Galactose Production and Disposal in Patients with GALT Deficiency

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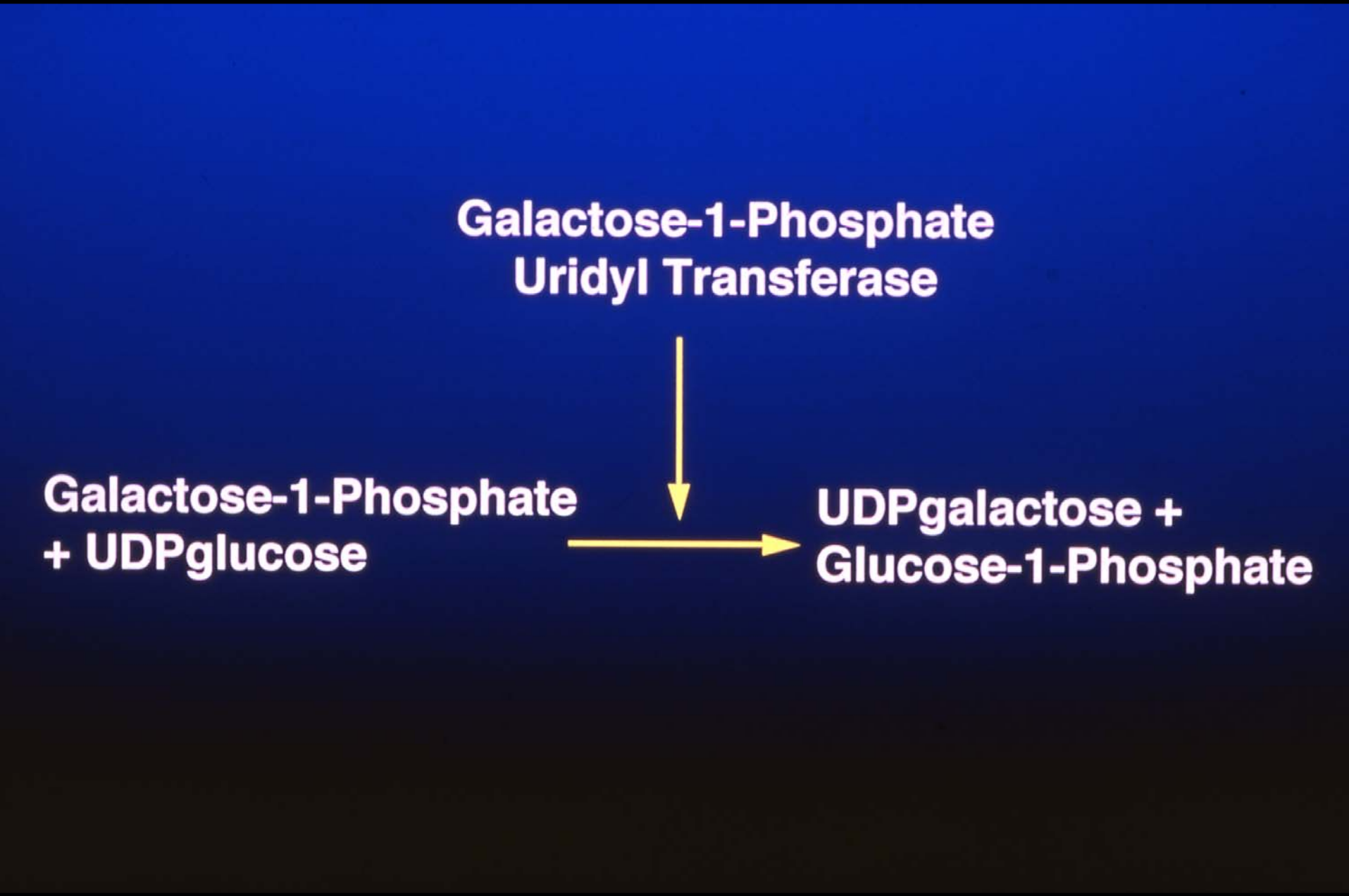
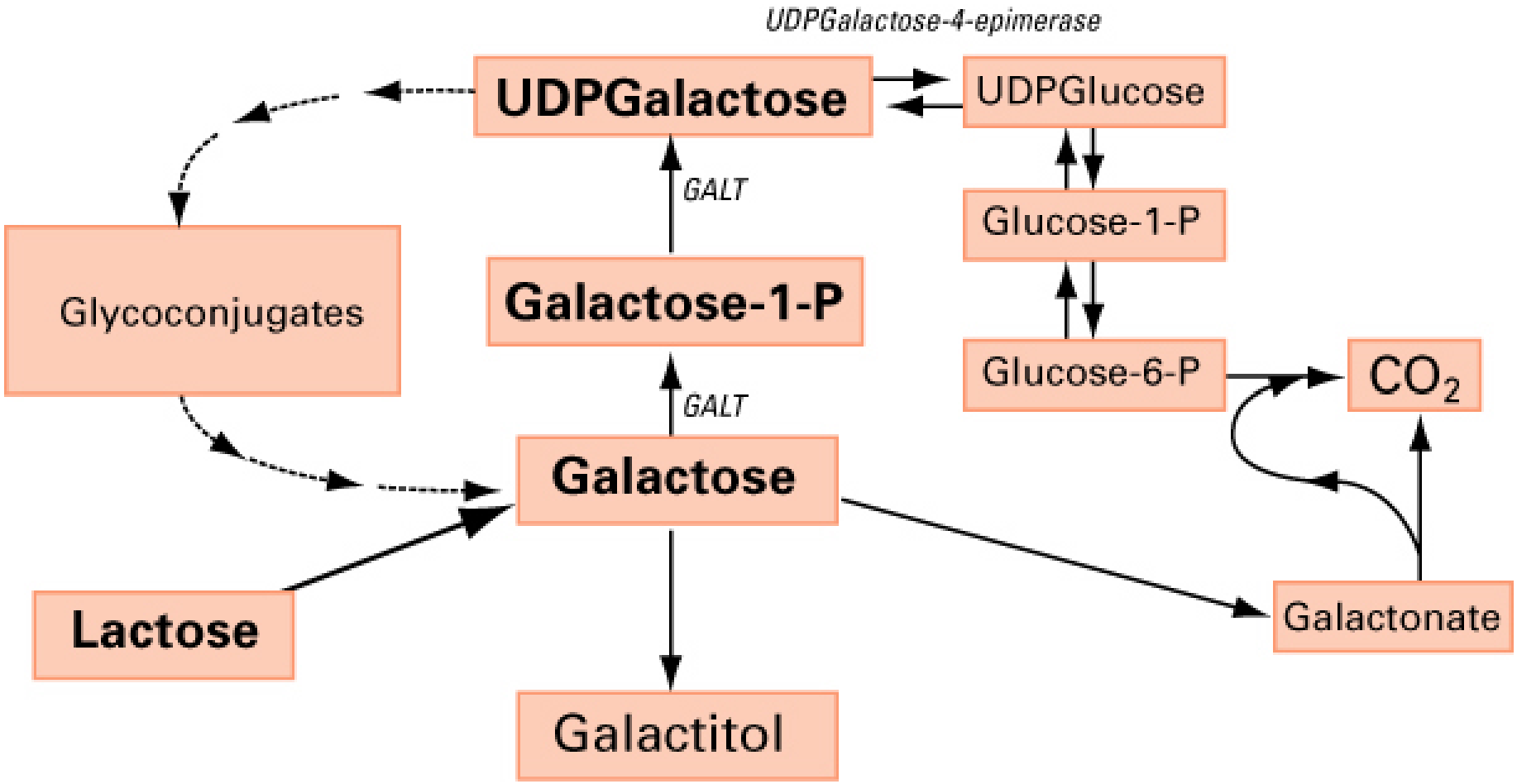
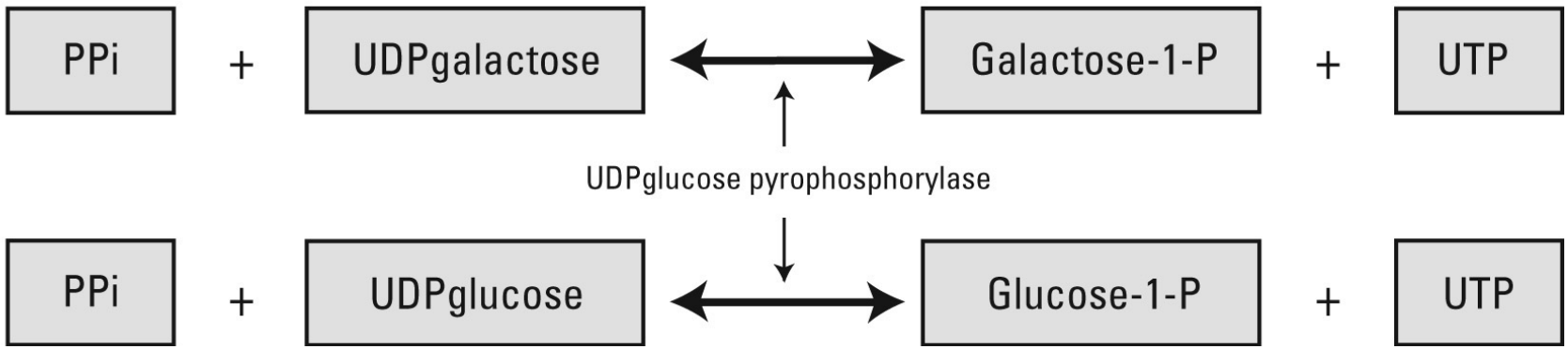

Galactose-1-Phosphate
Uridyl Transferase
Galactose-1-Phosphate
+ UDPglucose
UDPgalactose +
Glucose-1-Phosphate


UDPGalactose-4-epimerase
UDPGalactose
UDPGlucose
GALT
Glucose-1-P
Glycoconjugates
Galactose-1-P
GALT
Glucose-6-P
CO_2
Galactose
Lactose
Galactonate
Galactitol


PPi
+
UDPgalactose
Galactose-1-P
+
UTP
UDPglucose pyrophosphorylase
PPi
+
UDPglucose
Glucose-1-P
+
UTP

Galactose Appearance Rate (GAR) in patients with GALT deficiency

- Infants and children, 1 month-14 years old:

  1.34 +/- 0.53 mg / kg / h (n=17)

