Systems Biology

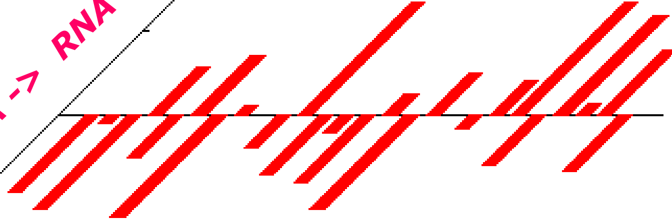
Systems biology studies biological systems by systematically perturbing them (biologically, genetically, or chemically); monitoring the **gene**, **protein**, and **informational** pathway responses; integrating these data; and ultimately, formulating mathematical models that describe the structure of the system and its response to individual perturbations.

Ideker, T., T. Galitski, and L. Hood. 2001. A new approach to decoding life: systems biology. Annu. Rev. Genomics Hum. Genet. 2: 343-372.

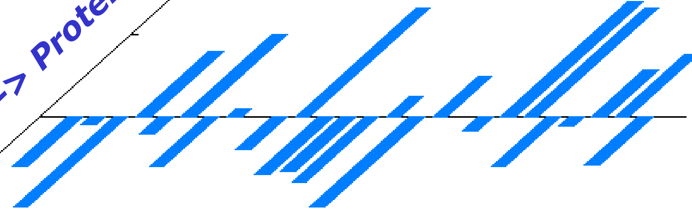
How do we retain emphasis on function ?

Joanne K. Kelleher, jkk@mit.edu

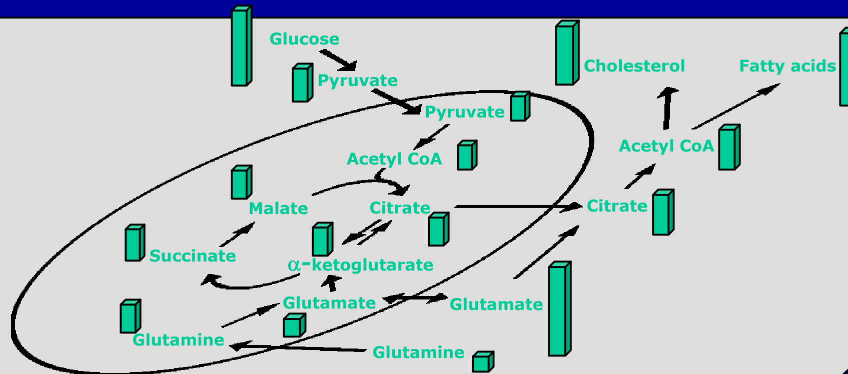
GENOME



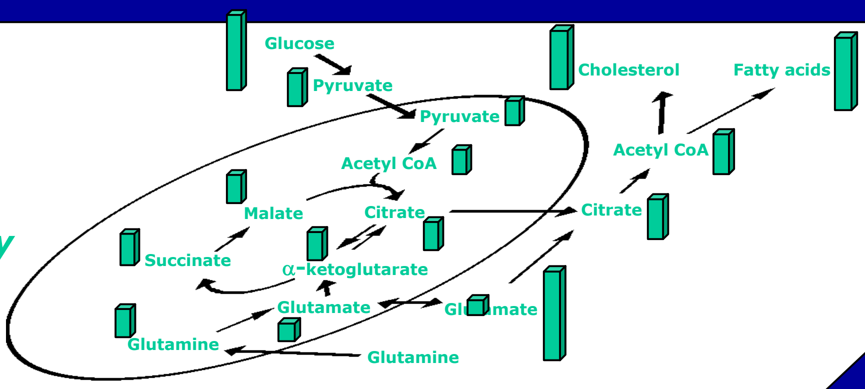
Homeostasis not possible. Not satisfactory to Physiologist.



Physiology or Metabolism



Dark days for the metabolites

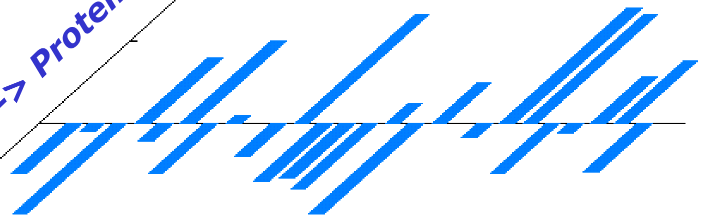


Physiology

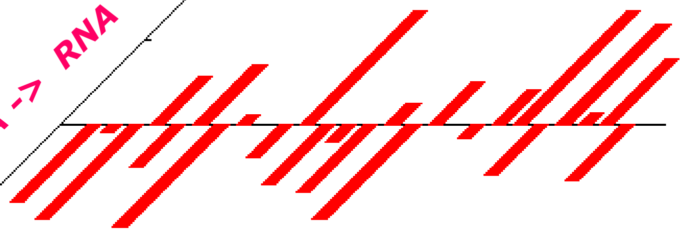
or

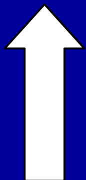
Metabolism

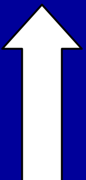
The Proteome

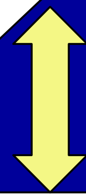


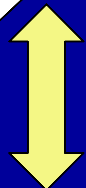
The Transcriptome

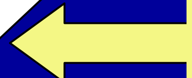


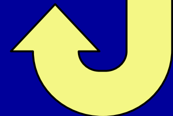


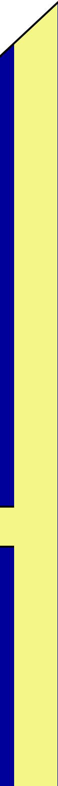












GENOME

Metabolites and metabolism have not held a prominent place in Biomedical research in the past two decades.

Two factors may drive rebirth of Metabolism in Human Physiology

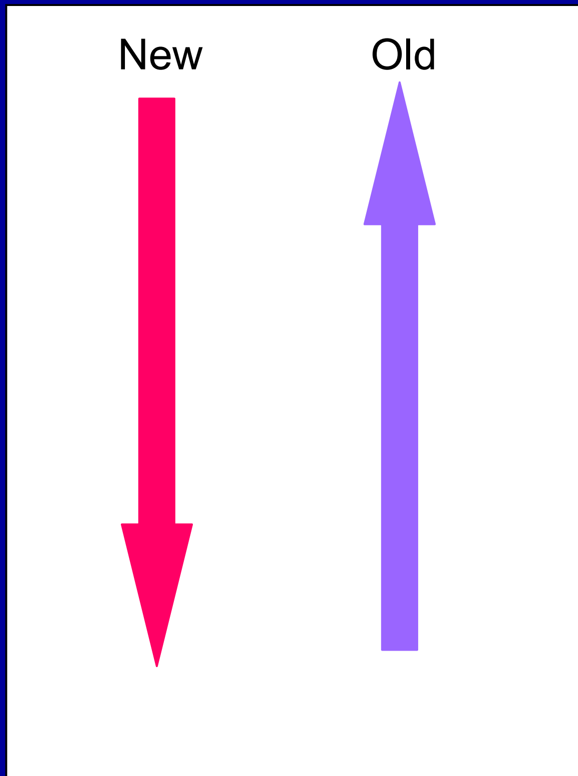
1. Role of metabolites in homeostasis at gene level of control

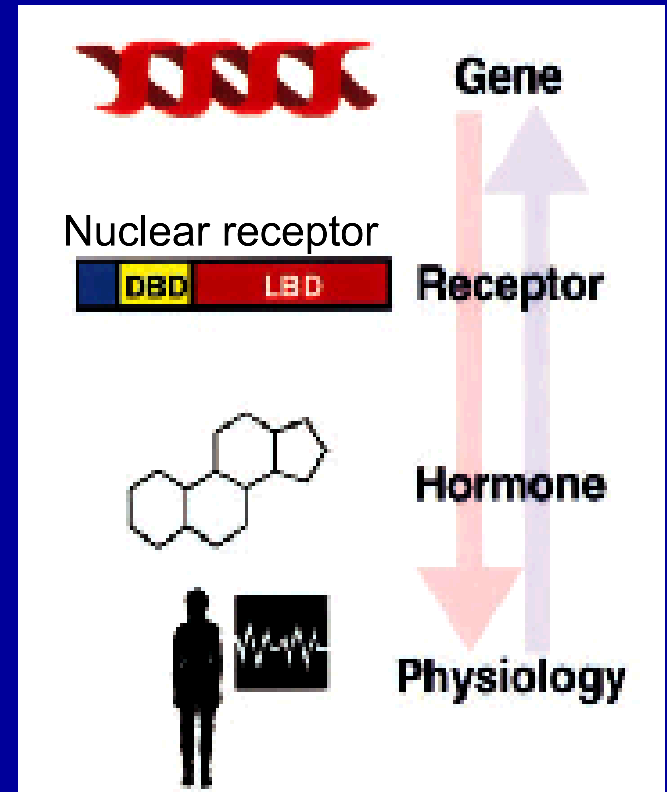
2. Complexities of mammalian physiology revealed by knockout models

Orphan Nuclear Receptors:

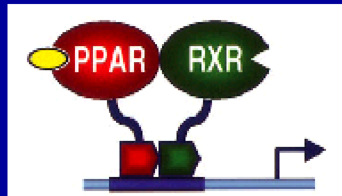
Shifting Endocrinology into Reverse

Kliewer, et al., 1999. Science 284: 757.

