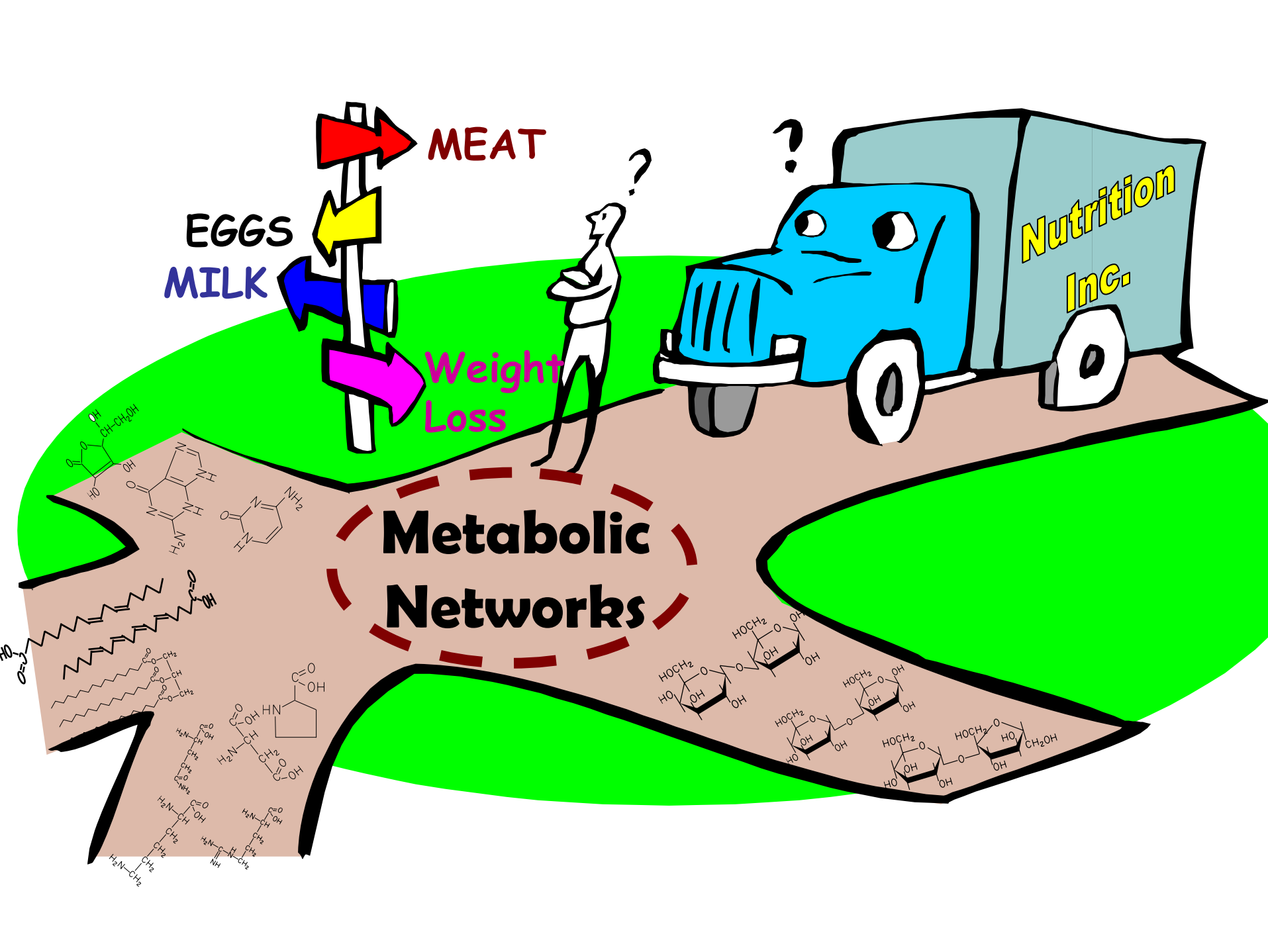
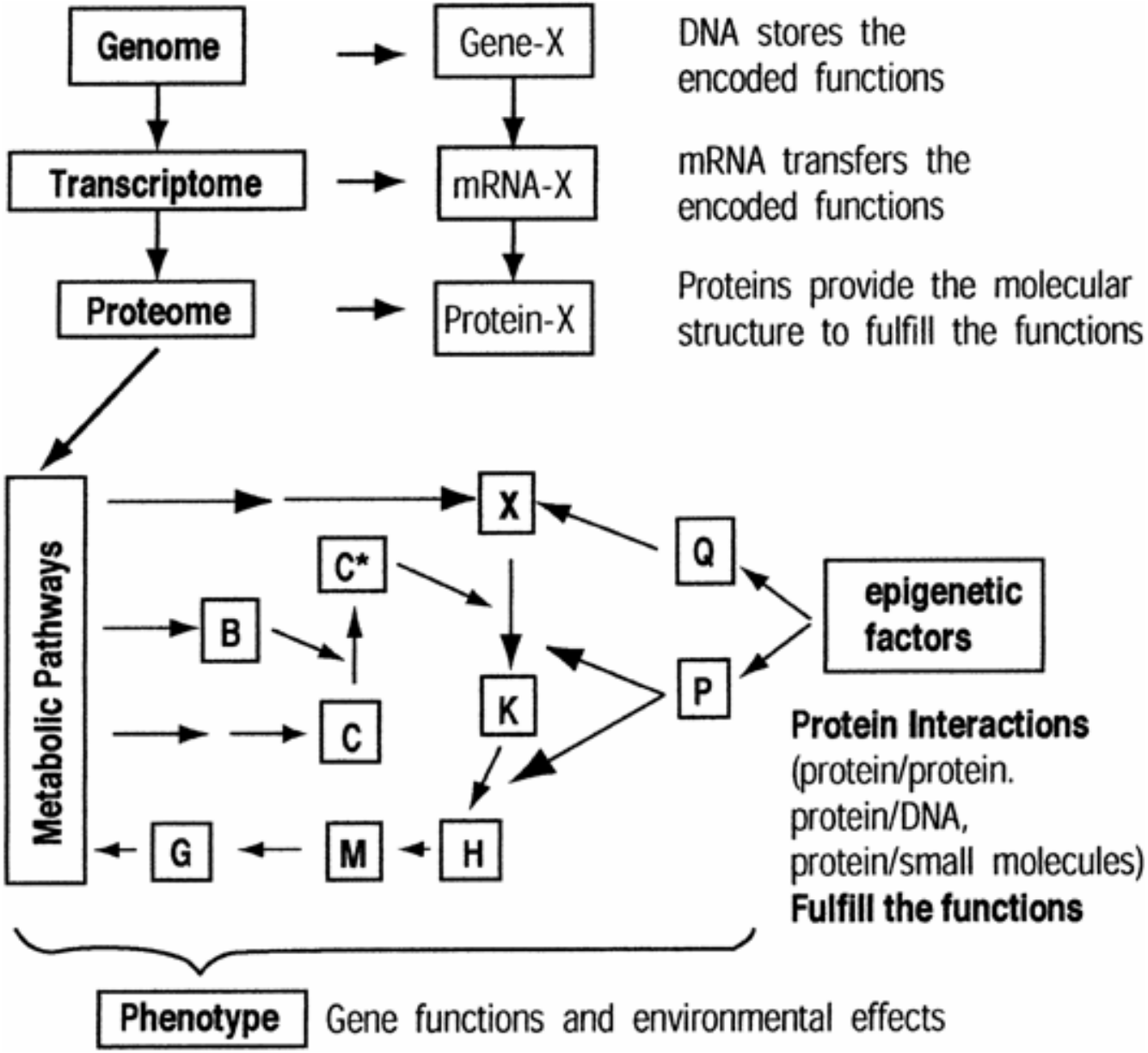
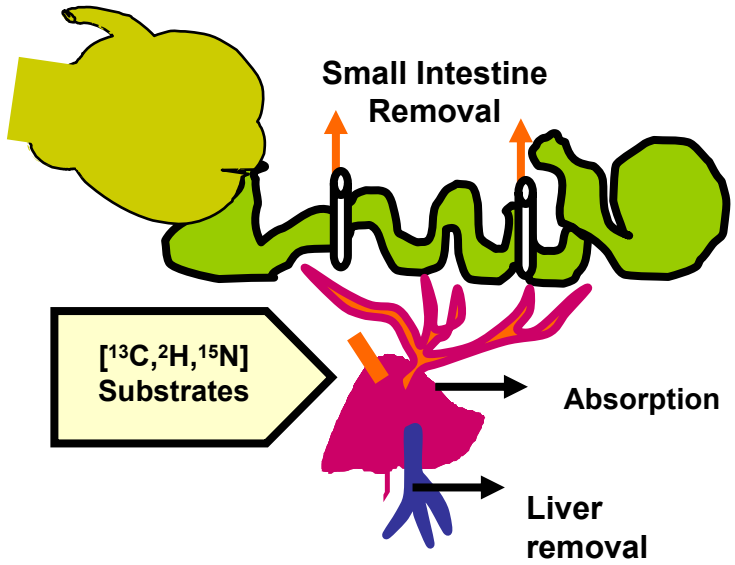
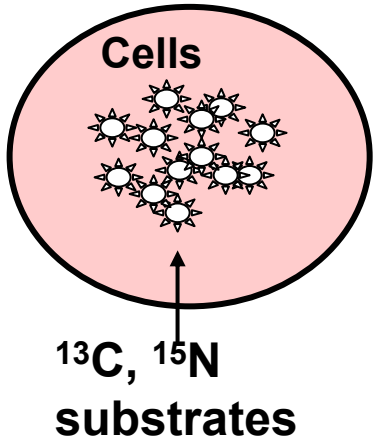

MEAT
EGGS
MILK
Weight Loss
Nutrition Inc.
Metabolic Networks


Genome
Transcriptome
Proteome
Gene-X
mRNA-X
Protein-X
DNA stores the encoded functions
mRNA transfers the encoded functions
Proteins provide the molecular structure to fulfill the functions
Metabolic Pathways
X
C*
B
C
K
G
M
H
Q
P
epigenetic factors
Protein Interactions
(protein/protein.
protein/DNA,
protein/small molecules)
Fulfill the functions
Phenotype
Gene functions and environmental effects

Tracers at the whole body or tissue level :

Organ balance and tissue metabolism





Utilization of tracers to study metabolism at the whole body / tissue level

Metabolic fate of carbohydrates from milk replacer in heavy milk calves
J.J.G.C. van den Borne, G.E. Lobley, J.W. Blum, W.J.J. Gerrits

Measurement of fatty acid oxidation in swine using ^{13}C labeled fatty acids
E. Van Heugten, J.J.G.C. van den Borne, M.W.A. Verstegen, J. van Milgen, W.J.J. Gerrits

Mathematical analysis of human [$^{13}CO_2$]-breath test results: Post prandial fate of amino acids is related to their dietary form
V.V.A.M. Schreurs, J.A. Nolles, K. Krawielitzki, J. Bujko

Effect of graded dietary tryptophan levels on [1-^{13}C]leucine oxidation and nitrogen deposition in growing pigs
U. Hennig, P. Junghans, J. Bartelt, C. Relandeau, A. Tuchscherer, C.C. Metges

Endogenous nitrogen flows in the digestive tract of lactating dairy cows: comparison between estimations using total ^{15}N versus ^{15}N-amino acid isotope dilution
D.R. Ouellet, R. Berthiaume, R. Martineau, H. Lapierre

Nutrient tracing studies

A multiple-isotope approach to studying arginine metabolism in neonatal piglets
K.L. Urschel, M. Rafii, P.B. Pencharz, R.O. Ball

Mutations in metabolic pathways, what role does genetic background play?
J. Marini, A. Erez, B. Lee

The use of glutamine and glutamate for gluconeogenesis and non-essential amino acid synthesis in late term chicken embryos .
N.E. Sunny, J. Adamany, S.L. Owens, B.J. Bequette