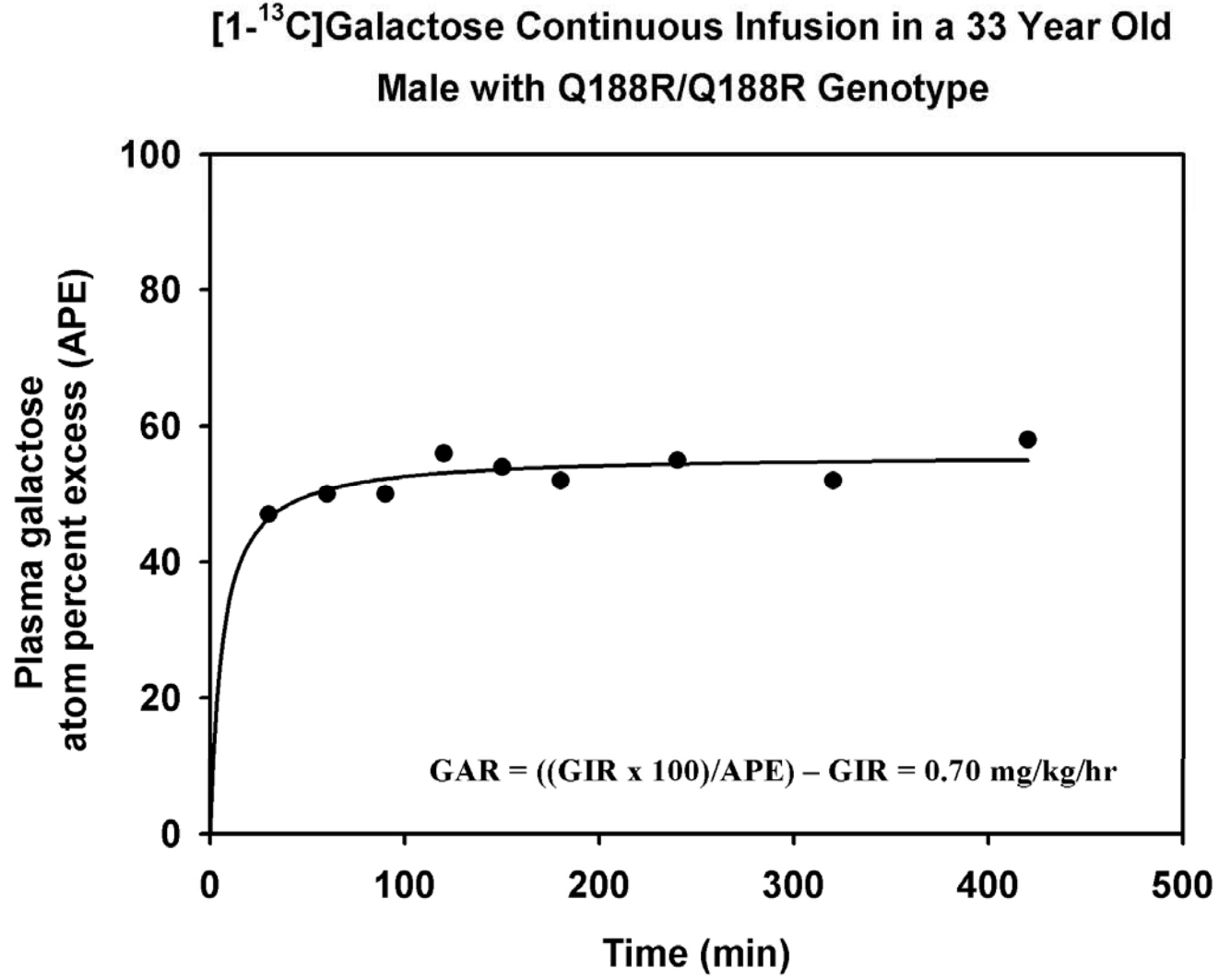
  (7.4 umol/kg/h)

- Adults, 19-33 years old:

  0.56 +/- 0.01 mg / kg / h (n=5)

  (3.1 umol/kg/h)

Berry et al, MGM: 81, 2004


[1-13C]Galactose Continuous Infusion in a 33 Year Old Male with Q188R/Q188R Genotype
Plasma galactose atom percent excess (APE)
100
80
60
40
20
0
0
100
200
300
400
500
Time (min)
GAR = ((GIR x 100)/APE) – GIR = 0.70 mg/kg/hr

Continuous Infusion Method (CIM)

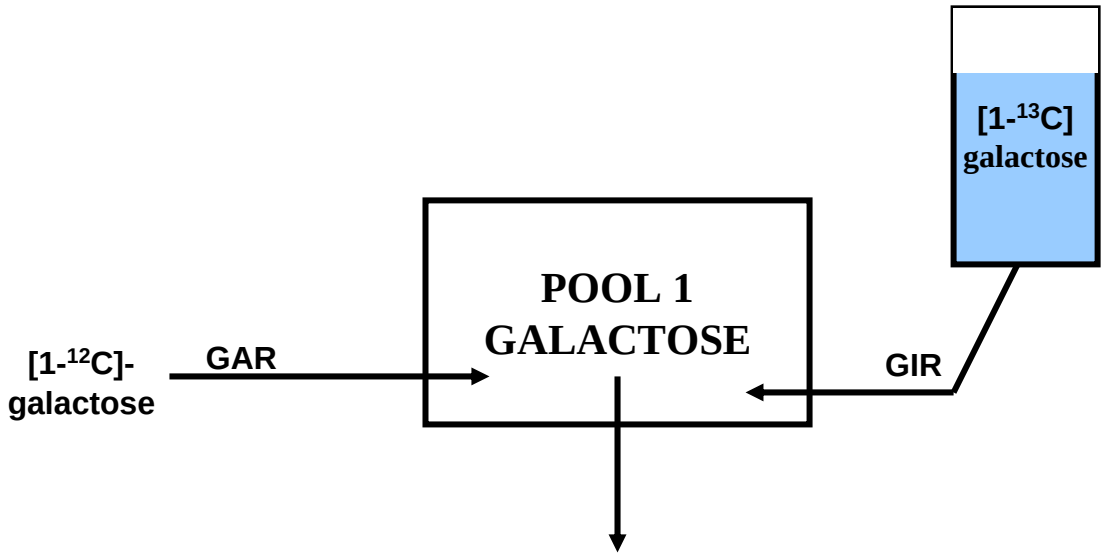
Administer 7 mg/kg body weight priming dose (or 0-10 mg/kg), followed by a continuous infusion of 0.76 mg/kg/h (or 0.2-5 mg/kg/h)

Genotypes of the Human Subjects

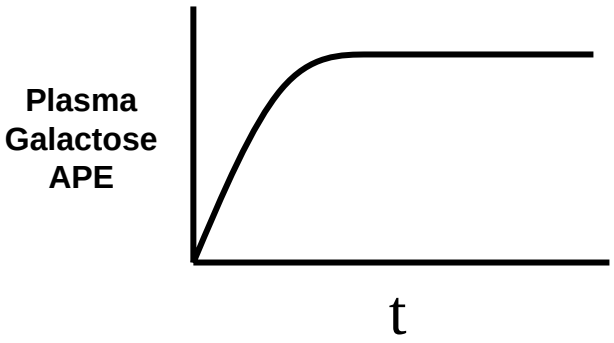
Genotype	Number
Q188R/Q188R	12
Q188R/H184Q	2
Q188R/Q344K	1
Q188R/D98H	1
Q188R/R123Q	1
Q188R/M142K	1
Q188R/S135L	1
Q188R/unknown	3
N/N	6
Total	28

Limitations of the Continuous Infusion Method

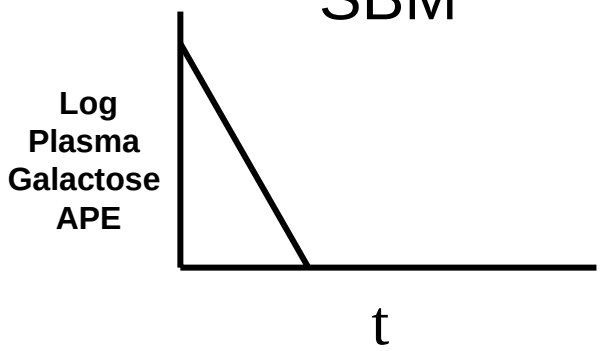
- Establishment of steady-state labeling of galactose in body pool(s)
- Tracer must not perturb body pool(s) size(s)
- No recycling of label, eg [1-^{13}C]-galactose $\rightarrow$ [1-^{13}C]-glucose $\rightarrow$ [1-^{13}C]-galactose



CIM



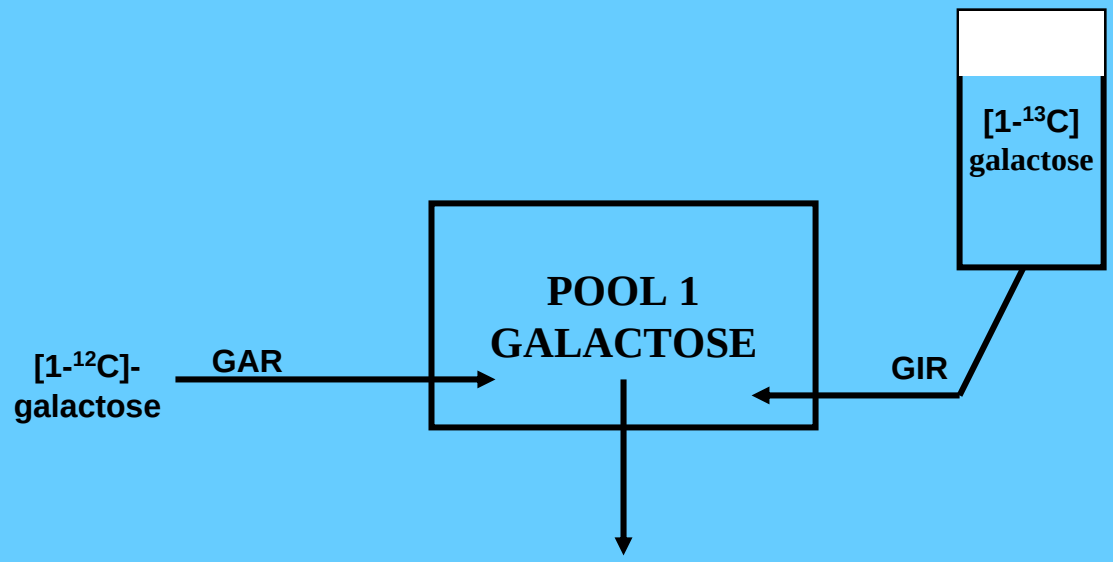
SBM

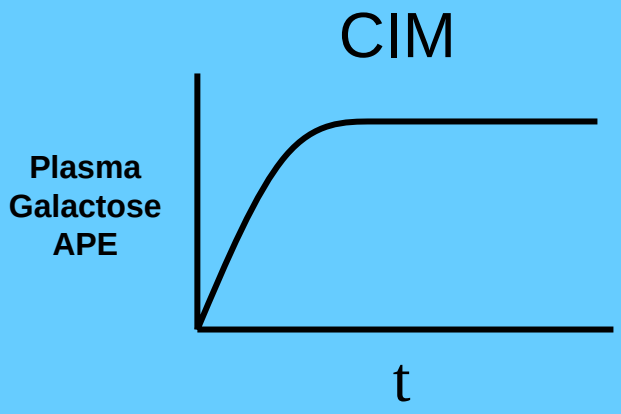


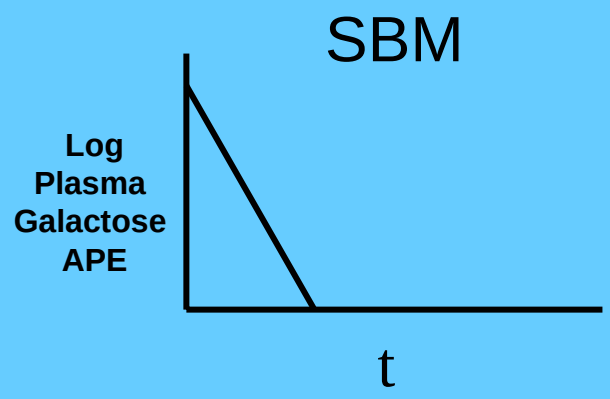
$$\frac{[1\text{-}^{13}C]\text{-Galactose}}{[1\text{-}^{13}C]\text{- + }[1\text{-}^{12}C]\text{-Galactose}} = \frac{GIR}{GIR + GAR}$$

