

# Tracers to study human metabolism in vivo

**Bettina Mittendorfer** 

Washington University School of Medicine

St. Louis, MO



# Goal(s)

What is a tracer?

Basic prinicples for application

Basic nomenclature

Analysis

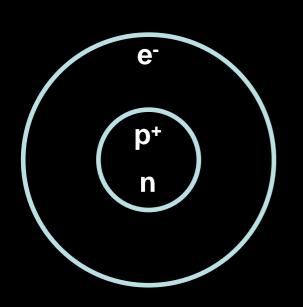
### What is a tracer?

Tracer = a substance added to the system to trace out a metabolic pathway (radio-active vs. stable isotope)

Tracee = the native substrate being traced

# Stable isotope labeled tracers

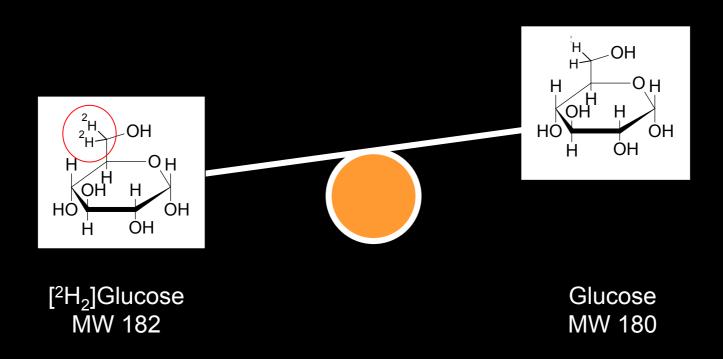
**Isotopes** = elements (i.e., same number of  $p^+$ ) with different numbers of neutrons; hence different atomic weight (i.e.,  $\sum p^+$  and n)



Element	Stable isotope	Atomic weight	Atom % natural abundance
Н	1	1.008	99.985
	2	2.014	0.015
С	12	12.000	98.89
	13	13.003	1.11
N	14	14.003	99.63
	15	15.000	0.37
O	16	15.995	99.76
	18	17.999	0.204

# Stable isotope labeled tracers

Stable isotope labeled tracer = structurally identical molecule that differs in isotopomer distribution, and therefore molecular mass, from tracee

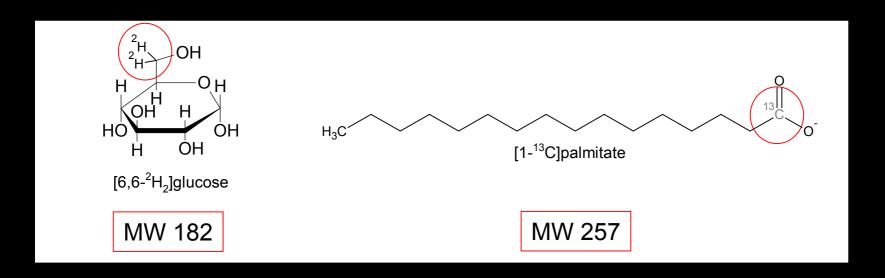


# Stable isotope labeled tracers

### Some examples

 $[6,6-^2H_2]$ glucose  $\Rightarrow$  <sup>1</sup>H in position 6 of glucose exchanged for <sup>2</sup>H

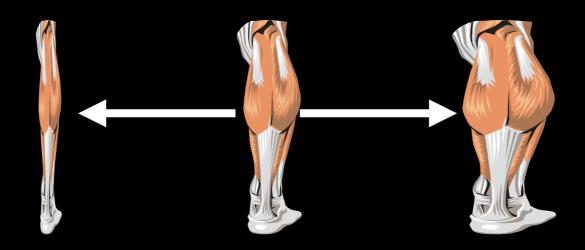
 $[1-^{13}C_1]$  palmitic acid  $\Rightarrow$   $^{12}C$  in position 1 in palmitic acid exchanged for  $^{13}C$ 



# Why do we like tracers?

Because we can measure metabolic flux.

For example,



Muscle mass ↓

Muscle mass ↑

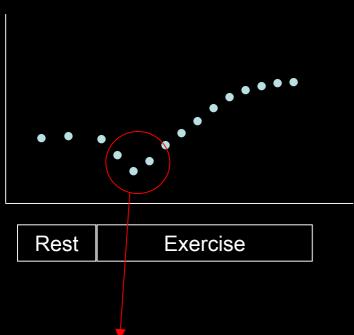
Why?