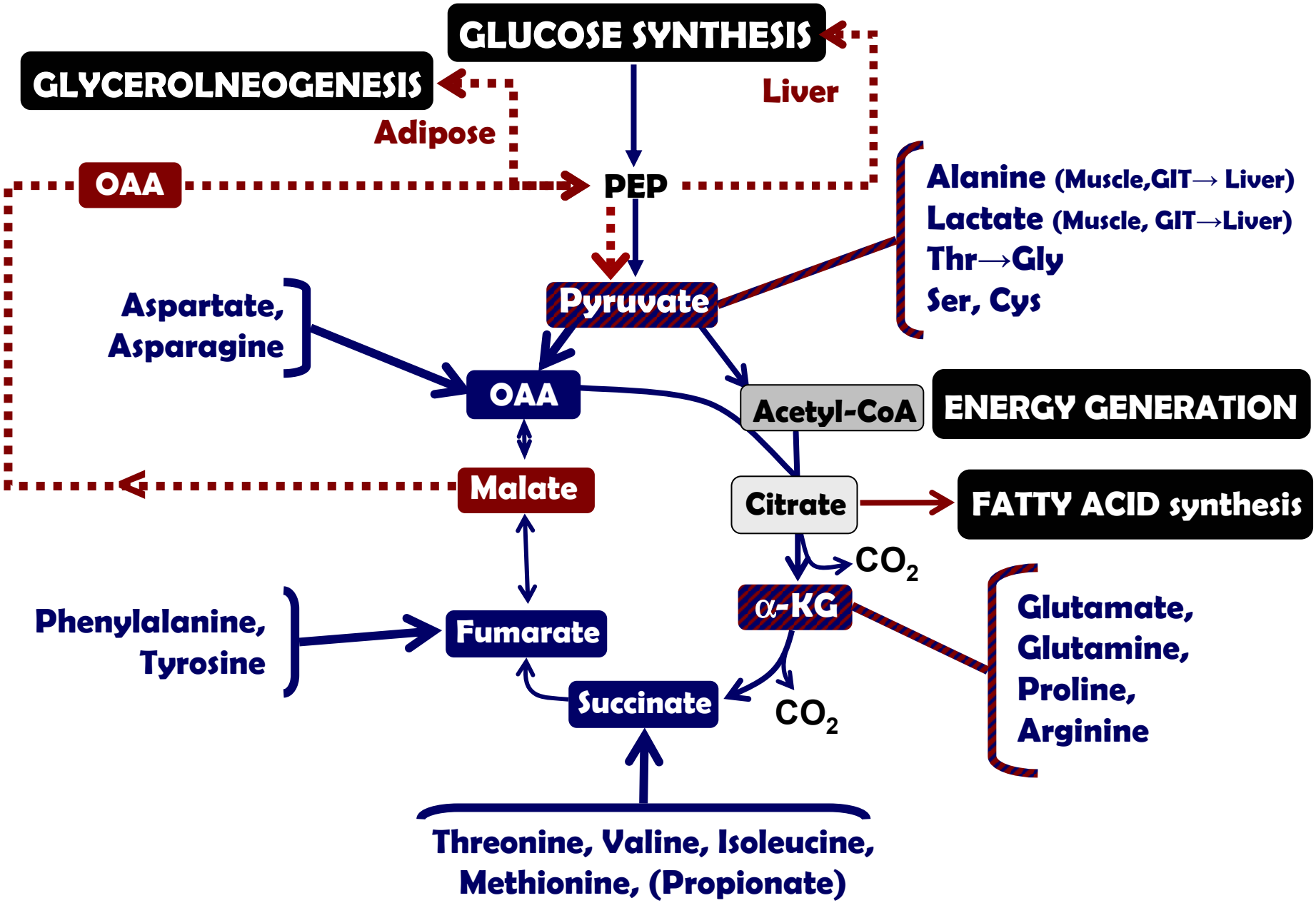
Association of various methodologies (including tracers)

Gut function of pigs in relation to weaning
P.J.A. Wijtten, H. Bouritius, P.R.T. Bonekamp, G.J. Witte, J.J. Verstijnen, TA.AT.G. van Kempen

Combination of two approaches to study mammary use of glucose for lactose synthesis in dairy cows: net mammary balance and RNA levels
M. Boutinaud, M.H. Ben Chedly, E. Delamaire, J. Guinard-Flament

Molecular markers for a delicate balance between protein and lipid metabolism in subcutaneous fat tissu of beef cow
A. Shabtay, H. Eitam, A. Orlov, Y. Aharoni, I. Izhaki, A. Brosh

How are the Inputs ( ) and Outputs ( ) Coordinated?



What are some BIG questions in Nutrient Metabolism?

What are some BIG questions in Nutrient Metabolism?

What is metabolic efficiency or inefficiency?

What approaches can be used to measure metabolic efficiency or inefficiency?

What are the metabolic networks that underlie the efficiency or inefficiency of protein and energy utilization?

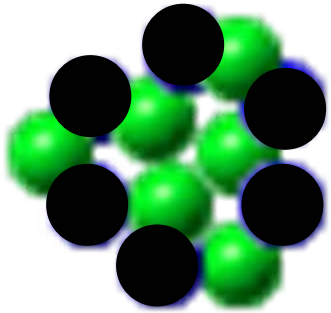
Stable isotopes
and
Mass Spectrometry

Table 1. Isotopes commonly used in biological research

Common stable	Rare stable	Radioactive
^{1}H	^{2}H (0·02 %)	^{3}H
^{12}C	^{13}C (1·1 %)	^{14}C
^{14}N	^{15}N (0·37 %)	^{13}N*
^{16}O	^{18}O (0·04 %)	^{11}O*

* No long-lived radioisotopes of these elements.

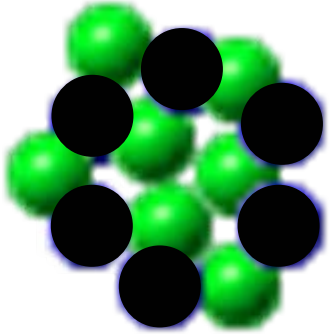
What is a Stable vs Radio-Isotope?



^{12}C

Protons – 6

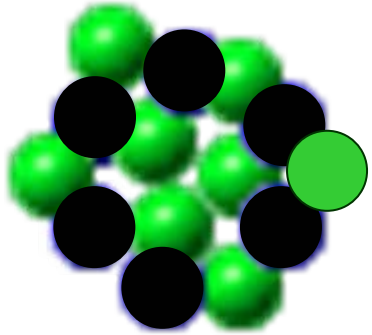
Neutrons - 6



^{13}C

Protons – 6

Neutrons - 7



^{14}C

Protons – 6

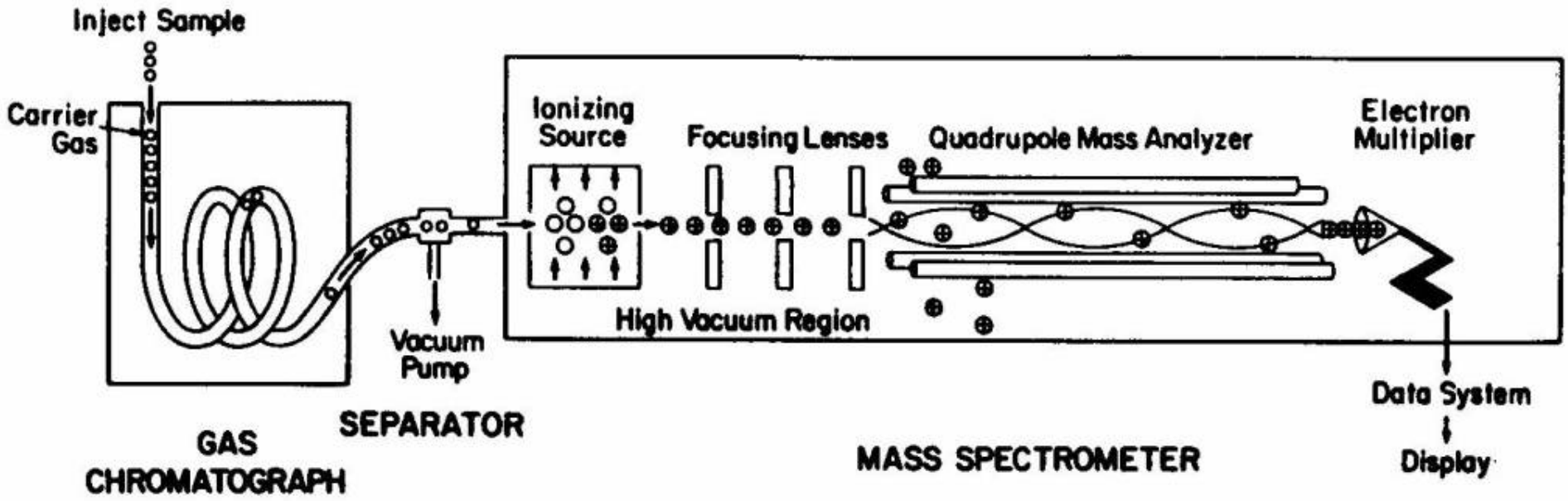
Neutrons - 8

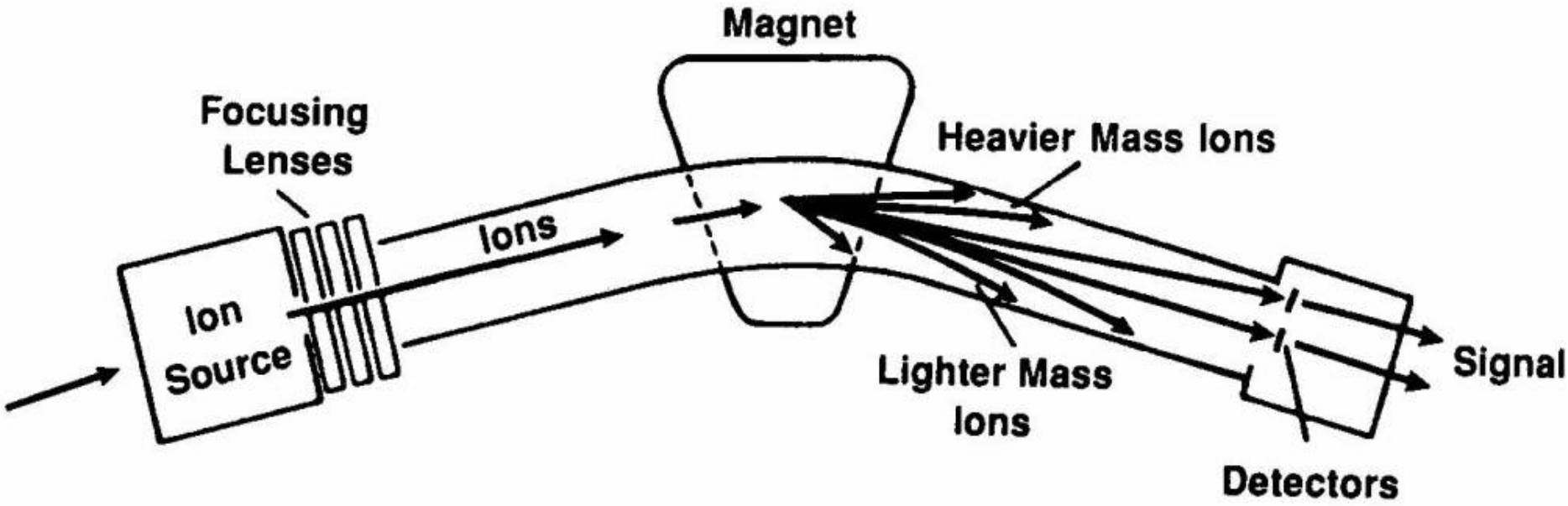
Element	Stable isotope	Percent natural abundance	Element	Stable isotope	Percent natural abundance
H	1	99.985	Fe	54	5.82
	2	0.015		56	91.66
				57	2.19
C	12	98.89		58	0.33
	13	1.11			
			Zn	64	48.89
N	14	99.63		66	27.81
	15	0.37		67	4.11
				68	18.57
O	16	99.76		70	0.62
	17	0.037			
	18	0.204	Se	74	0.87
				76	9.02
S	32	95.0		77	7.58
	33	0.76		78	23.52
	34	4.22		80	49.82
				82	9.19
			Si	28	92.21
				29	4.90
				30	3.09

Measuring Isotopic abundance of Biomolecules

Isotope Ratio Mass Spectrometry
Combustion-IRMS
Gas Chromatography-C-IRMS
GC-MS
Liquid Chromatography-MS
HPLC-MS
ESI-MS
ESI-MS/MS
LC-MS/MS
Nuclear Magnetic Resonance
LC-NMR