GAR = Galactose Appearance Rate
GIR = Galactose Infusion Rate

CIM = Constant Infusion Method
SBM = Single Bolus Method







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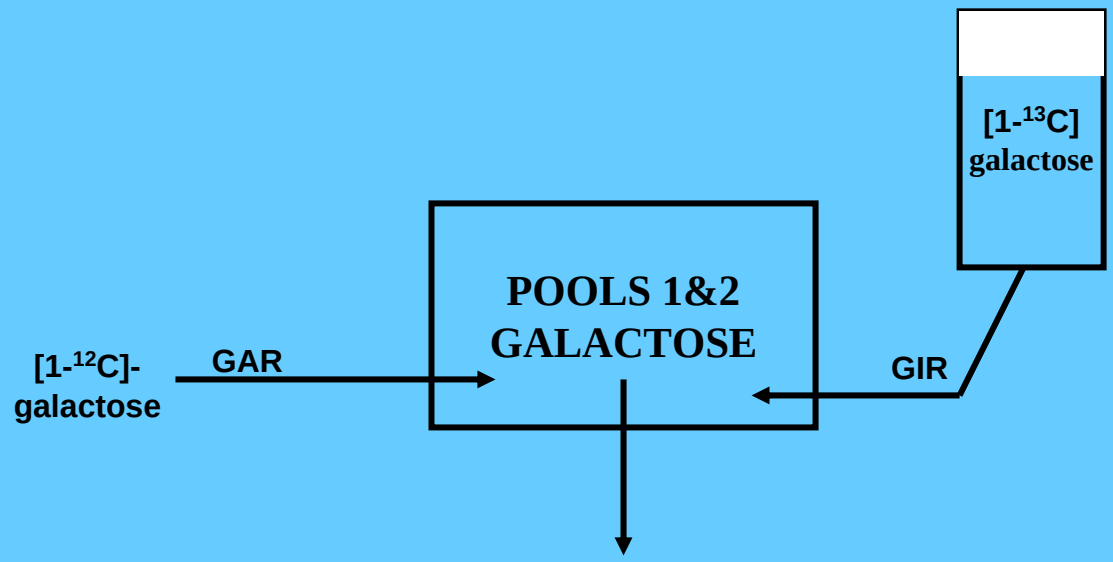

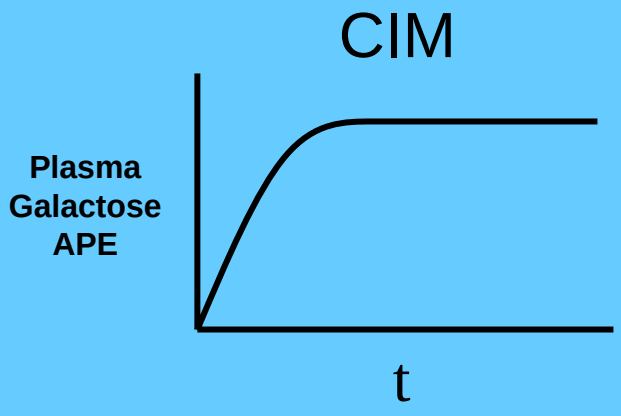

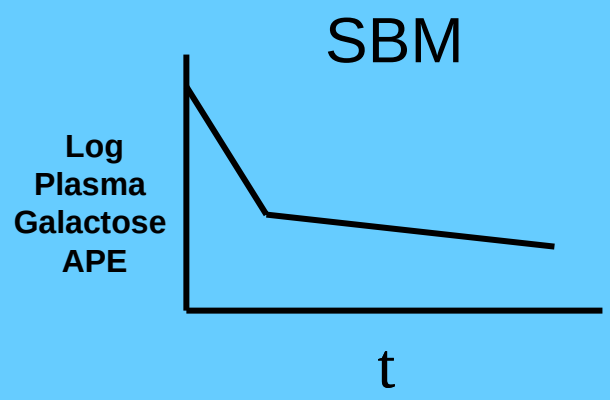

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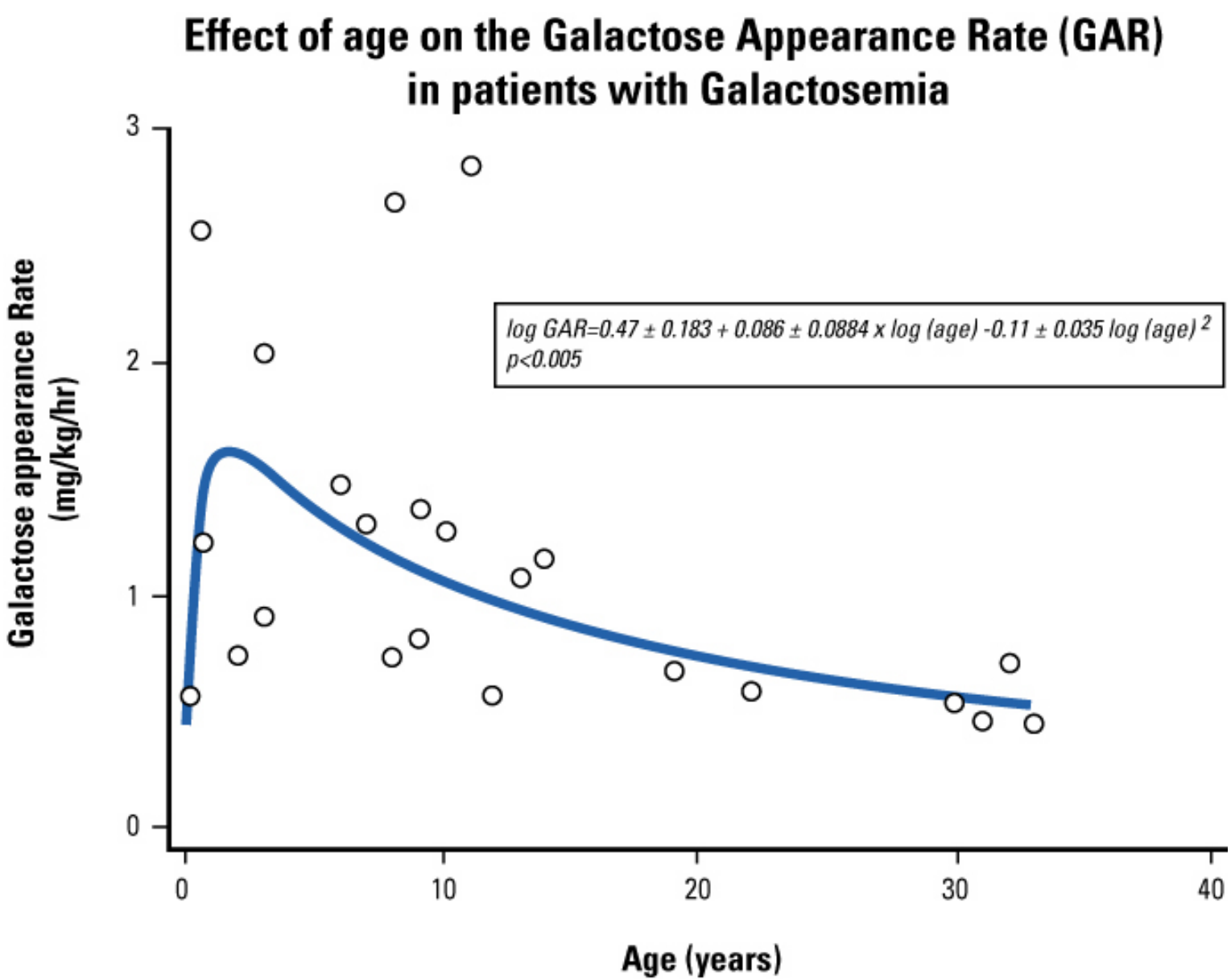
Method

- Aldononitrile / pentaacetate derivitization of plasma galactose
- Selected ion monitoring (SIM) of 212 and 213 m/z ion fragments by GC/MS
- Calculation of ^{13}C enrichment of galactose above baseline time zero sample

Does the GAR in infants and children with galactosemia show a developmental pattern that may be linked to the growth rate?


Effect of age on the Galactose Appearance Rate (GAR) in patients with Galactosemia
Galactose appearance Rate (mg/kg/hr)
3
2
1
0
0
10
20
30
40
Age (years)
log GAR=0.47 ± 0.183 + 0.086 ± 0.0884 x log (age) -0.11 ± 0.035 log (age) 2
p<0.005