Is it in/decreased breakdown, in/decreased synthesis, both?

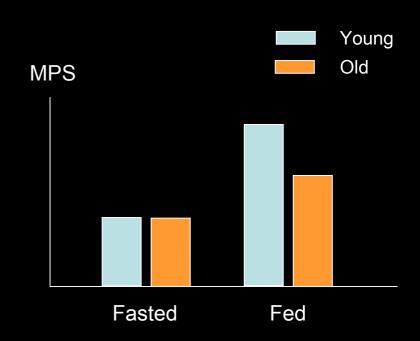
# FFA flux and plasma concentration during exercise

# Muscle protein synthesis and breakdown with aging

### FFA conc



FFA appearance in plasma ↑ but FFA uptake by muscle ↑ ↑ Hence, FFA conc in plasma ↓



# Assumptions that need to be fulfilled when using tracers

Tracer is physically, chemically, and metabolically indistinguishable from tracee

Metabolic kinetics are independent of the tracer used

Provides a massless perturbation to the system to avoid counter regulatory responses

### Basic principles for the use of tracers

Dilution (e.g., lipolysis, proteolysis)





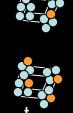


Degree of tracer dilution → rate of delivery of tracee

 $\mathsf{t}_2$ 

Incorporation into macromolecule
(precursor product relationship
e.g., muscle protein, lipoproteins)



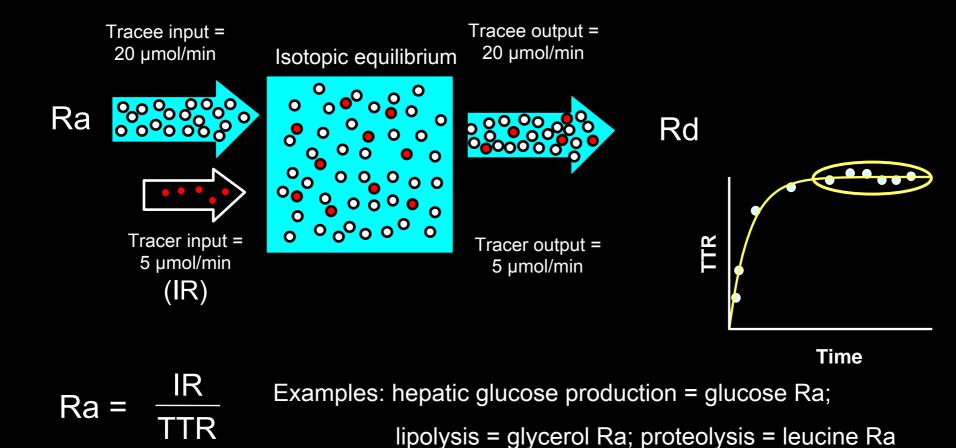


Degree of tracer incorporation → rate of synthesis

Tracer conversion (fatty acid  $\rightarrow CO_2$ )

They can be used at whole-body level, across organs, across body sections (limb), tissue specific (e.g., muscle, skin)