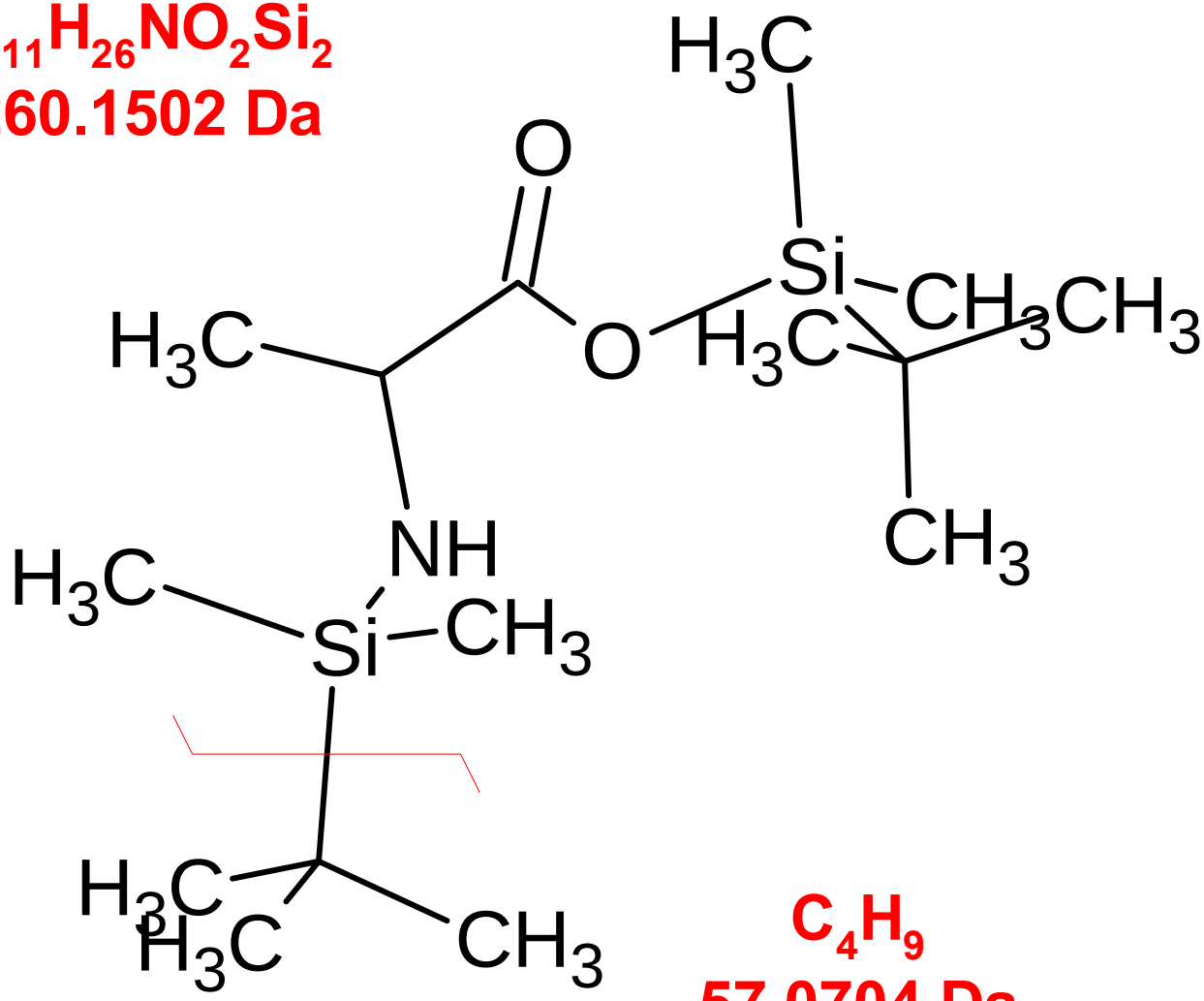
Gas Chromatography-Mass Spectrometer




Magnet
Focusing Lenses
Heavier Mass Ions
Ions
Ion Source
Lighter Mass Ions
Signal
Detectors

tBDMS-Alanine (MW 317.2)

$C_{11}H_{26}NO_2Si_2$
260.1502 Da



C_4H_9
57.0704 Da

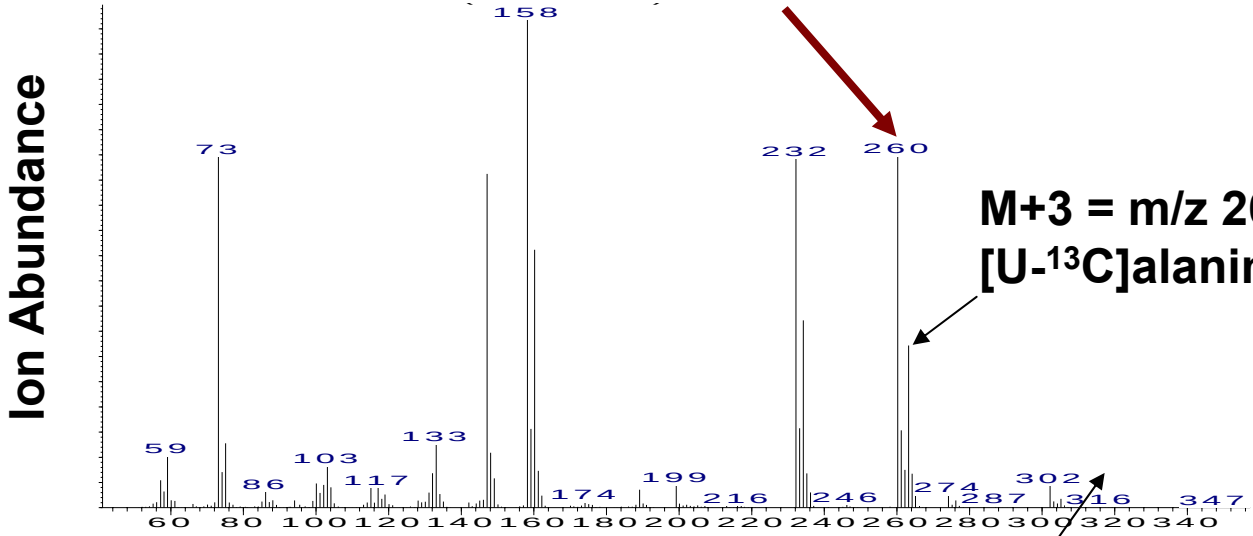
tBDMS-Alanine

Electron Ionisation (EI)

Fragmentation

'hard' ionization

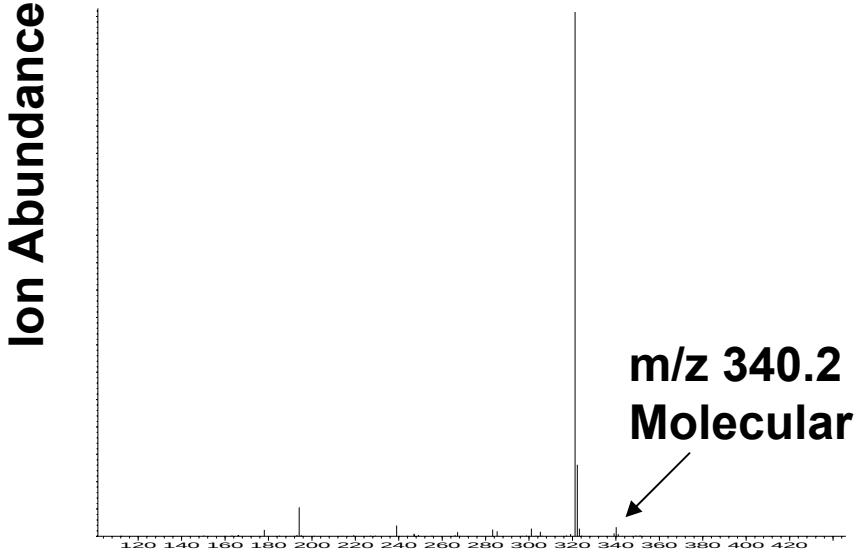
M+0 = m/z 260.2



M+3 = m/z 263.2
[U-^{13}C]alanine

m/z 317.2
Molecular ion

M+0 = m/z 321.2



m/z 340.2
Molecular ion

HFBA-Alanine

Negative Chemical Ionisation (NCI)

Fragmentation

'soft' ionization

Composition of Alanine-HFBA derivative

atom	#
C	11
F	6
O	3
N	1
H	13

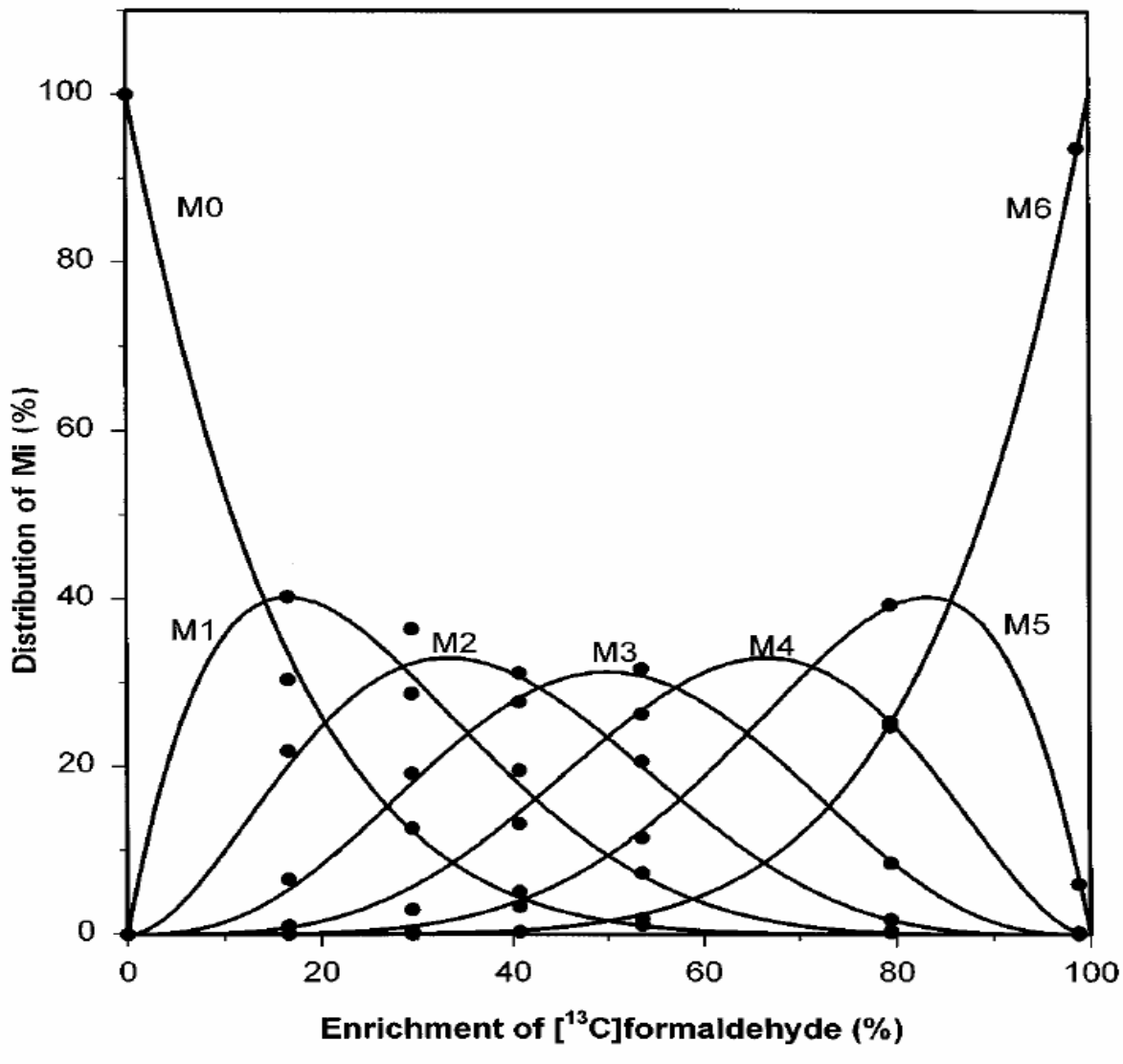
Probabilities

	M+1	M+2	M+3
C	0.0111	0.000123	1.37×10^{-7}
O (+1)	0.00037	1.37×10^{-6}	5.07×10^{-11}
O (+2)		0.00204	
N	0.0036		
H	0.00015	2.25×10^{-8}	3.38×10^{-12}