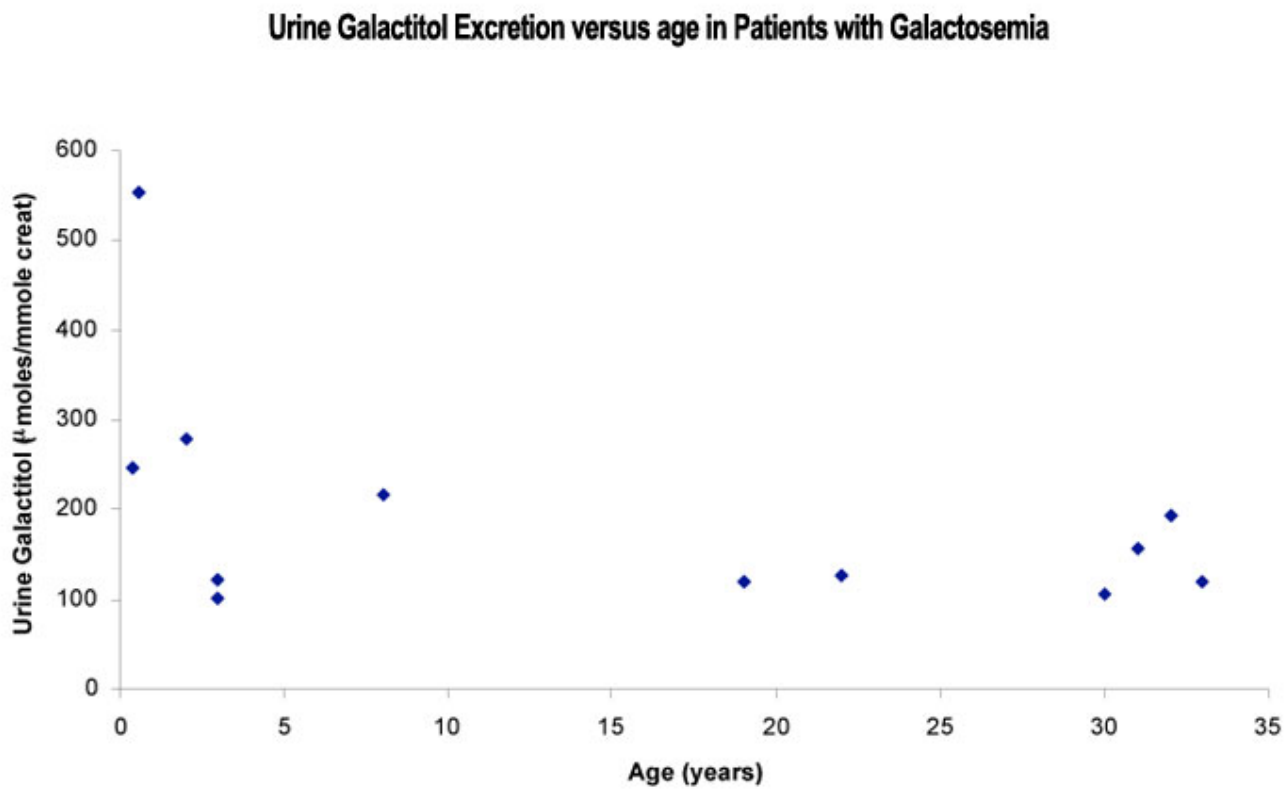
SUMMARY

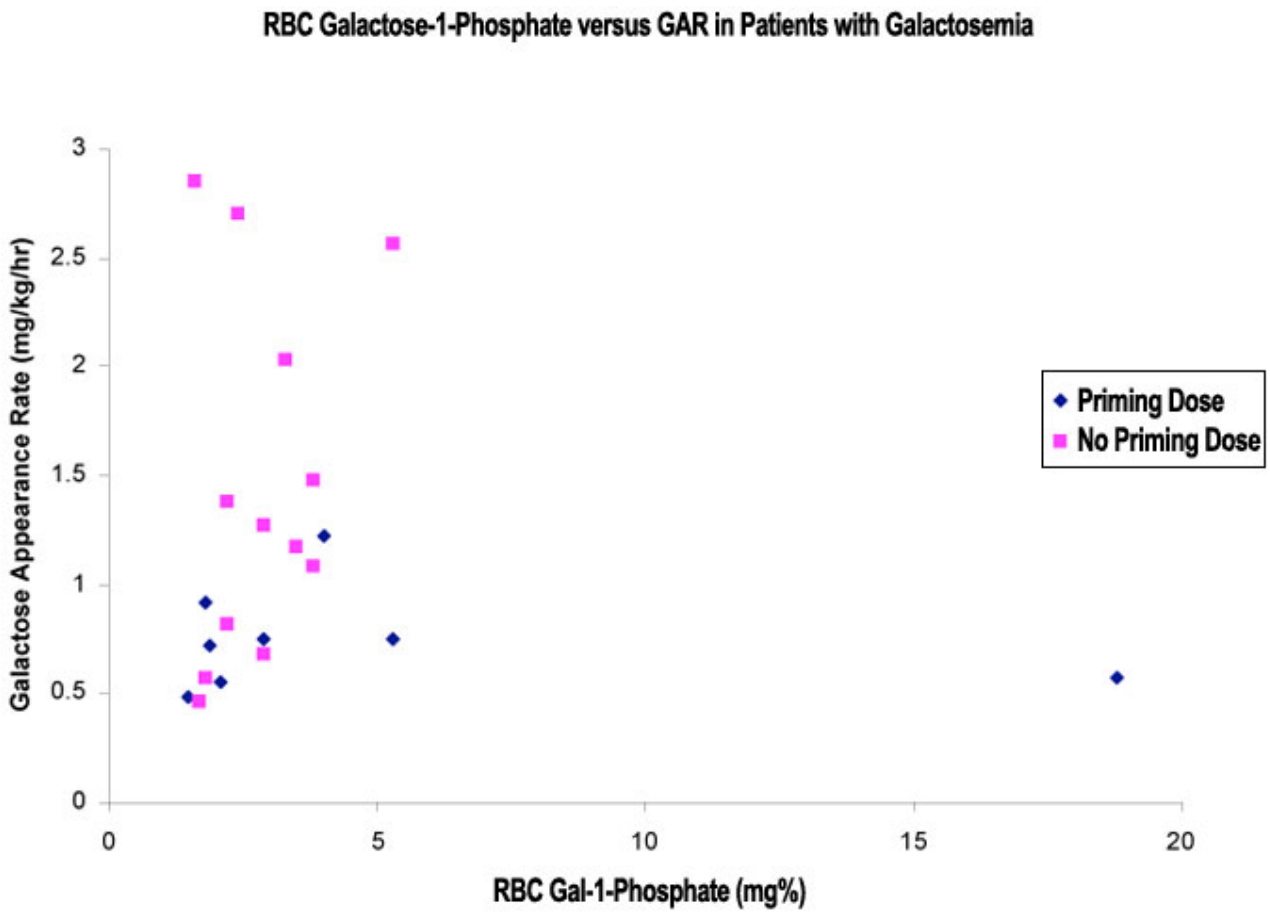
The galactose appearance rate (GAR) is approximately 1 gram per day in adults with galactosemia, and is even higher in infants and children per body weight.

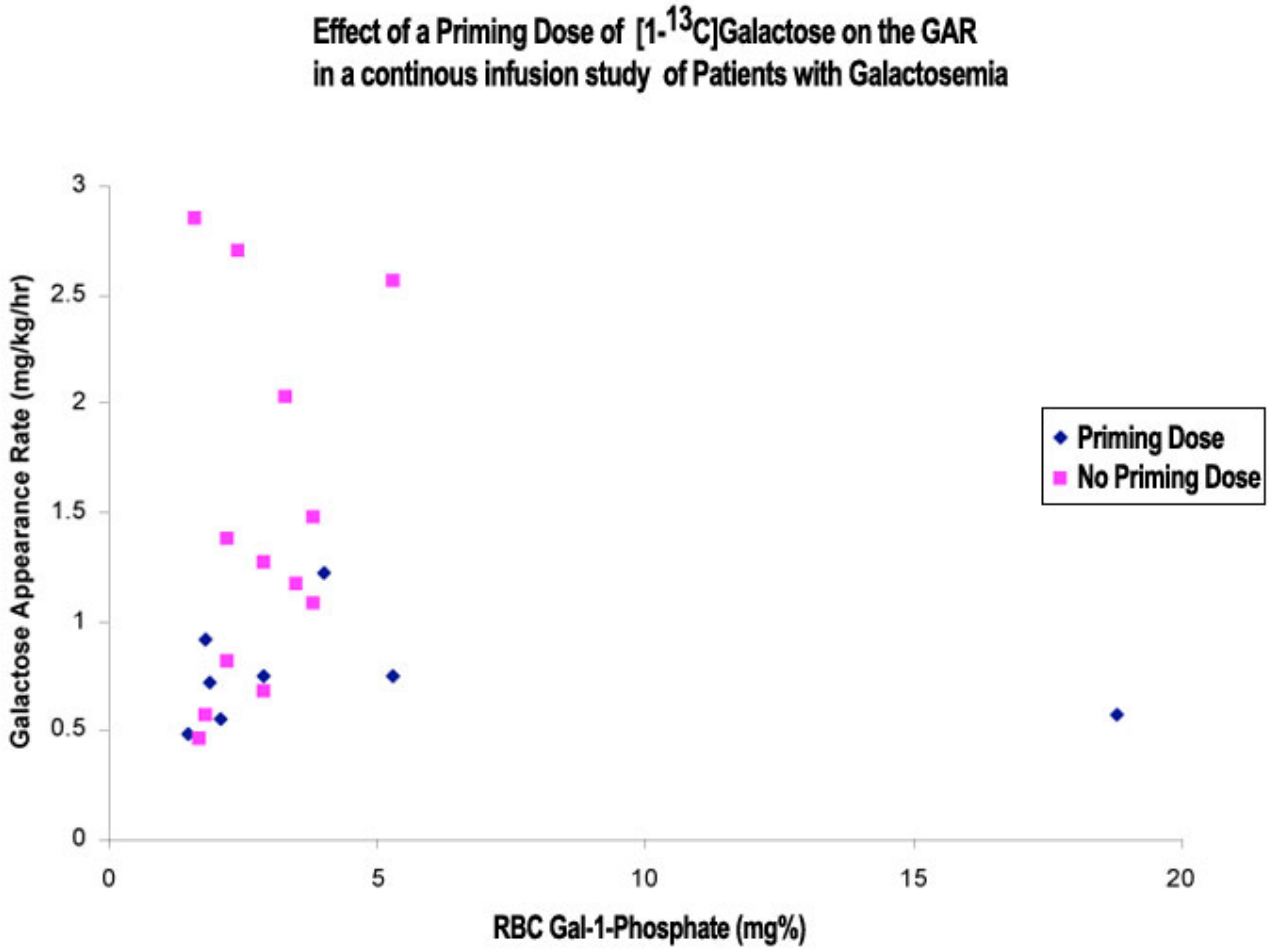
The GAR may be the biochemical basis for the persistent elevation of galactose-1-phosphate despite dietary galactose restriction.

