

*The Steady State Approximation and Flux  
in Functioning Tissues*

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# The CAC at equilibrium

- Supply a carbon-13 labeled nutrient that makes acetyl-CoA
- Assumption: the CAC has reaches a steady state
  - The pool sizes are constant
  - The enrichments are constant

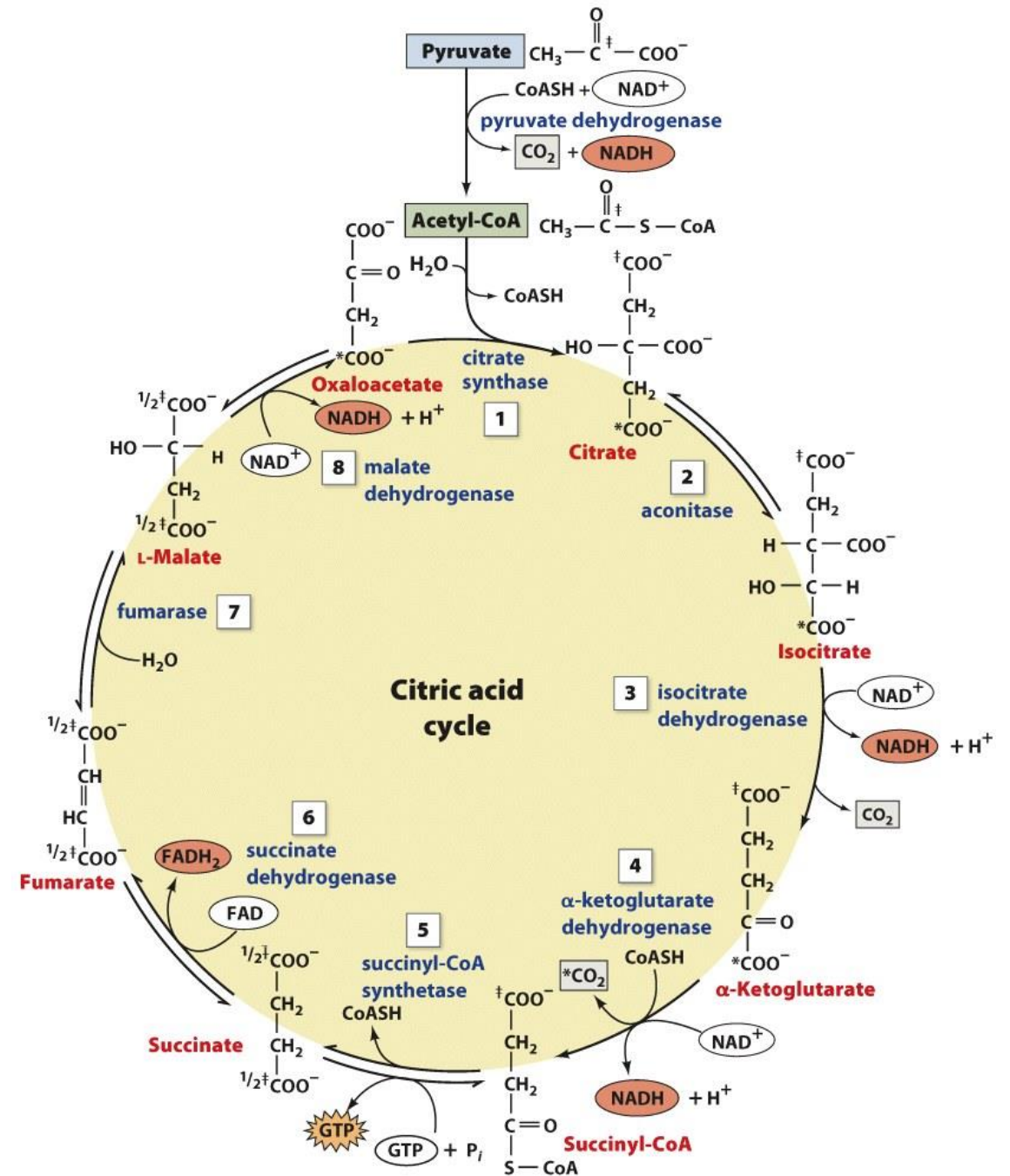
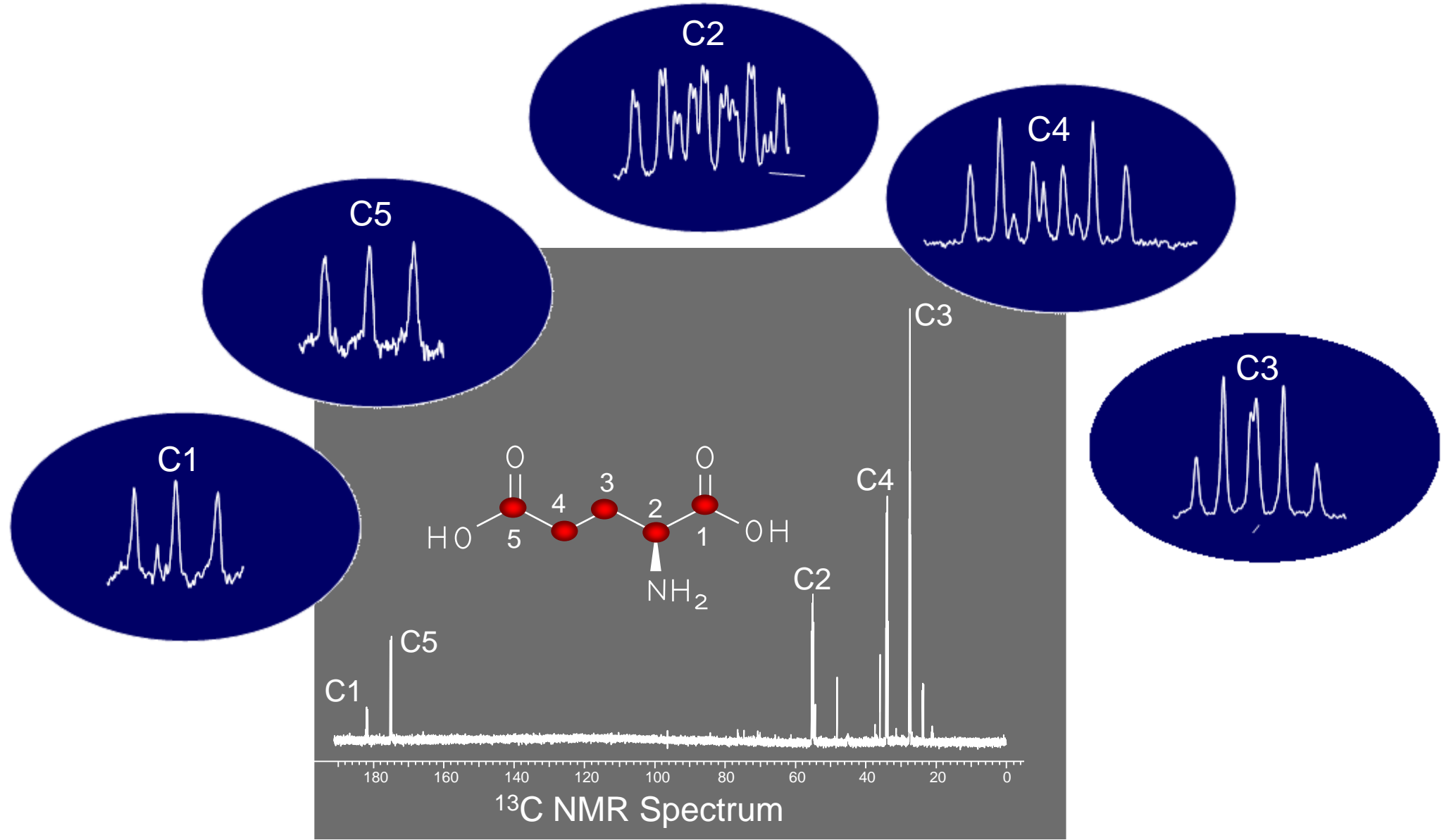


Figure 17-2

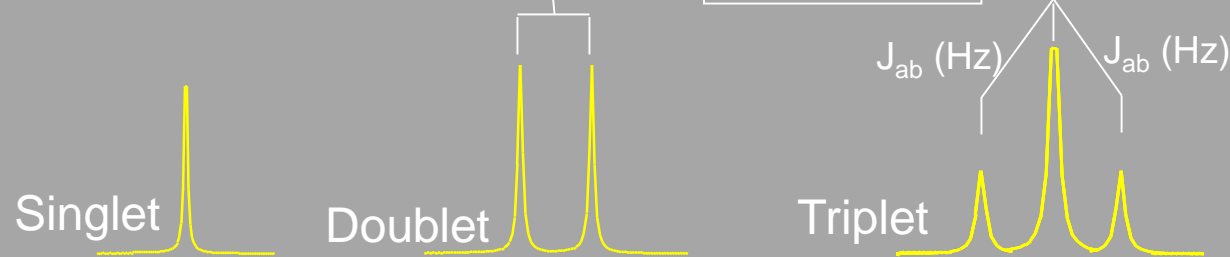
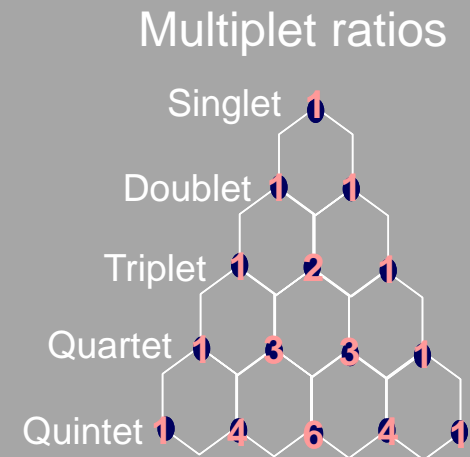
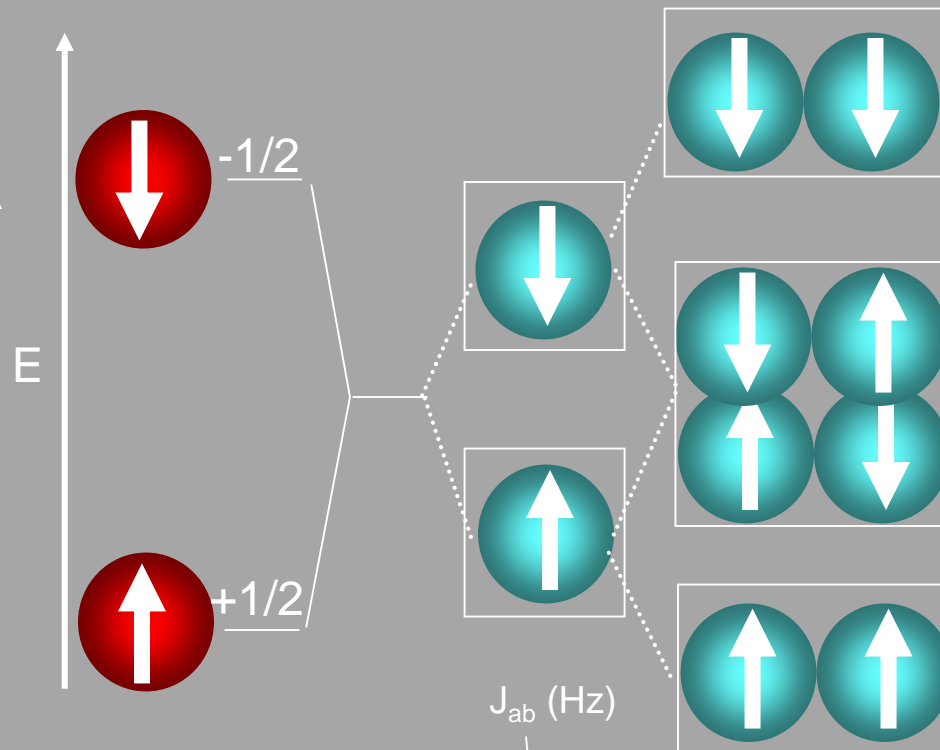
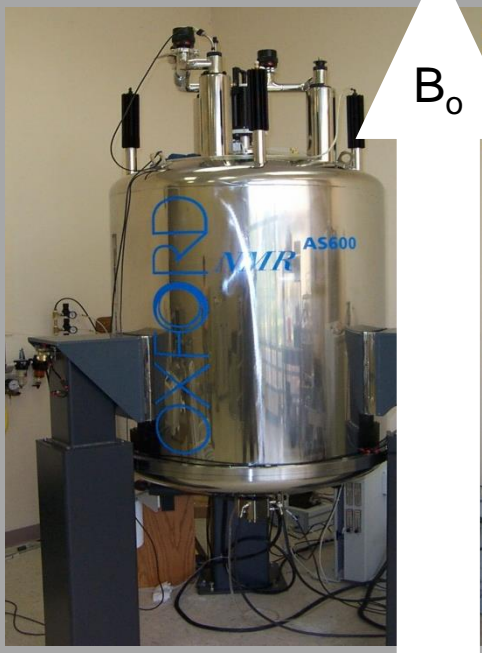
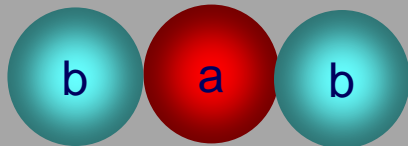
# Idea: Use carbon-13 isotopomer information

