

Galactose oxidation using ^{13}C in healthy and galactosemic children

D.R. Resende-Campanholi¹, G. Porta², E. Ferrioli³, K. Pfrimer³,
L.A. Del Ciampo¹ and J.S. Camelo Junior¹

¹Departamento de Puericultura e Pediatria, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

²Unidade de Hepatologia Pediátrica, Instituto da Criança Prof. Pedro de Alcântara, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brasil

³Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

Abstract

Galactosemia is an inborn error of galactose metabolism that occurs mainly as the outcome of galactose-1-phosphate uridylyltransferase (GALT) deficiency. The ability to assess galactose oxidation following administration of a galactose-labeled isotope ($1\text{-}^{13}\text{C}$ -galactose) allows the determination of galactose metabolism in a practical manner. We aimed to assess the level of galactose oxidation in both healthy and galactosemic Brazilian children. 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 $^{13}\text{CO}_2$ 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 $1\text{-}^{13}\text{C}$ -galactose to all children. The molar ratios of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ were quantified by the mass/charge ratio (m/z) of stable isotopes in each air sample by gas-isotope-ratio mass spectrometry. In sick children, the cumulative percentage of ^{13}C 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. The study found a significant difference in galactose oxidation between children with and without galactosemia, demonstrating that the breath test is useful in discriminating children with GALT deficiencies.

Key words: Galactosemias; Isotope labeling; Children; Breath tests

Introduction

Galactosemia (Online Mendelian Inheritance in Man database code 230400) is an inborn error of galactose metabolism caused by a deficiency of one of the three main enzymes involved in galactose metabolism (1). Classic galactosemia (GALT), the predominant form, is secondary to a severe reduction or absence of galactose-1-phosphate-uridylyltransferase (EC 2.7.7.12) (2,3).

The main clinical manifestations, which usually appear during the neonatal period and are frequently life-threatening, include failure to thrive, vomiting, diarrhea, jaundice, hepatic dysfunction or hepatomegaly, and severe hemolytic anemia (1,4,5).

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 Brazil, 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).

The treatment of the disease is based on dietary intervention. When a clinical suspicion of galactosemia exists, or in asymptomatic cases with a positive neonatal screening, galactose must be removed from the diet even before biochemical confirmation of the diagnosis. Unfortunately, despite dietary restriction of galactose intake, some patients with GALT deficiency eventually show persistent increases in erythrocyte concentration of galactose-1-phosphate and in the excretion of urinary galactitol (10). Gitzelmann et al. (11) suggested that endogenous galactose production is the mechanism underlying this occurrence, producing a chronic state of "self-intoxication".

The use of stable isotopes of carbon to show the galactose oxidation profile has improved our knowledge of galactose metabolism. Isotope tracing has shown that

Correspondence: J.S.Camelo Junior: <jscamelo@fmrp.usp.br>.

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both patients with variant genotype for galactosemia and healthy individuals synthesize similar quantities of galactose, and patients with more severe genotypes have a considerably reduced ability to metabolize the galactose that is produced.

The oxidation profile of galactose was studied by breath test analysis in a group of Brazilian children to better understand its metabolism. The specific objectives were to assess, for the first time, the capacity for galactose oxidation in Brazilian children with and without galactosemia and to determine, using a receiver operating characteristic (ROC) curve, whether there is a cut-off point whereby the breath test can discriminate among individuals with and without galactosemia.

Material and Methods

Study population

A total of seven galactosemic children ranging in age from 1 to 7 years were included in the study. Four were from Hospital das Clínicas, Faculdade de Medicina de Ribeirão Preto, USP, and three were followed up at the Instituto da Criança, USP. We also selected a control group of 21 age- and gender-matched healthy volunteer children, followed up at the Clínica de Pediatria at Hospital das Clínicas de Ribeirão Preto, USP, 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, Hospital das Clínicas, Faculdade de Medicina de Ribeirão Preto, USP, Brazil; Protocol HCRP #2450/2011), CNS/MS resolution (#196/96), and the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all parents or legal guardians before study enrollment.