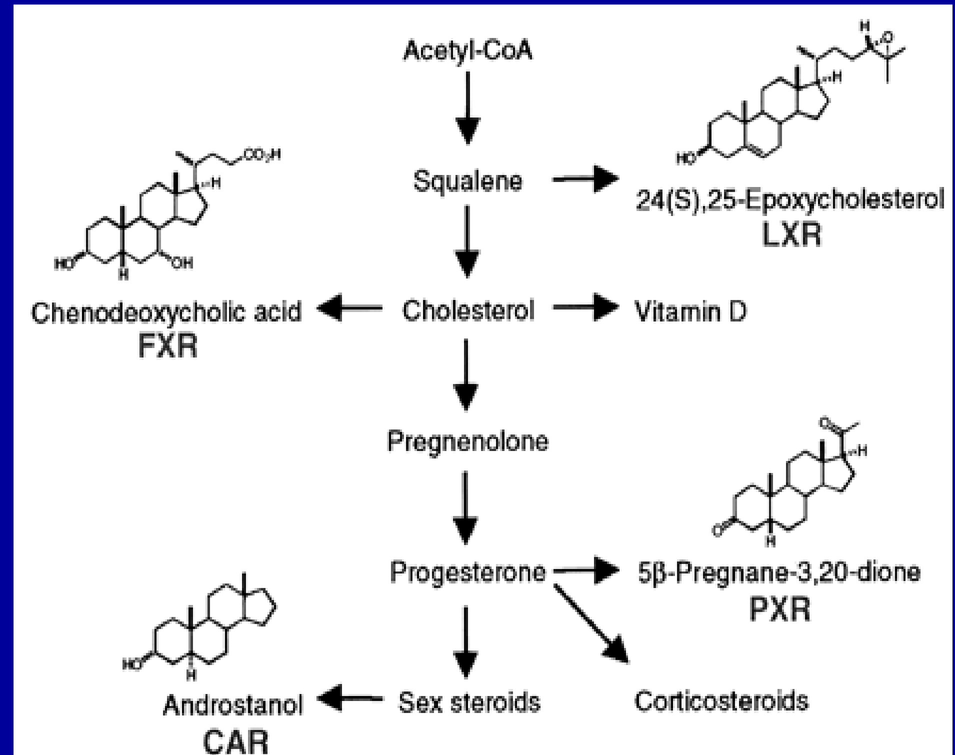


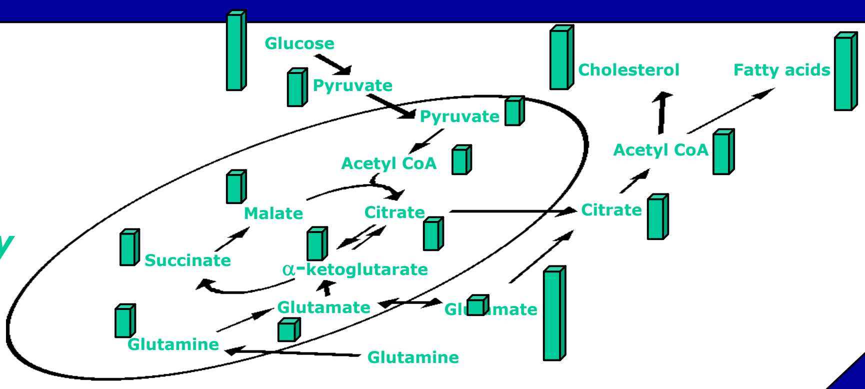


Metabolites directly affect gene expression in mammalian cells



=



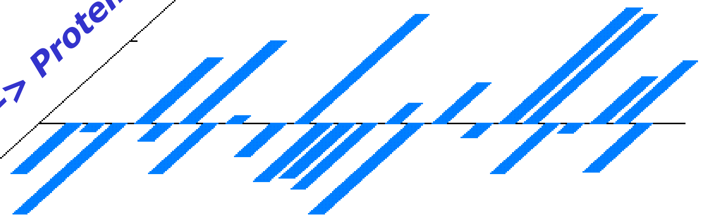


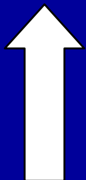
Physiology

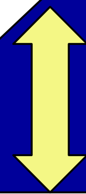
or

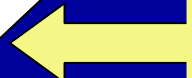
Metabolism

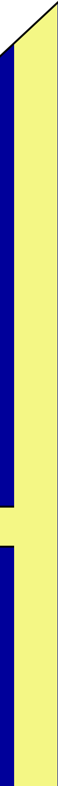
The Proteome

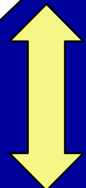




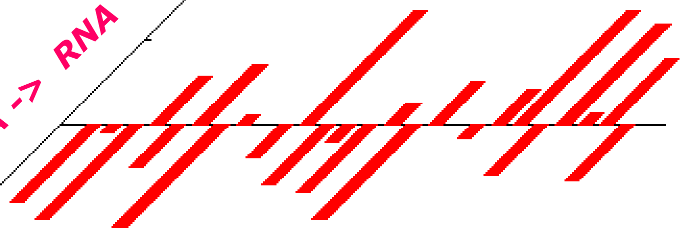


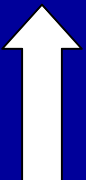


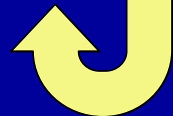




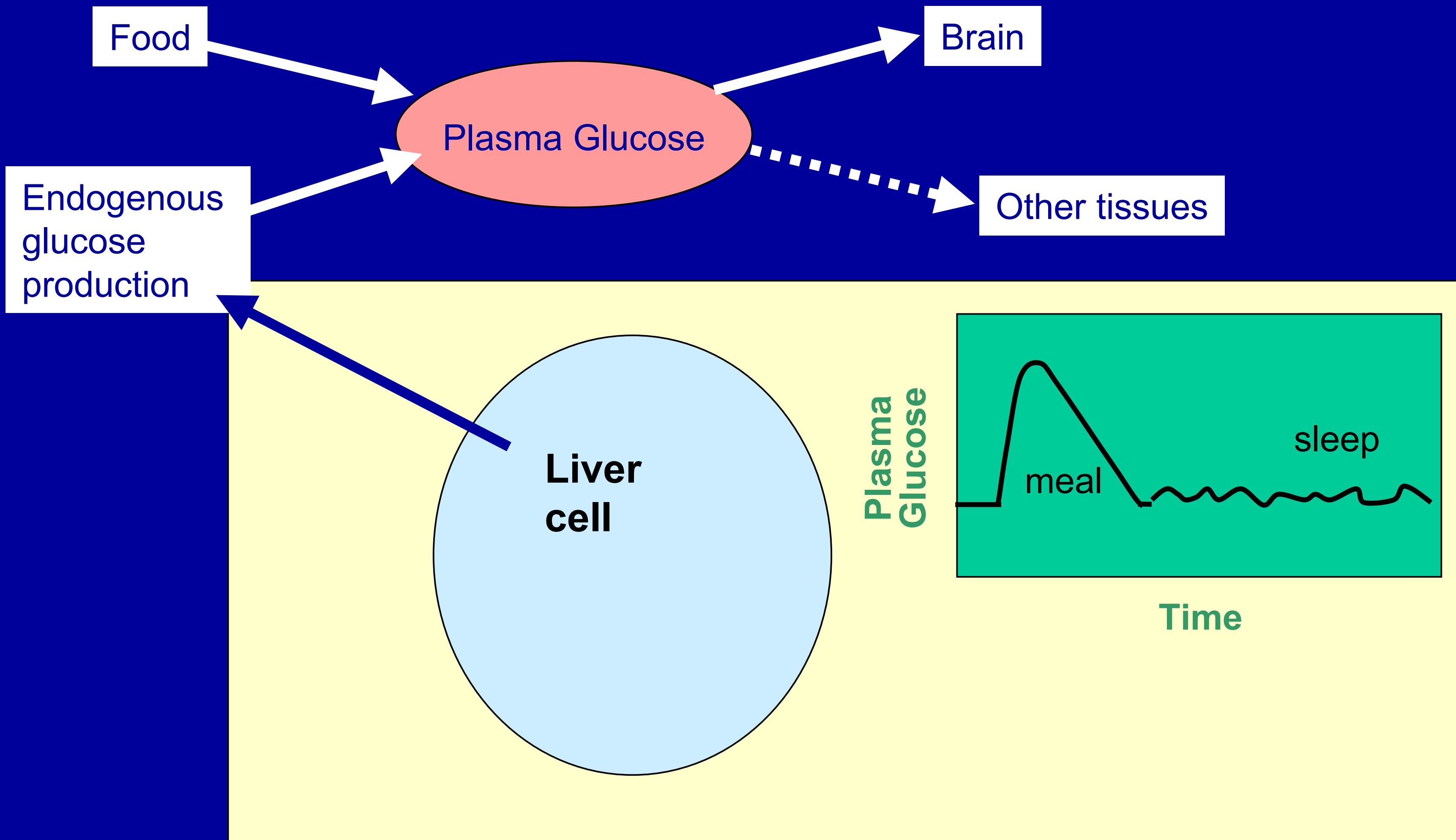
The Transcriptome

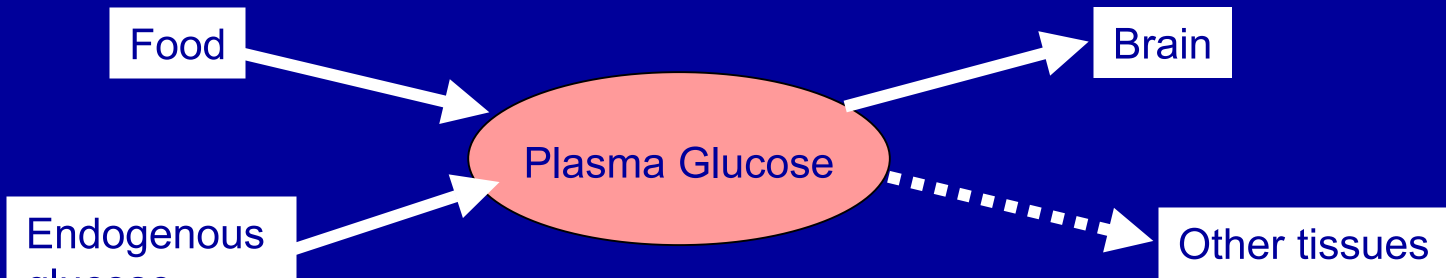


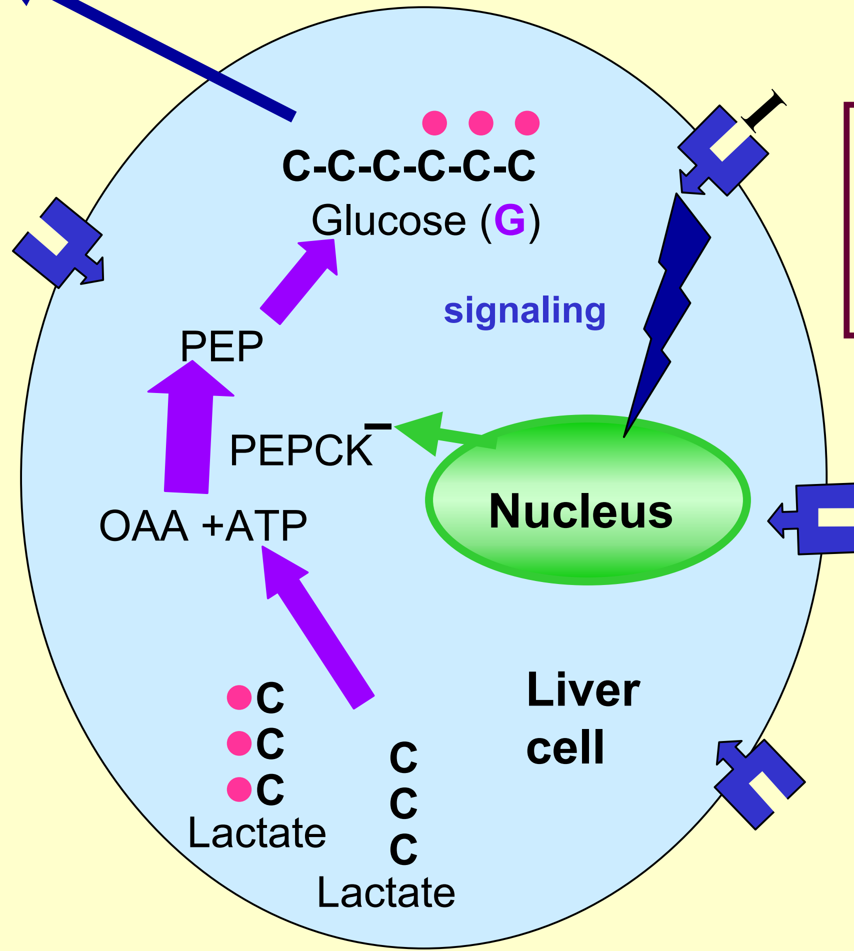




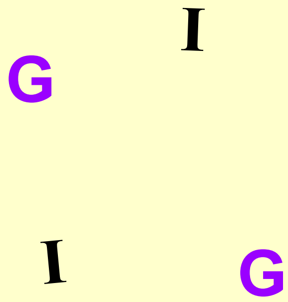
GENOME

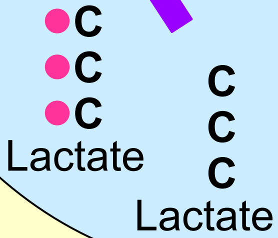

Food
Brain
Plasma Glucose
Endogenous glucose production
Other tissues
Liver cell
Plasma Glucose
meal
sleep
Time