- The presence of j-couplings provides direct information about carbon-13 positional labeling in the target molecule
- Our knowledge of unique atom mappings allows the spectra to be predicted based on the supplied substrate

# The Resonance of an NMR Signal Depends on The Chemical Environment of the Nuclei

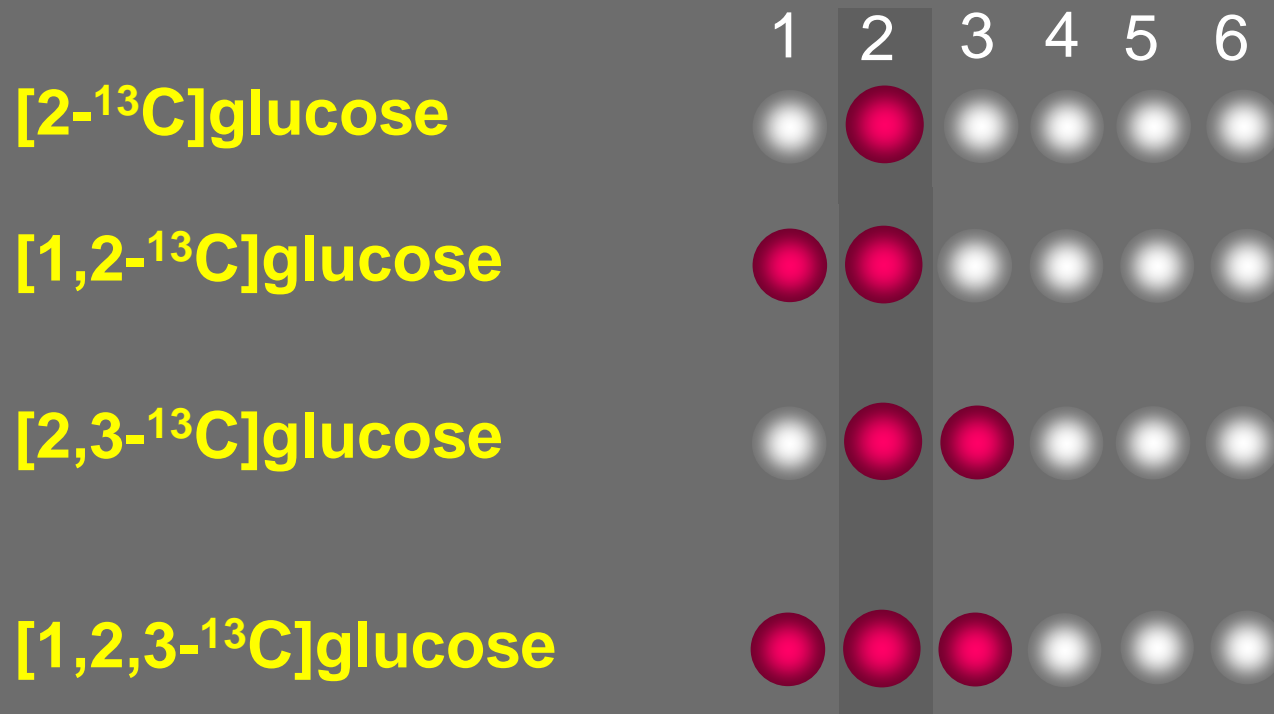


# Spin Coupling (*spin=1/2*)

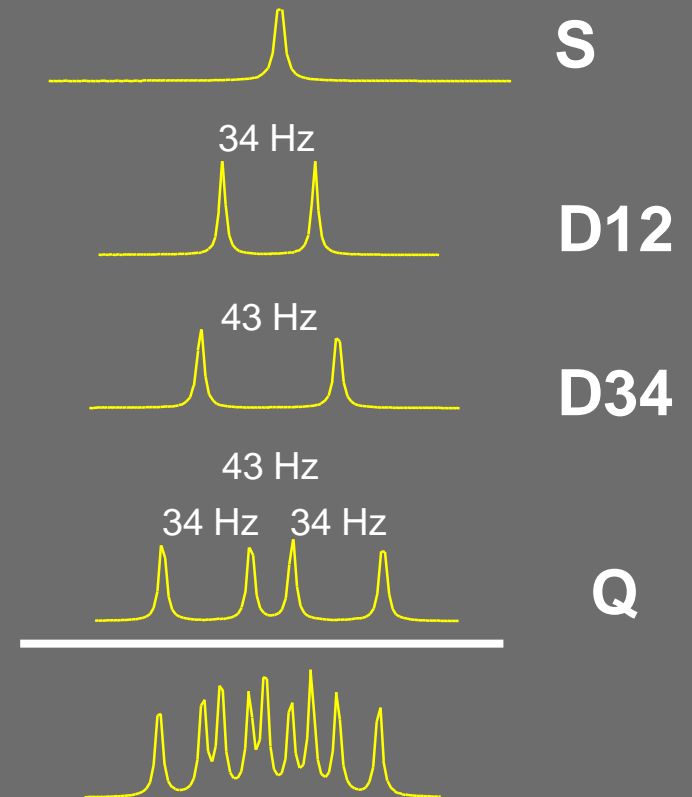


# $^{13}\text{C}$ Spin Coupling and Positional Isotopomer Analysis

## Glucose Isotopomers



## NMR Spectrum C2



# Application of Magnetic Resonance to Metabolic Flux

$^{31}\text{P}$  magnetic resonance can be used to detect ATP

Cohn M and Hughes TR. J Biol Chem. 1960;235(11):3250-3.

Metabolism of  $^{13}\text{C}$  labeled substrates can be detected by  $^{13}\text{C}$  NMR

Eakin RT et al. FEBS Lett. 1972;28(3):259-64.

Noninvasive, nondestructive approaches to cell bioenergetics

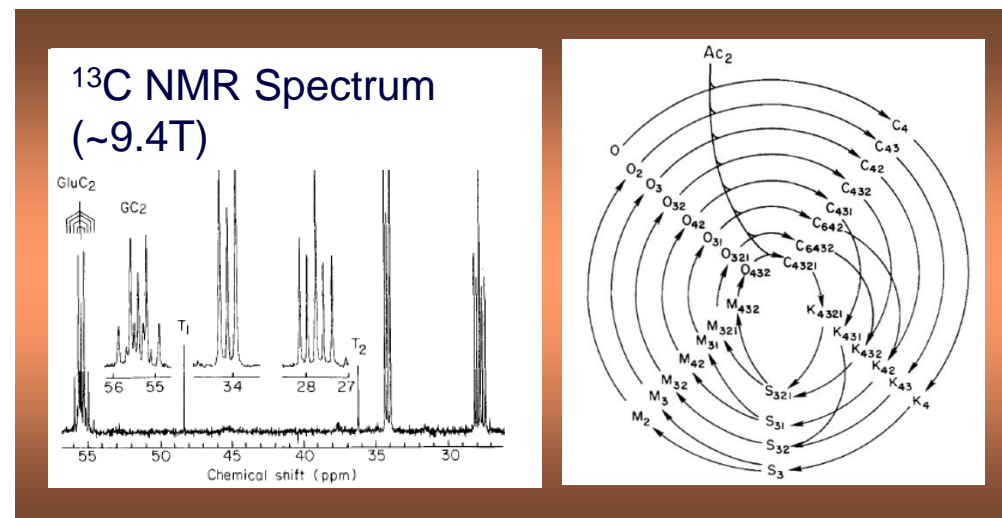
Chance B et al. PNAS. 1980;77(12):7430-4.

Incorporation of  $^{13}\text{C}$  into glutamate provides information about flux in the TCA cycle

Bailey IA, et al. FEBS Letters 1981;123:315-318.

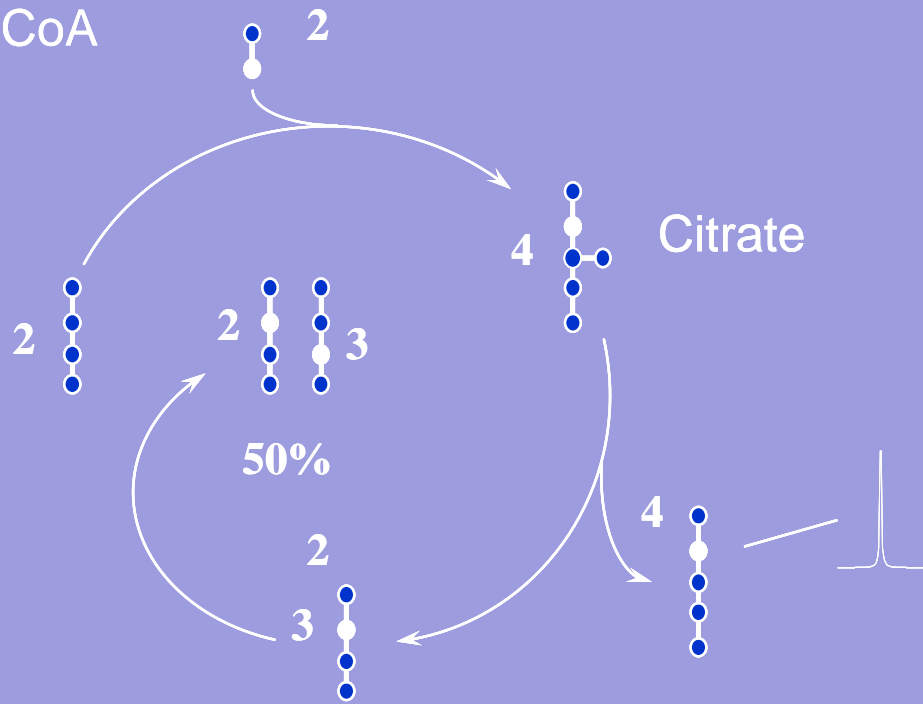
Mathematical analysis of isotope labeling in the citric acid cycle

Chance EM, et al. JBC 1983;258:13785-13794.



