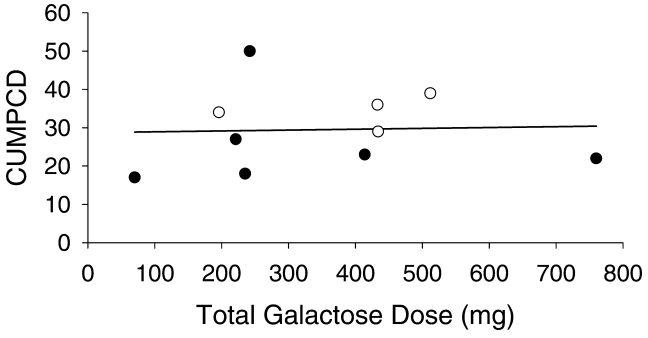

Fig. 3. Relationship between the total dose of galactose in milligrams administered to the subjects over a 24-h period of time and the CUMPCD. The patients who received a continuous intravenous infusion are shown as open circles. The rest of the subjects received an oral or an intravenous bolus alone and are noted as solid black circles.

eliminated 23% of the total 1835 mg of [1-^{13}C]galactose given over the 24-h period of time. The continuous infusion study was performed immediately after the 24-h bolus study. Since the time 0 sample of the infusion study still reflects the increased enrichment of ^{13}C in CO_2, the enhanced enrichment value of $^{13}CO_2$ was used as the baseline for all subsequent enrichment calculations over the 24-h period of time. Patient #7 was studied at both 33 and 35 years of age. In this individual, after 1440 min of a continuous intravenous infusion of [1-^{13}C]galactose, the CUMPCD was 22% and after 1980 min was 42%. Two years later, the CUMPCD after 24 h of the infusion was 30%. In the 21-year-old male, the continuous infusion was administered for 16 h only, and the CUMPCD was 20 and 38% at 480 and 960 min, respectively, during the infusion and 58 and 99% at 1440 and 2850 min, respectively, well after the infusion had stopped.

Discussion

The data provide evidence that patients with galactosemia eliminate a sizeable fraction of the administered isotopically labeled galactose in expired air as carbon dioxide when their breath tests are extended for 24 h or longer. In three of the subjects it was 50% or more and in one it had actually reached 99% at 48 h. The oxidation of galactose is an important route of disposal of galactose even in the patient with classic galactosemia. A scheme depicting a putative model of the economy of galactose in patients with galactosemia is shown in Fig. 4. Input into a theoretical whole body galactose pool shown in the center of the figure comes from diet and endogenous production, probably via turnover of glycoproteins, glycosaminoglycans, and galactocerebrosides. The central route of disposal, the classic Leloir pathway with GALT, will be completely absent in the patient with a GALT

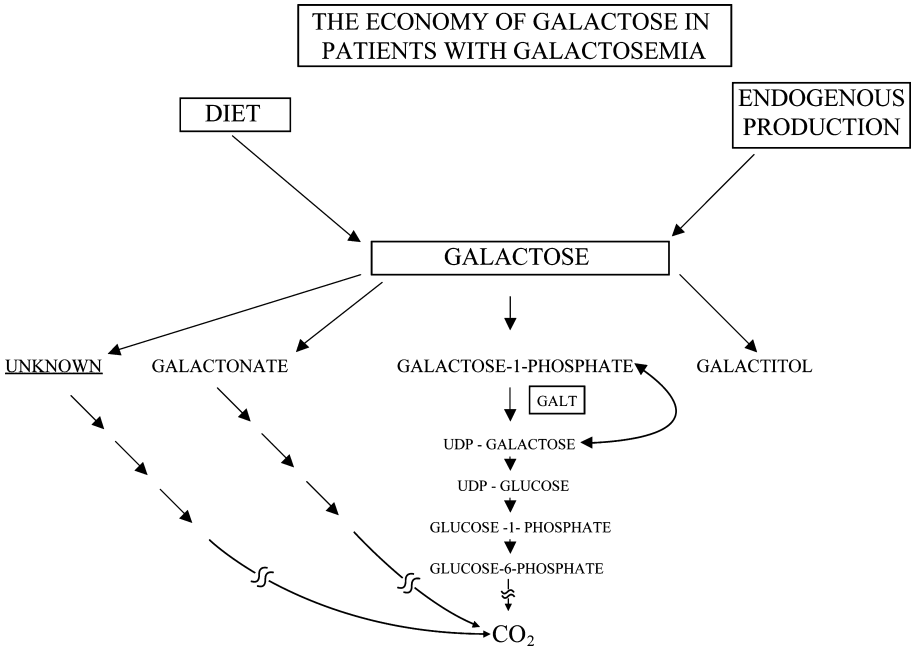

Fig. 4. Galactose in the galactose pool occurs from diet and endogenous production from the turnover of glycoproteins and complex carbohydrates and lipids. The amount entering the pool is balanced by that leaving the pool via (1) urinary excretion of galactitol an end product of metabolism formed by aldose reductase, (2) the formation of galactonate by galactose dehydrogenase and its excretion in urine, (3) the oxidation to CO_2 by further metabolism of galactonate [5], the formation of galactose-1-P and its subsequent disposition by residual GALT as UDP glucose pyrophosphorylase [24] to CO_2. The possibility of other unknown pathways also exists.

deletion/deletion genotype. (Patient #1 in Table 1, the youngest patient and the one with the lowest CUMPCD at 24 h.)