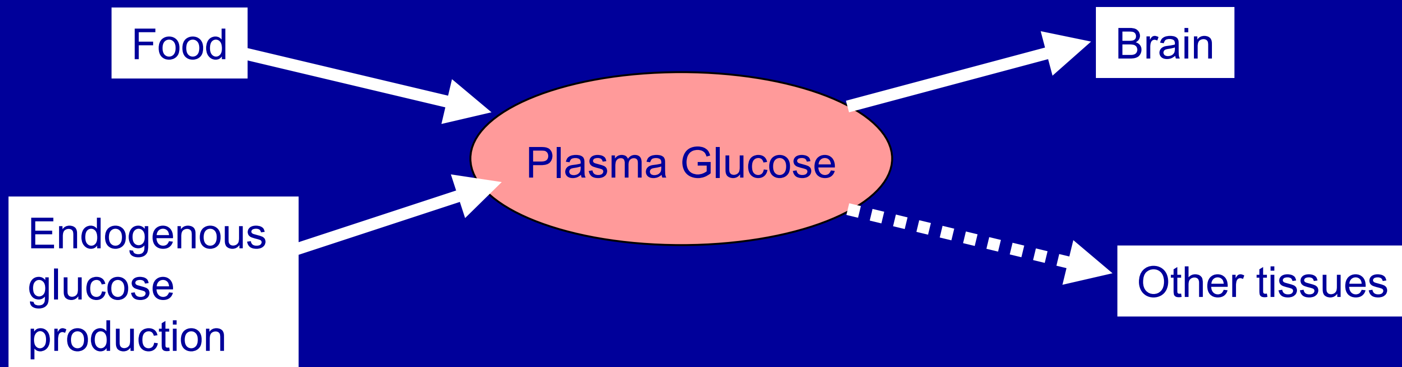




Glucose	Insulin	PEPCK
high	high	low
low	low	high







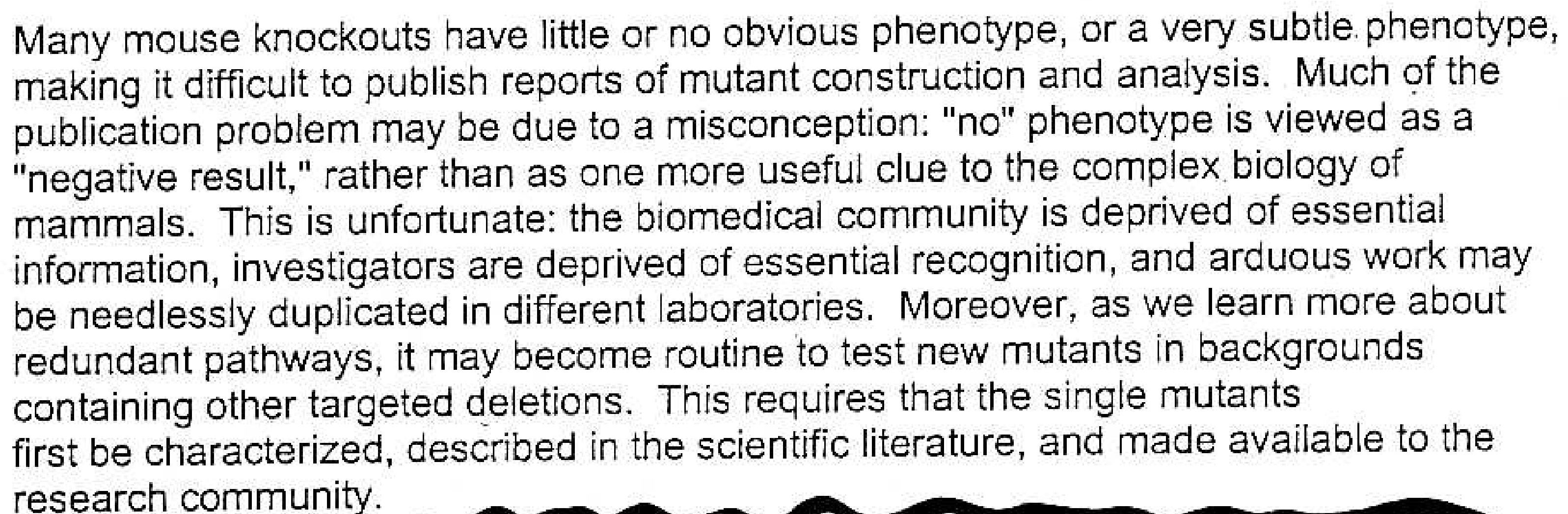
She, P., M. Shiota, K. D. Shelton, R. Chalkley, C. Postic, and M. A. Magnuson. 2000. Phosphoenolpyruvate carboxykinase is necessary for the integration of hepatic energy metabolism. Mol. Cell Biol. 20: 6508-6517.

Abstract: We used an allelogenic Cre/loxP gene targeting strategy in mice to determine the role of cytosolic phosphoenolpyruvate carboxykinase (*PEPCK*) in hepatic energy metabolism.

Mice that lack this enzyme die within 3 days of birth, while mice with at least a 90% global reduction of *PEPCK*, or a liver-specific *knockout* of *PEPCK*, are viable.

Surprisingly, in both cases these animals remain euglycemic after a 24-h fast.

However, mice without hepatic *PEPCK* develop hepatic steatosis after fasting despite up-regulation of a variety of genes encoding free fatty acid-oxidizing enzymes. Also, marked alterations in the expression of hepatic genes involved in energy metabolism occur in the absence of any changes in plasma hormone concentrations. Given that a ninefold elevation of the hepatic malate concentration occurs in the liver-specific *PEPCK* *knockout* mice, we suggest that one or more intermediary metabolites may directly regulate expression of the affected genes. Thus, hepatic *PEPCK* may function more as an integrator of hepatic energy metabolism than as a determinant of gluconeogenesis



SCIENCE VOL 284 25 JUNE 1999

Call for Papers

A NEW SECTION OF MCB: "MAMMALIAN GENETIC MODELS WITH MINIMAL OR COMPLEX PHENOTYPES"

Many mouse knockouts have little or no obvious phenotype, or a very subtle phenotype, making it difficult to publish reports of mutant construction and analysis. Much of the publication problem may be due to a misconception: "no" phenotype is viewed as a "negative result," rather than as one more useful clue to the complex biology of mammals. This is unfortunate: the biomedical community is deprived of essential information, investigators are deprived of essential recognition, and arduous work may be needlessly duplicated in different laboratories. Moreover, as we learn more about redundant pathways, it may become routine to test new mutants in backgrounds containing other targeted deletions. This requires that the single mutants first be characterized, described in the scientific literature, and made available to the research community.

Macroscopic Physiology, evaluating the roles of metabolites

What happens when we move form cells to tissues to mammalian organisms

Tissues have more than one type of cell.

Input ant output to tissues may not be easy to measure.

Mammalian organisms consume a variety of energy sources.

Mammals have many interacting cells and tissues that control affect physiology

Opportunities for bioinformatics

Metabolic Physiologist would like to determine flux

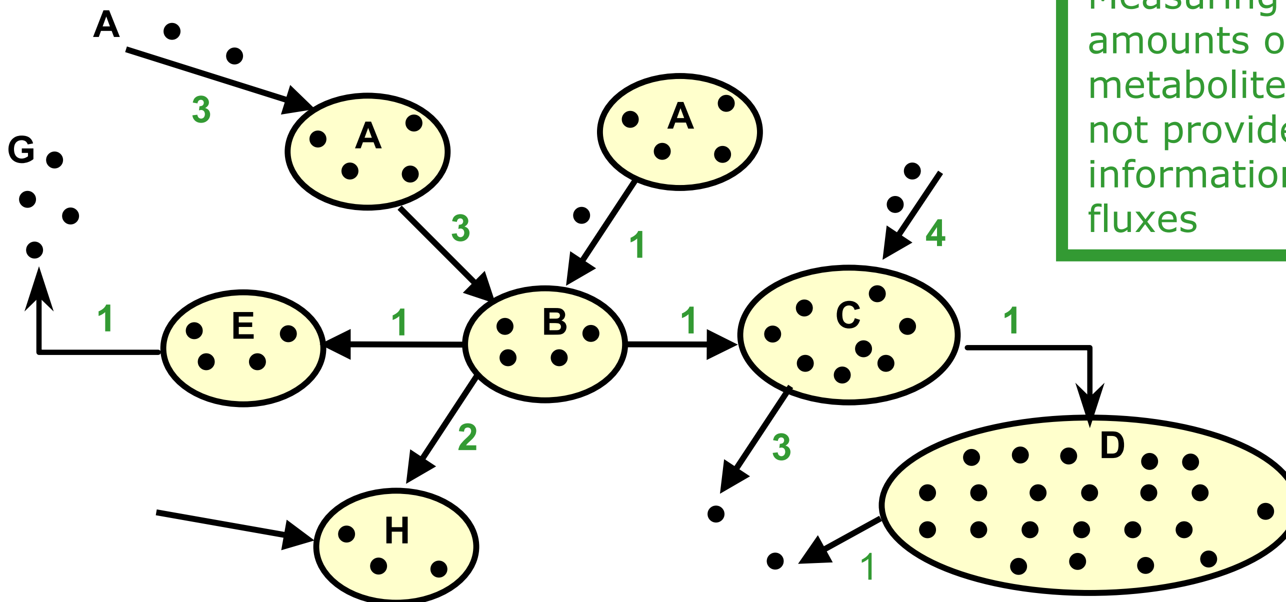
amounts (size of pool is relatively easy to determine)

fluxes are more difficult

Inter-organ fluxes : flux of glutamine from muscle to liver

Intracellular fluxes: lactate to glucose in the liver

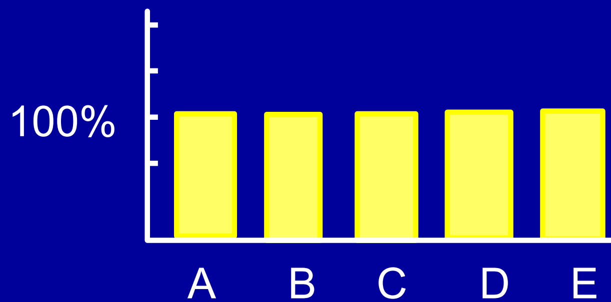
Intra-molecular fluxes: sources of acetyl moiety of citrate

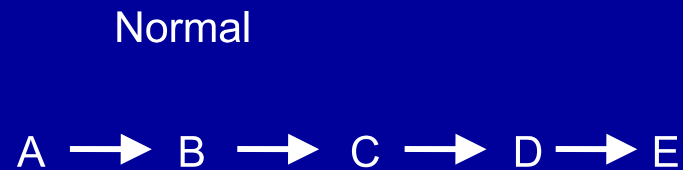


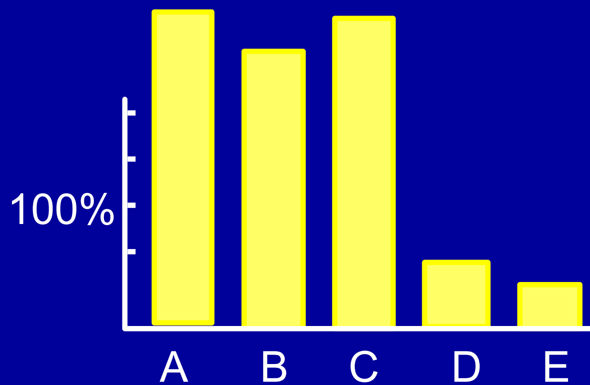
Measuring the amounts of metabolites does not provide information about fluxes

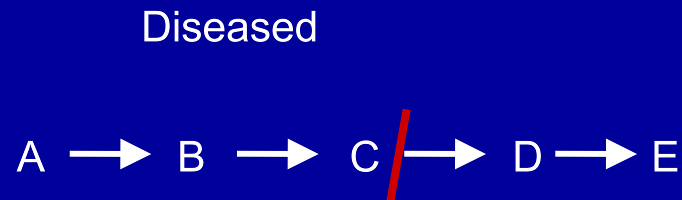
Analysis of Metabolite levels may reveal site of disease lesion or drug action

Cross over theorem.