Breath test

The breath test measured $^{13}\text{CO}_2$ enrichment in expired air before and after the oral administration of an aqueous solution containing 7 mg/kg $1\text{-}^{13}\text{C}$ -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 $^{13}\text{CO}_2$ in air

The molar ratio of $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ 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 in the Laboratório de Espectrometria de Massa of the Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, USP, using a Europa ANCA 20/20[®] mass spectrometer (UK). The results are reported as delta percent ($\Delta\%$) versus the PeeDee Belemnite (PDB) reference standard.

Enrichment was determined by subtracting the $\Delta\%$ PDB of each sample time from the basal $\Delta\%$ PDB. To determine the $\mu\text{M}/\text{min}$ of $^{13}\text{CO}_2$ released into the expired air, the rate of total body CO_2 production was measured using a combination of the Schofield and Weir equations (12,13).

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 $^{13}\text{CO}_2$ 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 ^{13}C -D-galactose retrieved as $^{13}\text{CO}_2$ in 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 σ^2 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).

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 $^{13}\text{CO}_2$ 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 %CO₂ enrichment with ¹³C at 120 min in children with galactosemia are reported in Table 1. Child A2, who has a classic homozygosity 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 μmol·h⁻¹·g⁻¹ Hb and a ¹³C 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 ¹³C enrichment ranging from 6.98 to 57.05% at 120 min, with a median of 18.83%.

Profile of CO₂ 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 (Figure 1).

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 (Table 2).

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 ¹³C 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 ¹³C in expired air originating

Table 1. Gene characteristics, enzyme activity and %CO₂ enrichment with ¹³C at 120 min in children with galactosemia (GALT).

Subject	Age (years)	Gender	GALT activity (μmol·h ⁻¹ ·g ⁻¹ Hb)	Mutation	%Enrichment at 120 min
A1	7	F	11	Q188R/IVS3+1	0.037
A2	7	M	Undetectable	Q188R/Q188R	-1.53*
A3	7	M	Undetectable	Q188R/S135L	4.05
A4	6	M	1.3	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

* The minus symbol indicates that CO₂ enrichment with ¹³C recovered in expired air at 120 min was lower than the baseline value. Thus, there was no enrichment.

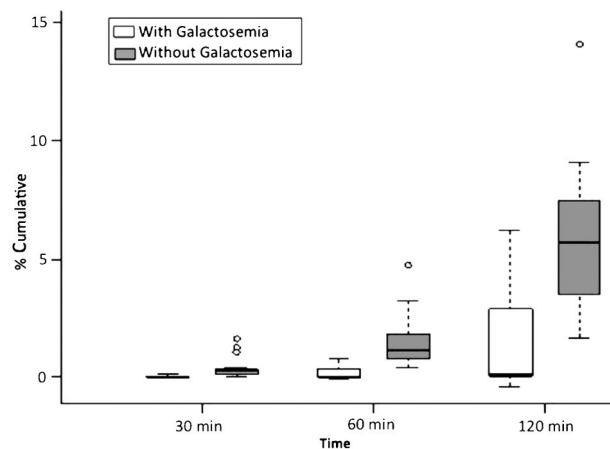


Figure 1. Boxplots of the variables of interest according to group and time: cumulative percent ^{13}C from ^{13}C -labeled galactose. Outliers are indicated by circles.

from labeled galactose 2 h after the ingestion of the solution containing $1\text{-}^{13}\text{C}$ -galactose.

Elsas et al. (14) 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 ^{13}C from galactose ranged from 8.47 to 28.23% at 120 min.

Barbouth et al. (15) 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.

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 $1.3\ \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}\ \text{Hb}$ but reached a $^{13}\text{CO}_2$ 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 $1\text{-}^{13}\text{C}$ -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 (15).