Proportions of Naturally Occurring Isotopologues

	M+1	M+2	M+3
C1	0.122100		
C2		0.006777	
C3			0.000226
C1N		0.000440	
C1H		0.000238	
C1O1		0.000136	

Matrix Algorithms for Correction of Mass Isotopomer Distribution

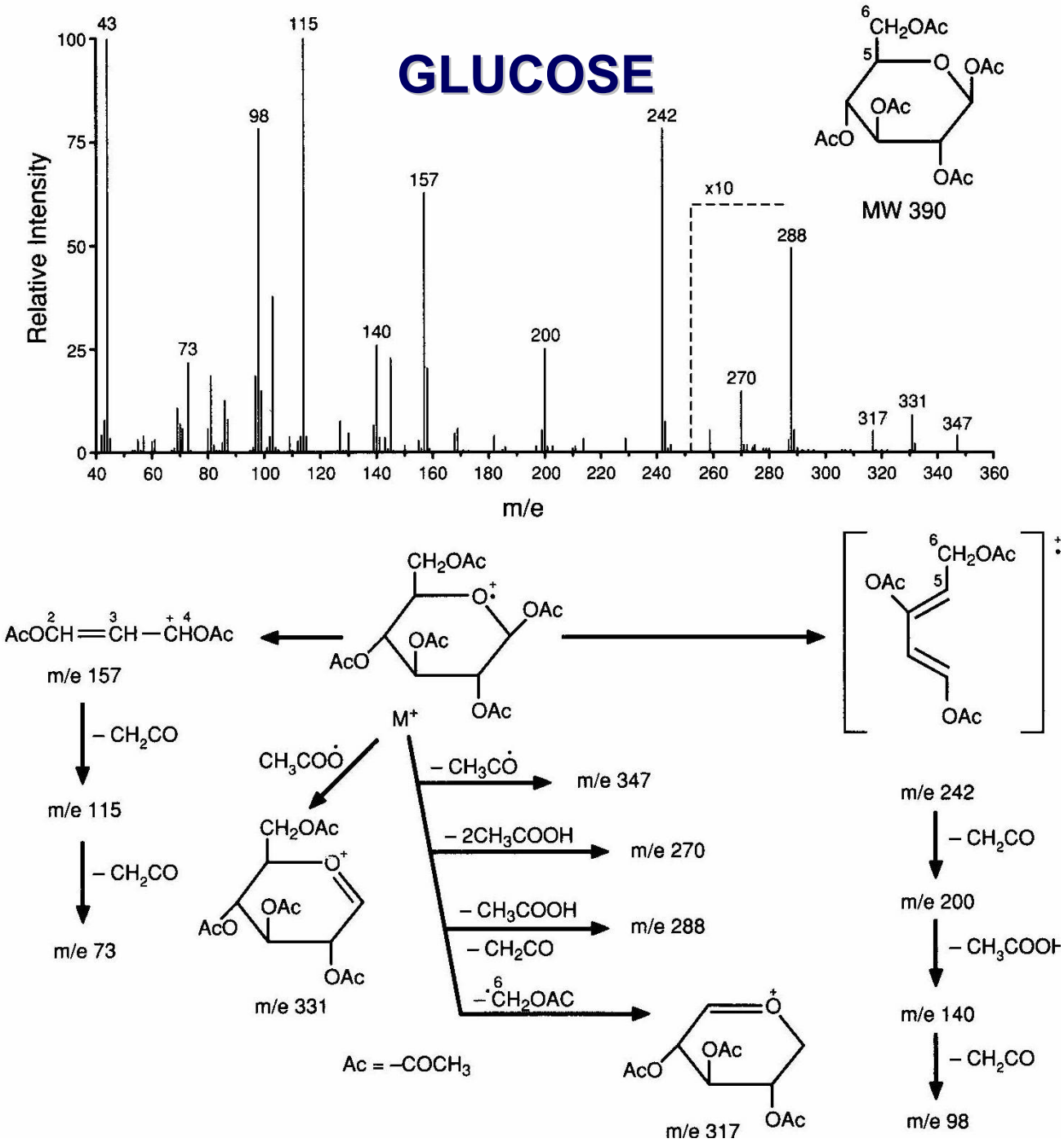


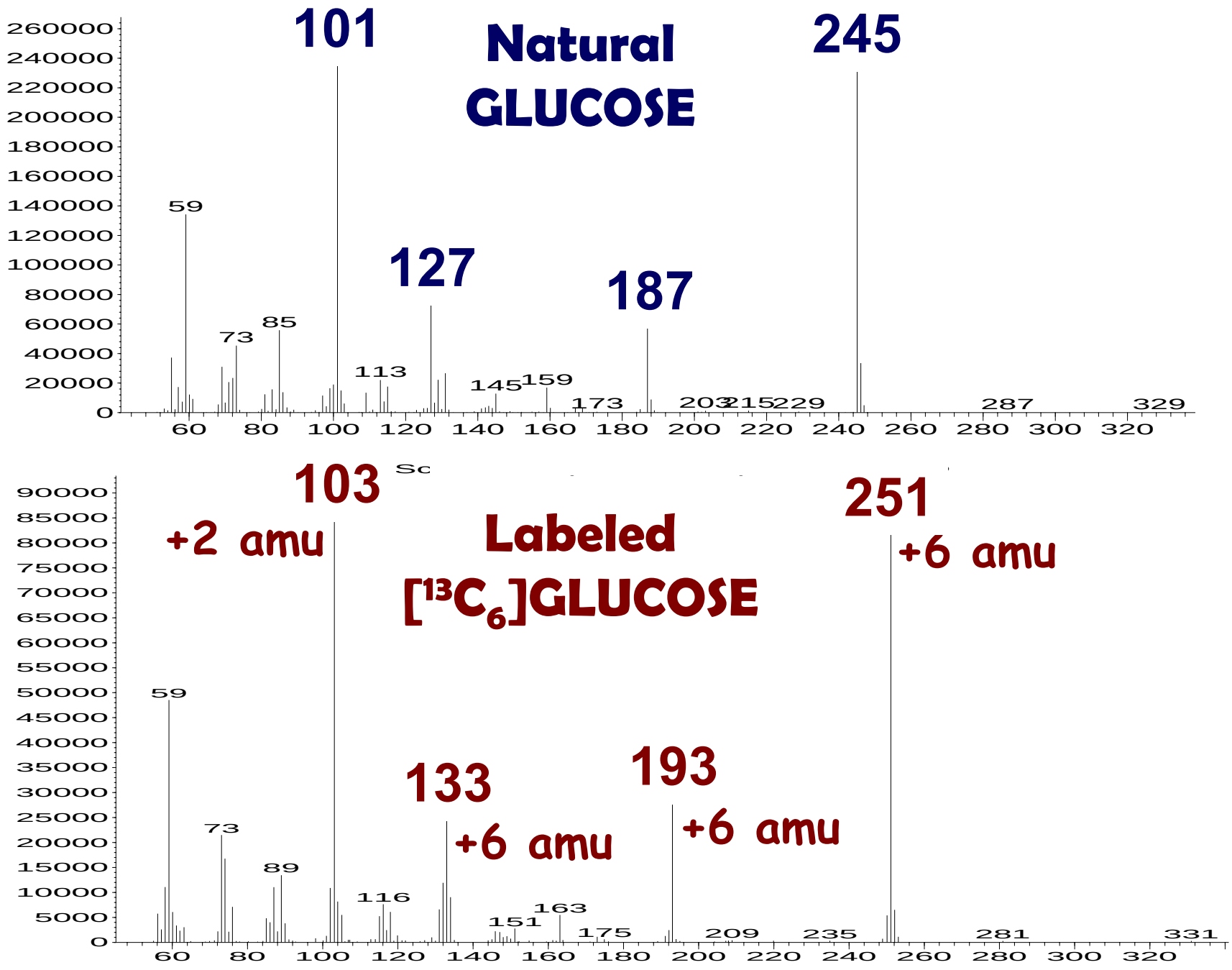
Tracers at the tissue level: Nutrient tracing studies

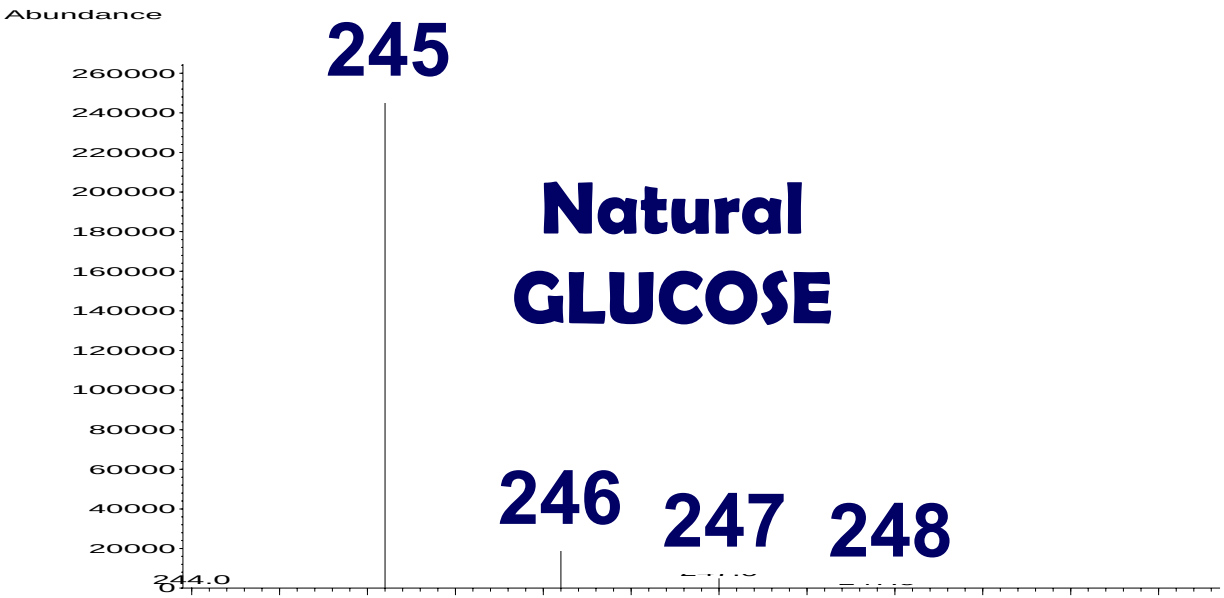
Mapping of Metabolism, Nutrients and Nutrient Requirements

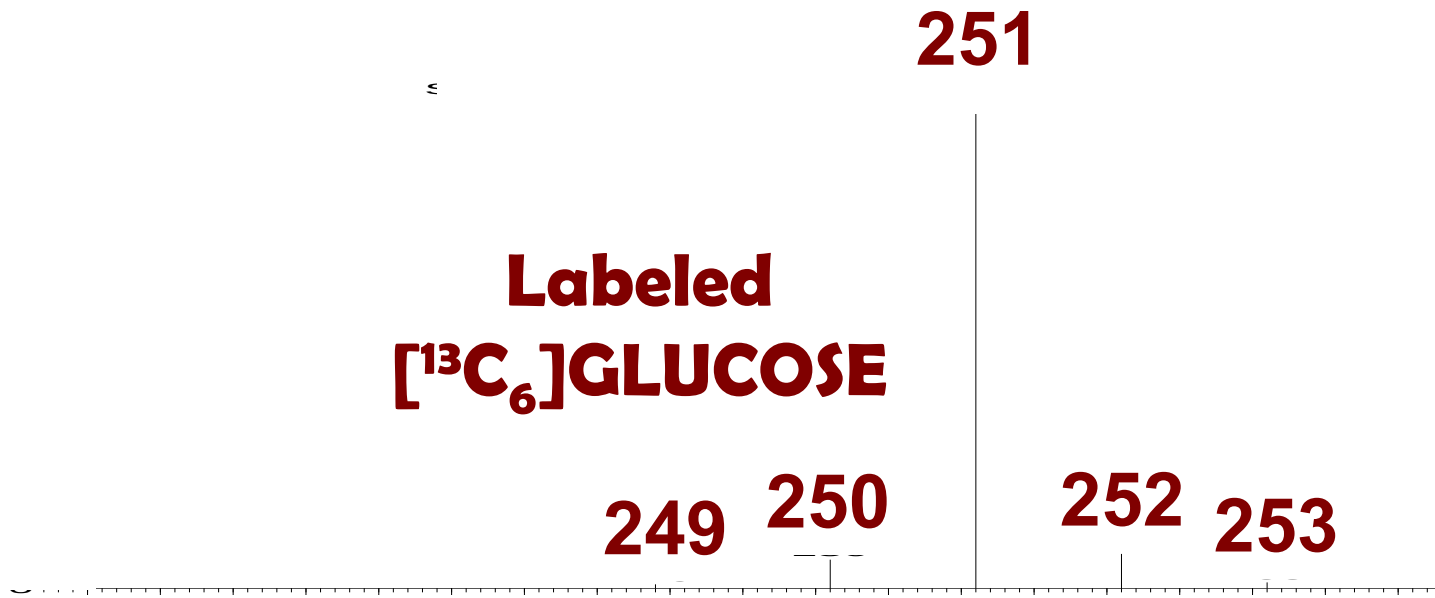
- Essential vs Non-essential nutrients: amino acids, pyrimidines, fatty acids, vitamins
- Dietary Precursors
- Gluconeogenic Substrates
- Urea-N sources and recycling
- Fatty Acid Synthesis and Lipogenesis: rates & precursors
- GlycerolneogenesisAbsorption and Metabolism of Amino Acids
- Glucose Metabolism and Recycling
- Krebs Cycle metabolism: rates, energy substrates, anaplerosis and cataplerosis balances
- Protein Synthesis
- Flux Networks or Metabolic Profiling

