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# Inborn Errors of Galactosemia Metabolism

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## ABSTRACT

**Objectives:** We aimed to assess the level of galactose oxidation in both healthy and galactosemic Middle East children.

**Subjects & Methods:** Twenty-one healthy children and seven children with galactosemia ranging from 1 to 7 years of age were studied. A breath test was used to quantitate 13CO2 enrichment in exhaled air before and at 30, 60, and 120 min after the oral administration of 7 mg/kg of an aqueous solution of 1-13C-galactose to all children. Lactose/galactose restricted formula is provided during the first ten days of life.

**Results:** The neonatal symptoms quickly resolve and the problems related to the mentioned symptoms usually disappear. Besides detecting infants with classic galactosemia, infants with other treatable forms of galactosemia as well as those who are carriers of galactosemia or galactosemic variant are identified by newborn screening. In sick children, the cumulative percentage of 13C from labeled galactose (CUMPCD) in the exhaled air ranged from 0.03% at 30 min to 1.67% at 120 min. In contrast, healthy subjects showed a much broader range in CUMPCD, with values from 0.4% at 30 min to 5.58% at 120 min.

**Conclusion:** The study found a significant difference in galactose oxidation between children with and without galactosemia.

**Key words:** Inborn Errors, Galactosemia, Metabolism.

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## INTRODUCTION

Galactosemia is an inborn error of metabolism, an inherited condition in which an enzyme, galactose-1-phosphate uridyltransfrase (GALT), in the body is either missing or not working well. In classic galactosemia, a sugar (called galactose) is not able to be broken down properly and it builds up in the body.[1]

Galactosemia is a rare genetic metabolic disorder which affects the metabolism of galactose.[2]

The three enzymes of the galactose metabolism pathway are galactokinase**,** galactose-1-phosphate uridyltransfrase (GALT), and uridindiphosphate (UDP) galactose-4-epimerase.[3] Although a deficiency of any of the three enzymes can lead to galactose accumulation in plasma, the term galactosemia is used specifically for GALT deficiency.[4] Profound GALT deficiency which is termed classic galactosemia occurs with a frequency of approximately 1 in 30000 to 1 in 60000 live births.[5] The occurrence of GALT varies according to the population's ethnic origin, with an incidence of 1:30,000 to 1:60,000 newborns in European and North American Caucasians.[6] In South Africa, its incidence is estimated at 1:14,400 newborns in the black population.[7] The incidence is much lower among Asians, with a frequency of less than 1:100,000 newborns.[8] In Middle East, with a population of highly mixed ethnicity, a study conducted in a sample of 60,000 neonates in the State of São Paulo found an incidence of galactosemia of 1:19,984 newborns.[9] Galactosemia is inherited as an autosomal recessive disorder which can be caused by over one hundred mutations. The most common one is Q188R which can induce complete loss of ability to process galactose; yet, other mutations can only diminish the process and the Duarte variant (N314D) has enzyme activity of about 50% of the normal level and usually produces no clinical manifestation.[10]

Classic galactosemia (G/G) is a severe disease which its symptoms are typically seen in second half of the first week of life. The most common initial signs of GALT deficiency are poor growth, irritability, lethargy, vomiting and poor feeding; persistent jaundice may also be seen in the first few weeks of life.[11] By continuing lactose ingestion, multi-organ toxicity syndrome ensues; it is associated with liver disease which can progress to cirrhosis, anemia, brain edema, and kidney damage.[12] Without treatment, mortality and morbidit rate in infants with galactosemia is high.[13] Thus, if lactose/galctose restricted formula is provided during the first ten days of life, the neonatal symptoms quickly resolve and the problems related to the mentioned symptoms usually disappear.[14] Although early diagnosis and treatment have improved the prognosis of galactosemia, patients may still have ovarian failure, mental retardation, speech dyspraxia and ataxia. Yet, mechanisms for these problems are still unknown. Some studies suggest that endogenous production of galactose could be accountable for these long term complications.[15] There are several techniques for the quantitative and qualitative detection of galactose in the blood.[16]