# Isotopic Dilution - Steady State Rate of Appearance (Ra) & Disappearance (Rd)

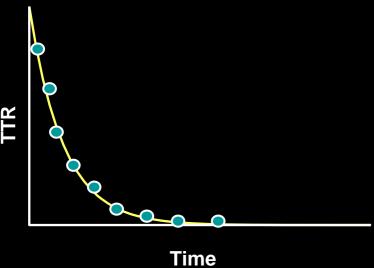


$$Rd = Ra + IR$$

glucose uptake = glucose Rd

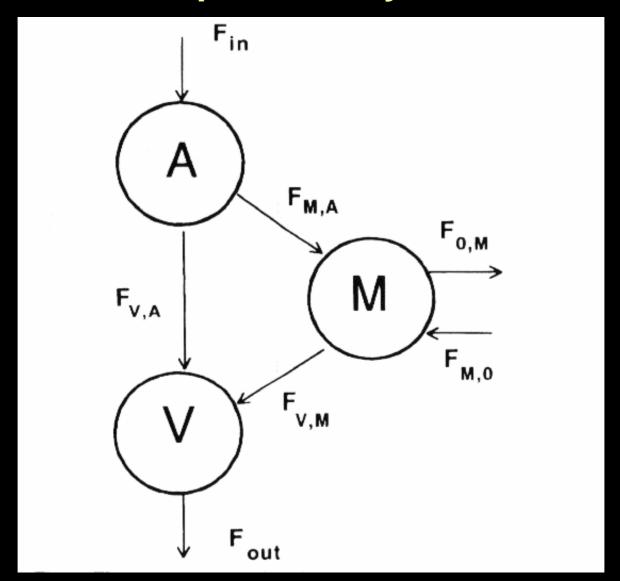
# Ra - bolus injection





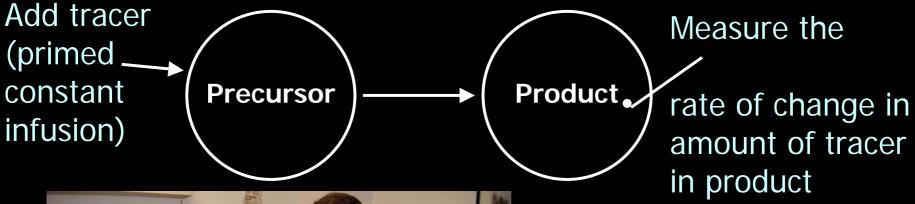
$$IE_t = IE_0 \bullet e^{-kt}$$

# Two/Three-pool steady-state model



# Incorporation into macromolecule (precursor product relationship)

e.g., muscle protein synthesis

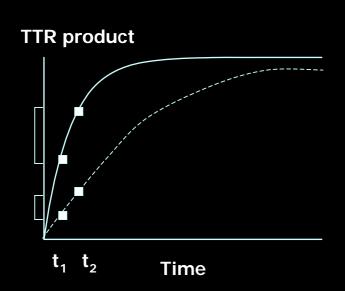






# Incorporation into macromolecule (precursor product relationship)

e.g., muscle protein synthesis



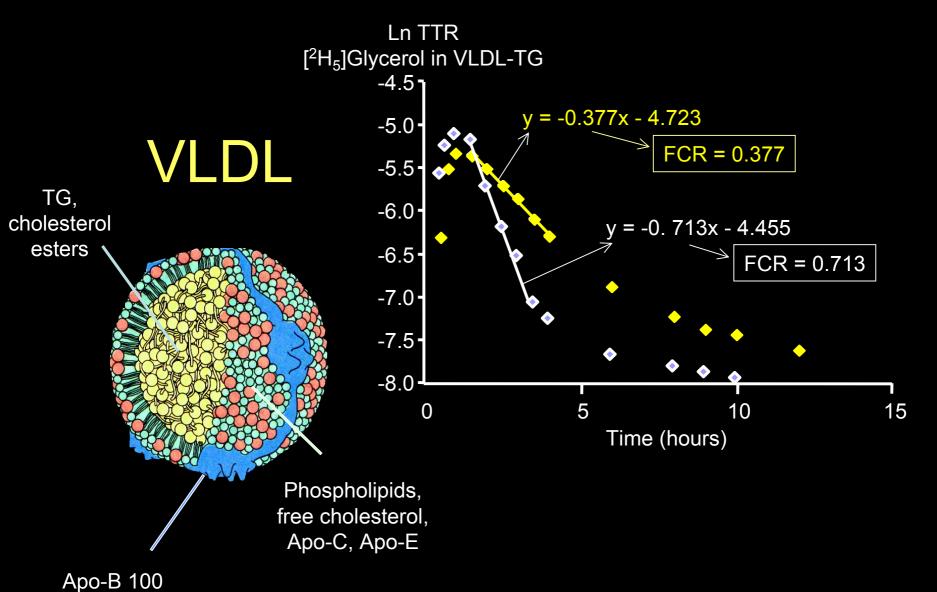
$$E_{t} = E_{p} (1 - e^{-kt})$$

$$\frac{dE_{t}}{dt} \Big|_{t=0} = E_{p} \cdot k$$

$$k = \frac{\text{initial slope}}{E_{p}}$$

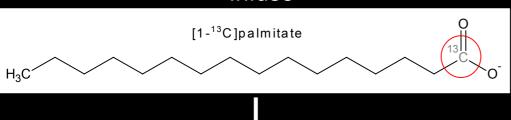
$$FSR \approx \frac{E_{t_{2}} - E_{t_{1}}}{(t_{2} - t_{1}) \cdot E_{p}}$$
Absolute production rate = FSR x pool size