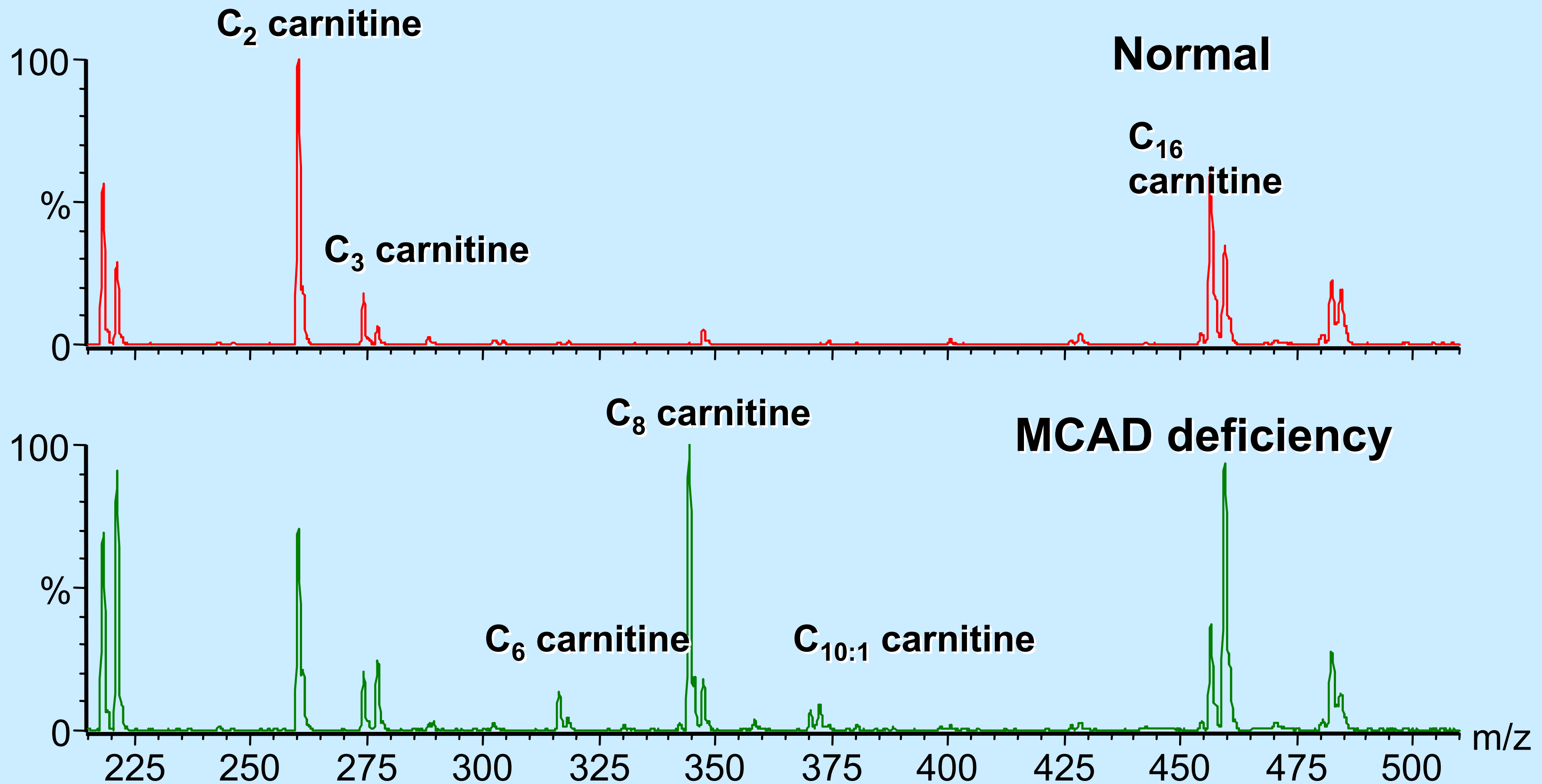


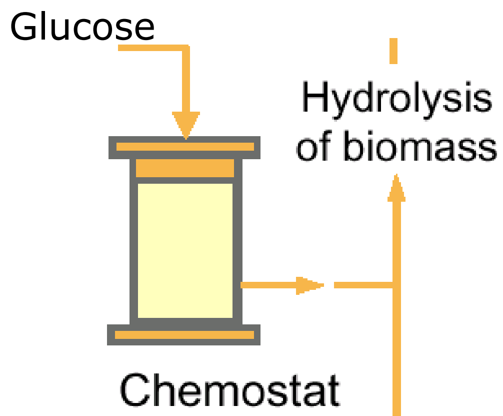


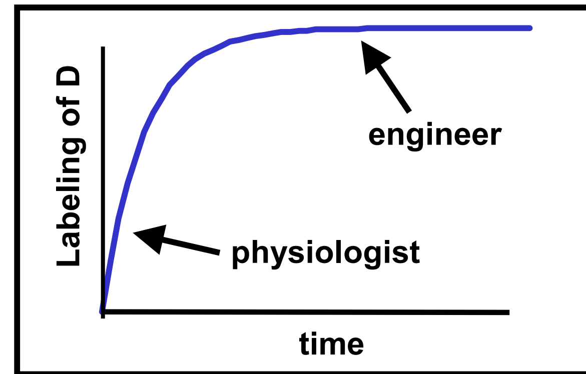


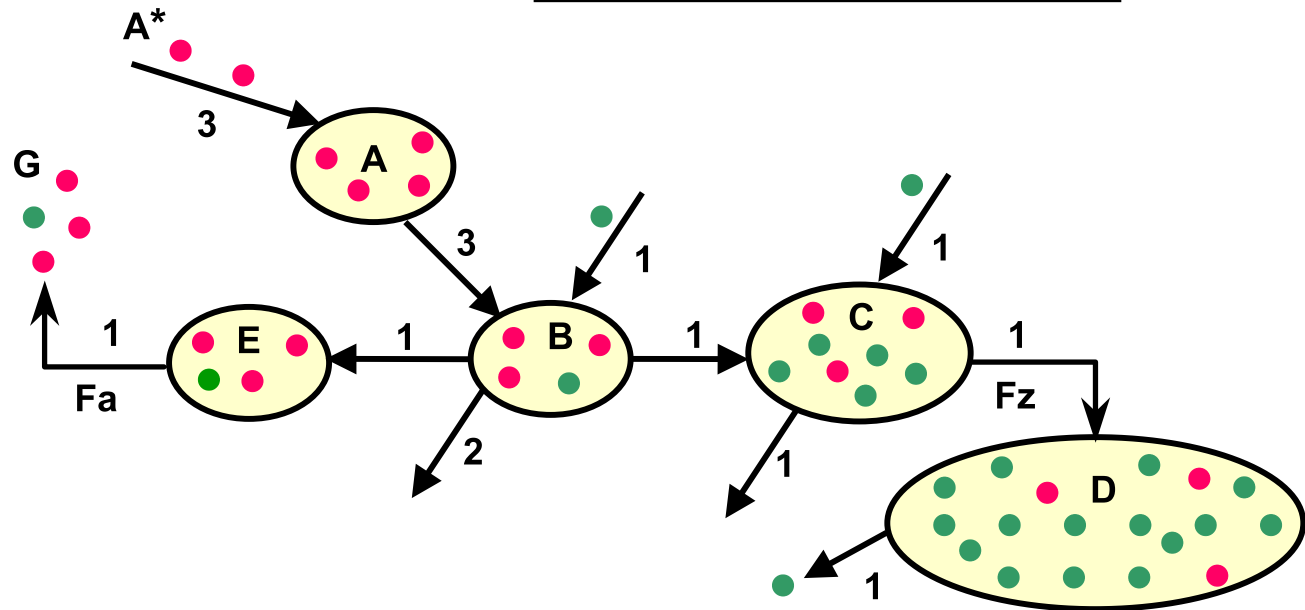
Medium Chain Acyl Dehydrogenase (MCAD) Deficiency.

A disease of muscle weakness detected by changes in urine metabolites.



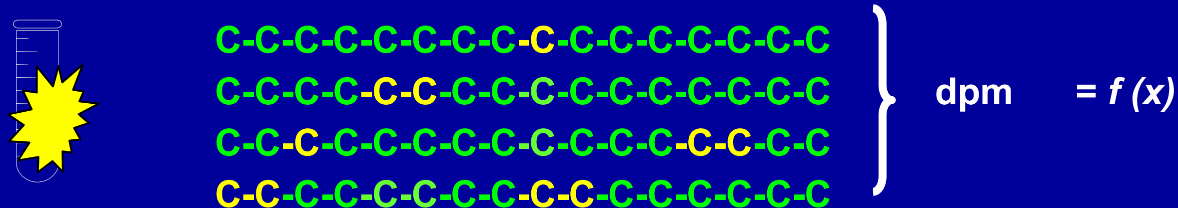




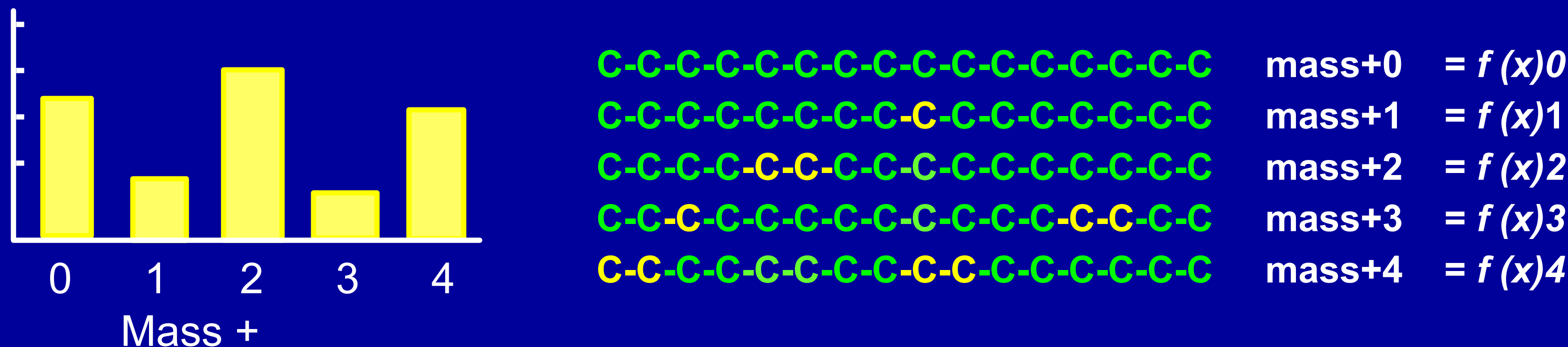


Sample problems: Whole body protein synthesis.
Rate of proliferation of specific cells (DNA synthesis).
Rate of glucose synthesis by the liver.
Rates of lipogenesis.