One approach of screening is measuring the GALT activity which mostly detects transferase deficiency irrespectively to prior dietary intake of galactose. Yet, it does not evaluate either epimerase deficiency or galactokinase deficiency. Another approach is to measure total galactose (galactose and galactose-1-phosphate) which depends on prior dietary intake, and thus, it evaluates all three enzyme deficiencies.[17] Besides detecting infants with classic galactosemia, infants with other treatable forms of galactosemia as well as those who are carriers of galactosemia or galactosemic variant are identified by newborn screening.[18]

## MATERIALS AND METHODS

### Study Population

A total of seven galactosemic children ranging in age from 1 to 7 years were included in the study. We also selected a control group of 21 age- and gender-matched healthy volunteer children, giving a ratio of three healthy children for each galactosemic child. Children with respiratory diseases, food allergies, and lactose intolerance were excluded. All procedures were carried out in accordance with the ethical standards of the responsible committee on human experimentation (Research Ethics Committee). Informed consent was obtained from all parents or legal guardians before study enrollment.

**Breath Test:** The breath test measured $^{13}CO_2$ enrichment in expired air before and after the oral administration of an aqueous solution containing 7 mg/kg 1-$^{13}$C-galactose. The children ingested the solution after a 2-h fast. After the first breath collection, additional samples were collected at 30, 60, and 120 min. For the collection of expired air samples, children older than 5 years of age breathed into test tubes using a straw; children younger than 5 years of age breathed normally for 1 min into test tubes positioned immediately below the nostrils. The samples were collected in triplicate and stored in a climatically controlled room until analyzed.

**Measurement of $^{13}CO_2$ in Air:** The molar ratio of $^{13}CO_2$ and $^{12}CO_2$was quantitated in each sample by the mass/charge (m/z) ratio of the stable isotopes using gas-isotope-ratio mass spectrometry. The analysis was carried out using a Europa ANCA 20/20¯ mass spectrometer (UK). The results are reported as delta percent (Δ%) versus the PeeDee Belemnite (PDB) reference standard. Enrichment was determined by subtracting the Δ% PDB of each sample time from the basal Δ% PDB. To determine the μM/min of $^{13}CO_2$ released into the expired air, the rate of total body $CO_2$ production was measured using a combination of the Schofield and Weir equations.

After basal energy expenditure (BEE) calculation using the Schofield equation, the 0.8 respiratory quotient was used to calculate $CO_2$ production using the Weir equation, which relates BEE to $CO_2$ production and $O_2$ consumption. The percentage of $^{13}CO_2$ recovered from the expired air at each time point was determined using the $CO_2$ production value and the isotope enrichment values. Next, the cumulative percentage of ingested 13C-d-galactose retrieved as 13CO2in expired air (CUMPCD) was calculated by the trapezoidal method at each sampling time.

**Statistical Analysis:** The ROC method was used to detect a cut-off point that would allow use of the breath test to diagnose patients with galactosemia. Sensitivity, specificity, and the area under the curve (AUC) were calculated for each cut-off point of the variables of interest. The analysis was carried out using the SAS software, version 9.2 (USA).

A mixed-effects linear model was used for the comparison of mean CUMPCD values. Mixed-effects linear models (random and fixed effects) were used for the analysis of data, in which the responses of the same individual are grouped and for which the assumption of independence between observations within each group was inadequate. The assumption for these models was that their residues had normal distributions with 0 mean and σ2 variance. A quadratic and logarithmic transformation was applied to the response variable in order to satisfy this assumption using the PROC MIXED procedure of the SAS 9.2 software (2008).