# Glycerol bolus and mono-exponential slope analysis for the determination of VLDL-TG secretion rate



### Substrate oxidation

### Infuse



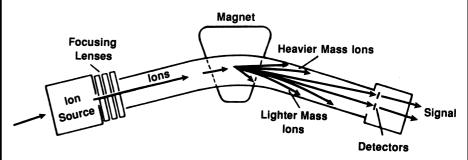
Measure

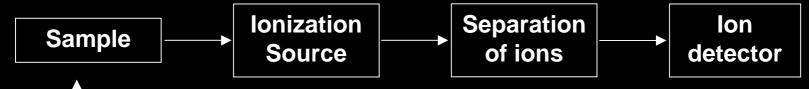
<sup>13</sup>CO<sub>2</sub> production (in breath, across organ/tissue etc)



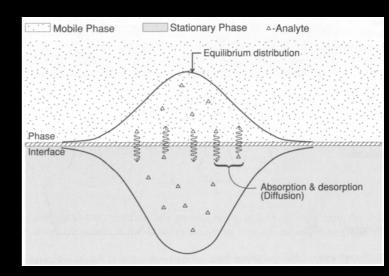


# Stable isotope labeled tracer analysis via Mass Spectrometry

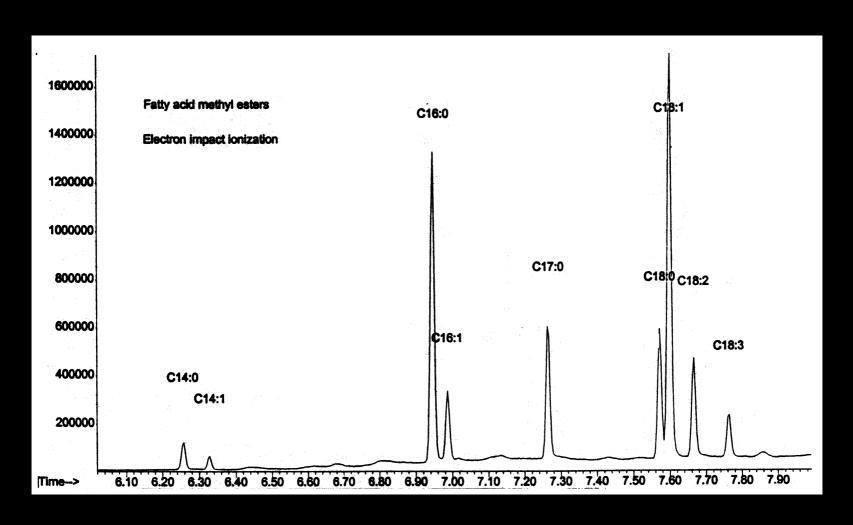


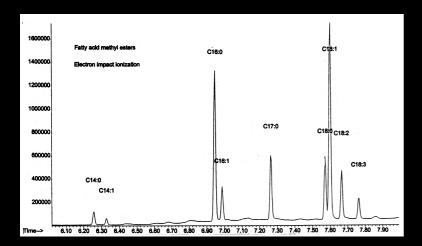


Gas Isotope Ratio MS (for H<sub>2</sub>, N<sub>2</sub>, CO<sub>2</sub>)
Gas Chromatograph/Liquid Chromatography MS (very versatile, suitable for complex molecules)
GC combustion IRMS



# Long chain fatty acid separation via GC

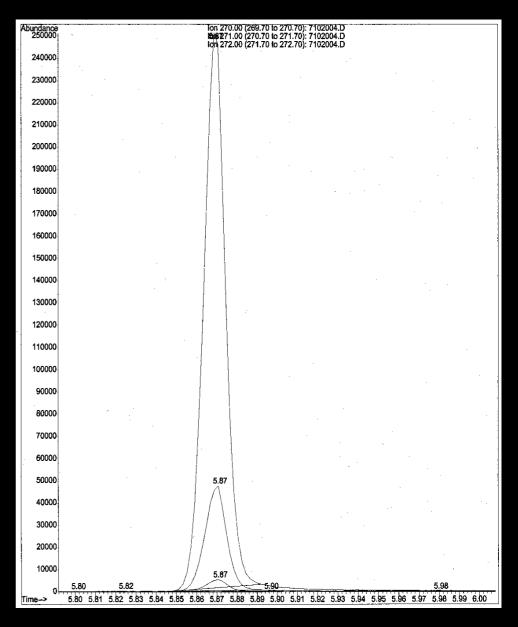




Selective ion monitiring (SIM) → Ratio of two peak areas

Tracer-tracee ratio (TTR) = m+z/m+0

APE or MPE = TTR / (TTR + 1)] = percent of sample that is enriched



#