Liquid scintillation counting of ^{14}C labeled compounds yields only one equation for total amount of labeled atoms. No information about unlabeled



"Organic" Mass spectrometry of ^{13}C labeled compounds and metabolic model yields one equation for the amount of each mass including unlabelled (mass+0)

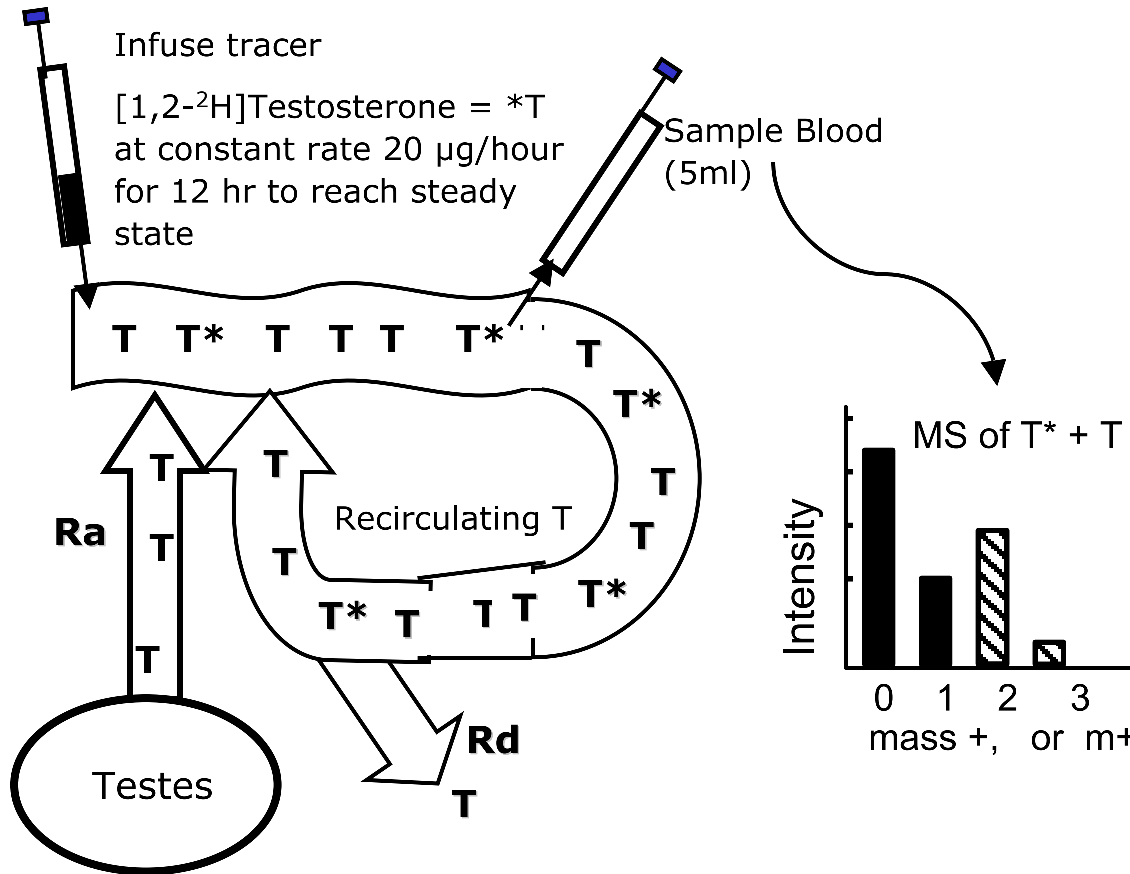


Although both ^{14}C and ^{13}C labeled compounds may be useful in metabolic studies. There are fundamental differences in the type of information available when these isotopes are detected by standard methods.

Radioisotopes are detected by Decay.
1 uCi = 2.2 * 10^6 dpm

Isotope	half life	decay constant	maximum SA
^{14}C	5730 years	1.209×10^{-4} /year	62.5 Ci/mole
^{11}C	20.4 min	3.40×10^{-2} /min	9.20×10^{9} Ci/mole
^{3}H	12.4 years	5.59×10^{-4} /year	2.88×10^{4} Ci/mole
^{35}S	87.4 days	7.93×10^{-3} /day	1.49×10^{6} Ci/mole
^{32}P	14.3 days	4.85×10^{-2} /day	9.13×10^{6} Ci/mole

Determination of Testosterone production rates by Stable Isotope Dilution Organic Mass Spectrometry



At t = 12 hr
Begin sampling

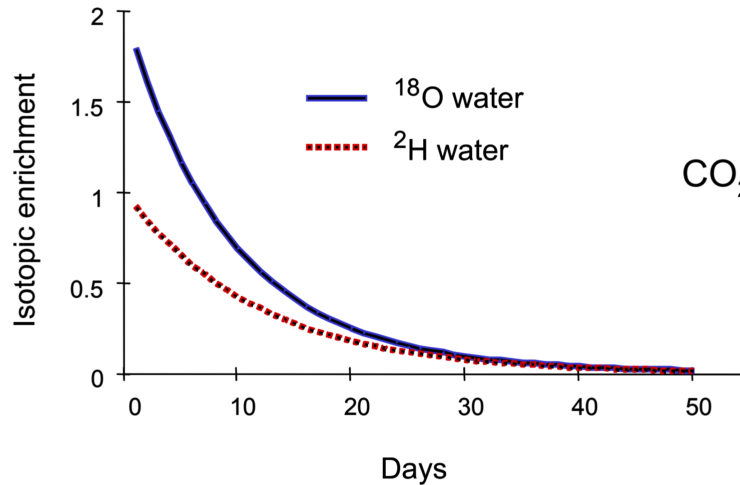
A Steady State method

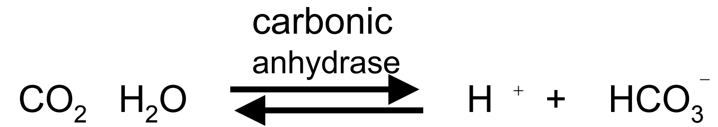
rapid changes in T production will be damped

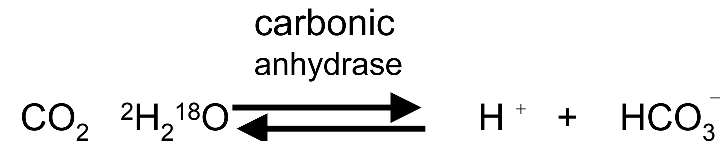
useful only for T production

Estimated T production rates in men of 64 to 101 µg/hour
in women of 3.6 to 6.0 µg/hour
Issue of lowering infusion rate

Doubly labeled water method for CO_2 production and for total energy expenditure







^{18}O lost, respiration $C^{18}O_2$ and as water $H_2{}^{18}O$

^{2}H lost as water $H_2{}^{18}O$

Rate of CO_2 production is computed from rate of ^{18}O loss in respiration

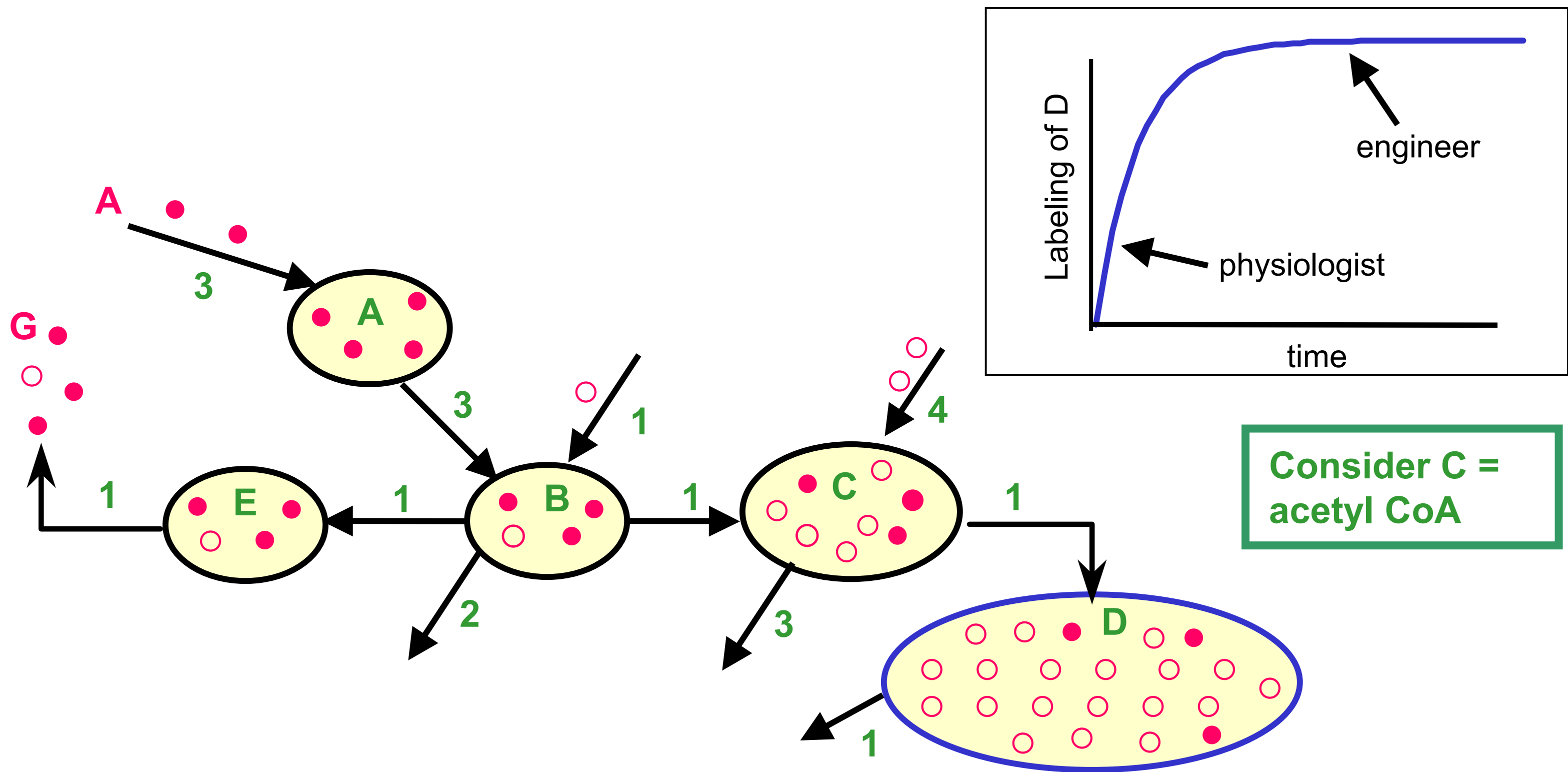
Isotopes are the key to determining metabolic fluxes.

A common ground for Metabolic Physiologists and Metabolic Engineers

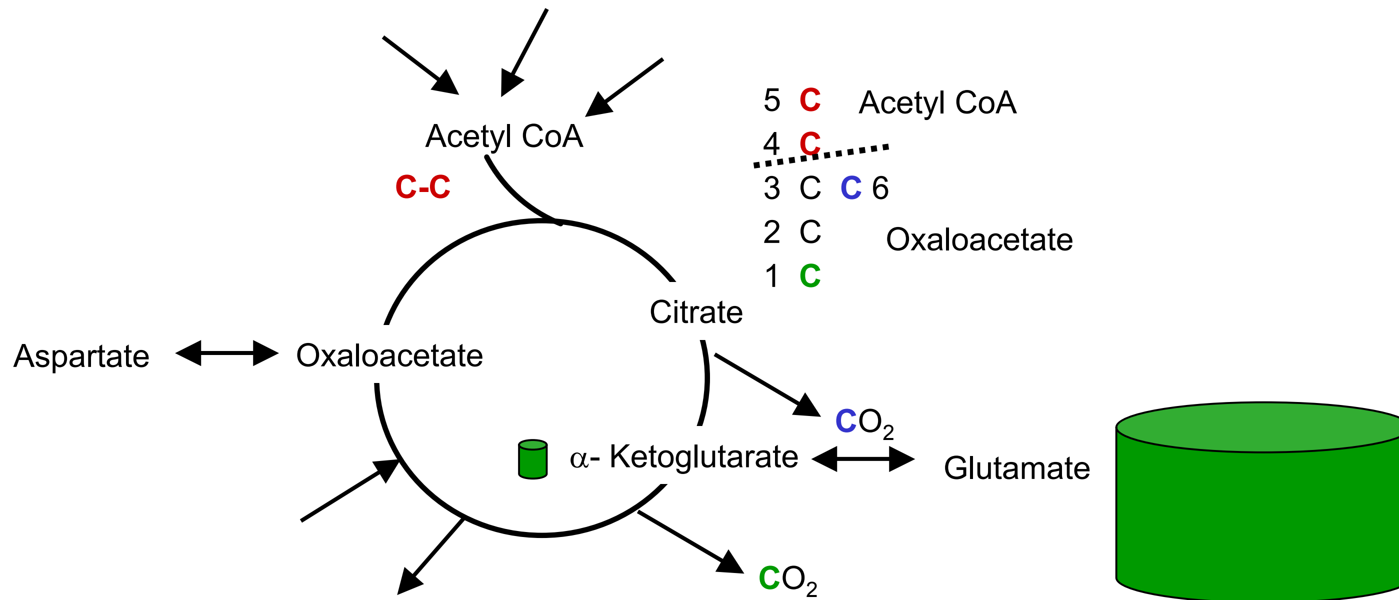
Many kinds of tracers may be used

Isotopes of various atoms ^{14}C, ^{13}C, ^{11}C, ^{2}H, ^{3}H, ^{15}N, ^{17}O, ^{18}O, ^{32}P, etc.