**Table 1: Gene characteristics, enzyme activity and %$CO_2$ enrichment with 13C at 120 min in children with galactosemia (GALT).**

| Subject | Age (years) | Gender | GALT activity (umol-h$^{-1}$-g$^{-1}$ Hb) | Mutation | % Enrichment at 120 min |
|---|---|---|---|---|---|
| **A1** | 7 | F | 11 | Q188R/IVS+1 | 0.037 |
| **A2** | 7 | M | Undetectable | Q188R/Q188R | -1.53* |
| **A3** | 7 | M | Undetectable | Q188R/S135L | 4.05 |
| **A4** | 6 | M | 13 | Unknown | 25.14 |
| **A5** | 1 | F | 2.0 | S135L/L275fs | 36 |
| **A6** | 6 | M | Undetectable | Q188R/K285N | 0.19 |
| **A7** | 1 | M | 3.0 | M1T/Q188R | 0.58 |

**Table 2: Results of group comparison at the various times using a mixed-effects linear model.**

| Group | Time | Group | Time | | | LL | UL |
|---|---|---|---|---|---|---|---|
| **A** | 1 | A | 2 | -2.03 | <0.01 | -3.25 | -0.81 |
| **A** | 1 | A | 3 | -6.12 | <0.01 | -7.34 | -4.90 |
| **A** | 2 | A | 3 | -4.09 | <0.01 | -5.31 | -2.87 |
| **H** | 1 | H | 2 | -3.09 | <0.01 | -3.81 | -2.37 |
| **H** | 1 | H | 3 | -5.97 | <0.01 | -6.67 | -5.26 |
| **H** | 2 | H | 3 | -2.88 | <0.01 | -3.59 | -2.16 |
| **A** | 1 | H | 1 | -5.15 | <0.01 | -7.33 | -2.97 |
| **A** | 2 | H | 2 | -6.21 | <0.01 | -8.39 | -4.03 |
| **A** | 3 | H | 3 | -4.99 | <0.01 | -7.17 | -2.82 |

Groups: A: affected group: H: healthy group: Time: 1: 30 min; 2: 60 min; 120 min. LL: lower limit; UL upper limit

**Figure 1: Boxplots of the variables of interest according to group and time: cumulative percent $^{13}CO_2$ from $^{13}C$-labeled galactose. Outliers are indicated by circles.**

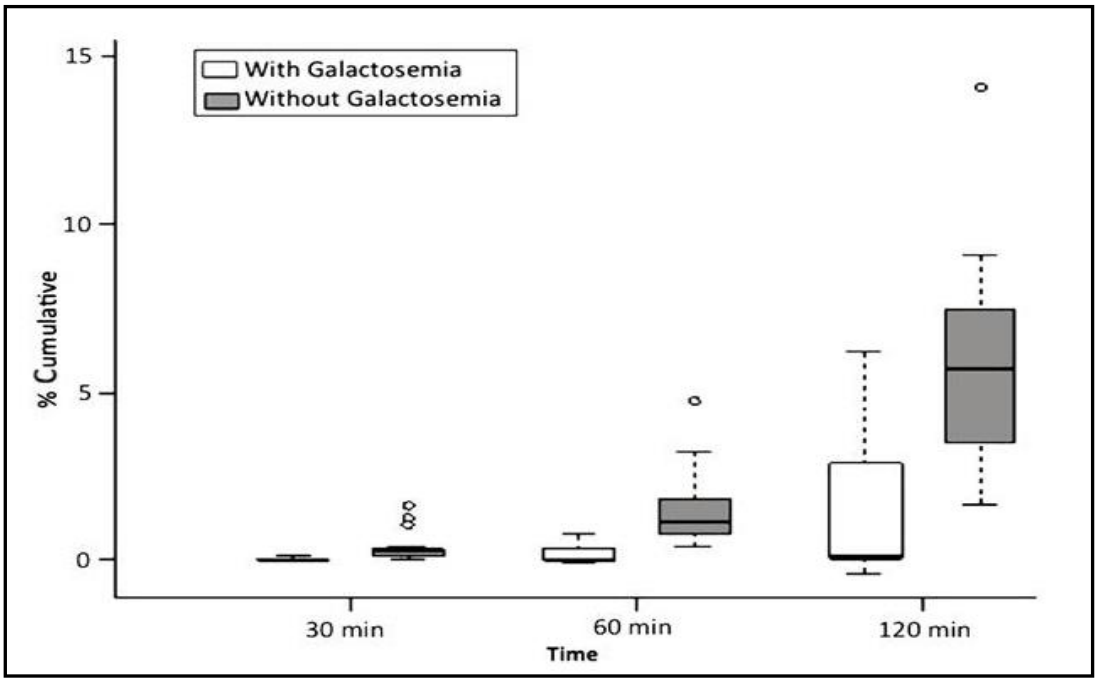
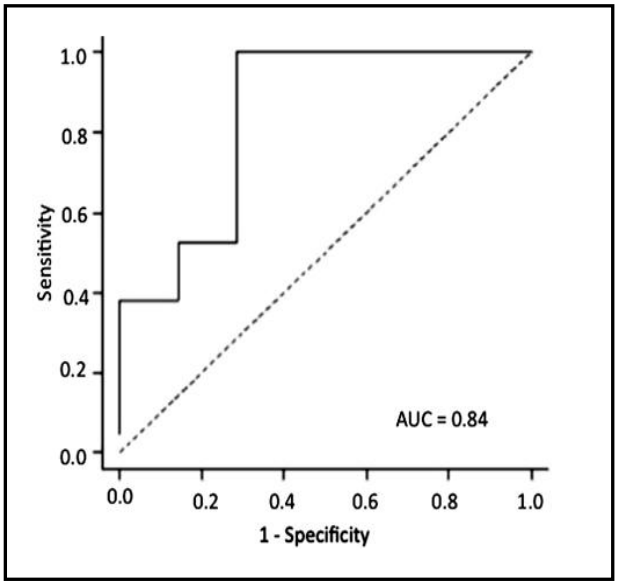