Fragmentology

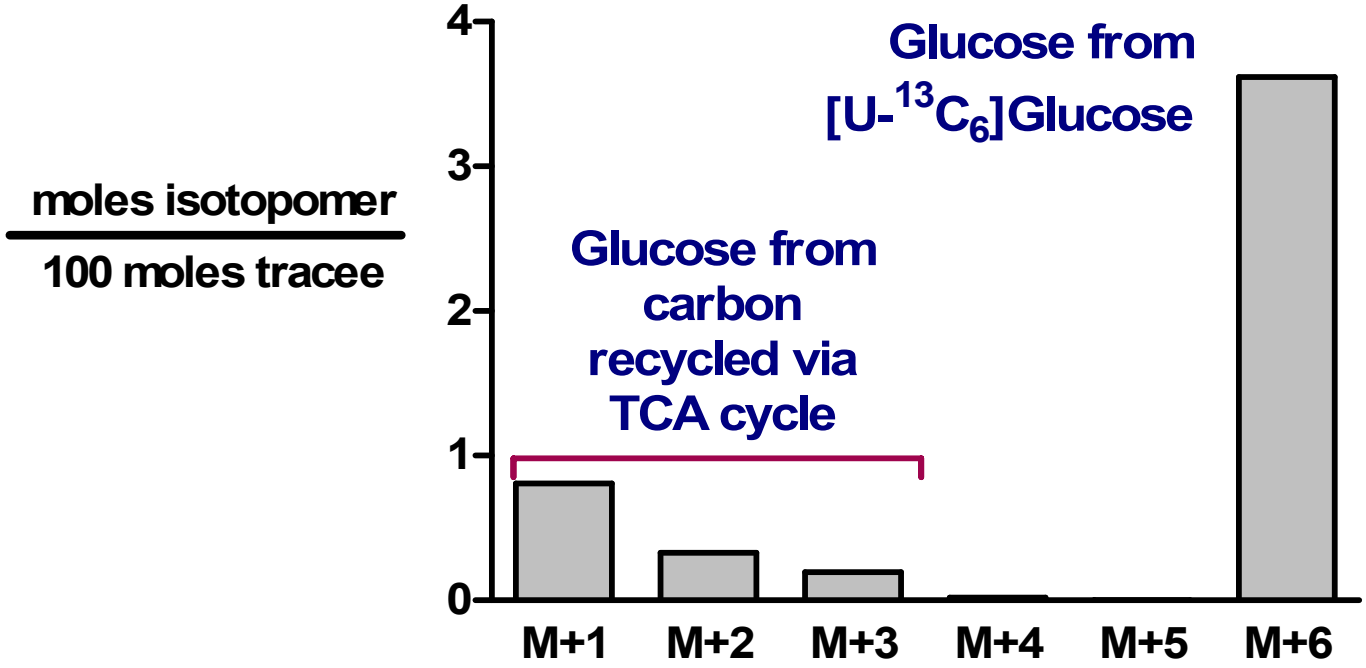



Natural
GLUCOSE
101
245
127
187
59
73
85
113
145
159
173
203
215
229
287
329
Labeled
[13C6]GLUCOSE
103
+2 amu
251
+6 amu
133
+6 amu
193
+6 amu
59
73
89
116
151
163
175
209
235
281
331
m/z-->

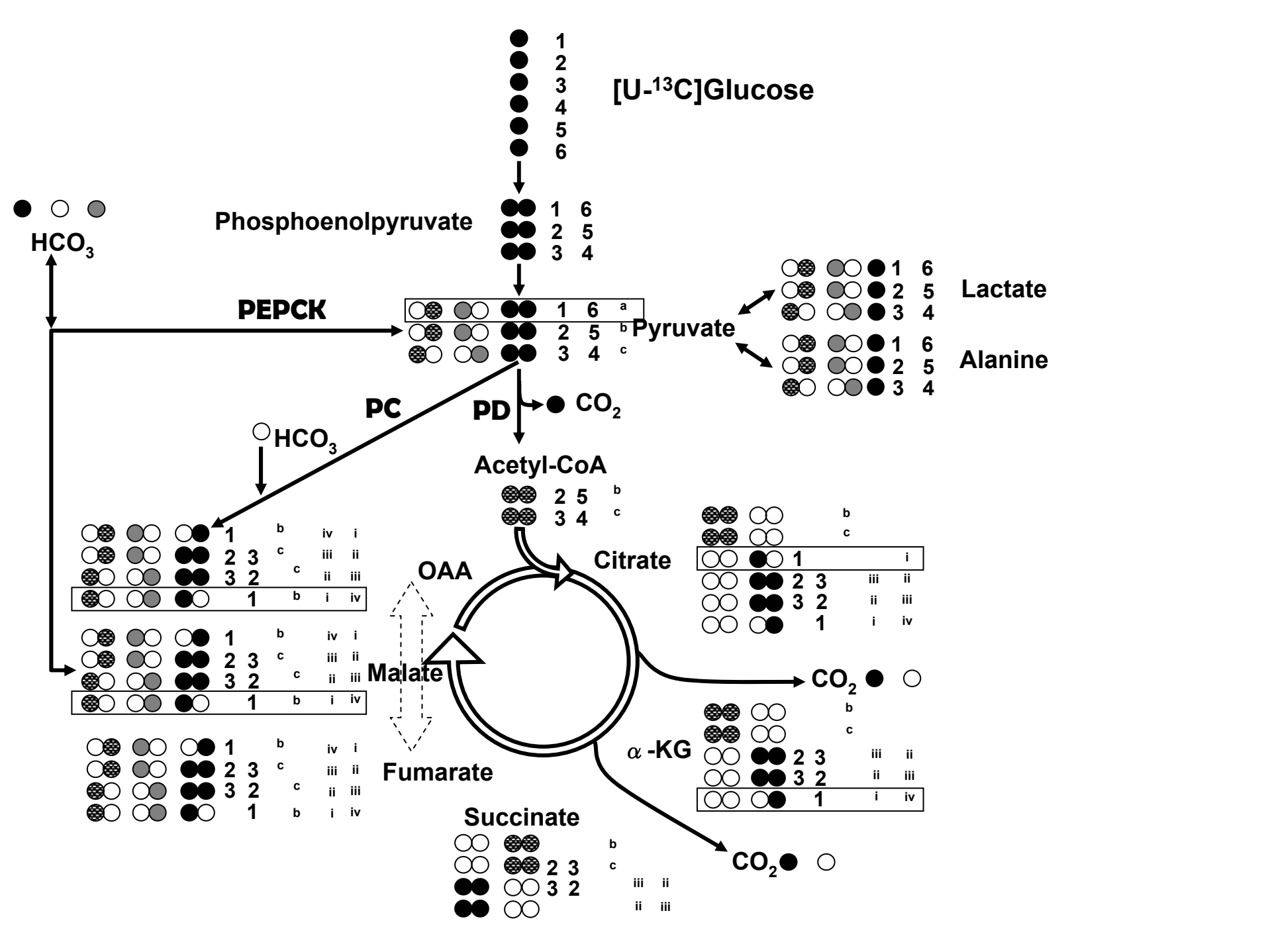

Abundance
245
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
244.0
Natural
GLUCOSE
246
247
248

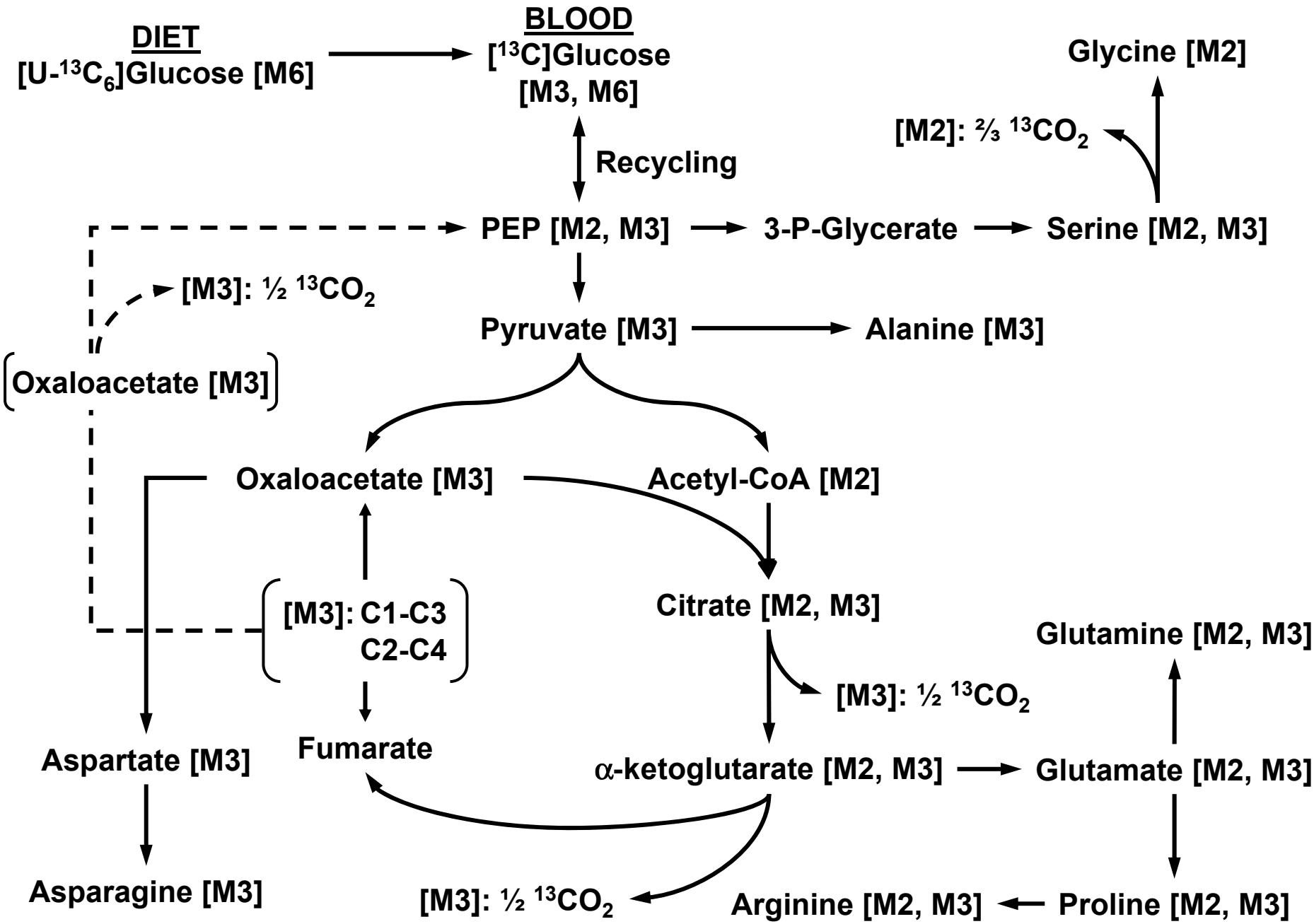

251
Labeled
[13C6]GLUCOSE
249
250
252
253

^{13}C-MIDA of Plasma Glucose (Sheep)