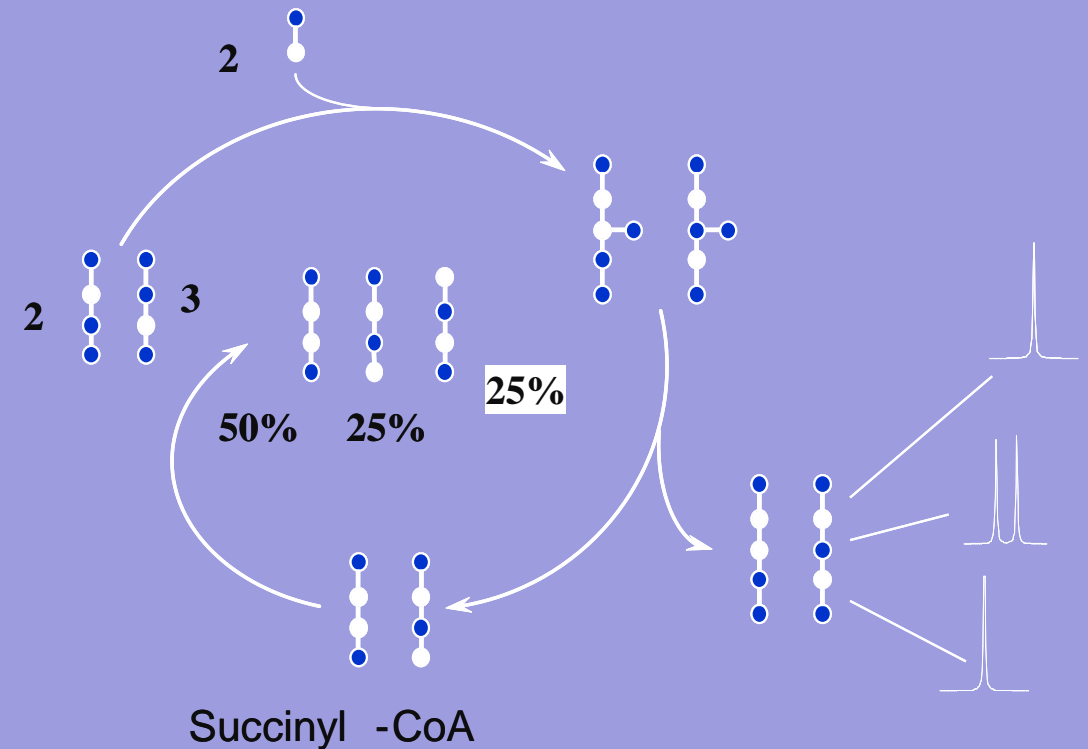
Chance EM, et al. JBC 1983;258:13785-13794.

Acetyl-CoA



Succinyl-CoA

2<sup>nd</sup> turn of TCA cycle



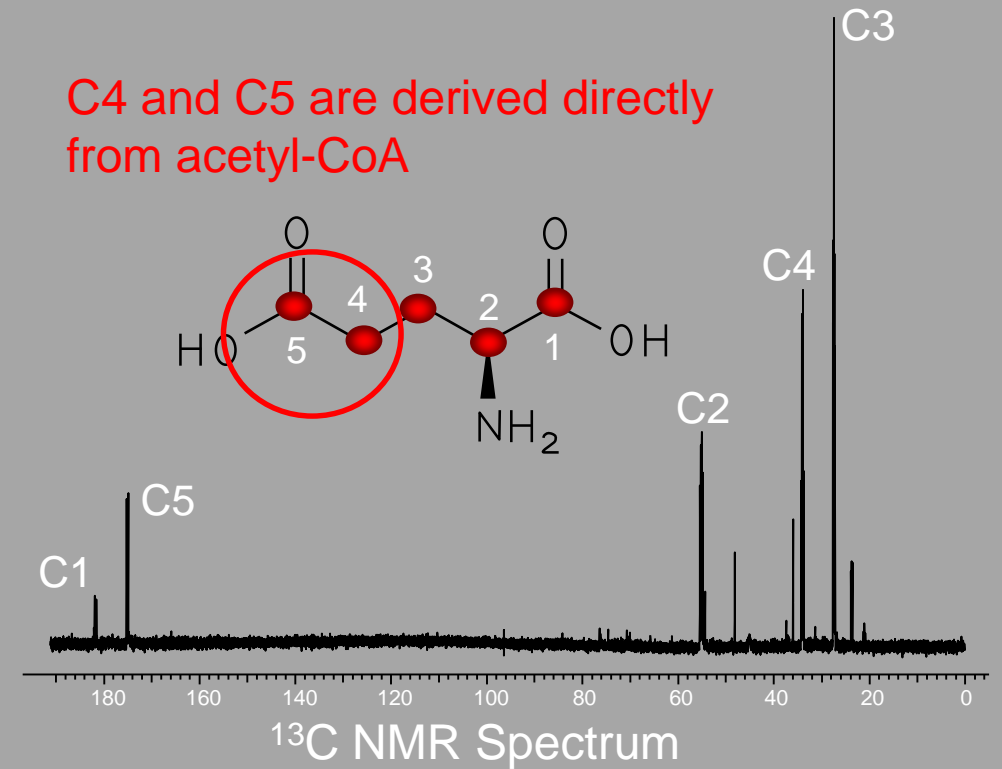
Succinyl -CoA

1<sup>st</sup> turn of TCA cycle



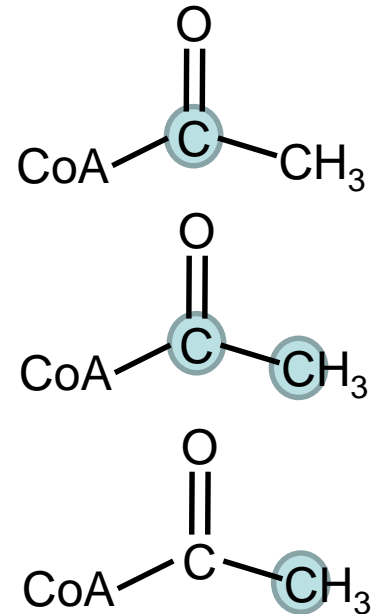
# Direct Analysis of the C4 and C5 positions of glutamate

- Substrate selection is a primary metabolic marker of a healthy heart
- The healthy adult heart uses fatty acids for 60-70% of its acetyl-CoA production in the fed state
- The remainder is provided by glucose
- Ketones important in fasting



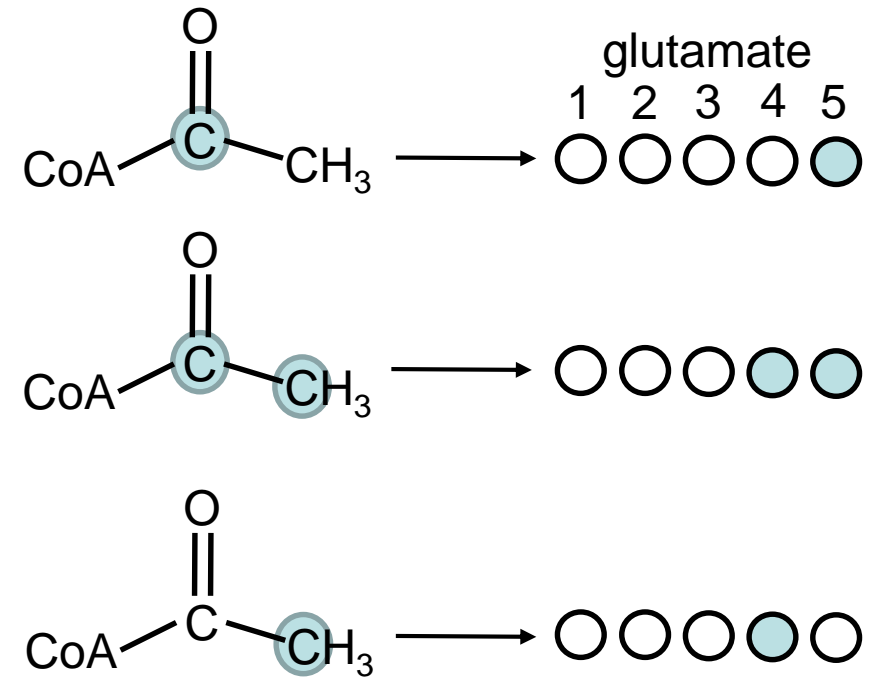
# Chemical Selectivity of NMR

- Multiple different carbon-13 labeling patterns can be used simultaneously
- [1,3- $^{13}\text{C}_2$ ]acetoacetate will produce [1- $^{13}\text{C}$ ]acetyl-CoA
- [U- $^{13}\text{C}$ ]fatty acids will produce [1,2- $^{13}\text{C}_2$ ]acetyl-CoA
- [3- $^{13}\text{C}$ ]pyruvate will produce [2- $^{13}\text{C}$ ]acetyl-CoA



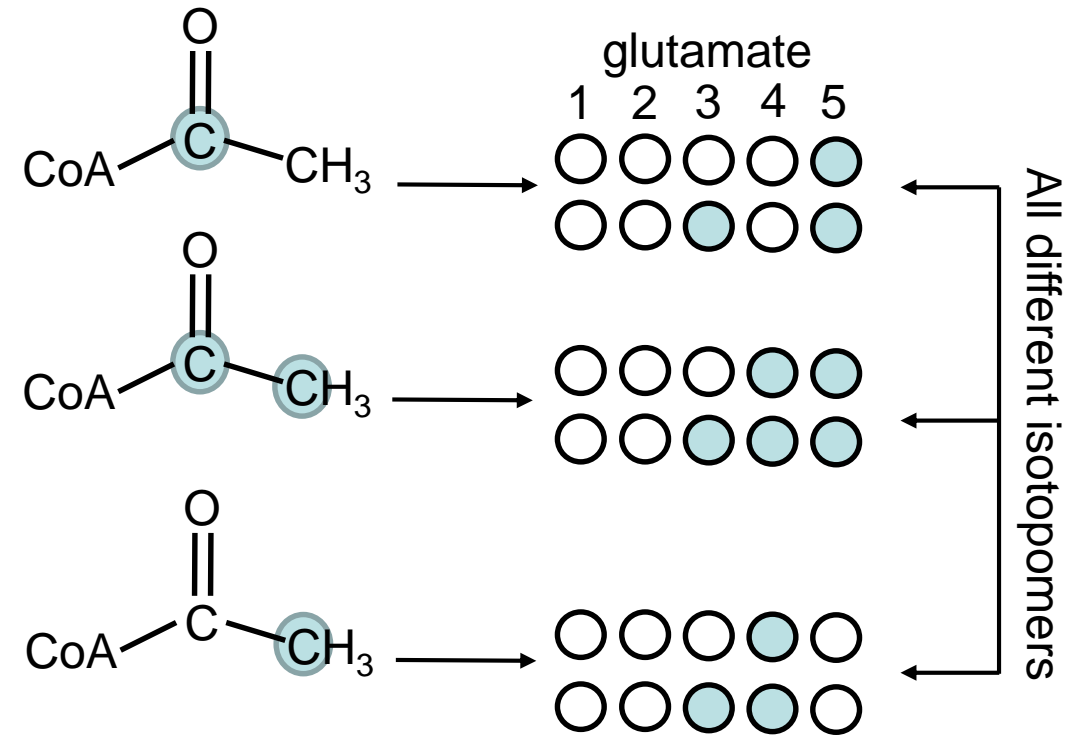
# First turn: acetyl-CoA incorporation into glutamate

- Give labeled substrates
- Harvest tissue and extract glutamate (PCA)
- Carbon-13 NMR and relative peak intensities