**Figure 2: Receiver operating characteristic (ROC) curve at 120 min.**




## RESULTS

### Characteristics of the Sample

Of the 7 children with the disease, 2 were girls and 5 were boys. The children ranged in age from 1 to 7 years, and their weight and body mass index (BMI) were within normal limits according to WHO 2007 growth curves. The galactosemic children differed from one another in enzyme activity and $^{13}CO_2$ enrichment at 120 min. The GALT activity values were obtained from the results of patient examinations carried out at the Laboratório de Genética Médica de Porto Alegre, Universidade Federal do Rio Grande do Sul. The mutations carried by these patients were identified in an ongoing study titled "Analysis of the genotypic profile of patients with classical galactosemia and study of the genotype-phenotype relationship", conducted by Dr Daniel Fantozzi in collaboration with his mentor Professor Wilson Araújo da Silva Júnior and his co-mentor Professor José Simon Camelo Jr. (unpublished data).

The genetic characteristics, enzyme activity and $\%CO_2$ enrichment with 13C at 120 min in children with galactosemia are reported in Table 1. Child A2, who has a classic homozygosis mutation (Q188R/Q188R), was unable to metabolize the ingested galactose, which was confirmed by the fact that the total carbon recovered from the ingested galactose at 120 min was lower than the baseline value, with a consequent negative final result.

Gene sequencing was not available for the child with an enzyme activity of 1.3 $\mu mol \cdot h^{-1} \cdot g^{-1}$ Hb and a $^{13}C$ enrichment of 25.14% because she was lost to follow-up at the hospital. It is important to note that the M1T, IVS3+1, and L275fs mutations found in the study by Fantozzi D, Camelo Jr JS, Turcato M, Molfetta GA, Souza CFM, Porta G, Steiner CE, and Silva WA (unpublished data) have not been previously described.

The 21 healthy gender- and age-matched control children had the same anthropometric profile as the affected children. Weight was a variable included in the statistical analysis for data correction. The control children showed $^{13}C$ enrichment ranging from 6.98 to 57.05% at 120 min, with a median of 18.83%.