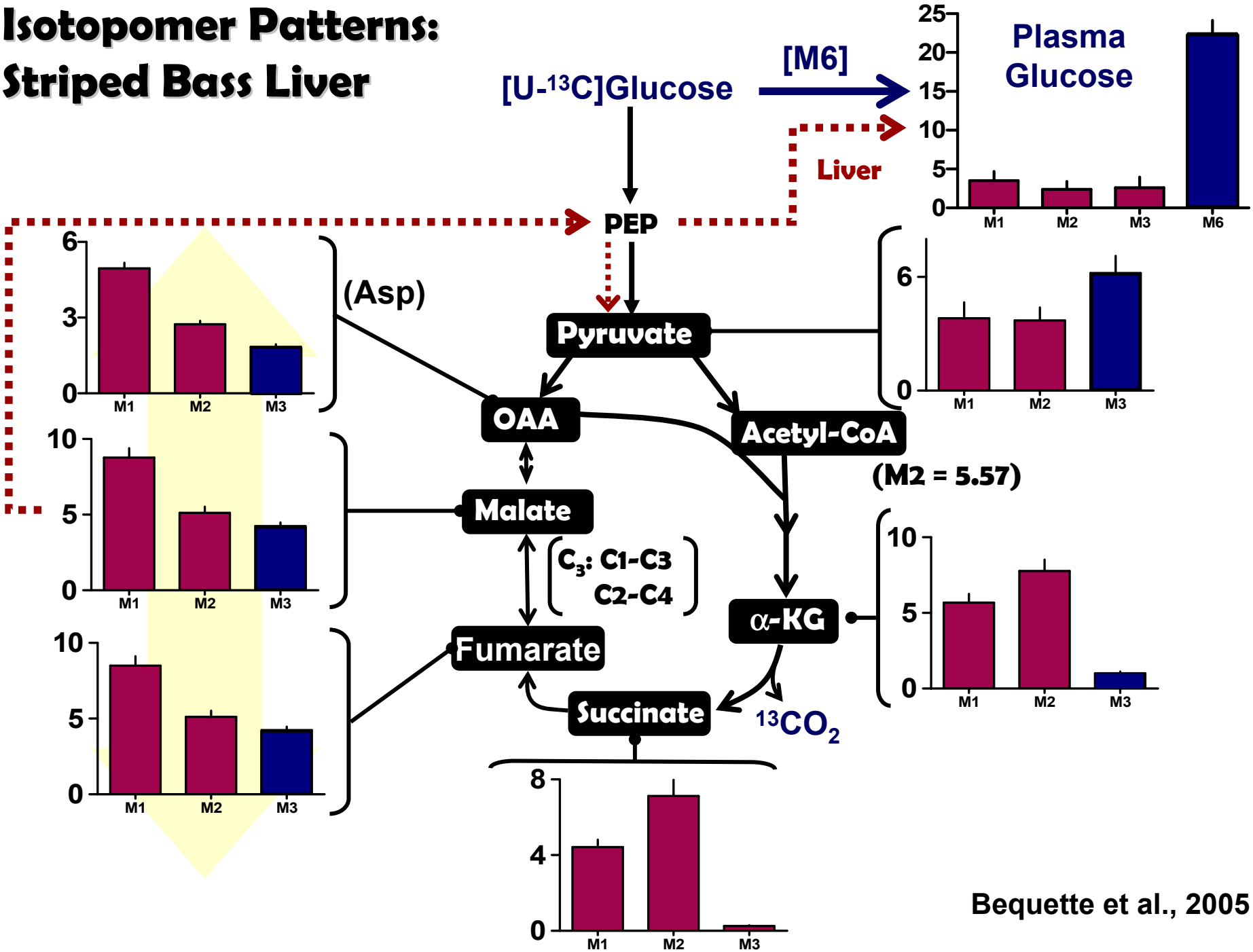


M+0: C-C-C-C-C-C = 100 (from unlabelled sources)
M+1: **C**-C-C-C-C-C = 0.81
M+2: **C-C**-C-C-C-C = 0.33
M+3: **C-C-C**-C-C-C = 0.20
M+4: **C-C-C-C**-C-C = 0.02
M+5: **C-C-C-C-C**-C = 0.00
M+6: **C-C-C-C-C-C = 3.62**


[U-13C]Glucose
Phosphoenolpyruvate
HCO3
PEPCK
Pyruvate
Lactate
Alanine
PC
PD
CO2
HCO3
Acetyl-CoA
Citrate
OAA
Malate
Fumarate
CO2
α -KG
Succinate
CO2

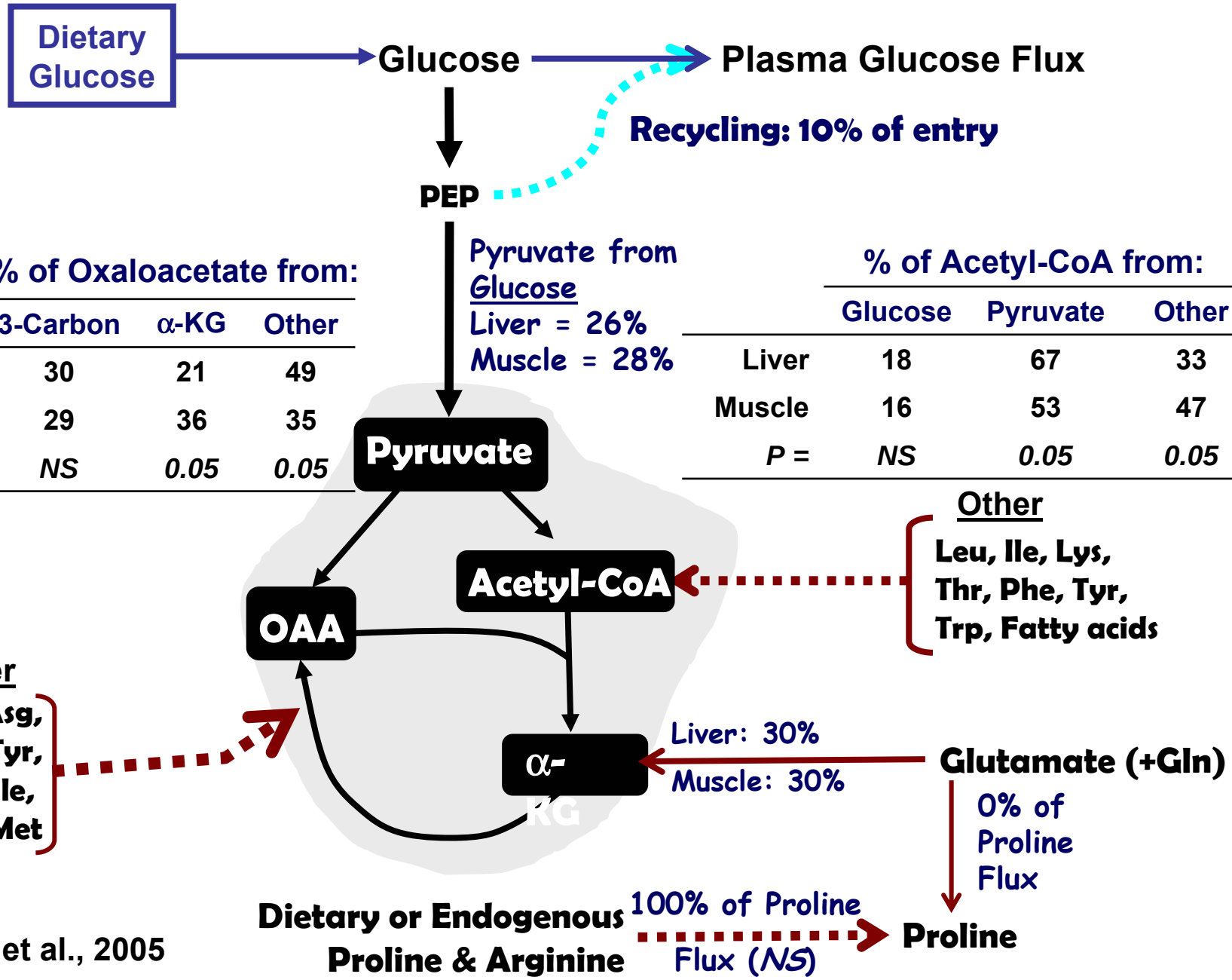

DIET
[U-13C6]Glucose [M6]
BLOOD
[13C]Glucose
[M3, M6]
Recycling
Glycine [M2]
[M2]: ⅔ 13CO2
PEP [M2, M3]
3-P-Glycerate
Serine [M2, M3]
[M3]: ½ 13CO2
Pyruvate [M3]
Alanine [M3]
Oxaloacetate [M3]
Oxaloacetate [M3]
Acetyl-CoA [M2]
[M3]: C1-C3
C2-C4
Citrate [M2, M3]
Glutamine [M2, M3]
[M3]: ½ 13CO2
Aspartate [M3]
Fumarate
α-ketoglutarate [M2, M3]
Glutamate [M2, M3]
Asparagine [M3]
[M3]: ½ 13CO2
Arginine [M2, M3]
Proline [M2, M3]

Isotopomer Patterns: Striped Bass Liver



Bequette et al., 2005

Striped Bass: Liver vs Muscle Metabolism



% of Oxaloacetate from:

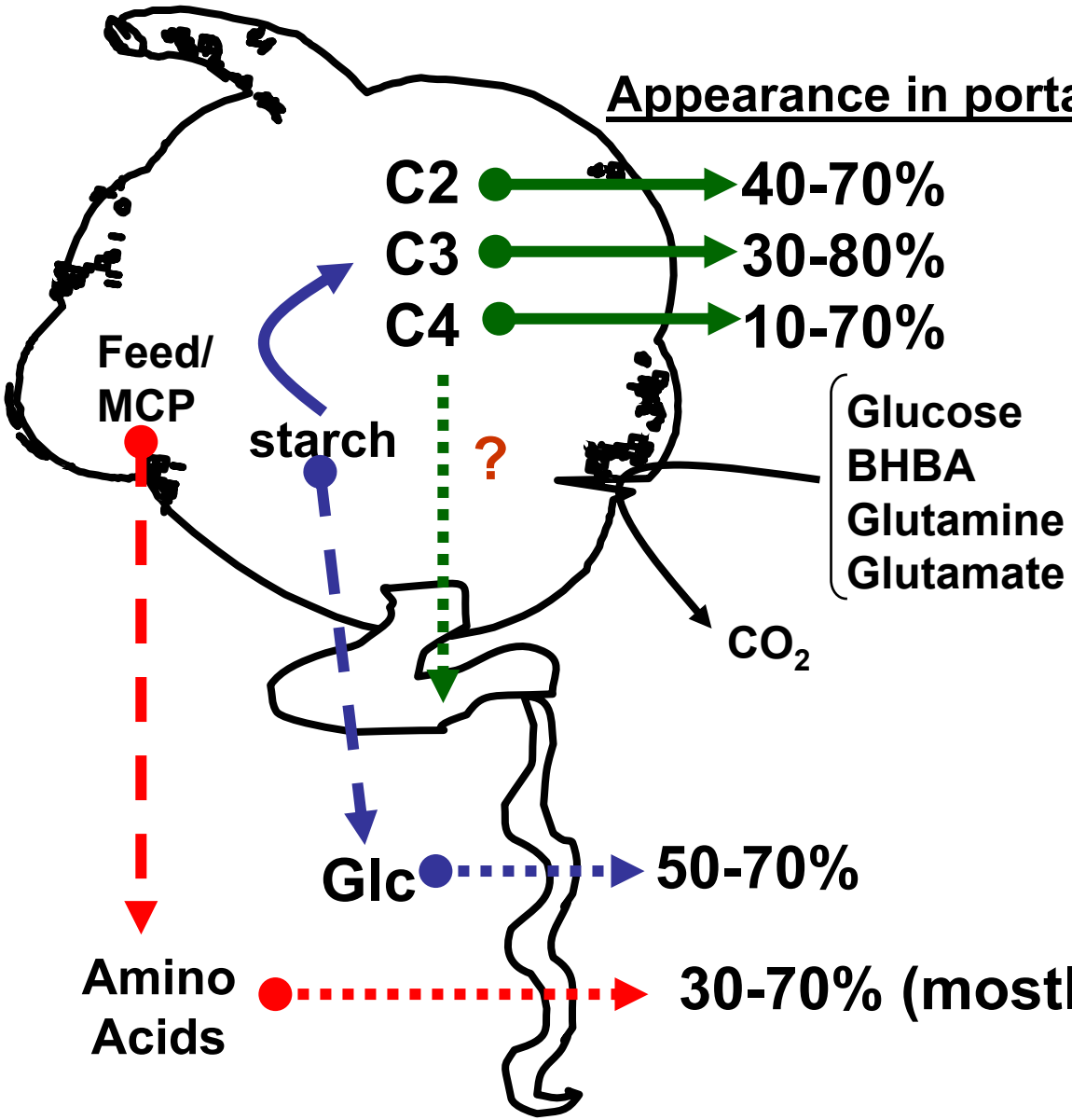
	3-Carbon	α-KG	Other
Liver	30	21	49
Muscle	29	36	35
P =	*NS*	*0.05*	*0.05*

% of Acetyl-CoA from:

	Glucose	Pyruvate	Other
Liver	18	67	33
Muscle	16	53	47
P =	*NS*	*0.05*	*0.05*

Bequette et al., 2005

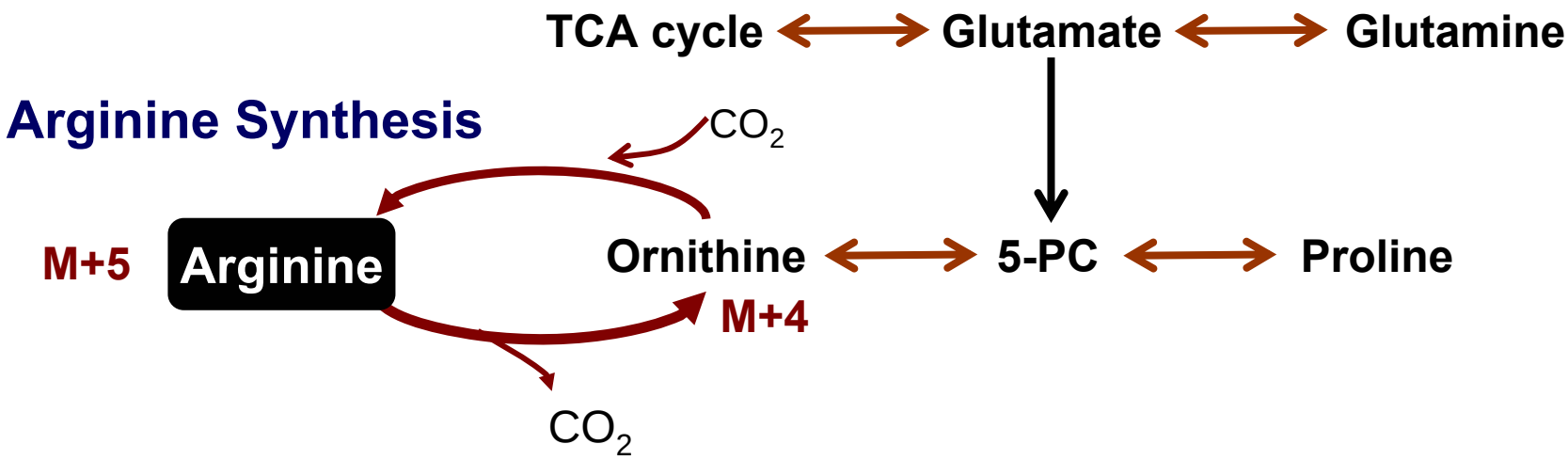
Carbon Substrate Utilization by the Pig or Ruminant Gut

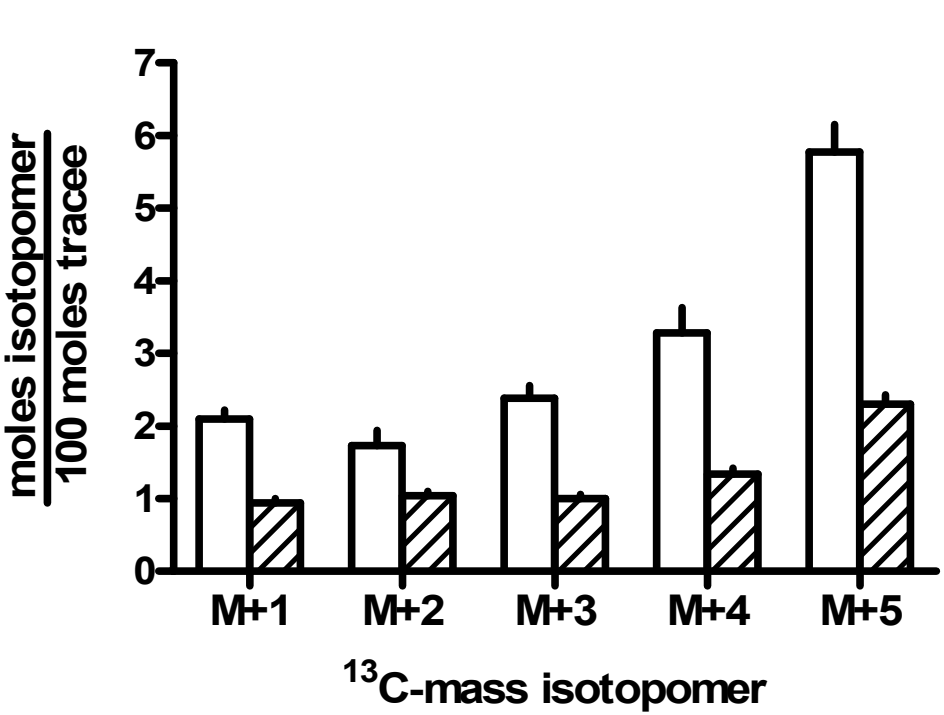


Why certain Substrates?

How are they replaced?

Piglet Intestines: Use of Glutamate and Glutamine



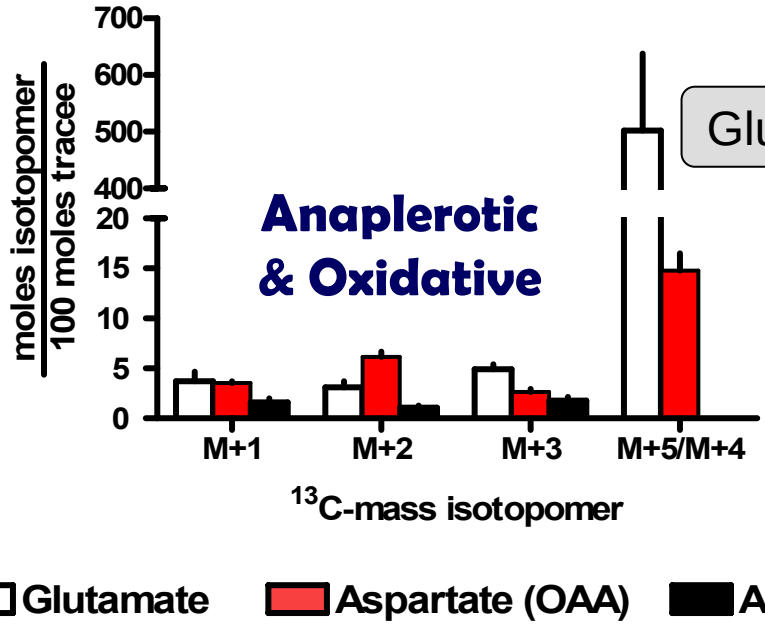


$1 - \frac{M+4}{M+5}$ = ~42% of Arginine synthesis via Glutamine, 58% via Ornithine cycle

Wu & Reeds, unpubl.

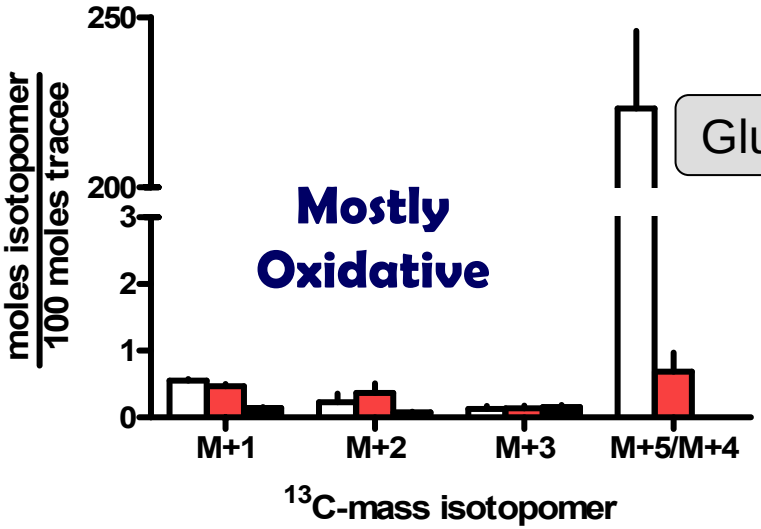
Incubation of Sheep Gut cells with 5 mM [U-^{13}C]Glutamine