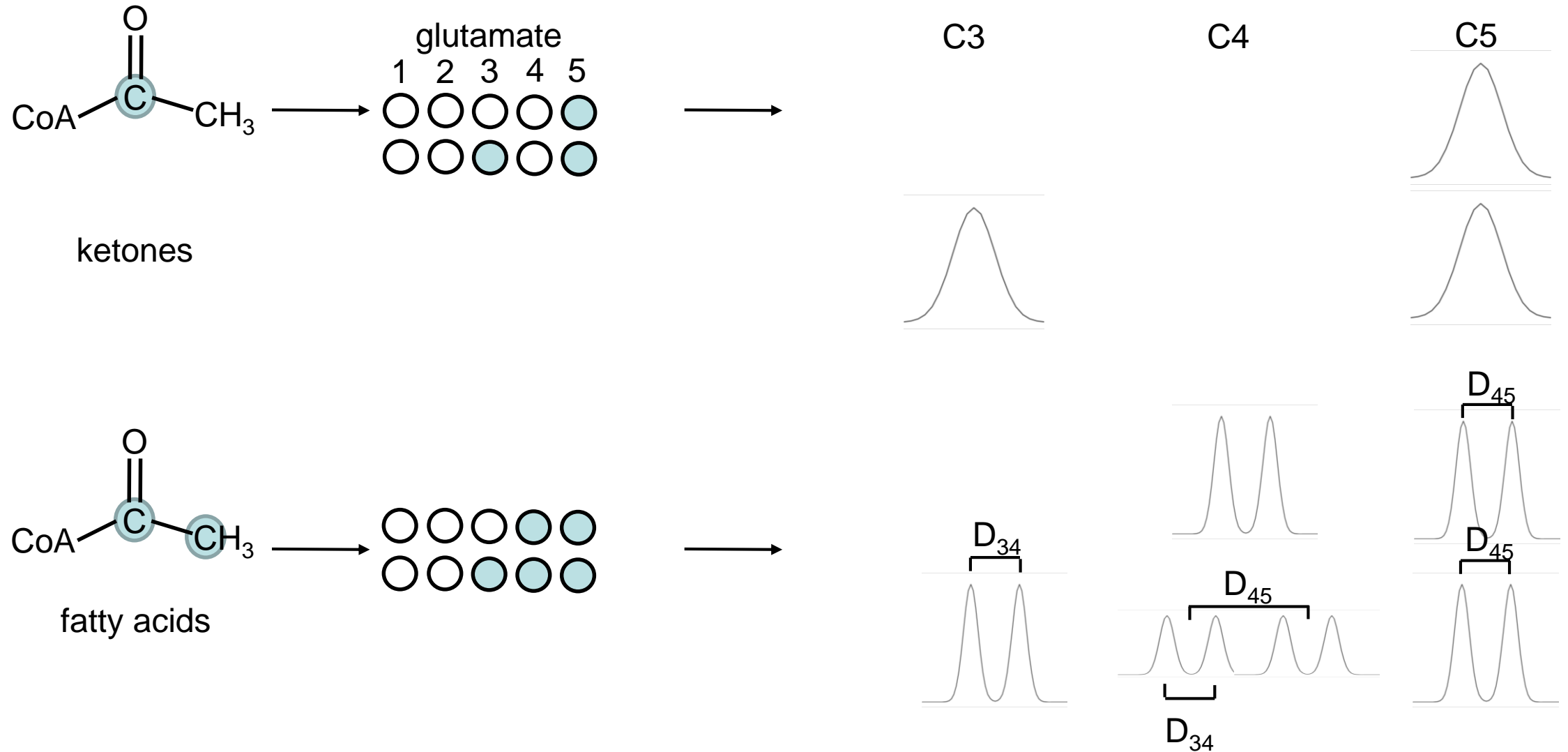


# Isotopomer distribution at equilibrium

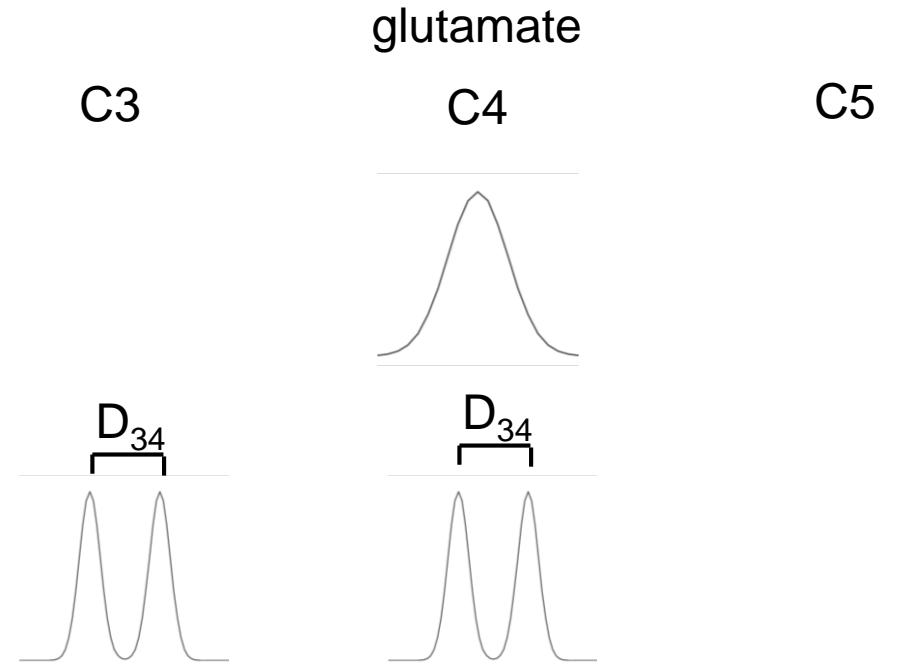
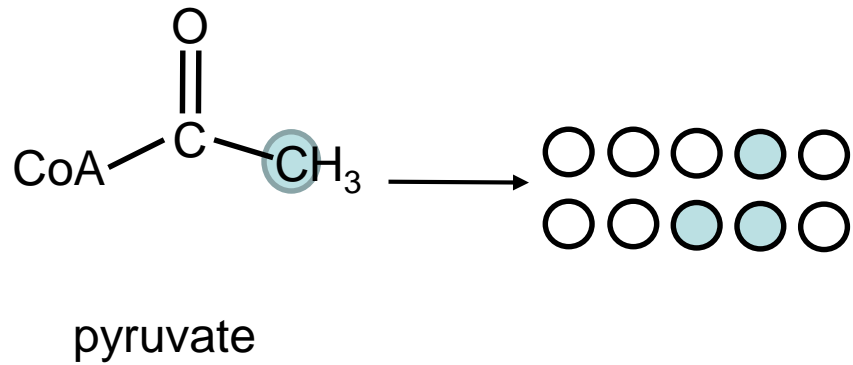
- Multiple turn of the cycle generate more complicated isotopomers
- Consider only isotopomers of glutamate that can additionally be labeled at the C3 position



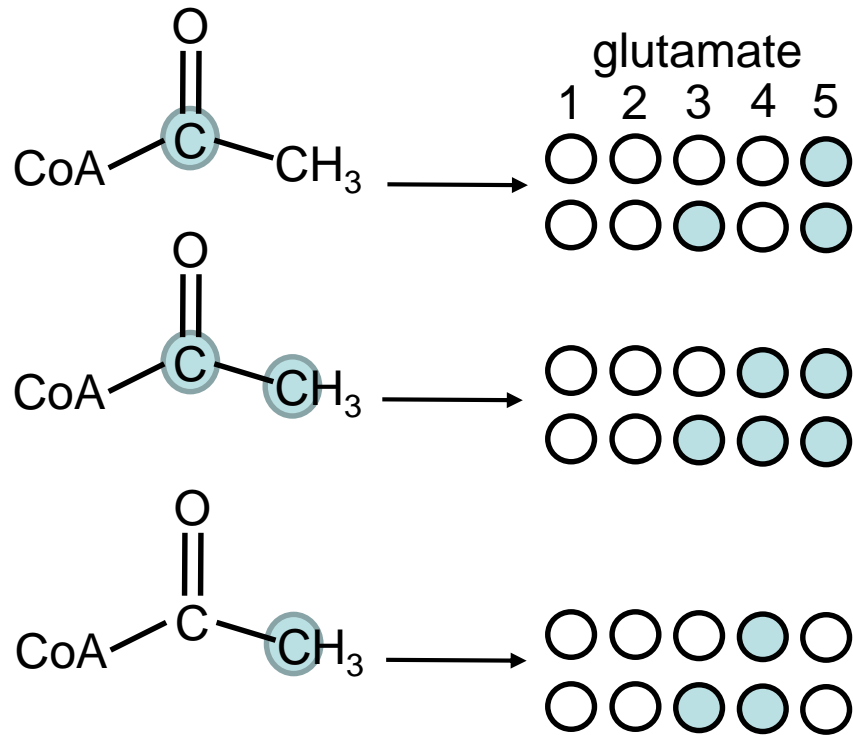
# Carbon-13 spectra of isotopomers



# Carbon-13 spectra of isotopomers, *cont.*



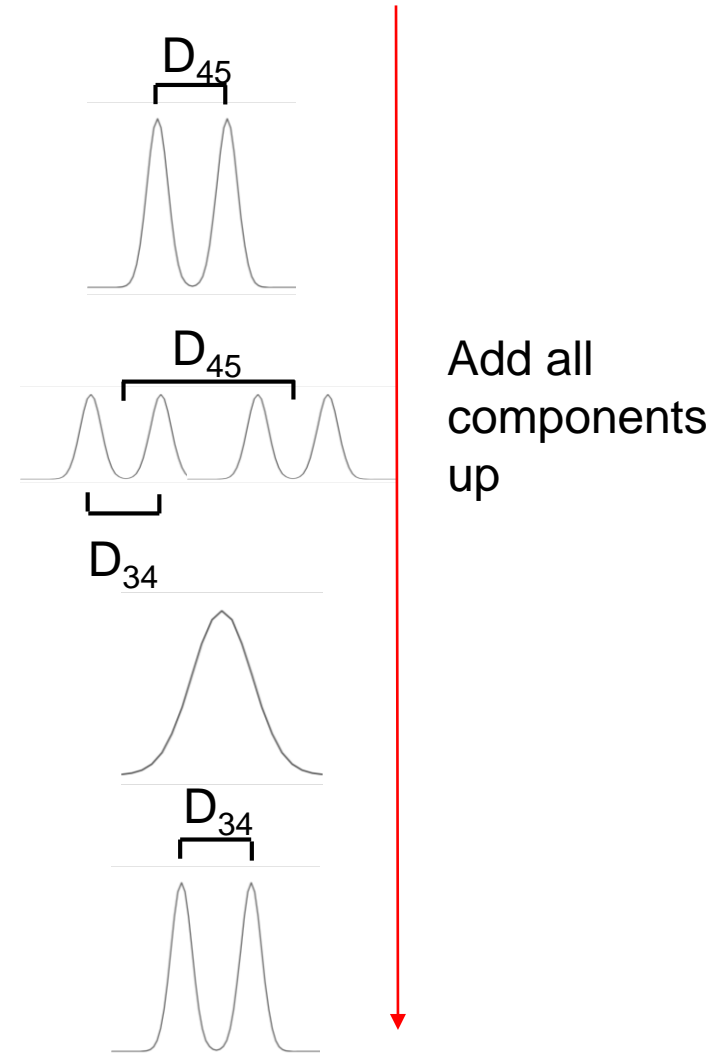
# Add up spectra (C4)