### Profile of $CO_2$ Enrichment and CUMPCD Recovered from Labeled Galactose

All galactosemic children showed low 13C enrichment in expired air and a consequent low CUMPCD, ranging from a mean of 0.03% at 30 min to 1.67% at 120 min. On the other hand, the healthy subjects showed higher 13C enrichment and CUMPCD, ranging from 0.4% at 30 min to 5.58% at 120 min. The difference in CUMPCD was calculated for the 2 groups at 30, 60, and 120 min. Data were compared between the affected and healthy groups and within the same groups. Since CUMPCD increases with time, the results within each group were negative numbers because they were calculated by subtracting the cumulative percentage at the longest time from the cumulative percentage at the shorter time.The calculation also resulted in negative numbers

in the between-groups comparisons because it involved the CUMPCD of the affected group minus the CUMPCD of the healthy group. This indicates that the cumulative percentage of 13C in expired air originating from labeled galactose in the affected group was always lower than that for the healthy group, with differences of 5.15% (95% CI=7.93-2.97) at 30 min; 6.21% (95% CI=8.39-4.03) at 60 min; and 4.99% (95% CI=7.17-2.82) at 120 min.

**Construction of the ROC Curve**

A cut-off point with good sensitivity and specificity was detected for this diagnostic test, albeit with greater sensitivity than specificity. At 120 min, the ROC curve had an AUC of 0.84, 0.95 sensitivity and 0.71 specificity at the cut-off point of 1.94 (Figure 2). However, it did not reach statistical significance.

## DISCUSSION

### Galactose Oxidation in Children With and Without Galactosemia

In the present study, there was a marked difference in galactose oxidation between children with and without galactosemia. In children without the disease, 5.58% of the $^{13}C$ was recovered from labeled galactose at 120 min, as opposed to an average of only 1.67% for the affected children. This corresponds to a 4.99% difference ($P<0.01$) between the affected and the healthy groups in cumulative percentage of $^{13}C$ in expired air originating from labeled galactose 2 h after the ingestion of the solution containing 1-$^{13}C$-galactose.[1]

Elsas et al. detected a CUMPCD of less than 1.48% at 120 min among affected individuals with more severe manifestations (i.e., classic galactosemia) and a CUMPCD ranging from 7.31 to 25.81% among patients with a variant phenotype. The values observed in both patient groups were lower than those in individuals without a diagnosis of the disease, where the percentage of 13C from galactose ranged from 8.47 to 28.23% at 120 min.

Barbouth et al. also detected a significant difference in the capacity of galactose oxidation between children with galactosemia and neonates without the disease. The authors reported a mean CUMPCD of 0.39% at 120 min in a group of galactosemic children 1 month to 12 years of age, whereas the corresponding values in 2- and 14-day-old neonates without the disease were 4.4 and 9.96%, respectively.[13]

Another important observation in this study was that patient A4, with low erythrocyte GALT activity, had an increased rate of galactose oxidation, similar to that in control children (Table 1). This child had a GALT activity of 1.3 μmol·h-1·g-1 Hb but reached a $^{13}CO_2$ enrichment of 25.14% at 120 min. This patient did not follow treatment correctly and thus was lost to follow-up and his mutation could not be described.

Some authors have related this finding to residual GALT activity in organs such as the liver, in which the synthesis and degradation of the mutant GALT protein reach an equilibrium differing from that reached in the erythrocytes. In addition, it should be pointed out that $CO_2$ enrichment with $^{13}C$ from 1-$^{13}C$-galactose depends on the total body quantity of the unlabeled precursor. Hence, treated galactosemic children must have a smaller amount of unlabeled galactose than untreated children.[4]

Berry et al. reported that variations in the hepatic capacity for galactose oxidation occur over extended time intervals in individuals with galactosemia, with CUMPCD ranging from 17 to 58% during a 1-day period. That result is not incompatible with the hypothesis that the residual hepatic GALT activity and/or GALT-independent genetic factors may determine the capacity of galactose oxidation in individuals with galactosemia. These authors stated that individuals with galactosemia, when evaluated for longer periods of time (5 to 8 h), are able to oxidize galactose in amounts close to those oxidized by normal individuals. Thus, the question is whether this oxidation is due to residual hepatic enzymatic activity or whether a still-unknown GALT-independent pathway exists. Indeed, recent studies have suggested that residual enzymatic activity exists in patients homozygous for the Q188R mutation (the classic form of the disease), whereas in patients with GALT gene deletion in homozygosis, an extremely rare mutation, the existence of residual GALT activity would be impossible. Other possibilities would be the GALT-independent pathways, including the galactonate and UDP-glucose pyrophosphorylase pathways, in addition to some still unknown pathways as part of galactose metabolism. It is important to identify and characterize all proteins involved in this pathway at the molecular level, as their use may be of help in finding ways that would increase galactose tolerance in galactosemic patients.[1]