Duodenal Mucosal



Glutamate: 19% from Glutamine

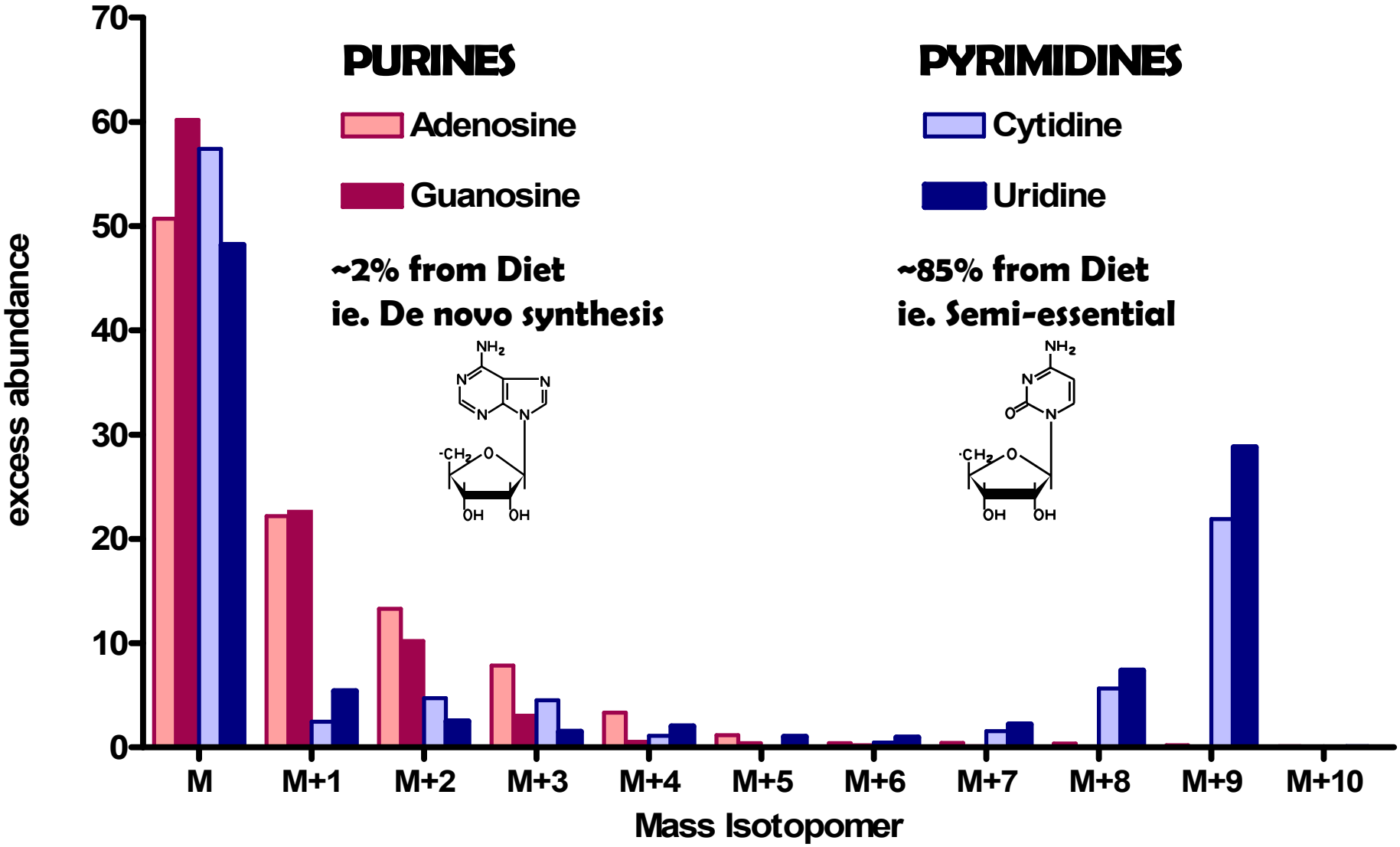
Rumen Epithelial



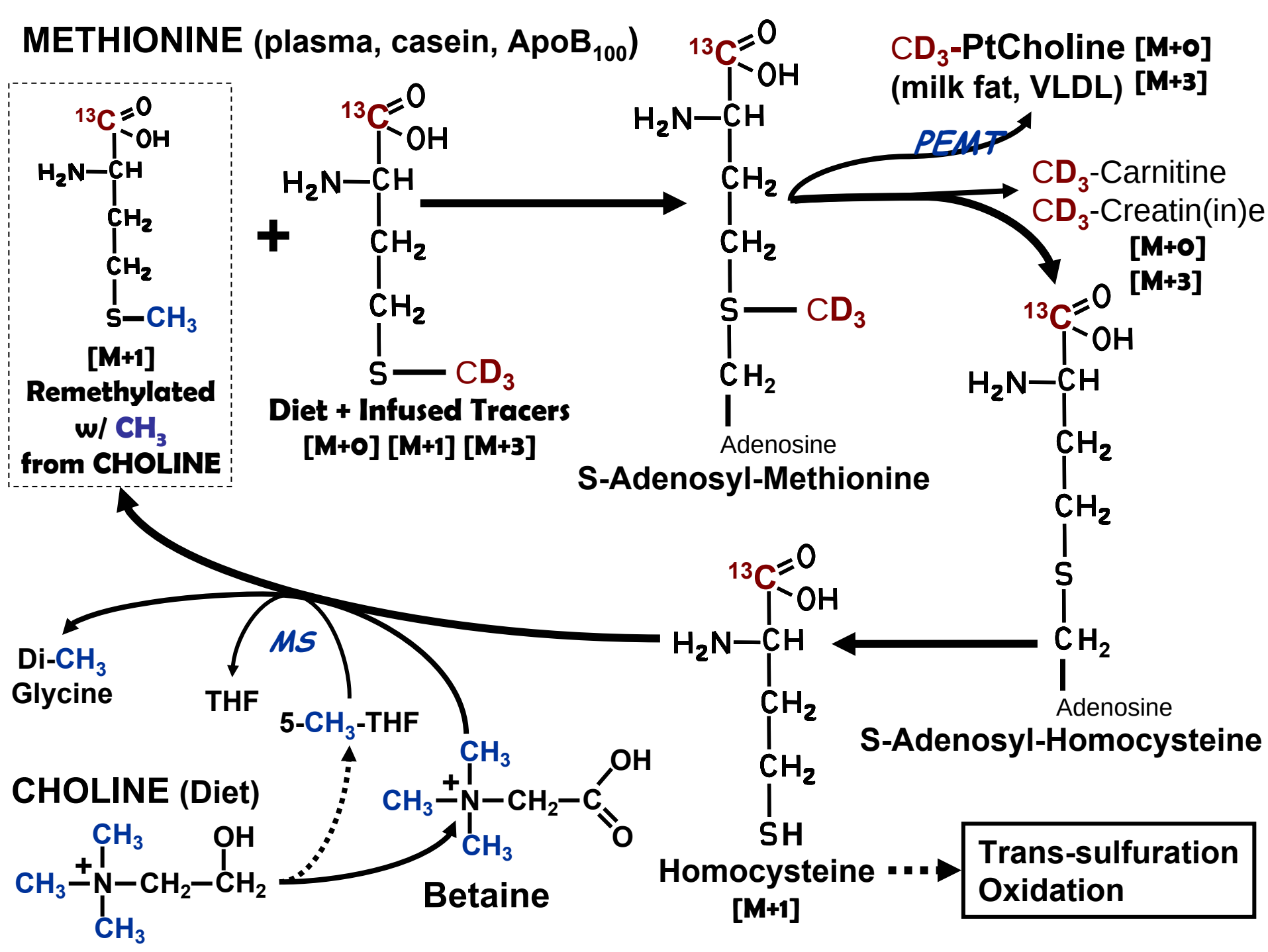
Glutamate: 64% from Glutamine

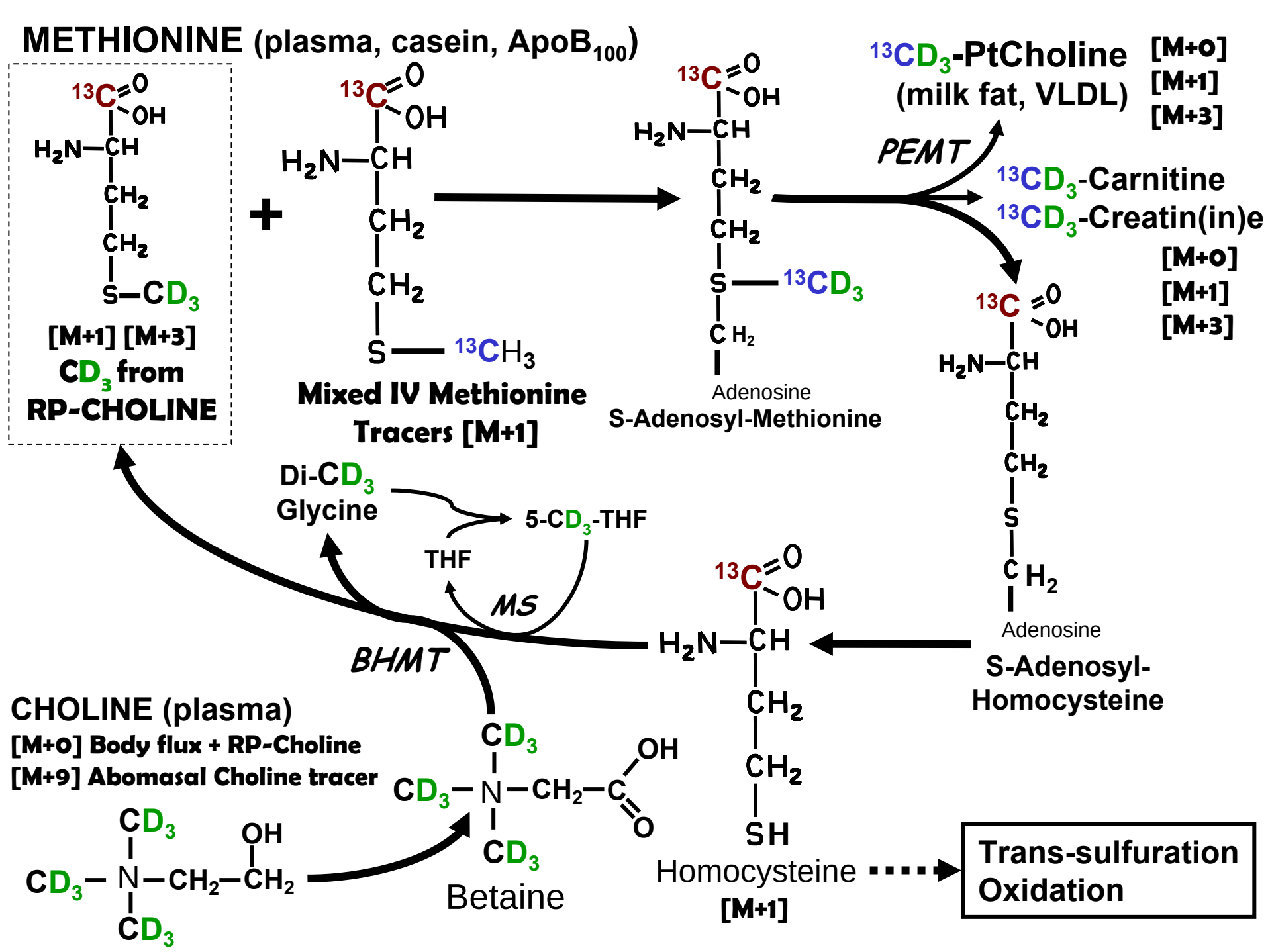
Bequette et al., 2004

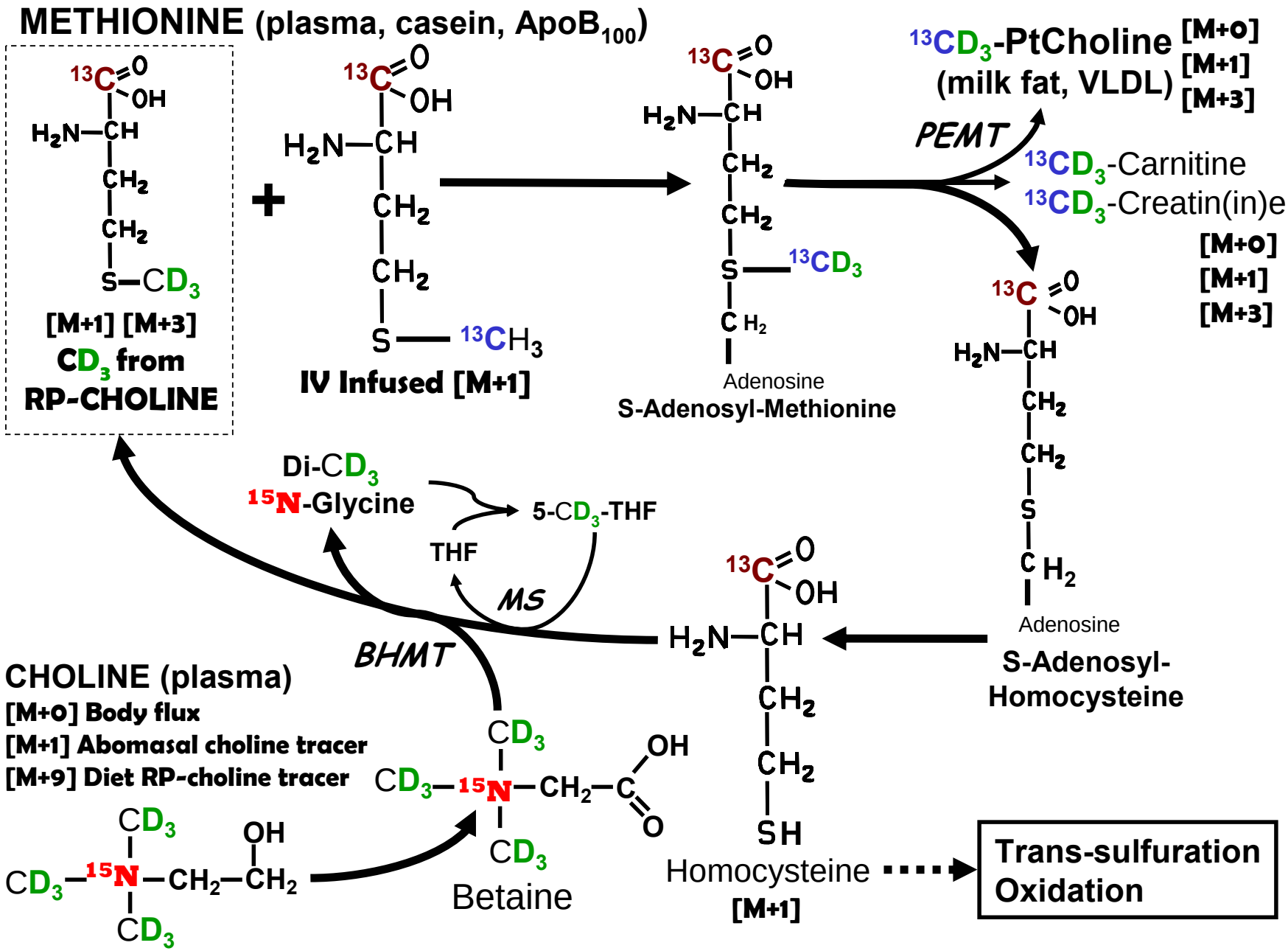
Dietary Essentiality of Pyrimidines in a Laying Hen



Berthold et al., 1995


METHIONINE (plasma, casein, ApoB100)
[M+1]
Remethylated
w/ CH3
from CHOLINE
Diet + Infused Tracers
[M+0] [M+1] [M+3]
Adenosine
S-Adenosyl-Methionine
CD3-PtCholine [M+0]
(milk fat, VLDL) [M+3]
PEMT
CD3-Carnitine
CD3-Creatin(in)e
[M+0]
[M+3]
Adenosine
S-Adenosyl-Homocysteine
Homocysteine
[M+1]
Trans-sulfuration
Oxidation
MS
Di-CH3
Glycine
THF
5-CH3-THF
CHOLINE (Diet)
Betaine


METHIONINE (plasma, casein, ApoB100)
[M+1] [M+3]
CD3 from
RP-CHOLINE
Mixed IV Methionine
Tracers [M+1]
Adenosine
S-Adenosyl-Methionine
13CD3-PtCholine
(milk fat, VLDL)
[M+0]
[M+1]
[M+3]
PEMT
13CD3-Carnitine
13CD3-Creatin(in)e
[M+0]
[M+1]
[M+3]
Adenosine
S-Adenosyl-
Homocysteine
Di-CD3
Glycine
5-CD3-THF
THF
MS
BHMT
CHOLINE (plasma)
[M+0] Body flux + RP-Choline
[M+9] Abomasal Choline tracer
Betaine
Homocysteine
[M+1]
Trans-sulfuration
Oxidation


METHIONINE (plasma, casein, ApoB100)
[M+1] [M+3]
CD3 from
RP-CHOLINE
IV Infused [M+1]
Adenosine
S-Adenosyl-Methionine
13CD3-PtCholine
(milk fat, VLDL)
[M+0]
[M+1]
[M+3]
PEMT
13CD3-Carnitine
13CD3-Creatin(in)e
[M+0]
[M+1]
[M+3]
Adenosine
S-Adenosyl-
Homocysteine
Di-CD3
15N-Glycine
5-CD3-THF
THF
MS
BHMT
CHOLINE (plasma)
[M+0] Body flux
[M+1] Abomasal choline tracer
[M+9] Diet RP-choline tracer
Betaine
Homocysteine
[M+1]
Trans-sulfuration
Oxidation