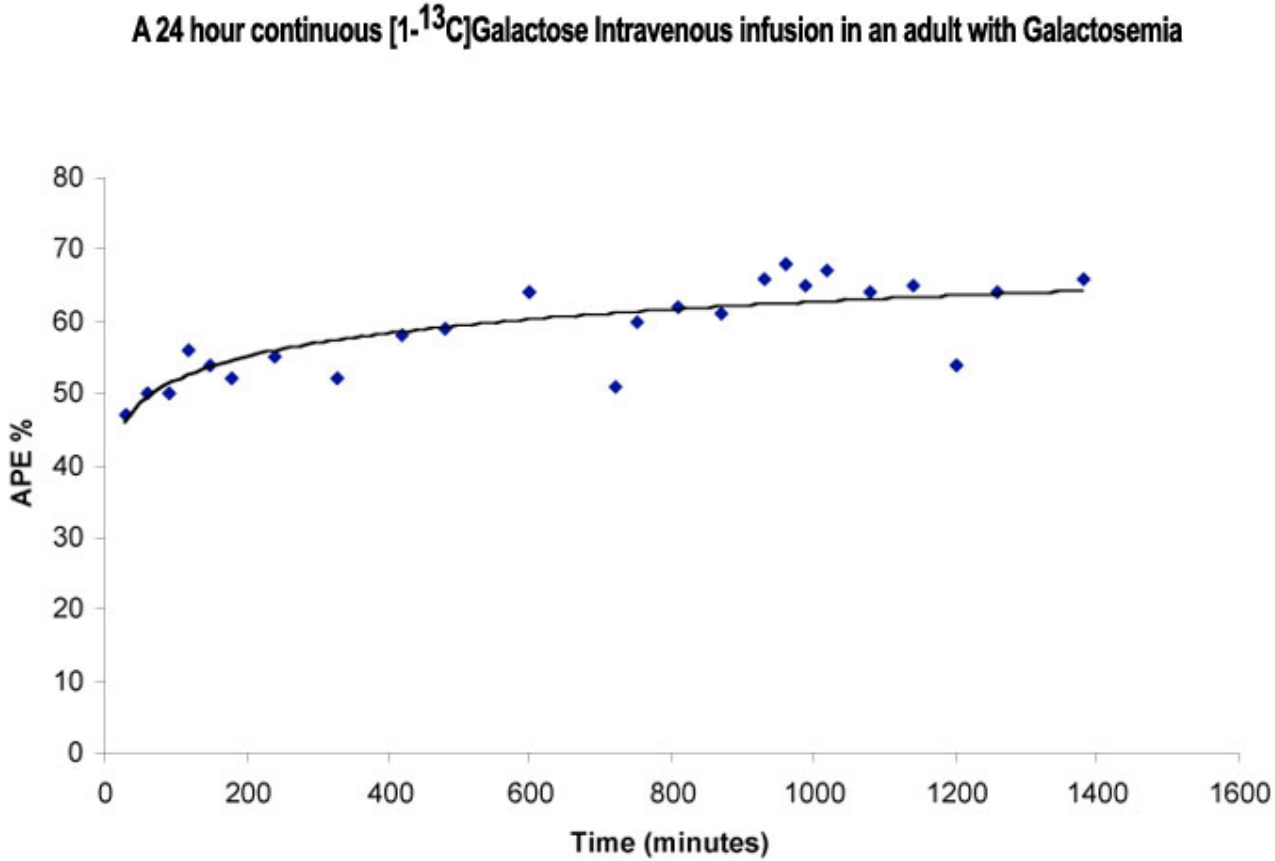
HYPOTHESIS

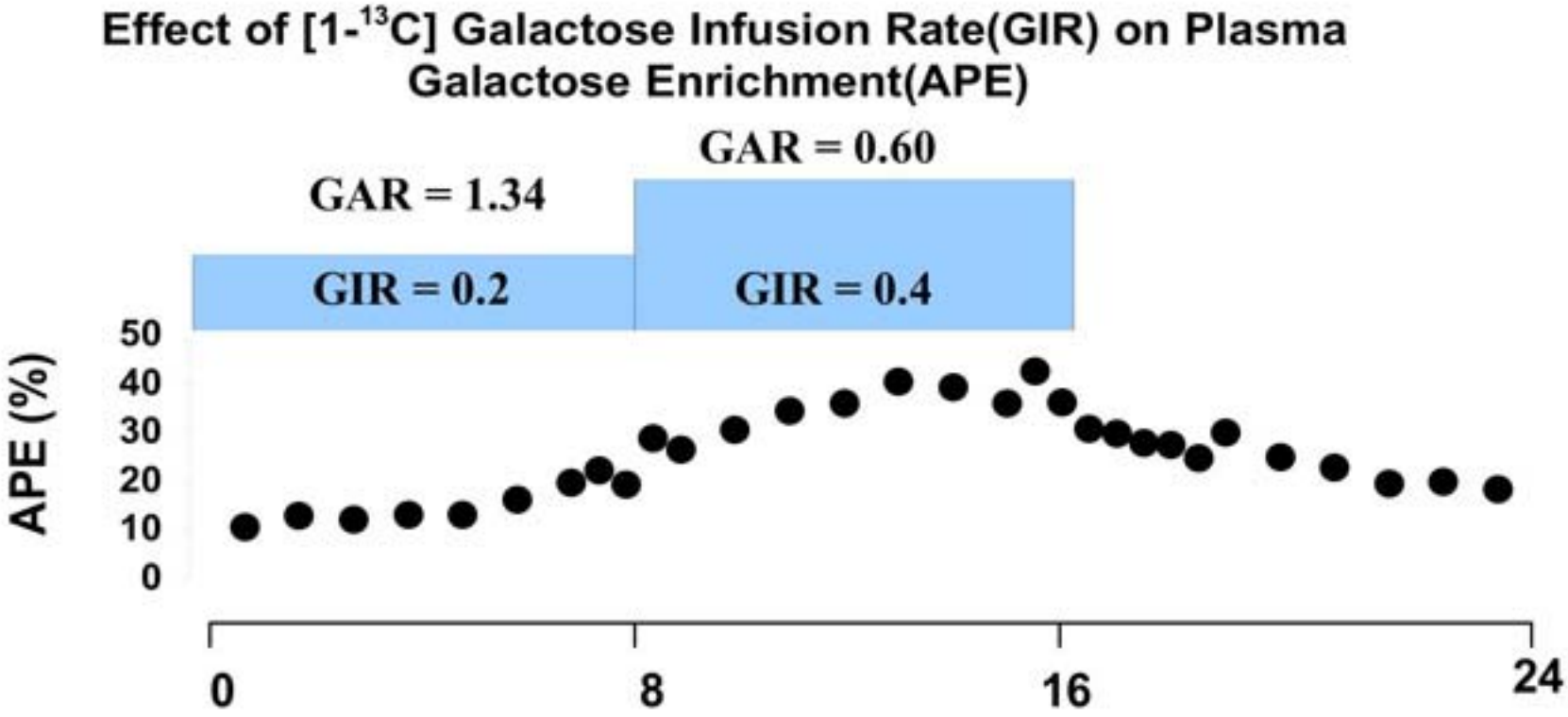
The rate of de novo synthesis of galactose, a rate that may be linked to the rate of turnover of glycoconjugates, is decreased compared to controls during the fetal and/or newborn period because of accumulation of the substrate in the GALT reaction.

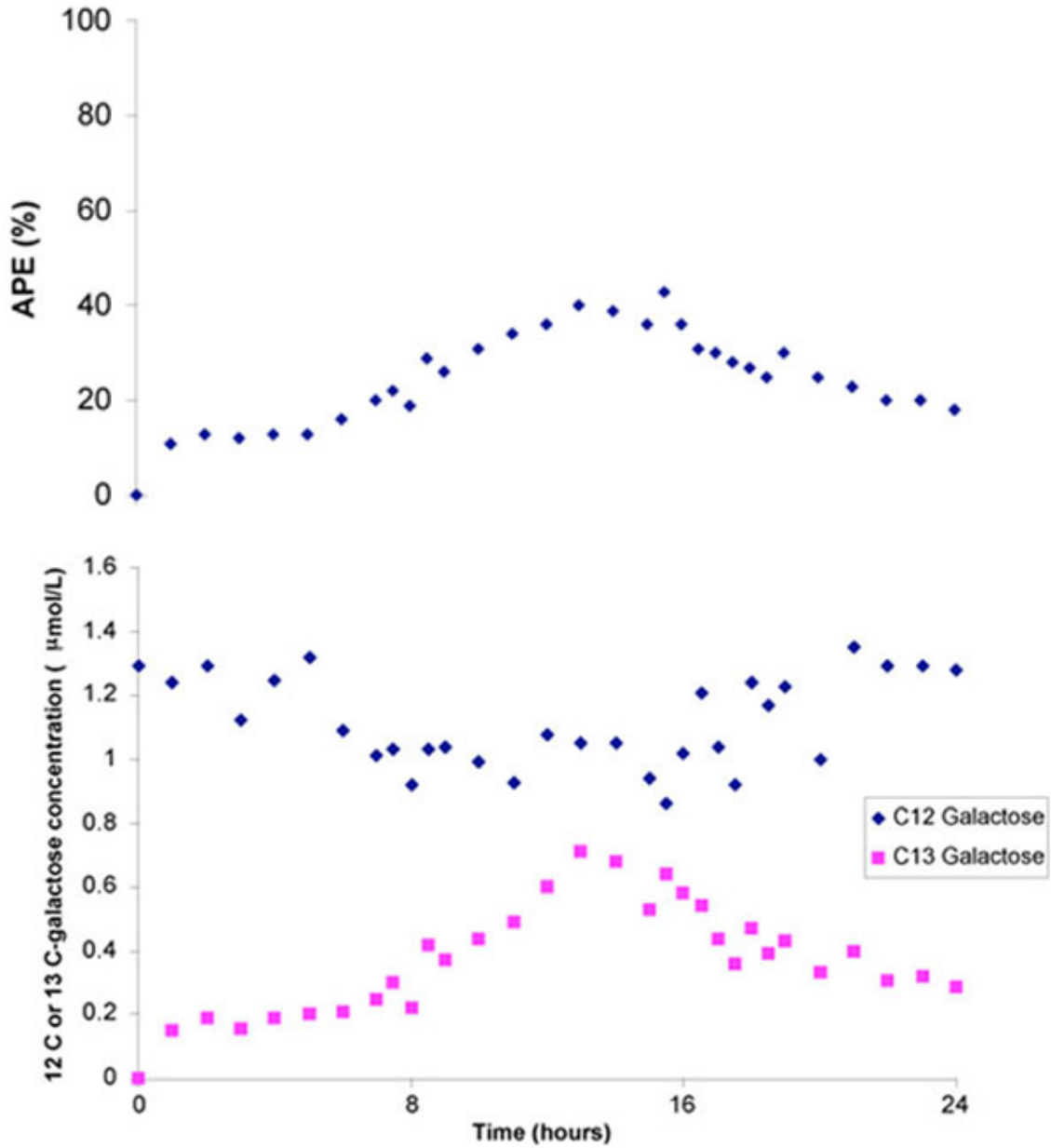

Urine Galactitol Excretion versus age in Patients with Galactosemia
Urine Galactitol (μ moles/mmole creat)
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
Age (years)


RBC Galactose-1-Phosphate versus GAR in Patients with Galactosemia
Galactose Appearance Rate (mg/kg/hr)
3
2.5
2
1.5
1
0.5
0
0
5
10
15
20
RBC Gal-1-Phosphate (mg%)
Priming Dose
No Priming Dose

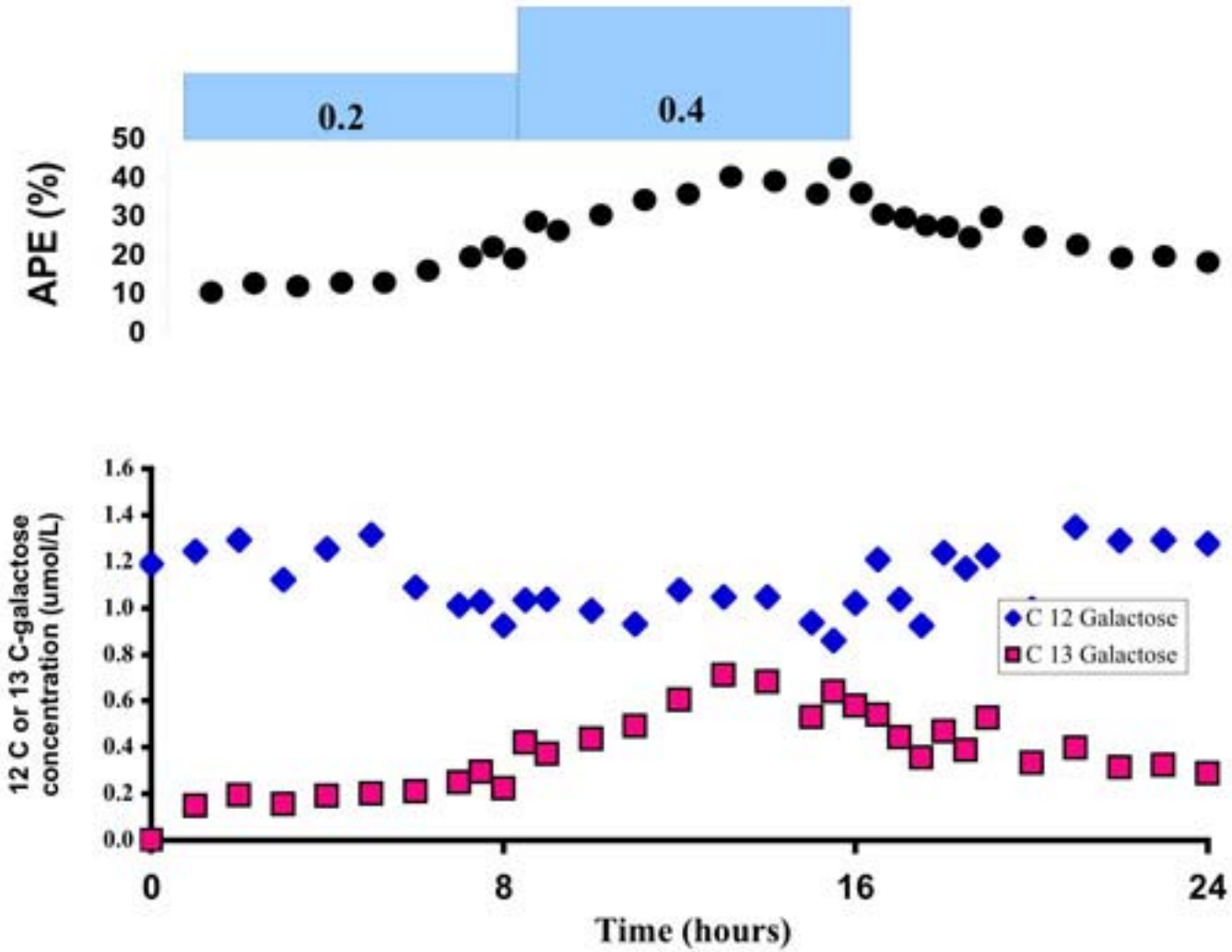

Effect of a Priming Dose of [1-13C]Galactose on the GAR
in a continous infusion study of Patients with Galactosemia
Galactose Appearance Rate (mg/kg/hr)
RBC Gal-1-Phosphate (mg%)
0
0.5
1
1.5
2
2.5
3
0
5
10
15
20
Priming Dose
No Priming Dose