### Detection of Galactosemia by Breath Testing

When the cumulative $^{13}CO_2$ percentage in expired air from the ingested 1-$^{13}C$-galactose was plotted on a ROC curve at the 120 min time point, a cut-off point of 1.94% was detected, with an AUC of 0.84, 0.95 sensitivity and 0.71 specificity. However, the study sample size of 28 children was not large enough to reach statistical significance in order to determine whether this galactose oxidation value could be used as a cut-off point for the screening of galactosemic patients. Even so, the value was highly suggestive of a positive diagnosis of the disease. In a study of 18 affected and 14 healthy subjects, Barbouth et al. tried to validate use of the ROC curve at 120 min. Those authors detected a cut-off point of 1.17%, with 0.97 sensitivity and 0.96 specificity, but they also did not achieve statistical significance because of their small sample size.[13]

### Phenotypic Expression and Breath Test

The M1T, IVS3+1 and L275fs mutations identified in the present study have not been described previously. The carriers' phenotypic expression of these mutations, as well as the GALT activity and the mechanisms of galactose oxidation, may be differentiated in these patients and require further study for a better understanding. The genetic heterogeneity of the Middle Eastern population that has been documented clearly contributes to understanding the phenotypic heterogeneity observed in Middle eastern children with galactosemia.[16]

According to Berry et al. and Lai, the breath test with the ingestion of 1-$^{13}C$-galactose is useful for the determination of the metabolic phenotype of children with GALT deficiency, permitting the degree of deficiency of metabolic galactose oxidation in those patients to be established, and to assess the severity of the mutation in question. Other studies have demonstrated that patients with classic galactosemia eliminated less than 2% of the ingested 1-$^{13}C$-galactose bolus as $^{13}CO_2$ in expired air at 120 min and have reported that a cut-off point of 5% for CUMPCD was significant for discriminating the clinical course of patients with GALT deficiency.

Barbouth et al. have suggested that if less than 2% $^{13}CO_2$ is recovered in the breath test 2 h after the ingestion of labeled galactose, then the children should start ingesting soy milk instead

of cow's or mother's milk until the result of traditional screening tests is obtained. On this basis, it would not be necessary to collect blood samples, and the intervention could be made before neurological or hepatocellular damage occurs.

In this study, the breath test results revealed that the indicated enzyme activity was not always a good predictor of how much galactose the child was able to oxidize. It shows that a child with an enzyme activity of 11 $\mu mol \cdot h^{-1} \cdot g^{-1}$ Hb had a 0.037 % $^{13}CO_2$ enrichment in expired air, whereas a child with undetectable enzyme activity had a 4.05% enrichment. The specificity of the breath test was probably lower than that reported in the study by Barbouth et al. because of these variations. According to Berry et al. the breath test is more useful for the determination of the patients who manifest the disease, or who manifest much like patients with the classic mutation (Q188R/Q188R). The test does not reveal which patients with a normal oxidative capacity at 2 h will manifest abnormal galactose tolerance should they consume foods that are a source of galactose. The recovery of $^{13}CO_2$-labeled galactose was greater in healthy children than in galactosemic children. However, the sample size of 28 children was not enough to reach statistical significance in order to determine whether this galactose oxidation value could be used as a cut-off point for screening galactosemic patients.

On the other hand, the breath test following ingestion of 1-$^{13}$C-galactose is useful for permitting the determination of the degree of deficiency in total body galactose oxidation and assessment of the severity of the mutation.

On this basis, the breath test, in addition to permitting the doctor to determine whether a child with a known genotype has an oxidative phenotype similar to that of patients with more severe disease, can also be a starting point for determining the quantity of galactose that a child can ingest, which will improve quality of life and increase the variability of the diet.[16]

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