There was no obvious difference in the CUMPCD between classic patients with a Q188R/Q188R or deletion/deletion genotypes and other combination of mutations such as Q188R/V151A, Q188R/M142K, and Q188R/Q344K. In the limited number of patients, there did not appear to be substantial differences in the rates of elimination depending on mode of administration of the bolus dose or whether the labeled sugar was infused intravenously over many hours. Of interest, the 14-year-old male with galactosemia eliminated the same percentage (23%) of the dose of galactose (Fig. 1) even though the amount as an oral bolus was less than a quarter of the total amount of intravenously administered [1-^{13}C]galactose over 24 h (1835 vs 414 mg). Thus, our new long-term oxidation studies appear to match the earlier results of Segal et al. [3] using [1-^{14}C]galactose as a tracer, in that the CUMPCD remained relatively constant even though the subjects with galactosemia received as much as a gram of galactose or as little as a tracer dose alone.

Even in extended breath tests, whether administered orally or intravenously for at least 24 h, there was no relationship between the fractional elimination of the dose and the total amount of galactose given to the subject (Fig. 3). There may be little intrasubject variability in oxidative capacity as one of the subjects, an adult with the Q188R/Q188R genotype who was studied in three separate occasions, had remarkably similar results. In 24 h, he eliminated 29, 22, and 30% of the exogenous galactose as CO_2 at 30, 33, and 35 years of age, respectively. This is reminiscent of our earlier short-term oxidation studies in which we showed reproducibility in breath test results in the individual subject [6,7]. As seen in Figs. 2 and 3, there is a considerable variation in the apparent in vivo hepatic oxidative capacity with a CUMPCD at 24 h that ranges from 17–58%. This data is not incompatible with the hypothesis that residual hepatic GALT activity and/or non-GALT genetic factors determine the in vivo capacity for the individual subject with galactosemia.

This work extends our studies from the previous documentation of long-term oxidation in the patient with the extremely rare deletion/deletion genotype [18]. In that study, we were able to demonstrate in an unambiguous fashion that there is another pathway in man for oxidation of galactose to CO_2. As a consequence of this work in 10 more patients, it is clear that this substantial oxidation is not unique for Patient #1 that has 10 of the eleven GALT exons deleted [18]. As originally suggested by the older work using [1-^{14}C]galactose in breath test extended for 8–10 h [4], the majority, perhaps all, of patients with galactosemia can oxidize galactose in amounts that approaches what a normal individual can do in 5–8 h [6–8], if the breath test is carried out for a long enough period of time. The interesting question is

whether the oxidation is due to residual hepatic GALT enzyme activity or whether it is due to a non-GALT pathway as in the patient with a deletion/deletion genotype. Indeed, recent lymphoblast in vitro studies suggest that cells from Q188R homozygous patients may have residual GALT activity [23]. Yet, the GALT enzyme cannot be contributing to the galactose oxidative capacity in the liver of the rare patient with a homozygous deletion of the GALT gene. The obvious possibilities for the non-GALT pathways include the galactonate [5] and UDPglucose [UDPgalactose] pyrophosphorylase [24] pathways (Fig. 4). There may also be an unknown pathway, or at least one that we have not previously recognized to function in galactose metabolism. Based on 24-h urine analyses of adults with galactosemia and an endogenous production of 1 g galactose per day, we had estimated that the urinary excretion of galactitol and galactonate may account for 20 and 10%, respectively, of the galactose that is synthesized de novo on a daily basis [7]. The contribution of UDPglucose pyrophosphorylase activity to UDPgalactose synthesis and whole body galactose handling is unknown. This reversible enzyme may utilize both galactose-1-phosphate and UDPgalactose as substrates. It was originally hypothesized by Gitzelmann [25] to function in the reverse direction for de novo synthesis of galactose.

It is important to identify and characterize at a molecular level the proteins that are important in these pathways as augmentation of the relevant activity could improve the galactose tolerance of the patient with galactosemia. The relative contributions of different hepatic pathways may even vary from patient to patient. Knowledge concerning these previously poorly delineated or cryptic pathways may prove valuable for the future treatment of patients with GALT deficiency.

Acknowledgments

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