Berry et al. (16) 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

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

Group	Comparisons		Estimate	P	95%CI		
	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; 3: 120 min. LL: lower limit; UL: upper limit.

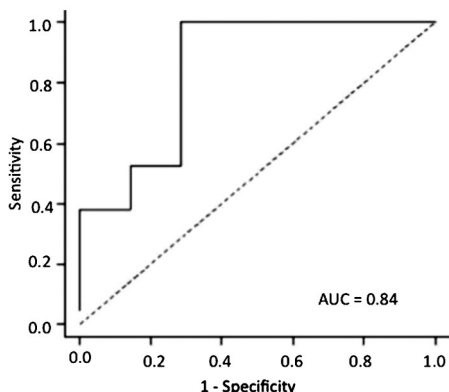


Figure 2. Receiver operating characteristic (ROC) curve at 120 min.

and UDP-glucose pyrophosphorylase pathways, in addition to some still unknown pathways as part of galactose metabolism (17). 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.

Detection of galactosemia by breath testing

When the cumulative $^{13}\text{CO}_2$ percentage in expired air from the ingested $1\text{-}^{13}\text{C}$ -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. (15) 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.

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 Brazilian population that has been documented clearly contributes to understanding the phenotypic heterogeneity observed in Brazilian children with galactosemia.

According to Berry et al. (18) and Lai (19), the breath test with the ingestion of $1\text{-}^{13}\text{C}$ -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 $1\text{-}^{13}\text{C}$ -galactose bolus as $^{13}\text{CO}_2$ in expired air at 120 min (20) and have reported that a cut-off point of 5% for CUMPCD was significant for discriminating the clinical course of patients with GALT deficiency (13,21,22).

Barbouth et al. (15) have suggested that if less than 2% $^{13}\text{CO}_2$ 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. Table 1 shows that a child with an enzyme activity of $11\ \mu\text{mol}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$ Hb had a 0.037% $^{13}\text{CO}_2$ 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. (15) because of these variations. According to Berry et al. (18), 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 $^{13}\text{CO}_2$ -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 $1\text{-}^{13}\text{C}$ -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.