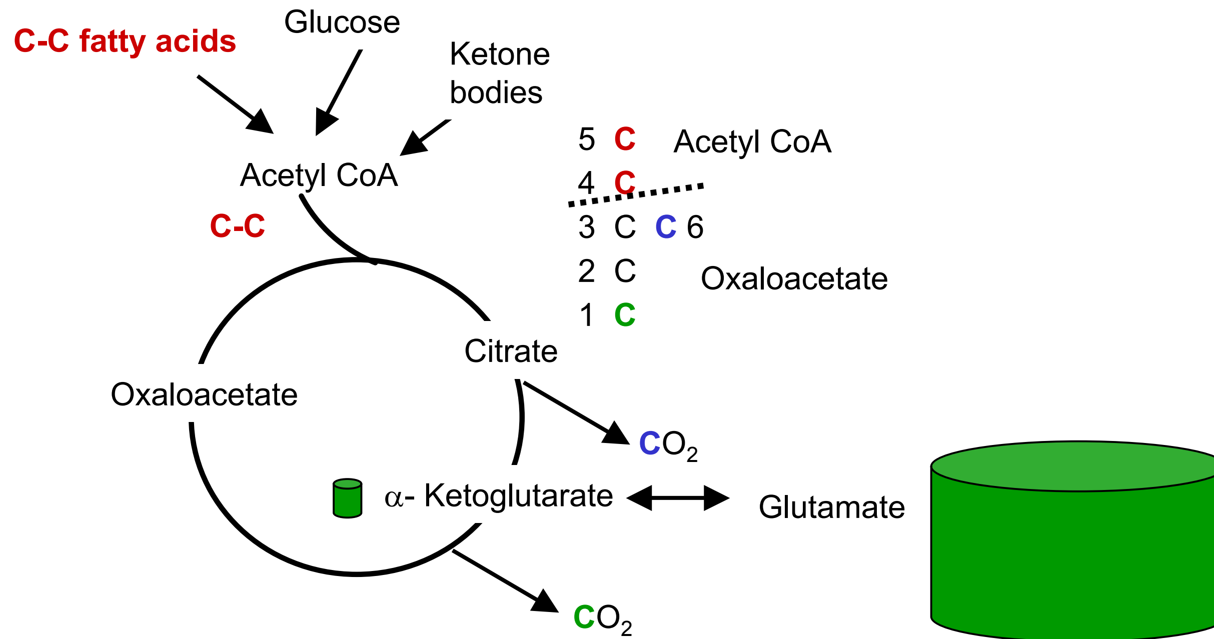
Isotopes detected by numerous methods



Measuring the rate of the TCA cycle in the heart with 13C and NMR

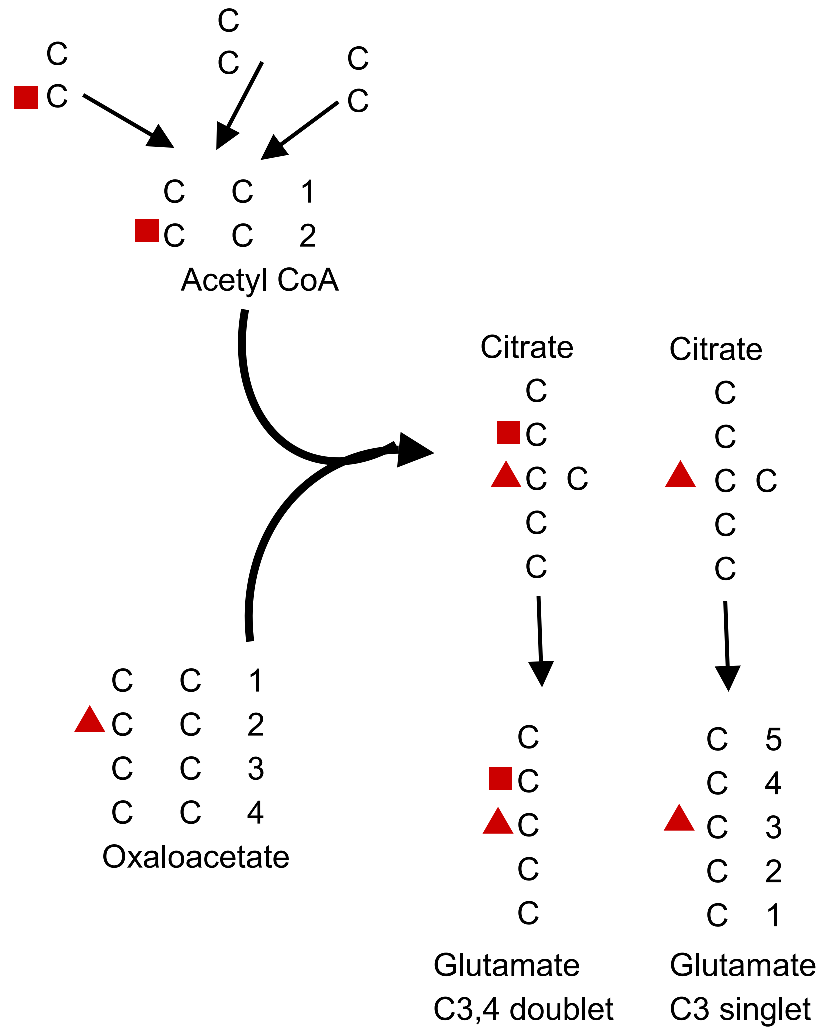


Measuring the rate of the TCA cycle in the heart with 13C and NMR



The heart is an omnivore and we cannot take a sample of heart
But we do have 13C tracers and NMR

Labeling of glutamate, visible in the NMR, in vivo
provides estimate for fraction acetyl CoA derived from labeled precursor



Many interesting biomolecules are products of condensation of identical subunits.

Condensation Polymerization

n A	→	1 B
2 lactate	→	1 glucose
16 acetate	→	1 palmitate
18 acetate	→	1 cholesterol + 9 C
6 mevalonate	→	1 cholesterol + 3 C
2 acetate	→	1 acetoacetate
? H from water	→	1 cholesterol or fatty acids
n hydroxyproline	→	1 collagen
n adenylic acid	→	1 poly A tail on Messenger RNA
n glucose	→	glycogen or glycosylated protein
n aa (X)	→	peptide ….XXX….

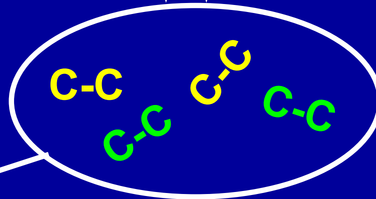
Isotopomer Spectral Analysis (ISA) of Fatty acid Biosynthesis

Media with [U-13C]Glucose
C-C-C-C-C-C

C-C Acetyl CoA

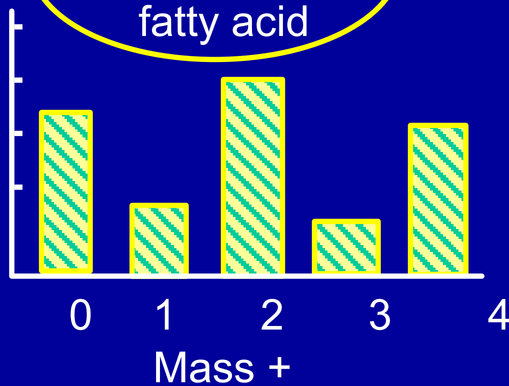
C-C (precursor dilution) C-C Natural sources of Acetyl CoA , 12C

D = ? 1-D ?



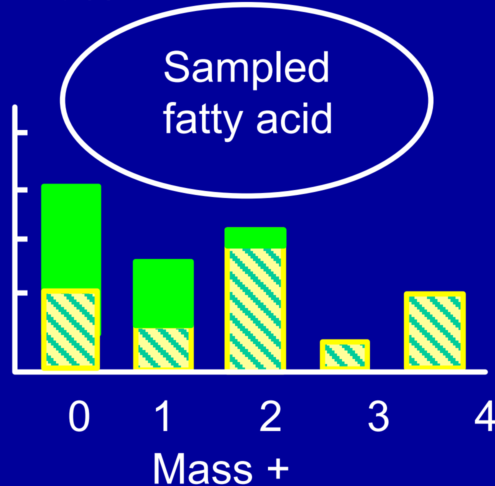
Intracellular lipogenic precursor Acetyl CoA

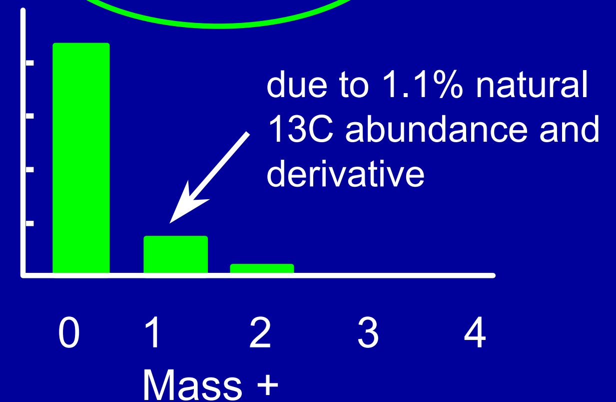
8 Acetyl CoA -> 1 Palmitate



(product dilution)

g(t) =? 1-g(t)