A 24 hour continuous [1-13C]Galactose Intravenous infusion in an adult with Galactosemia
APE %
0
10
20
30
40
50
60
70
80
0
200
400
600
800
1000
1200
1400
1600
Time (minutes)


Effect of [1-13C] Galactose Infusion Rate(GIR) on Plasma Galactose Enrichment(APE)
GAR = 0.60
GAR = 1.34
GIR = 0.2
GIR = 0.4
APE (%)
50
40
30
20
10
0
0
8
16
24


Effect of the [1-13C]Galactose infusion rate on Galactose Metabolite Levels and Enrichment
APE (%)
100
80
60
40
20
0
12 C or 13 C-galactose concentration (µmol/L)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
8
16
24
Time (hours)
C12 Galactose
C13 Galactose

Effect of [1-^{13}C] Galactose Infusion Rate on Plasma Galactose Enrichment and Levels

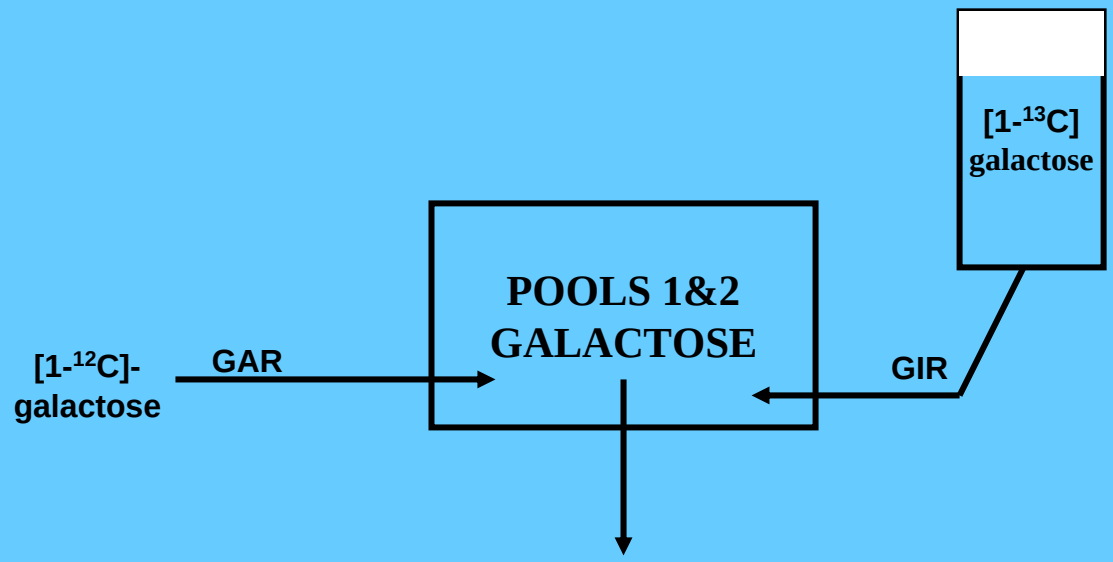


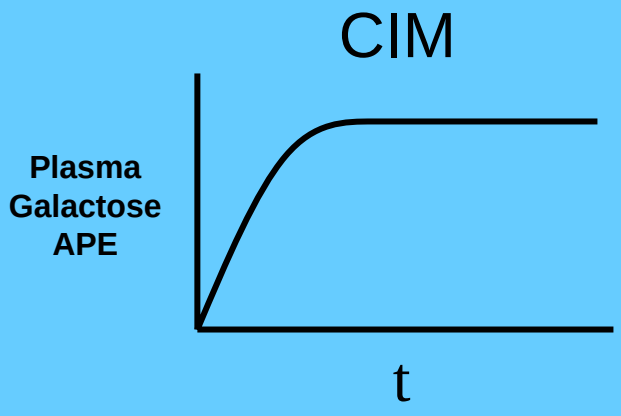
The Single Bolus Method is not subject to the limitations of the Continuous Infusion Method

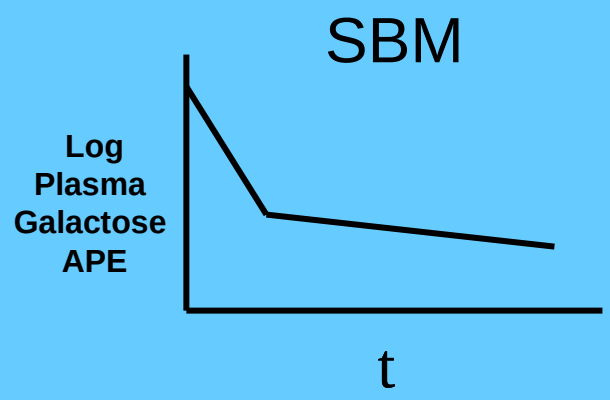
The Single Bolus Method also provides information on number of compartments and pool size(s)

Single Bolus Method

- Intravenous bolus of 7 mg (1-^{13}C)galactose / kg at time zero
- Obtain plasma samples at baseline and every 5-30 minutes up to 420 minutes for analyses of 13C enrichment
- Data on ^{13}Cgalactose / ^{12}Cgalactose versus time was subjected to compartmental analysis, a model with two exchanging compartments and elimination from the central compartment
- Model parameters were fitted directly to the plasma data using the WinSAAM computer program





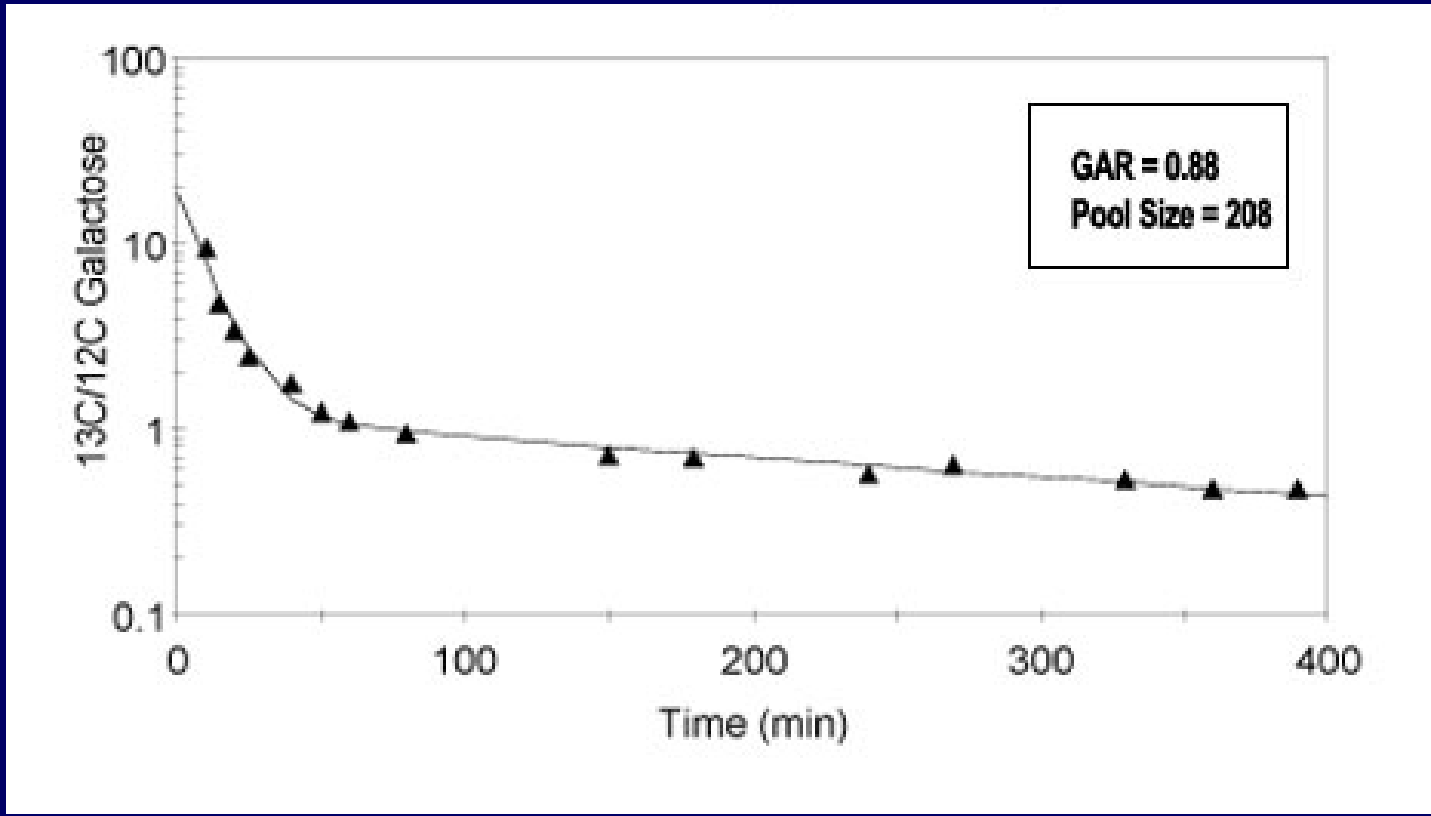


$$\frac{[1\text{-}^{13}C]\text{-Galactose}}{[1\text{-}^{13}C]\text{- + }[1\text{-}^{12}C]\text{-Galactose}} = \frac{GIR}{GIR + GAR}$$

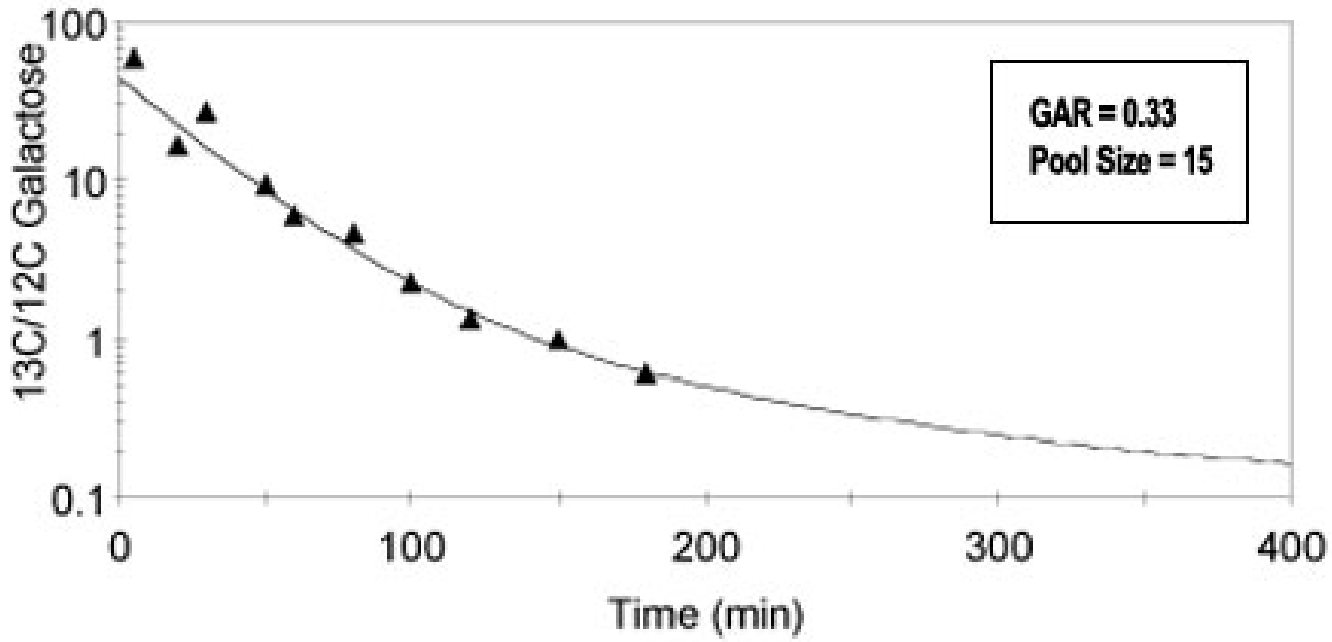
GAR = Galactose Appearance Rate
GIR = Galactose Infusion Rate