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References

- Schweitzer-Krantz S. Early diagnosis of inherited metabolic disorders towards improving outcome: the controversial issue of galactosaemia. *Eur J Pediatr* 2003; 162 (Suppl 1): S50-S53, doi: 10.1007/s00431-003-1352-2.
- Bosch AM. Classical galactosaemia revisited. *J Inherit Metab Dis* 2006; 29: 516-525, doi: 10.1007/s10545-006-0382-0.
- de Jongh WA, Bro C, Ostergaard S, Regenber B, Olsson L, Nielsen J. The roles of galactitol, galactose-1-phosphate, and phosphoglucomutase in galactose-induced toxicity in *Saccharomyces cerevisiae*. *Biotechnol Bioeng* 2008; 101: 317-326, doi: 10.1002/bit.21890.
- Kaufman FR, Bride-Chang C, Manis FR, Wolff JA, Nelson MD. Cognitive functioning, neurologic status and brain imaging in classical galactosemia. *Eur J Pediatr* 1995; 154: S2-S5, doi: 10.1007/BF02143794.
- Bosch AM, Grootenhuis MA, Bakker HD, Heijmans HS, Wijburg FA, Last BF. Living with classical galactosemia: health-related quality of life consequences. *Pediatrics* 2004; 113: e423-e428, doi: 10.1542/peds.113.5.e423.
- Clague A, Thomas A. Neonatal biochemical screening for disease. *Clin Chim Acta* 2002; 315: 99-110, doi: 10.1016/S0009-8981(01)00716-1.
- Henderson H, Leisegang F, Brown R, Eley B. The clinical and molecular spectrum of galactosemia in patients from the Cape Town region of South Africa. *BMC Pediatr* 2002; 2: 7, doi: 10.1186/1471-2431-2-7.
- Padilla CD, Dans LF, Estrada SC, Tamondong MR Jr, Lacleste JJ, Bernal RM. Cost-benefit analysis of newborn screening for galactosemia in the Philippines. *Southeast Asian J Trop Med Public Health* 2003; 34 (Suppl 3): 215-220.
- Camelo JS Jr, Fernandes MI, Maciel LM, Scrideli CA, Santos JL, Camargo AS Jr, et al. Galactosaemia in a Brazilian population: high incidence and cost-benefit analysis. *J Inherit Metab Dis* 2009; 32 (Suppl 1): S141-S149, doi: 10.1007/s10545-009-1112-1.
- Berry GT, Palmieri M, Gross KC, Acosta PB, Henstenburg JA, Mazur A, et al. The effect of dietary fruits and vegetables on urinary galactitol excretion in galactose-1-phosphate uridylyltransferase deficiency. *J Inherit Metab Dis* 1993; 16: 91-100, doi: 10.1007/BF00711320.
- Gitzelmann R. Formation of galactose-1-phosphate from uridine diphosphate galactose in erythrocytes from patients with galactosemia. *Pediatr Res* 1969; 3: 279-286, doi: 10.1203/00006450-196907000-00003.
- Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. *Hum Nutr Clin Nutr* 1985; 39 (Suppl 1): 5-41.
- Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 1949; 109: 1-9.
- Elsas LJ, Ellerine NP, Klein PD. Practical methods to estimate whole body leucine oxidation in maple syrup urine disease. *Pediatr Res* 1993; 33: 445-451, doi: 10.1203/00006450-199305000-00005.
- Barbouth DS, Velazquez DL, Konopka S, Wilkinson JJ, Carver VH, Elsas LJ. Screening newborns for galactosemia using total body galactose oxidation to CO₂ in expired air. *Pediatr Res* 2007; 62: 720-724, doi: 10.1203/PDR.0b013e3181598cdf.
- Berry GT, Reynolds RA, Yager CT, Segal S. Extended [¹³C] galactose oxidation studies in patients with galactosemia. *Mol Genet Metab* 2004; 82: 130-136, doi: 10.1016/j.yimgme.2004.03.003.
- Abraham HD, Howell RR. Human hepatic uridine diphosphate galactose pyrophosphorylase. Its characterization and activity during development. *J Biol Chem* 1969; 244: 545-550.
- Berry GT, Nissim I, Gibson JB, Mazur AT, Lin Z, Elsas LJ, et al. Quantitative assessment of whole body galactose metabolism in galactosemic patients. *Eur J Pediatr* 1997; 156 (Suppl 1): S43-S49, doi: 10.1007/PL00014271.
- Lai K, Langley SD, Dembure PP, Hjelm LN, Elsas LJ. Duarte allele impairs biostability of galactose-1-phosphate uridylyltransferase in human lymphoblasts. *Hum Mutat* 1998; 11: 28-38, doi: 10.1002/(SICI)1098-1004(1998)11:1<28::AID-HUMU5>3.0.CO;2-H.
- Berry GT, Singh RH, Mazur AT, Guerrero N, Kennedy MJ, Chen J, et al. Galactose breath testing distinguishes variant and severe galactose-1-phosphate uridylyltransferase genotypes. *Pediatr Res* 2000; 48: 323-328, doi: 10.1203/00006450-200009000-00010.
- Guerrero NV, Singh RH, Manatunga A, Berry GT, Steiner RD, Elsas LJ. Risk factors for premature ovarian failure in females with galactosemia. *J Pediatr* 2000; 137: 833-841, doi: 10.1067/mpd.2000.109148.
- Webb AL, Singh RH, Kennedy MJ, Elsas LJ. Verbal dyspraxia and galactosemia. *Pediatr Res* 2003; 53: 396-402, doi: 10.1203/01.PDR.0000049666.19532.1B.