glutamate

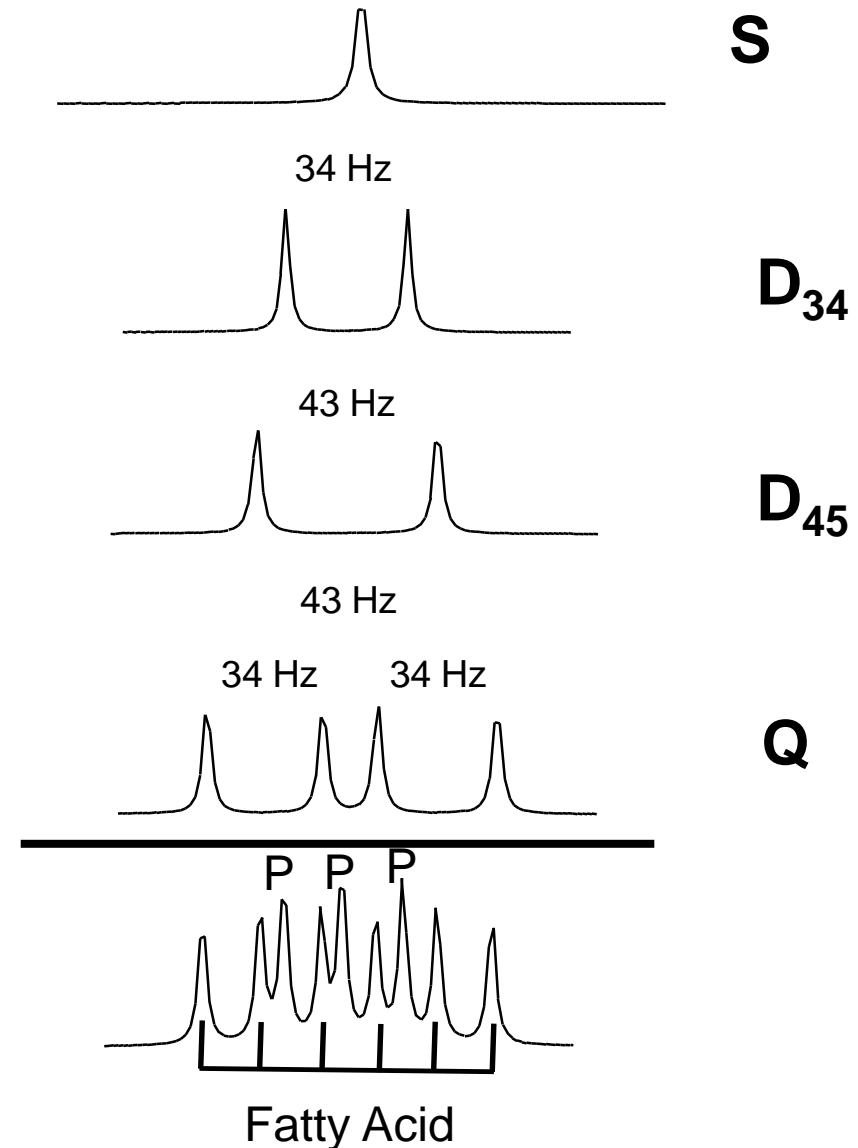
C4

ketones-No Contribution



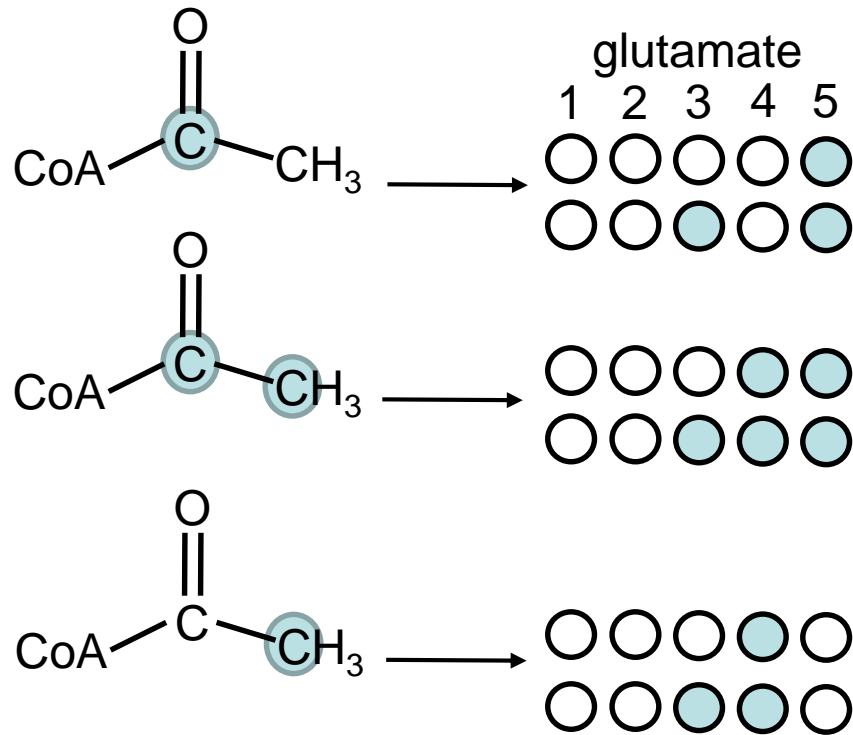
# Spectral Sum

- The sum of spectral components reports directly on the substrate selection
- The C4 position of glutamate with these substrates reports on FA versus pyruvate competition

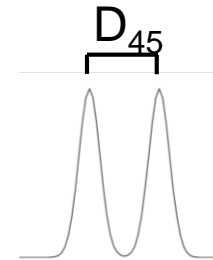
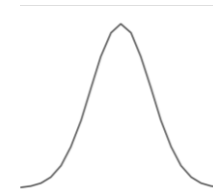




# Add up spectra (C5)

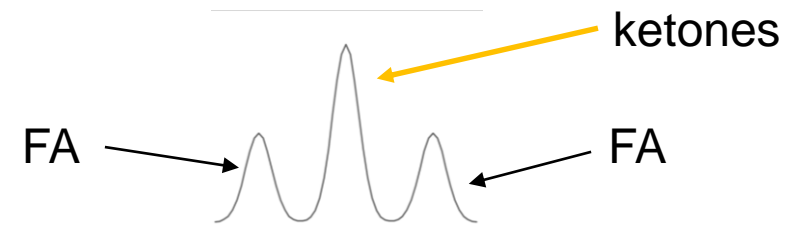


glutamate  
C5

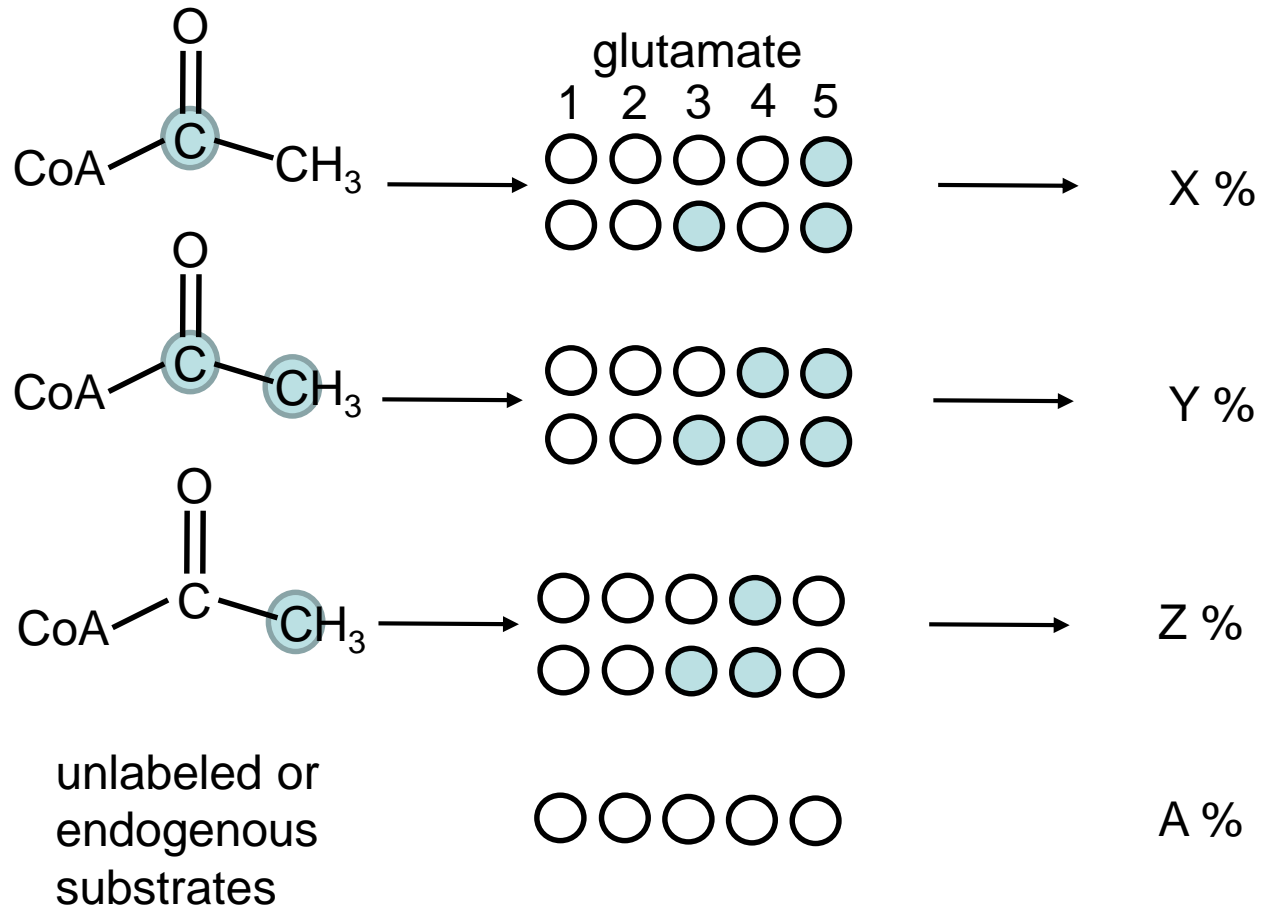


Add all  
components  
up

pyruvate-No Contribution



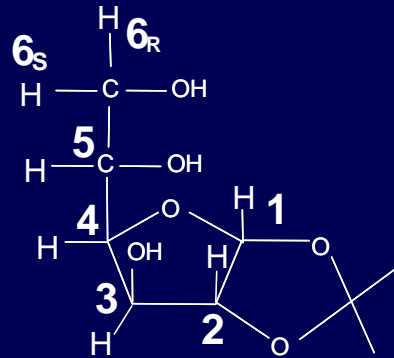
# Fractional Contributions



The ultimate outcome of the analysis is a fractional estimate of the contribution of each substrate to acetyl-CoA production.

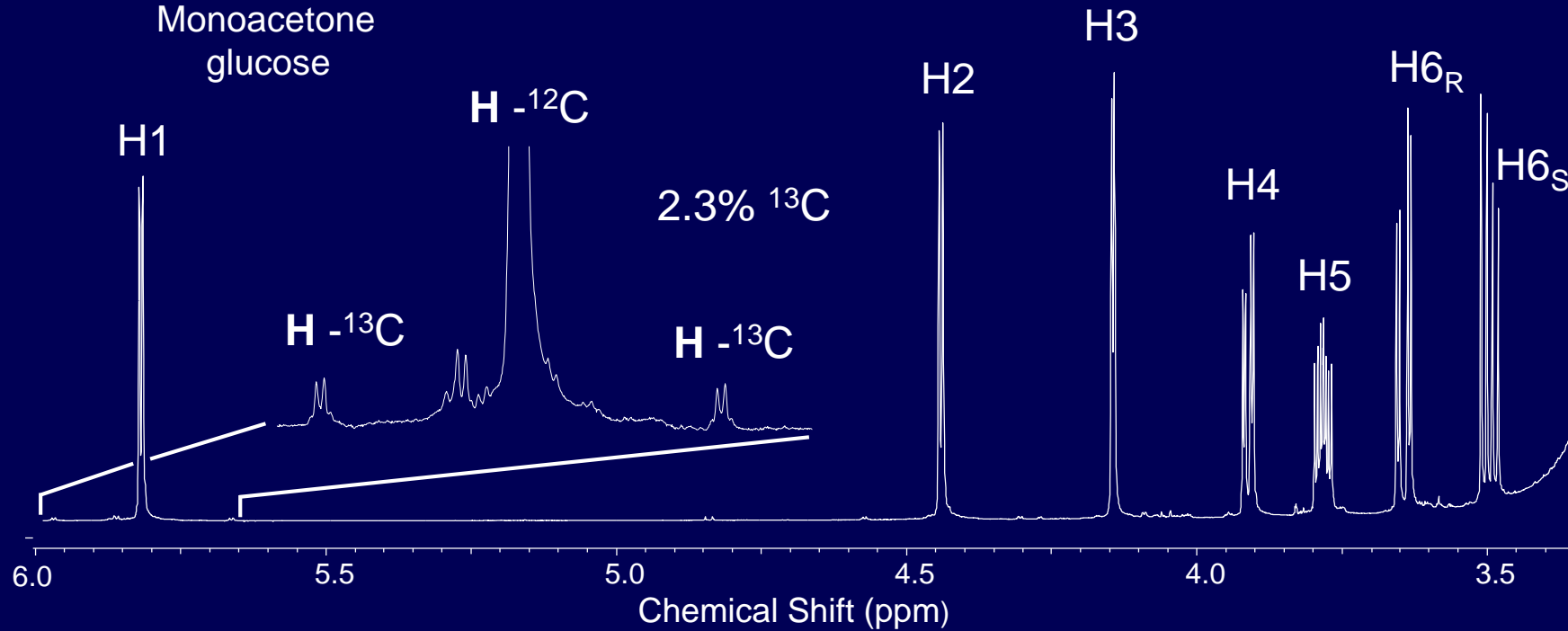
Substrate selection,  
Fuel switching,  
Metabolic switch, etc.