Composition of precursor (acetate) for biosynthesis is known

Tracer ^{13}C Acetate

$T_0 = 0.01$, **C-C**

$T_1 = 0.02$, **C-C**

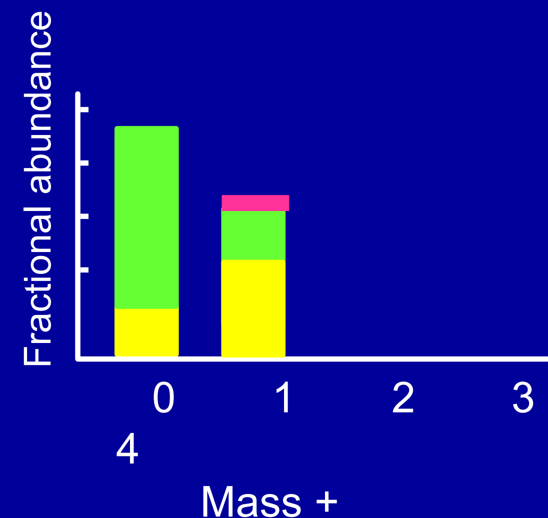
$T_2 = 0.97$, **C-C**

Natural Acetate

$N_0 = 0.9799$, **C-C**

$N_1 = 0.0200$, **C-C**

$N_2 = 0.0001$, **C-C**



Fractional abundance

$$\text{Mass+0} = g(t) * (D\,T_0 + (1-D)\,N_0)^8 + (1-g(t))\,N_0^8$$

New synthesis

pre-existing

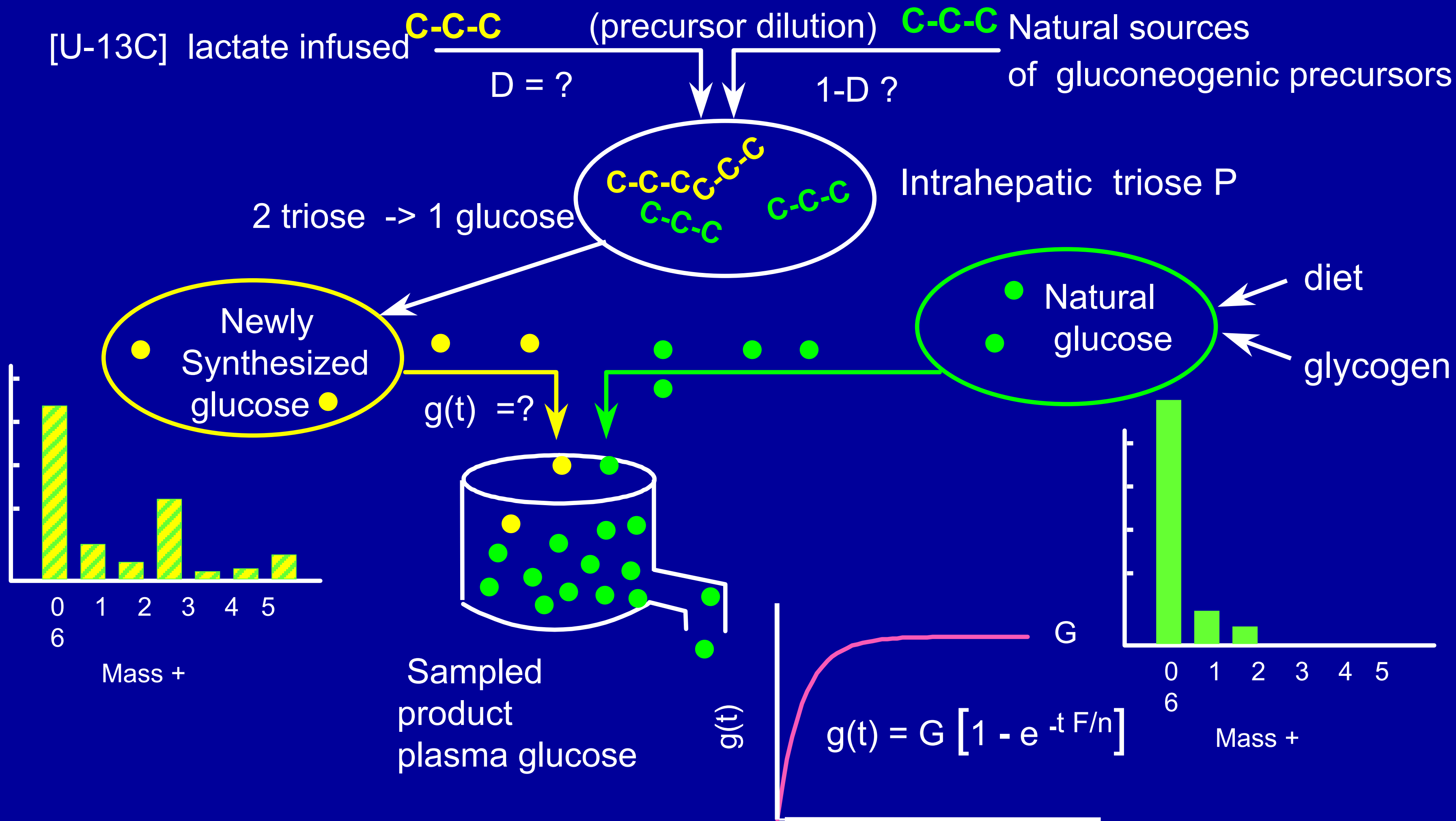
$$\text{Mass+1} = g(t) * 8 * (D\,T_0 + (1-D)\,N_0)^7 * (D\,T_1 + (1-D)\,N_1) + (1-g(t))\,8 * N_0^7 * N_1 + \text{derivative effect}$$

New synthesis

pre-existing

ISA vs MIDA ISA uses nonlinear regression to fit data to model. No correction for deriv prior to analysis. Derivative is included in the model.

Isotopomer Spectral Analysis of Gluconeogenesis

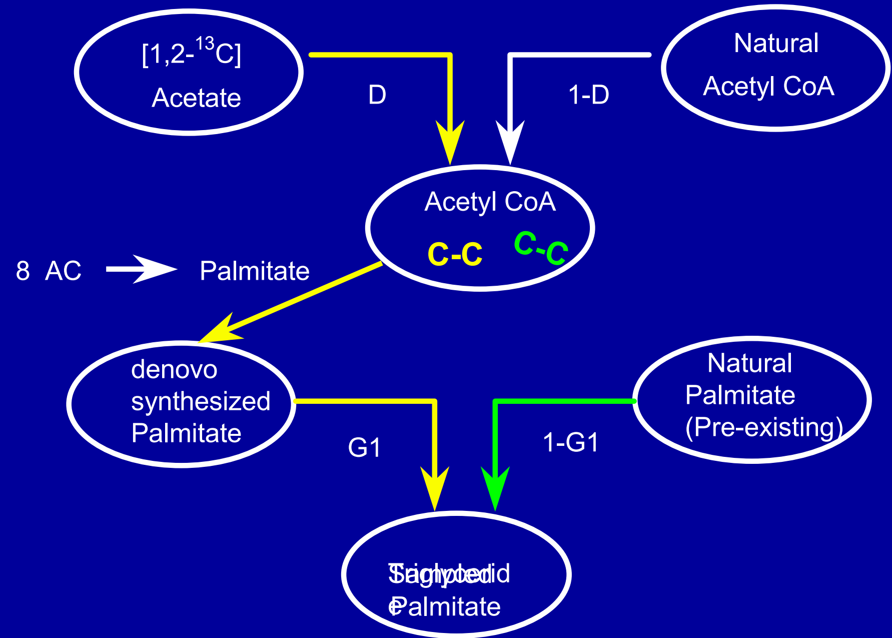


Hepatoma Cell Palmitate Synthesis

Media [1,2-13C]Acetate

Best fit, lowest error is:

D = 0.63 ± 0.004
g(t) = 0.52 ± 0.003



Experiment

MODEL

g(t) New Synthesis

1-g(t) Pre-existing

FRACTIONAL ABUNDANCE

0.80
0.60
0.40
0.20
0.08
0.06
0.04
0.02
0.00

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

MASS +

(Media) Labeled Acetate , 13C

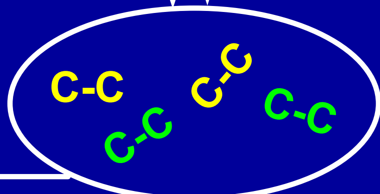
C-C

Natural sources of Acetyl CoA , 12C

C-C

D = ?

1-D ?



18 acetate ->1 squalene

new squalene

Squalene 30C

Lanosterol → Dihydrolanosterol

$\Delta^{8,24}$-dimethylsterol → Δ^{8} -dimethylsterol

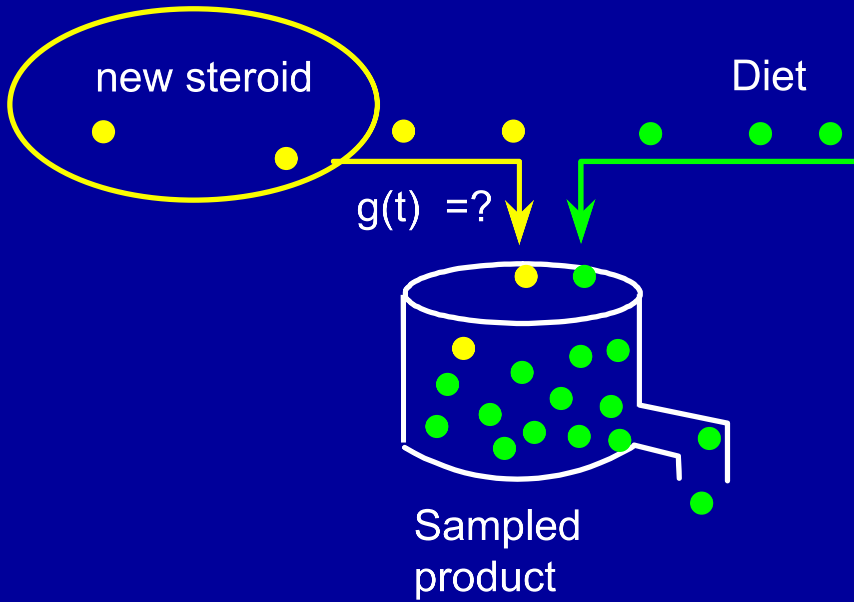
$\Delta^{8,24}$-monomethylsterol → Δ^{8}-monomethylsterol

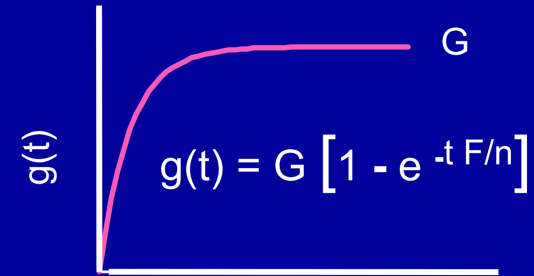
$\Delta^{8,24}$-cholestenol → Δ^{8}-cholestenol