CIM = Constant Infusion Method
SBM = Single Bolus Method

Single Bolus Method with [1-^{13}C]Galactose in a 30 Year Old Male with Q188R/Q188R Genotype

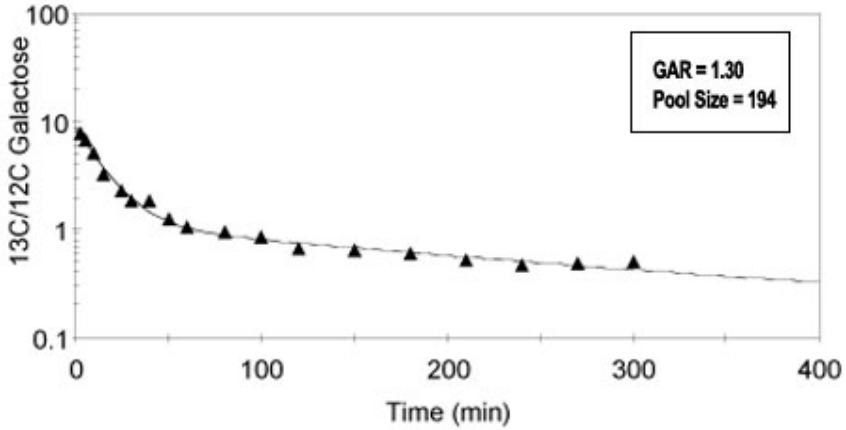


Single Bolus Method in a Normal 25 year old Female

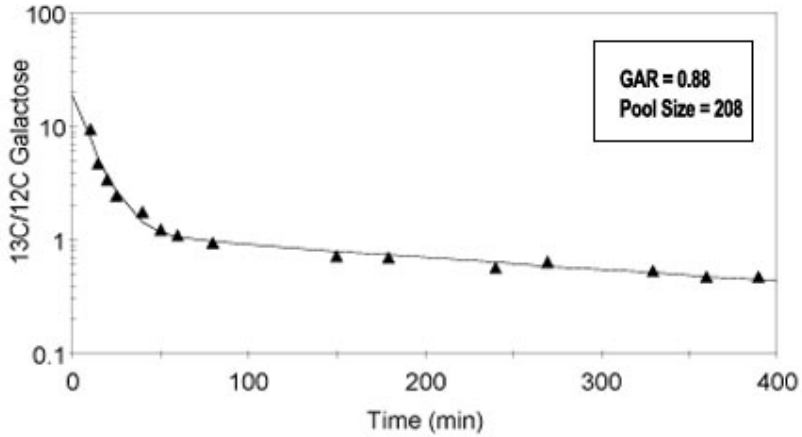


Single Bolus Studies with [1-^{13}C]Galactose

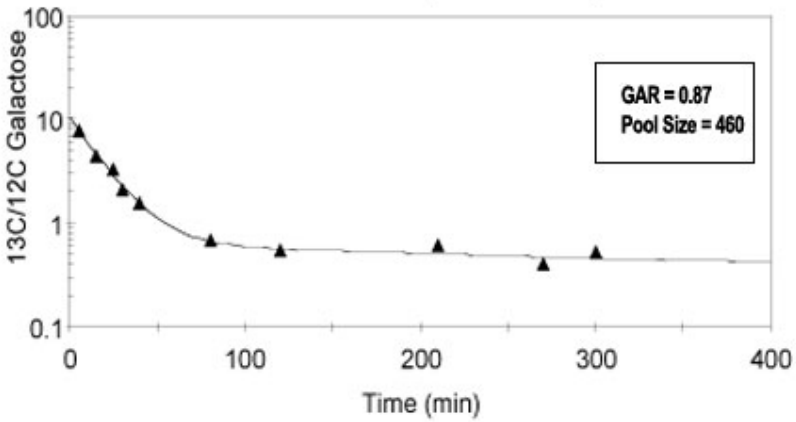
22 Year old Female (Q188R/Q188R)



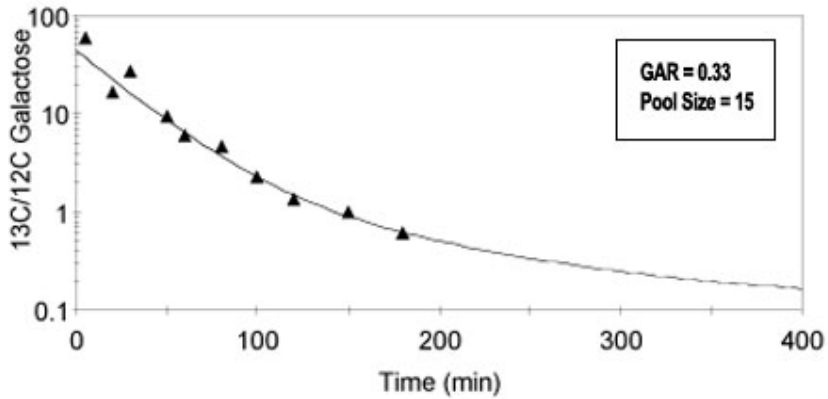
30 Year old Male (Q188R/Q188R)



31 Year old Female (Q188R/Q188R)



25 Year old Female (normal/normal)



Galactose Appearance Rate (GAR) in control subjects

Infants and children, 2 months-6 years old:

0.58-0.68 mg / kg / h (n=3)

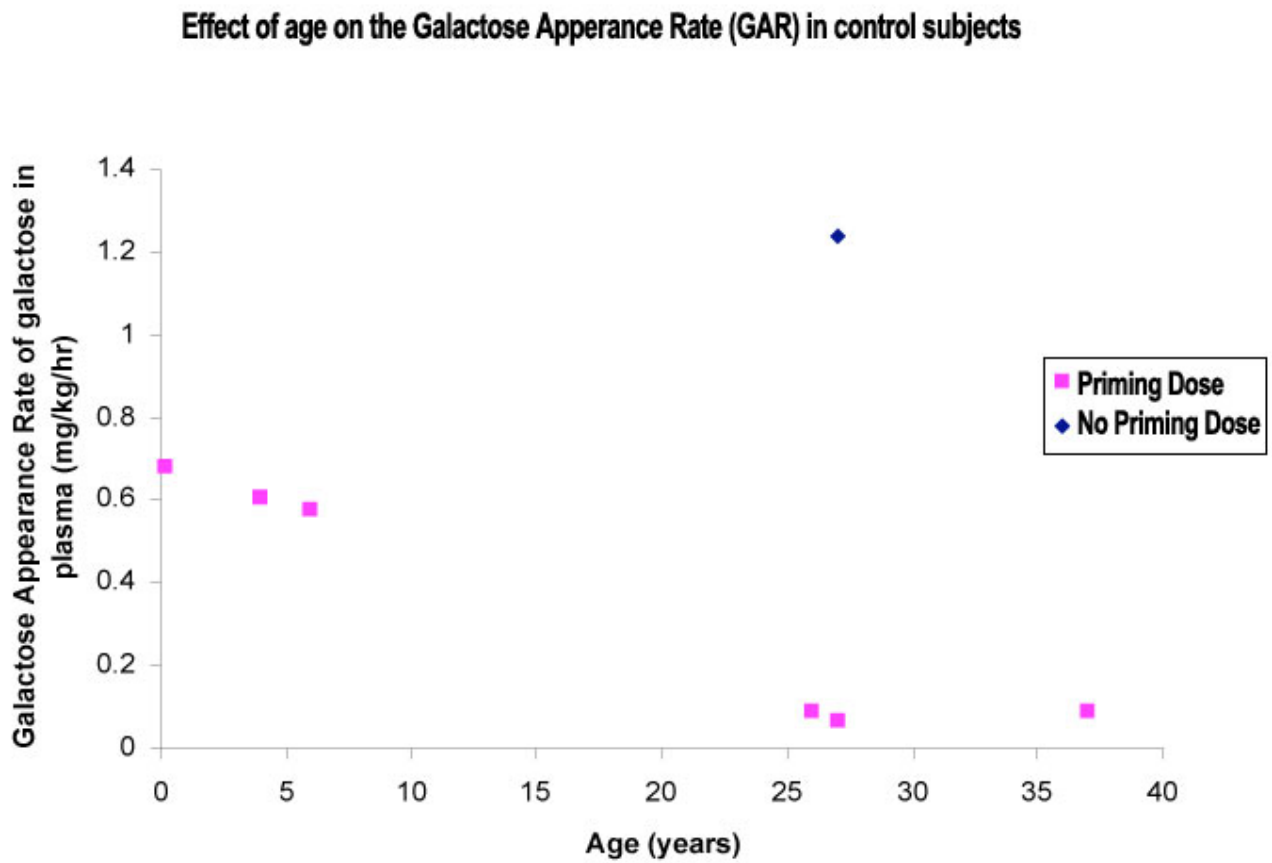
(3.2-3.8 umol/kg/h)

Adults, 26-37 years old:

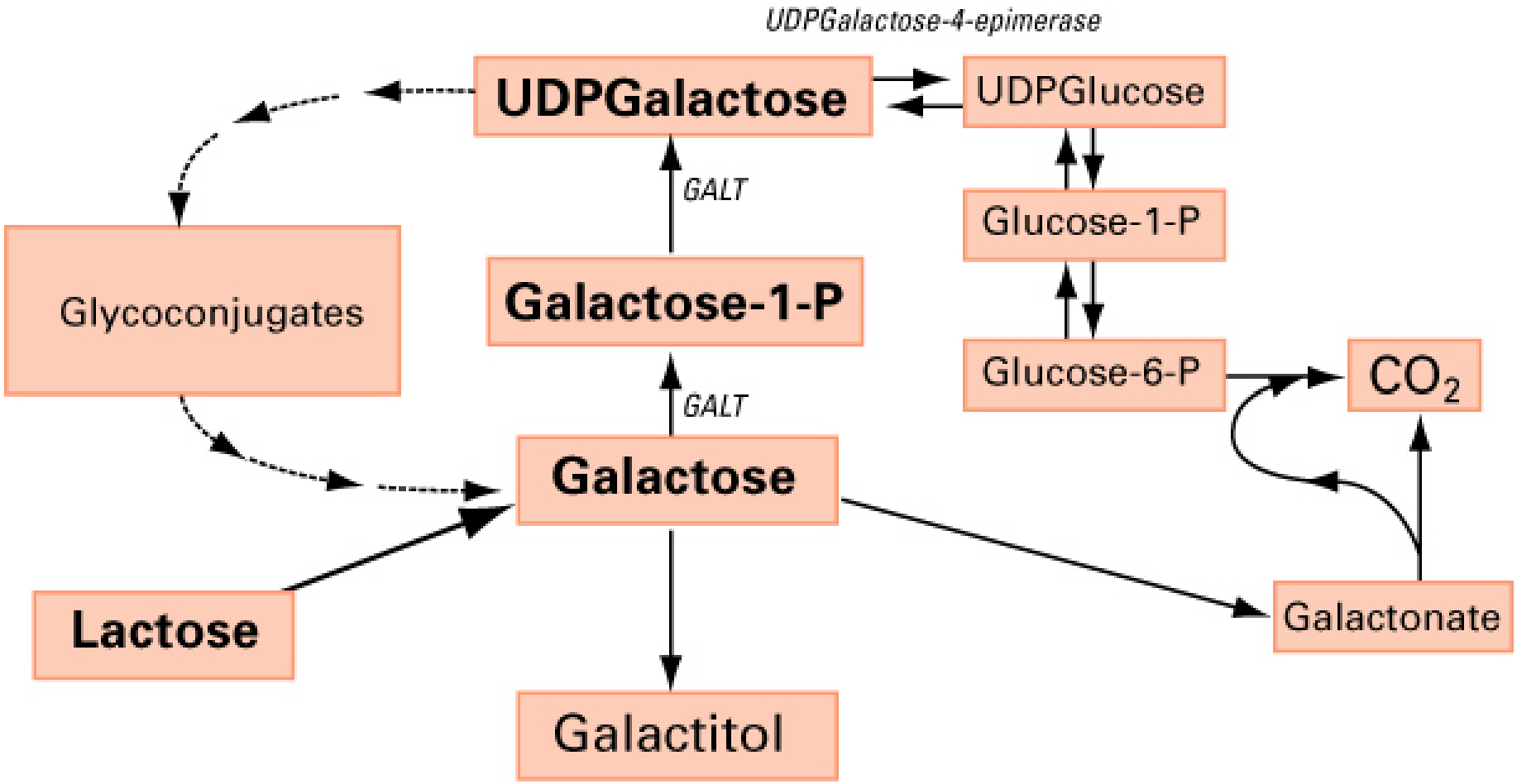
0.07-0.09 mg / kg / h (n=3)

(0.4-0.5 umol/kg/h)

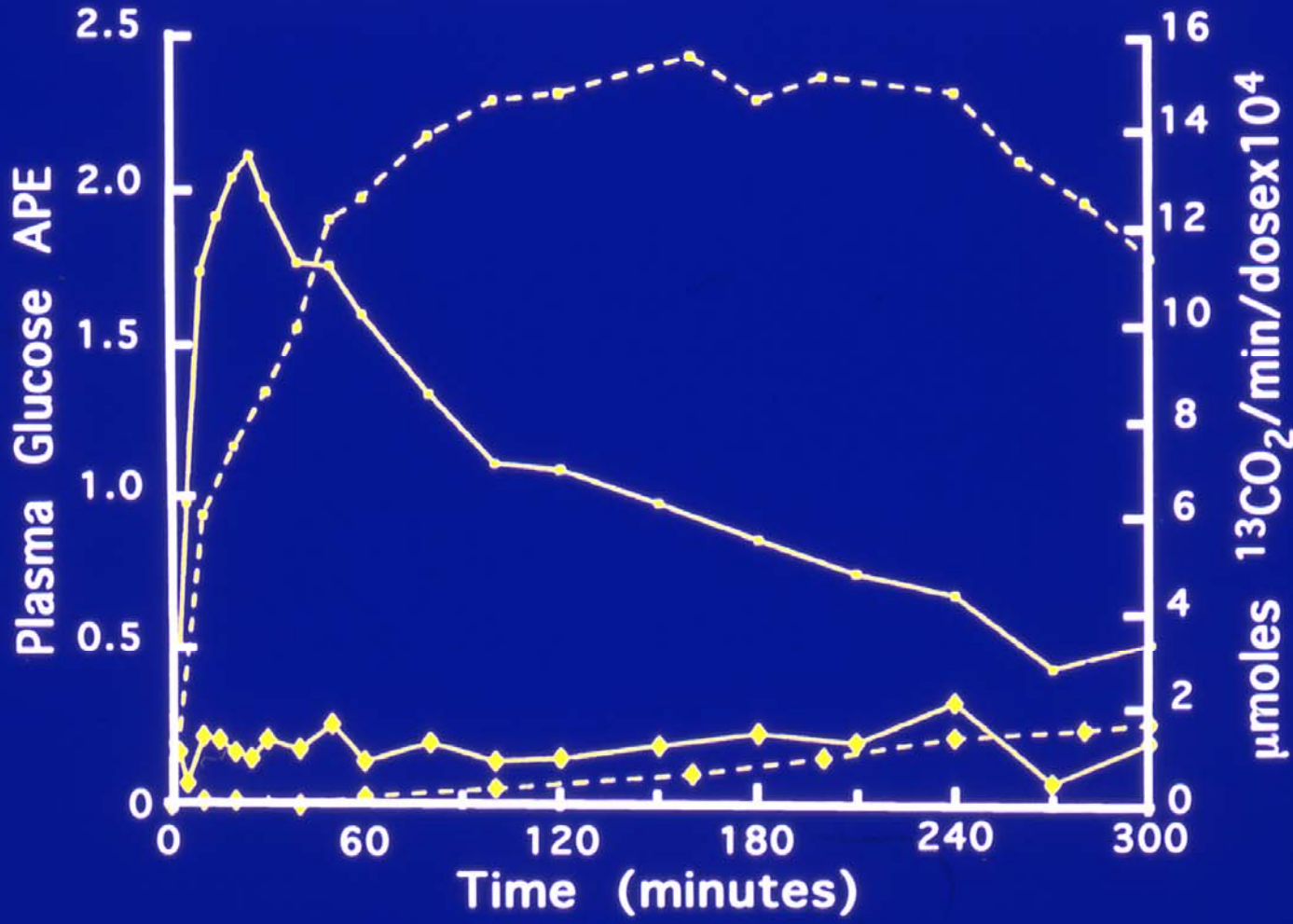
Berry et al, MGM: 81, 2004


Effect of age on the Galactose Apperance Rate (GAR) in control subjects
Galactose Appearance Rate of galactose in plasma (mg/kg/hr)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
35
40
Age (years)
Priming Dose
No Priming Dose

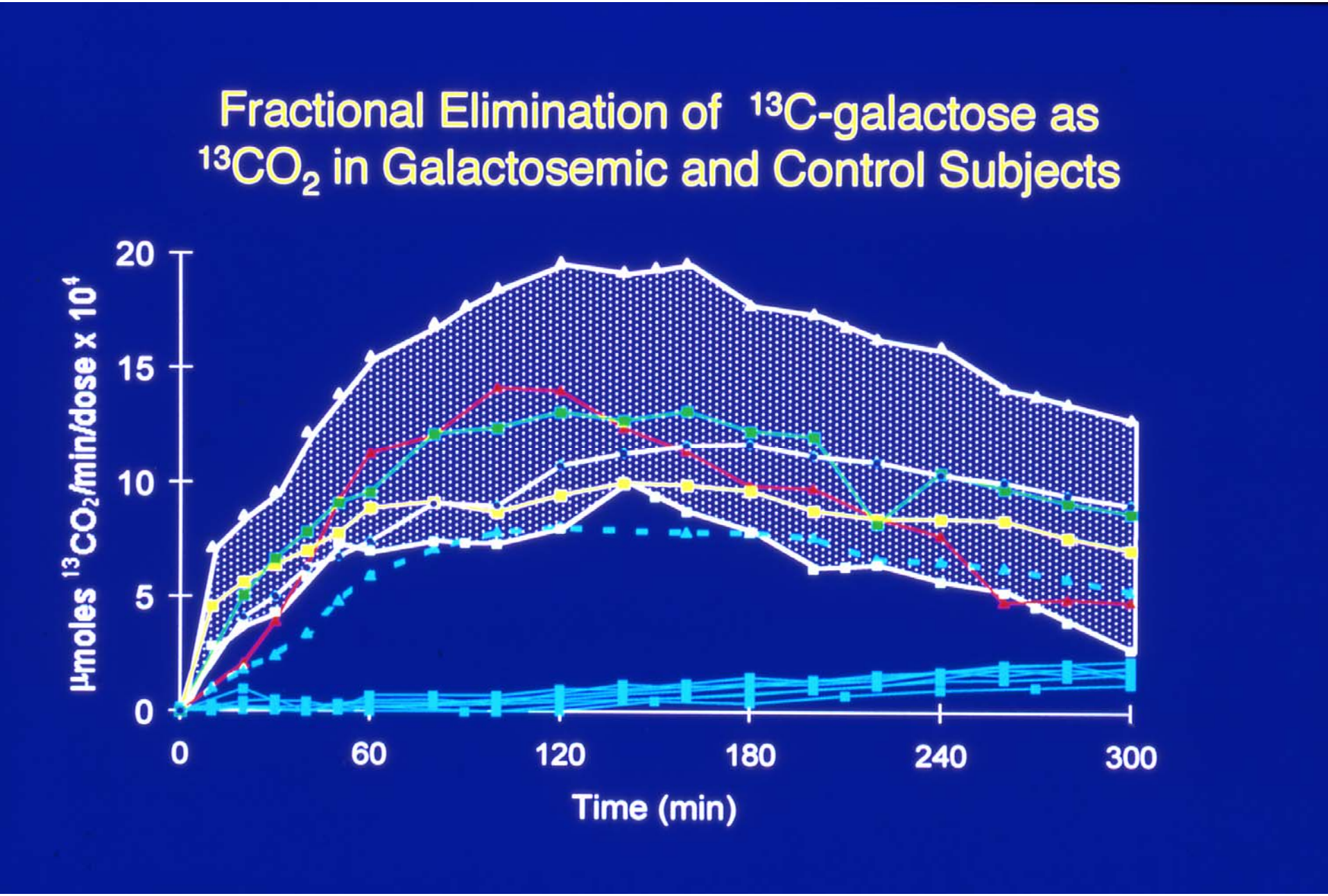
Establishment of chronic steady-state levels of RBC galactose-1-phosphate and urinary galactitol requires that the galactose disposal rate equal the galactose production rate in a patient on a galactose-free diet.


UDPGalactose-4-epimerase
UDPGalactose
UDPGlucose
GALT
Glucose-1-P
Glycoconjugates
Galactose-1-P
GALT
Glucose-6-P
CO_2
Galactose
Lactose
Galactonate
Galactitol

$^{13}CO_2$ Production and ^{13}C-Glucose Formation after IV ^{13}C-Galactose



Genotype-Phenotype correlations with ^{13}C-galactose breath testing


Fractional Elimination of ^{13}C-galactose as $^{13}CO_2$ in Galactosemic and Control Subjects
μmoles $^{13}CO_2$/min/dose x 10^4
20
15
10
5
0
0
60
120
180
240
300
Time (min)

Over 167 GALT gene mutations have been detected!

Conclusions

- The GAR varies with age in patients with galactosemia
- The GAR is higher in infants and children than in adults with galactosemia
- Administration of galactose over time may reduce the GAR in patients with galactosemia

Conclusions, cont.

- Using the continuous infusion method, the GAR was lower in controls than in patients with galactosemia
- Using the continuous infusion method, the GAR was highly variable especially in infants and children with galactosemia
- Because of the perturbing effects of the “tracer” itself, especially with time, in a continuous infusion, the single bolus method deserves further study in the determination of the GAR in man

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