# Carbon-13 Enrichment by $^1\text{H}$ NMR



Derived from human  
plasma glucose

Monoacetone  
glucose



# Chemical elements of CAC

- Now we have substrate selection worked out
- Each substrate provides a known number of NADH equivalents
- We measure the  $O_2$  consumption
- Therefore, we have the flux in the system in g tissue/mol/min
- FLUX

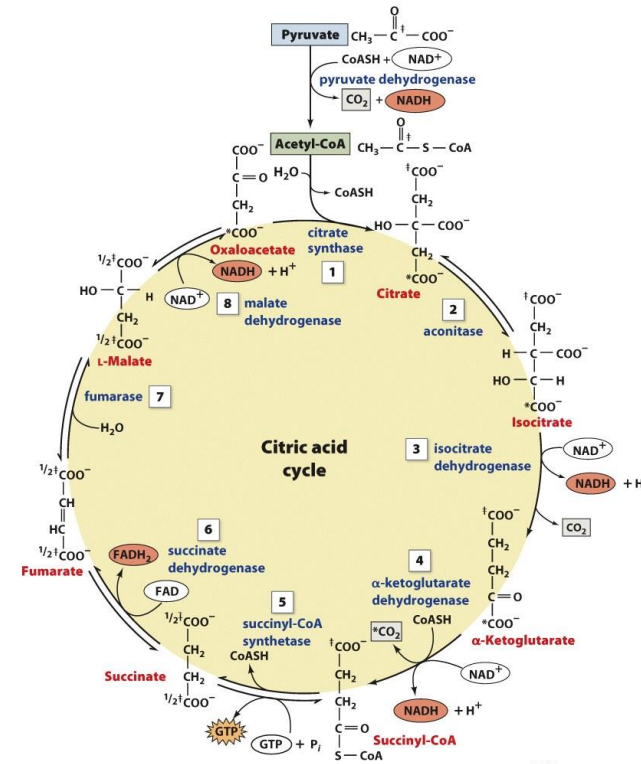


Figure 17-2  
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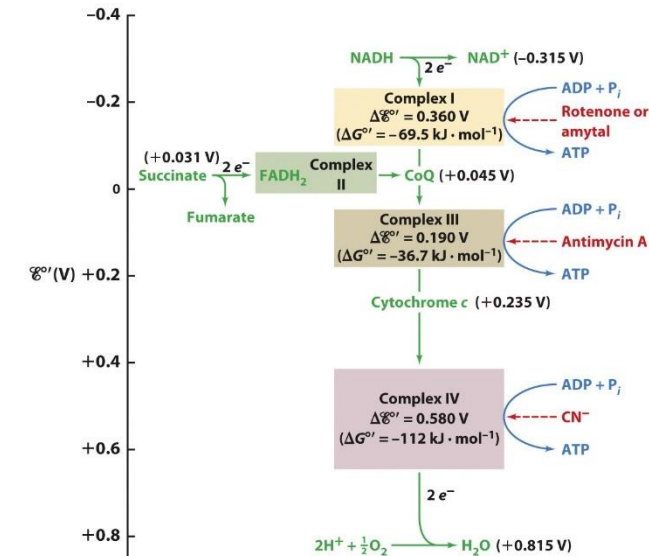
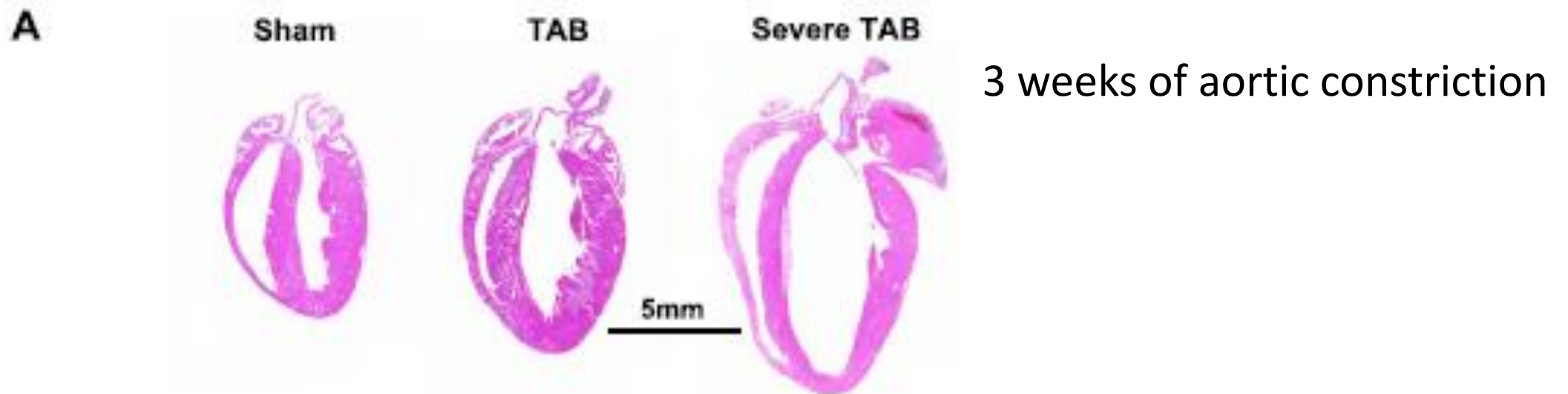


Figure 18-7  
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# A model of Congestive Heart Failure: the sTAC mouse (w/ Aslan Turer)

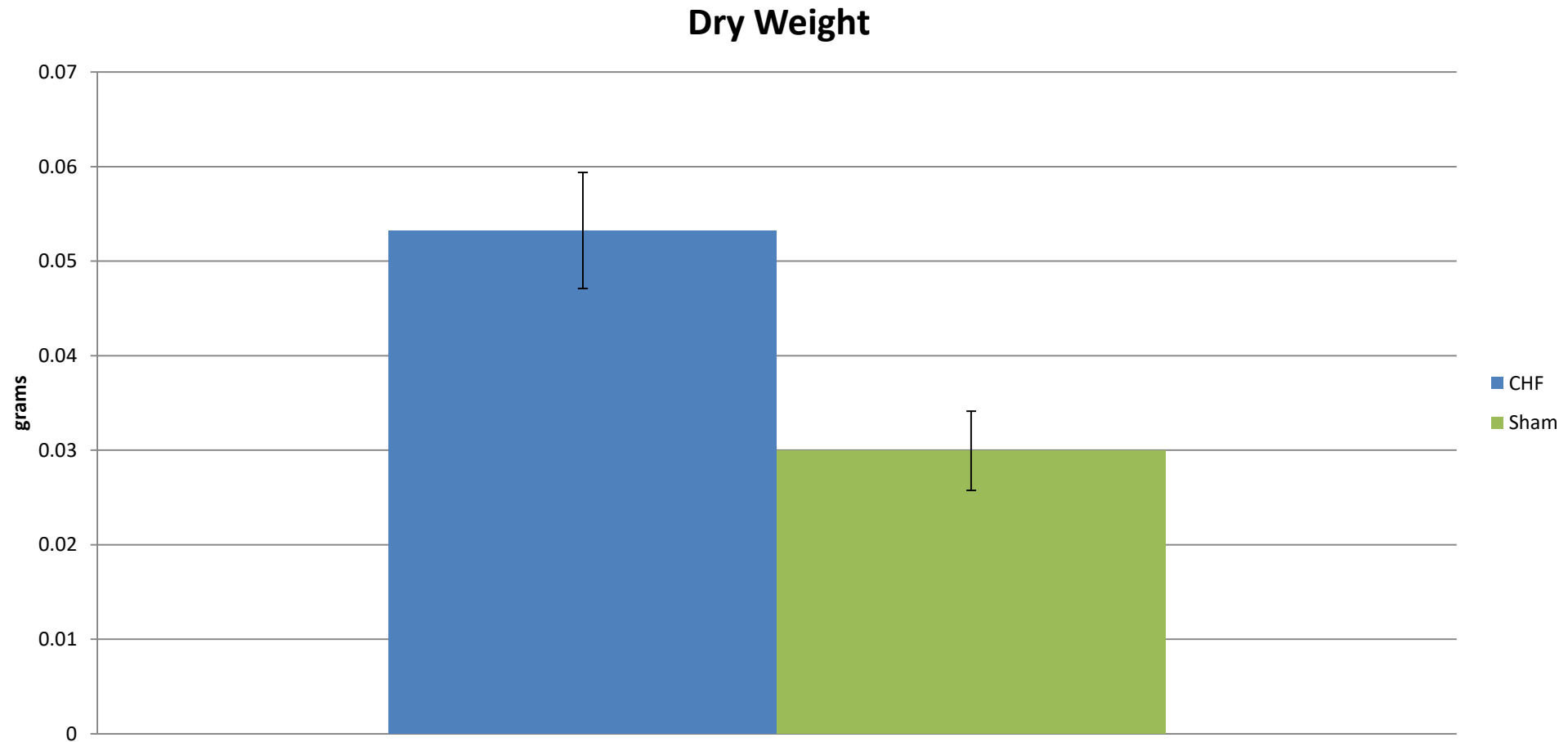
- Pressure overload hypertrophy model
  - thoracic aortic banding, specifically in the transverse aorta
- Hypothesis: In severe CHF, substrate selection will be different.