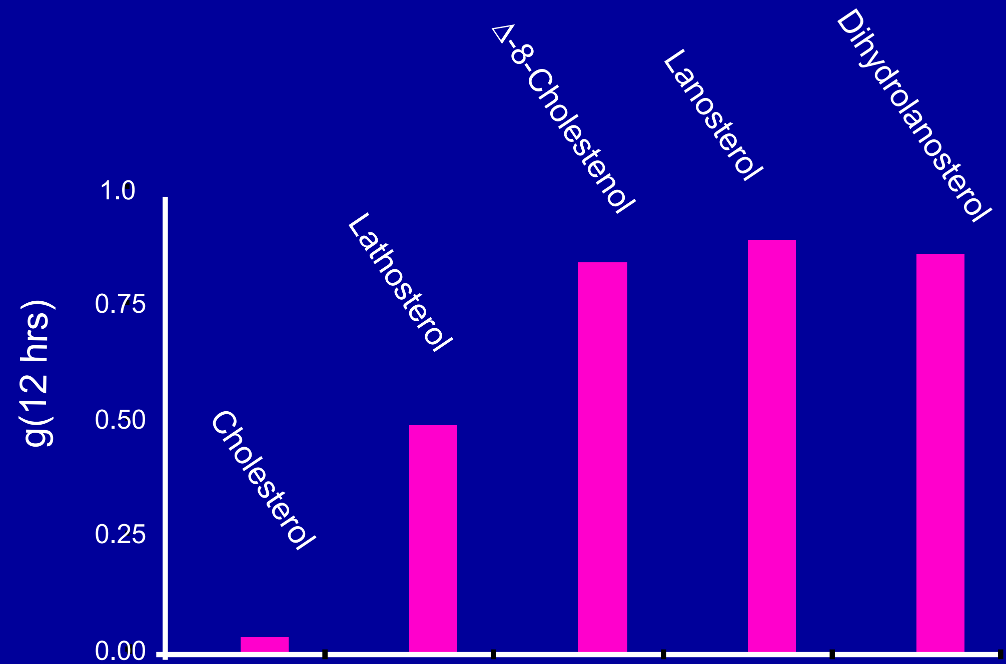
Lathosterol

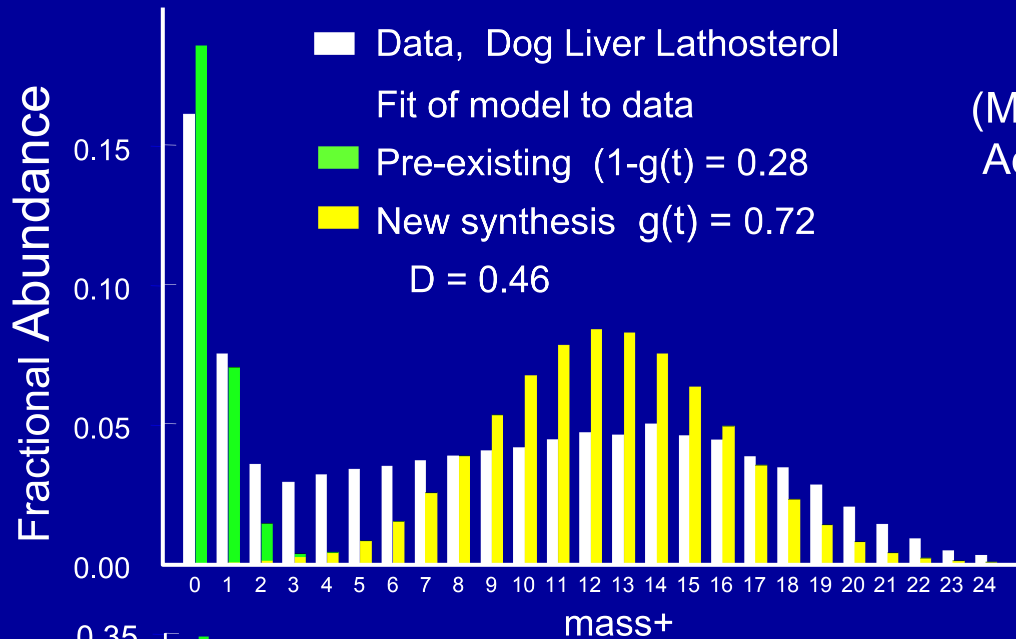
Desmosterol → Cholesterol 27C

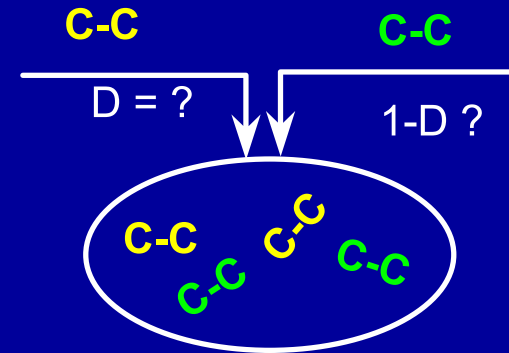
Isotopomer Spectral Analysis : g(12 hrs) values for Cholesterol and Its Precursors

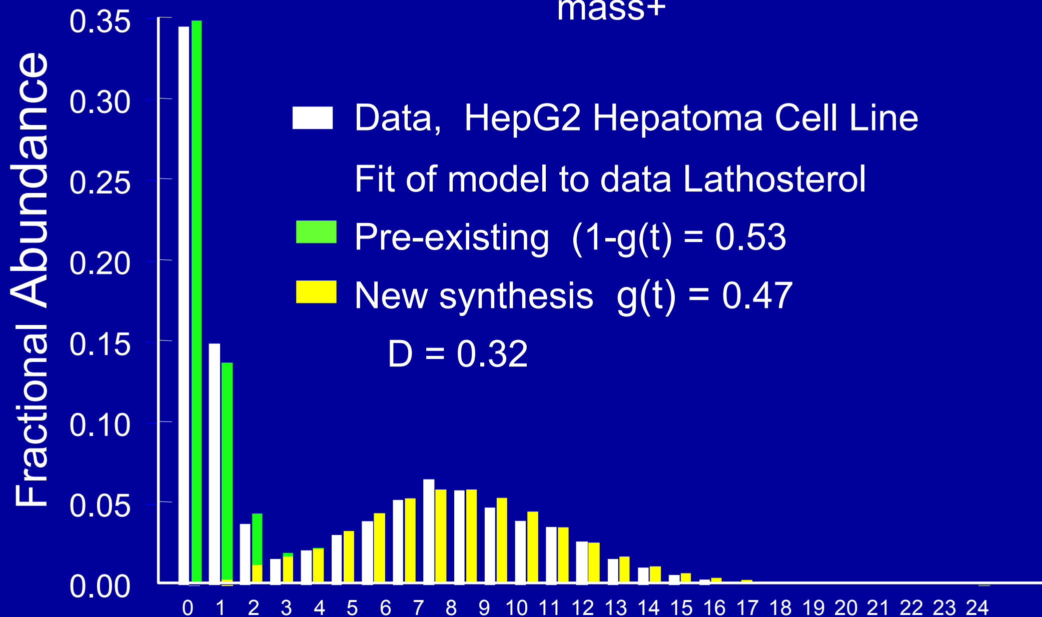




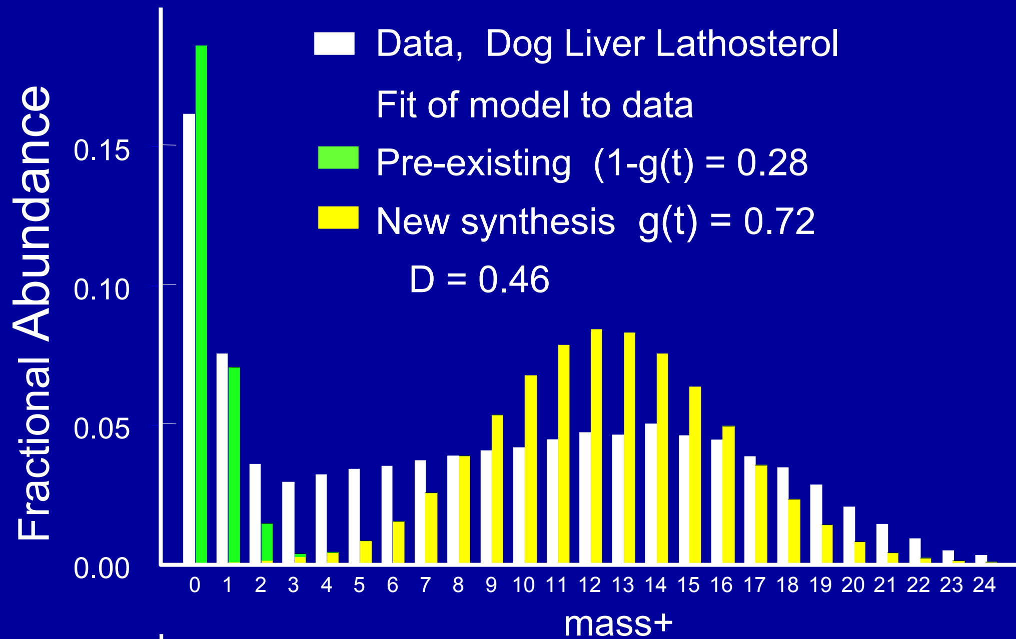






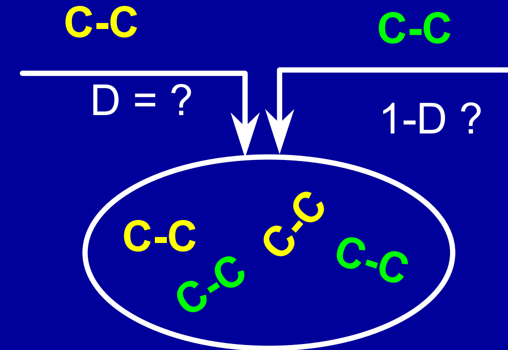


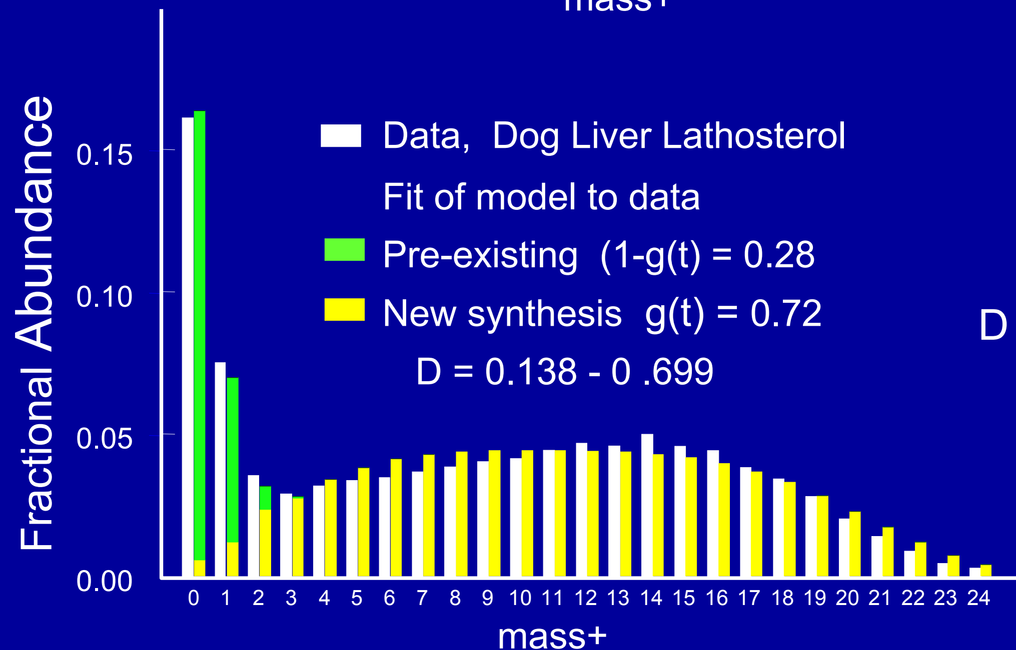
The standard form of the model assumes that D is constant . But this does not always appear to be true.



(Media) Labeled Acetate , 13C

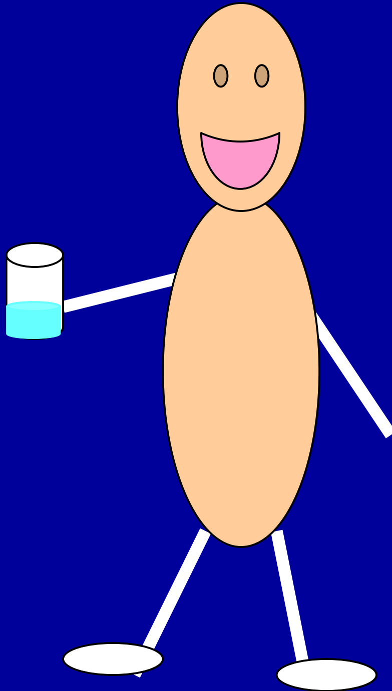
Natural sources of Acetyl CoA , 12C





D = 0.699

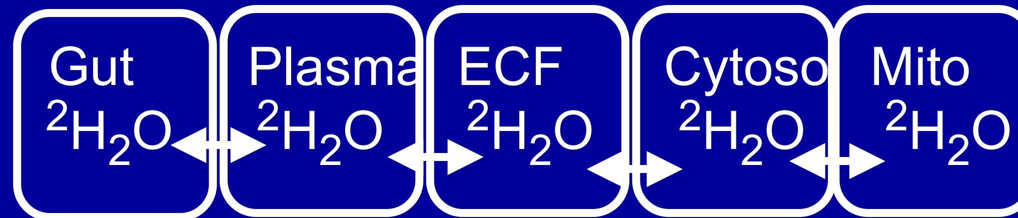
D = 0.138



Subject agrees to consume ^2H_2O to yield 0.35% total body water.

Because water equilibrates across cell membranes the “dilution problem” is simplified.

The enrichment of plasma water equals the enrichment of all body water pools



Also discuss breath tests   H. pylori infection diagnosis, fat malabsorption