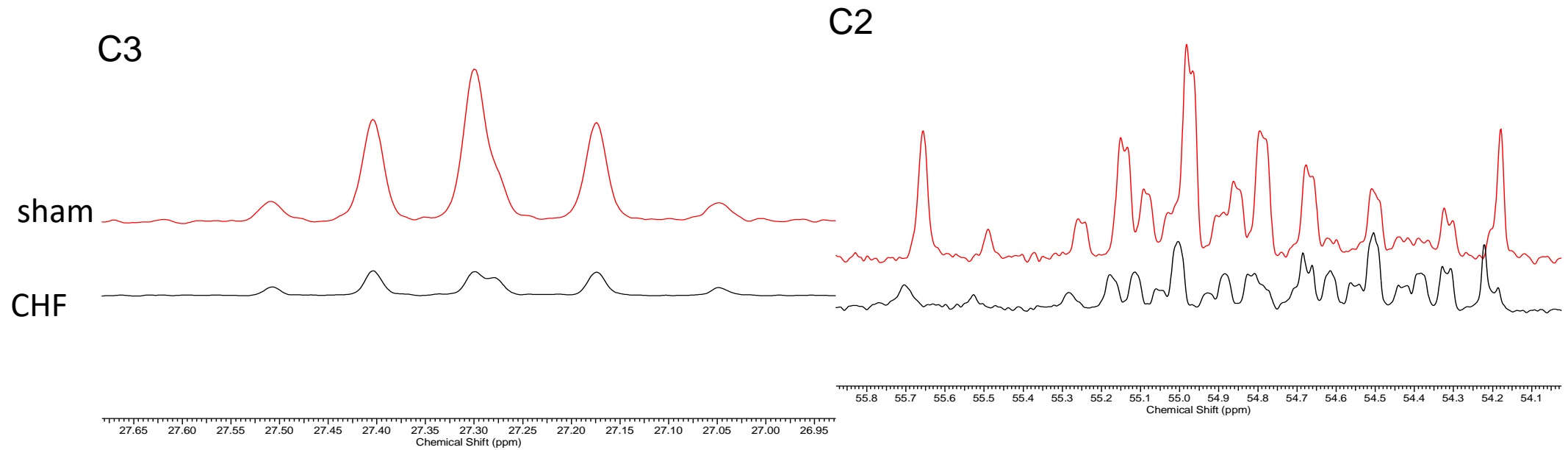
# *Experimental*

- Animals underwent surgery
  - 3 sham surgeries
  - 5 procedures
  - 3 weeks sTAC
- Perfusion Conditions
  - 8.2 mM [1,6-<sup>13</sup>C]glucose, .63 mM [U-<sup>13</sup>C]FA, .17 mM [1,3-<sup>13</sup>C]acetoacetate, 1 microunit/ml Insulin, 2% BSA
  - Langendorff perfused

# CHF leads to enlarged heart



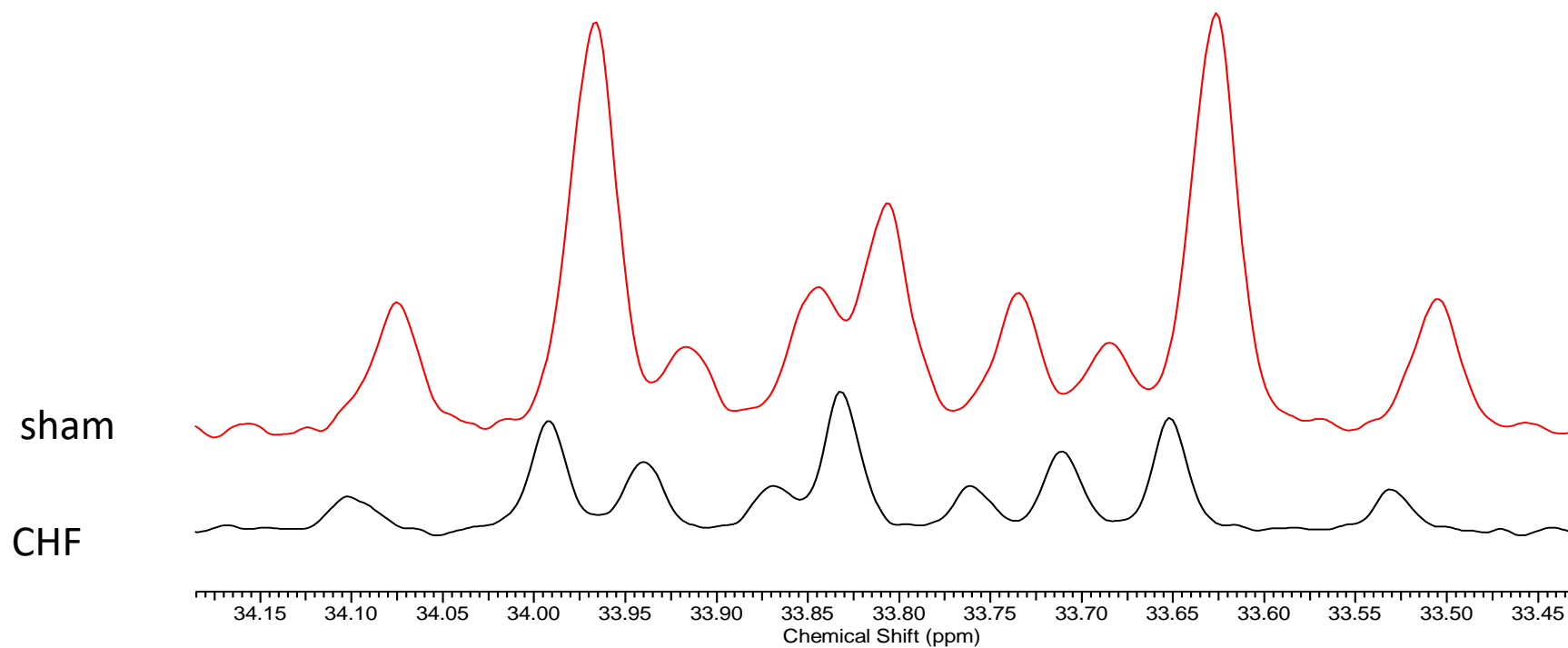
# C2 and C3-oxaloacetate labeling



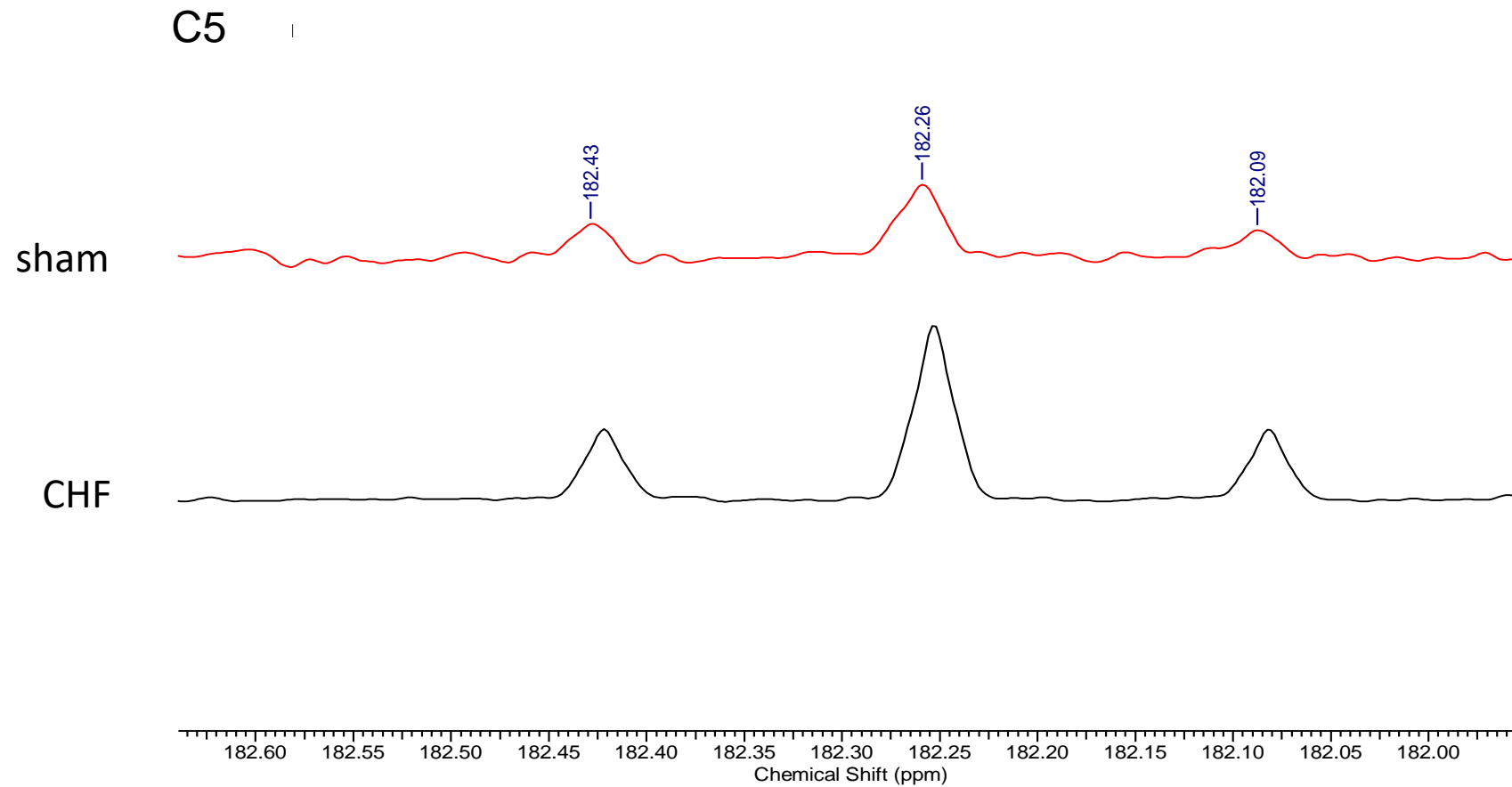


# C4- glucose versus fatty acids

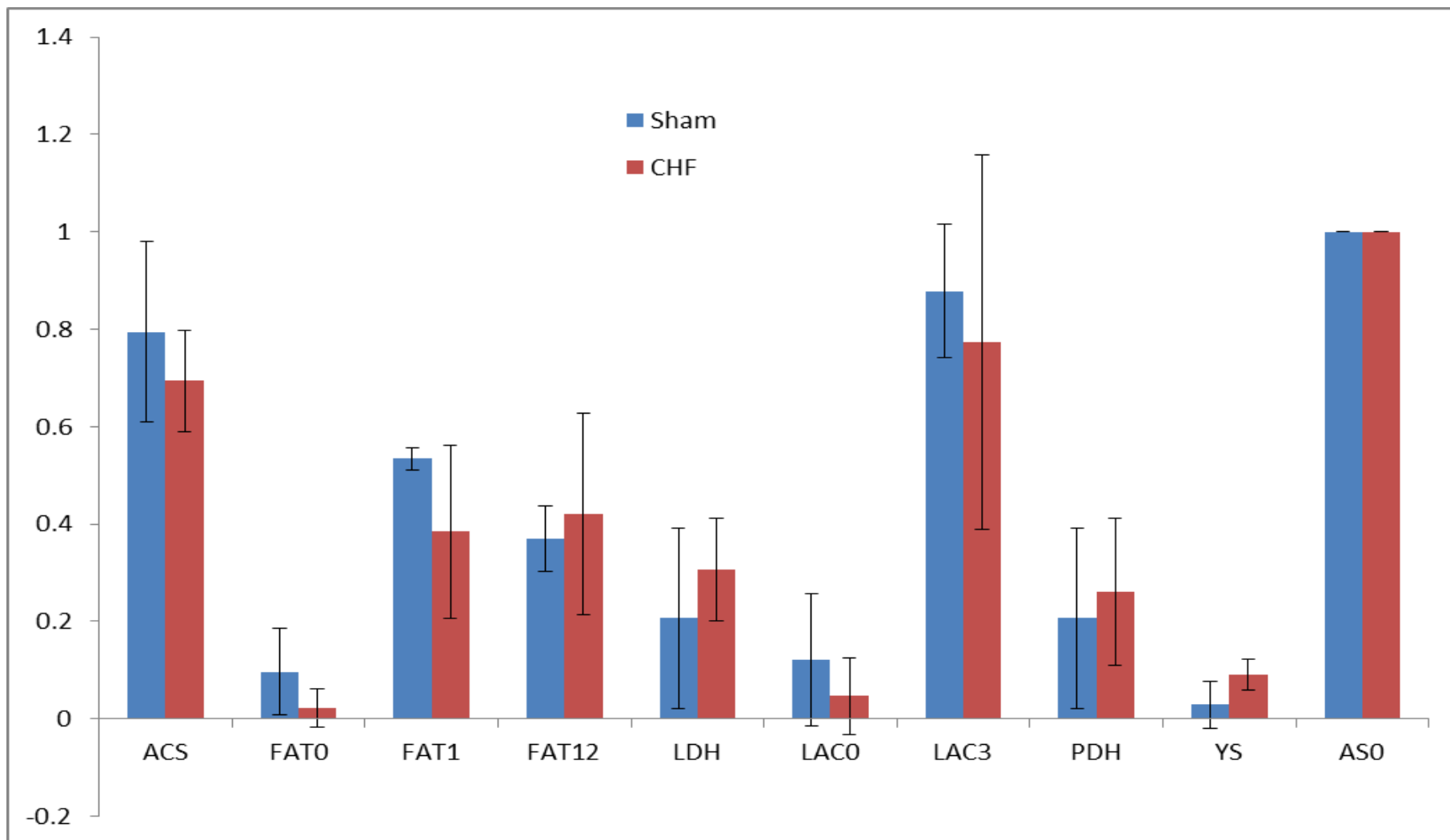
C4



# C5-ketone contributions

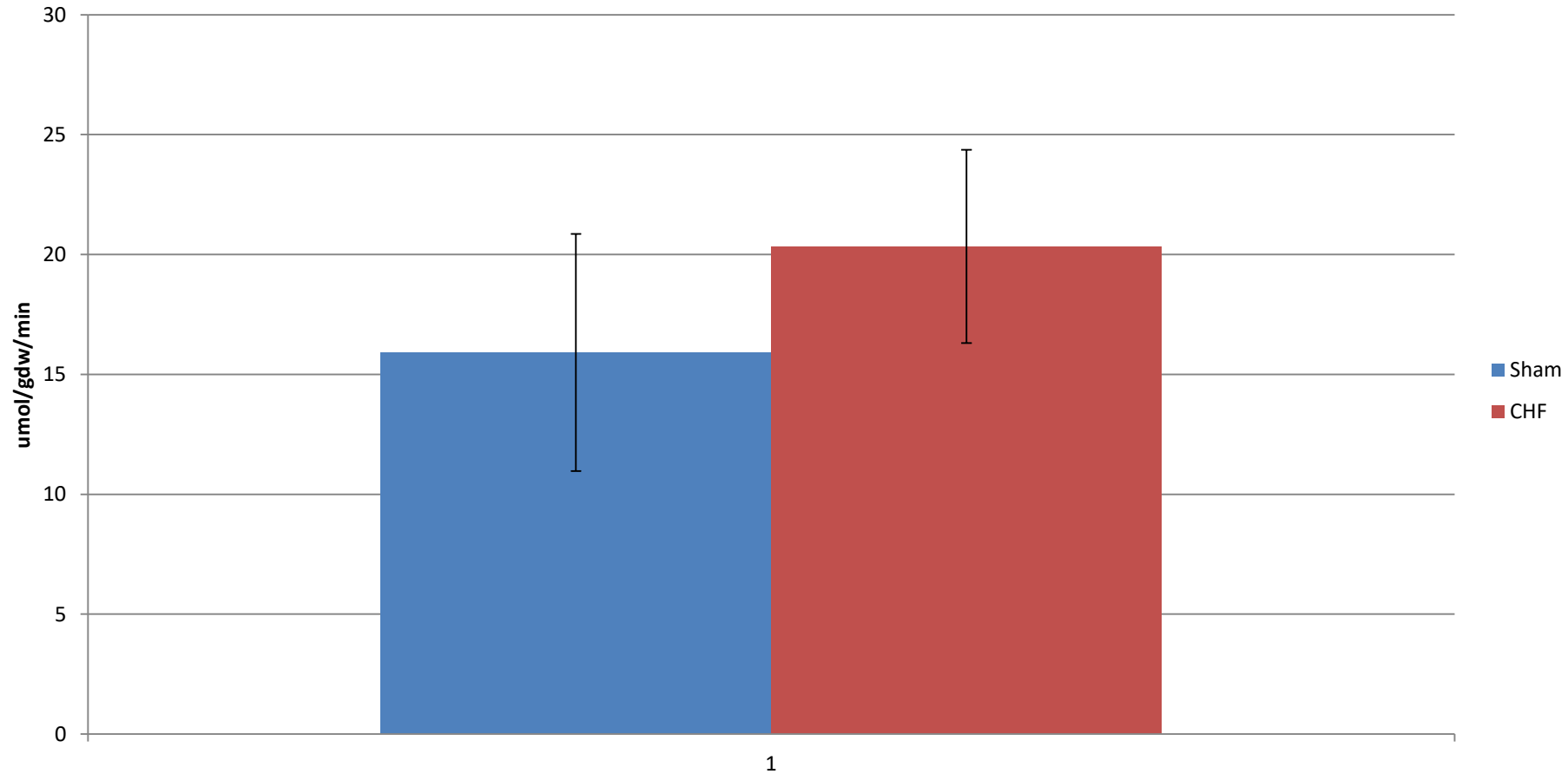


# Sham Vs. CHF



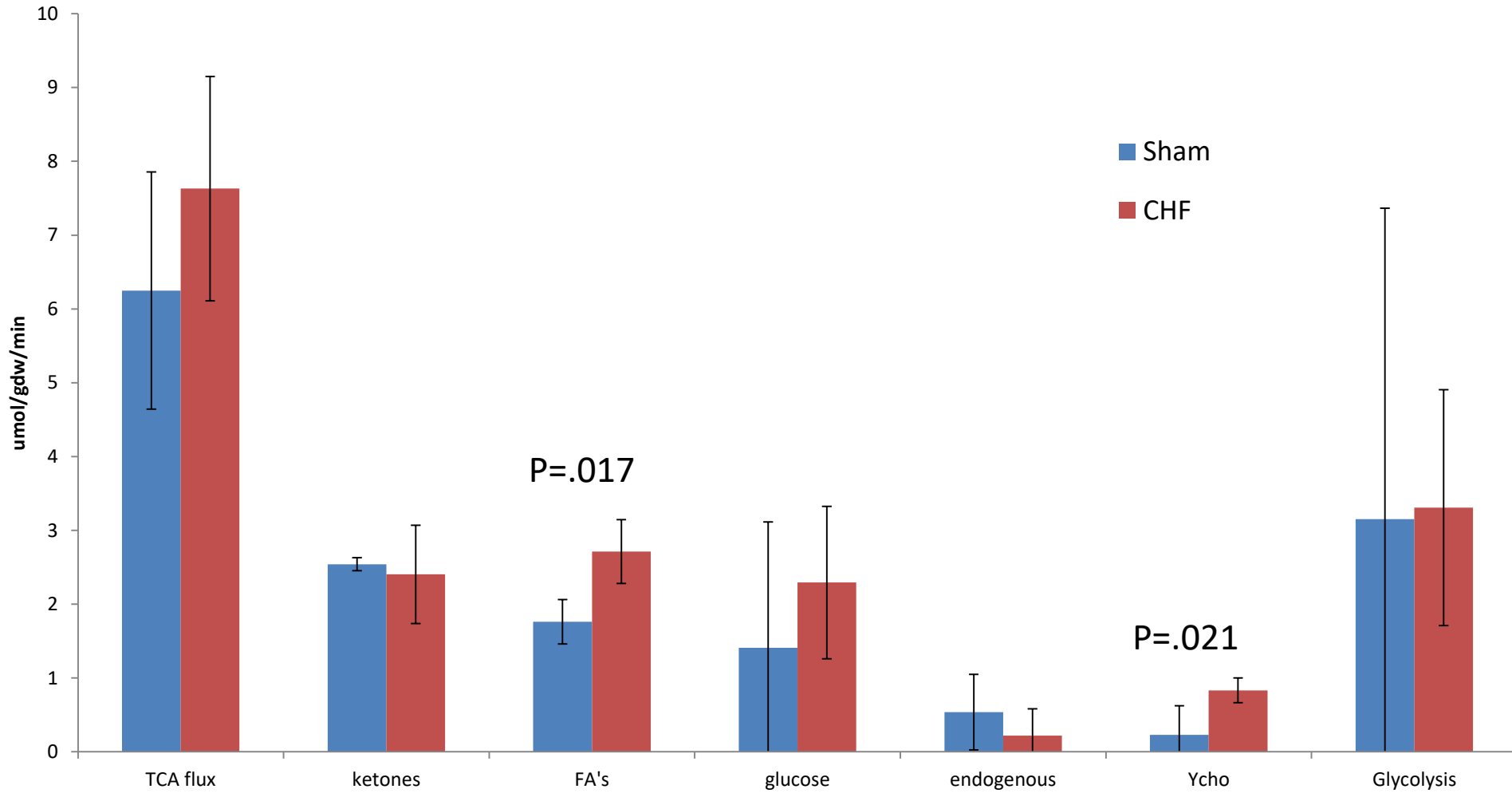
# Absolute Turnover

## O<sub>2</sub> Consumption



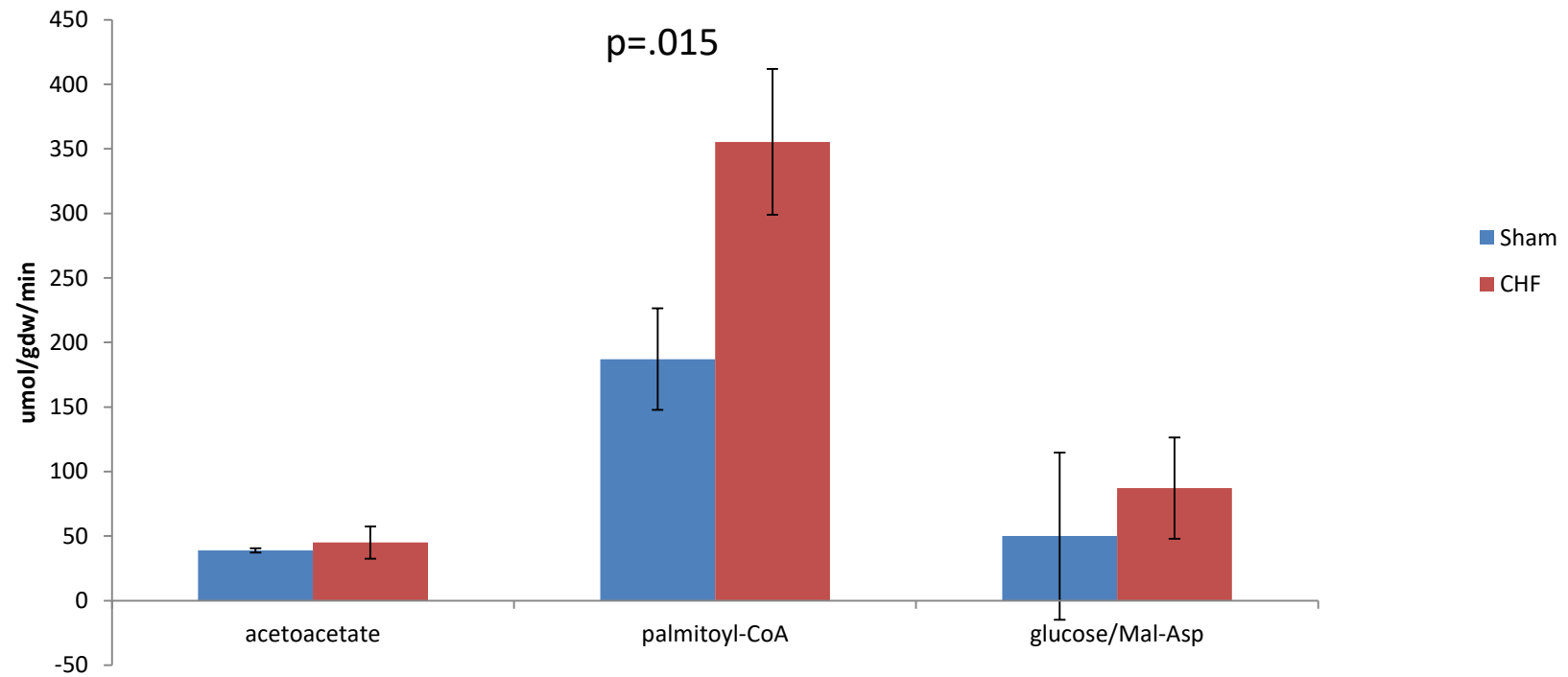
# Substrate Selection

## Absolute Flux



# ATP production

Sources of ATP, neglecting endogenous metabolites



## *Conclusions*

- Steady state approximation is a very powerful tool for understanding metabolic flux
- Substrate selection is linked to many disease states with particular importance in the heart
- Flux is a particular element of homeostasis that is gain being studied and appreciated