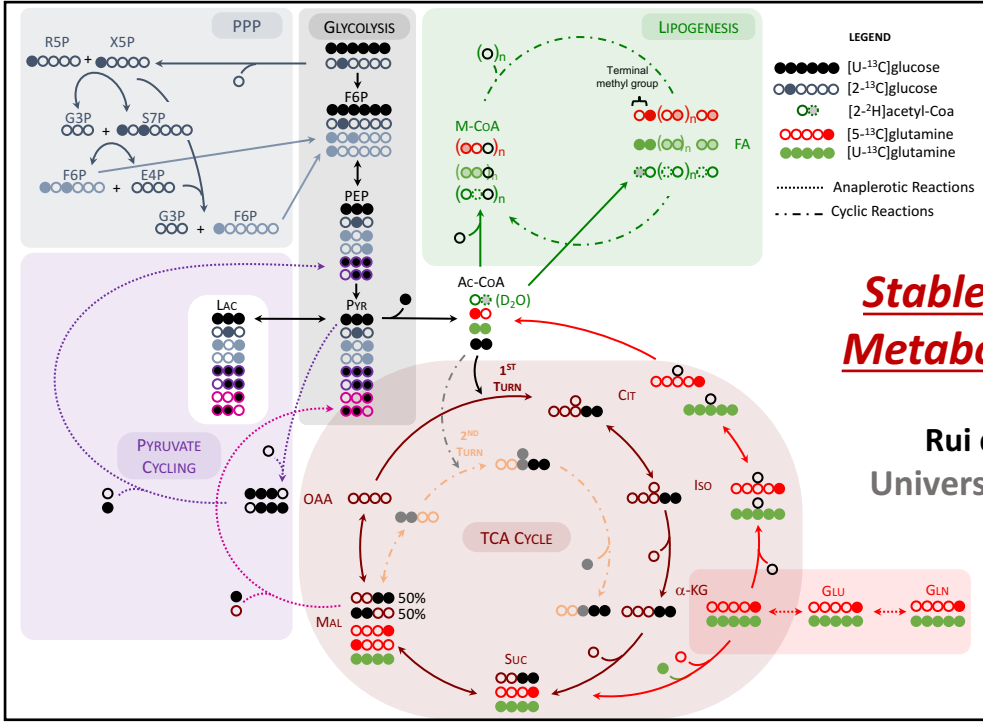


# Stable Isotopes in Metabolic Research

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University of Coimbra
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WILEY NMR IN BIOMEDICINE

RESEARCH ARTICLE

## Metabolic remodeling triggered by salivation and diabetes in major salivary glands

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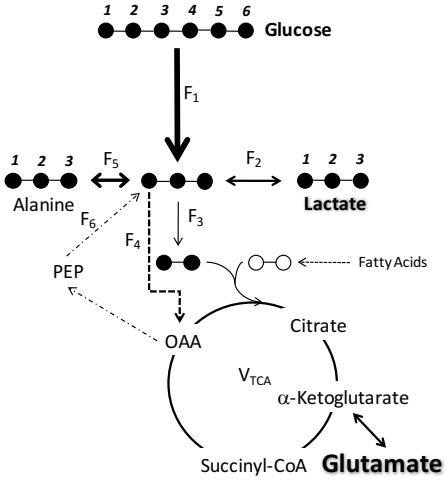
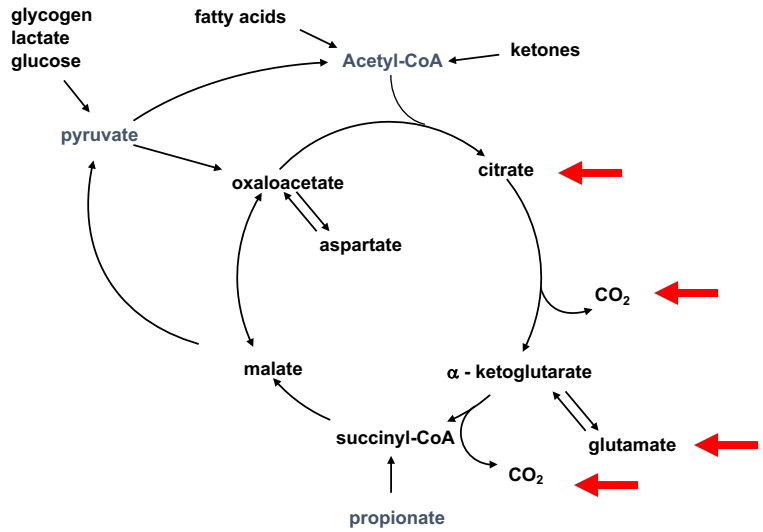
The metabolic profile of major salivary glands was evaluated by $^{13}C$ nuclear magnetic resonance isotopomer analysis ($^{13}C$ NMR-IA) following the infusion of [U-$^{13}C$]glucose in order to define the true metabolic character of submandibular (SM) and parotid (PA) glands at rest and during salivary stimulation, and to determine the metabolic remodeling driven by diabetes. In healthy conditions, the SM gland is characterized at rest by a glycolytic metabolic profile and extensive pyruvate cycling. On the contrary, the PA gland, although also dominated by glycolysis, also possesses significant Krebs' cycle activity and does not sustain extensive pyruvate cycling. Under stimulation, both glands increase their glycolytic and Krebs' cycle fluxes, but the metabolic coupling between the two pathways is further compromised to account for the much increased biosynthetic anaplerotic fluxes. In diabetes, the responsiveness of the PA gland to a salivary stimulus is seriously hindered, mostly as a result of the incapacity to burst glycolytic activity and also an inability to improve the Krebs' cycle flux to compensate. The Krebs' cycle activity in the SM gland is also consistently compromised, but the glycolytic flux in this gland is more resilient. This diabetes-induced metabolic remodeling in SM and PA salivary glands illustrates the metabolic need to sustain adequate saliva production, and identifies glycolytic and oxidative pathways as key players in the metabolic dynamism of salivary glands.

**KEYWORDS**
glycolysis, isotopomer analysis, Krebs' cycle, metabolism, salivary glands, salivary stimulation

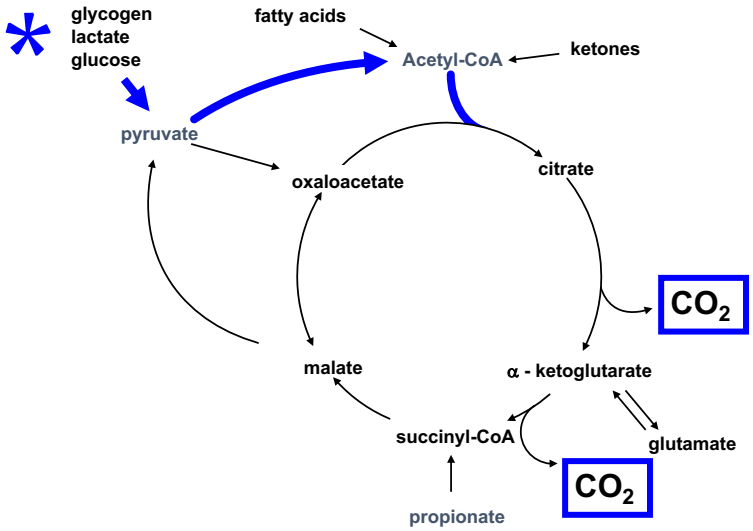
# Stable Isotopes in Salivary Gland Metabolic Research



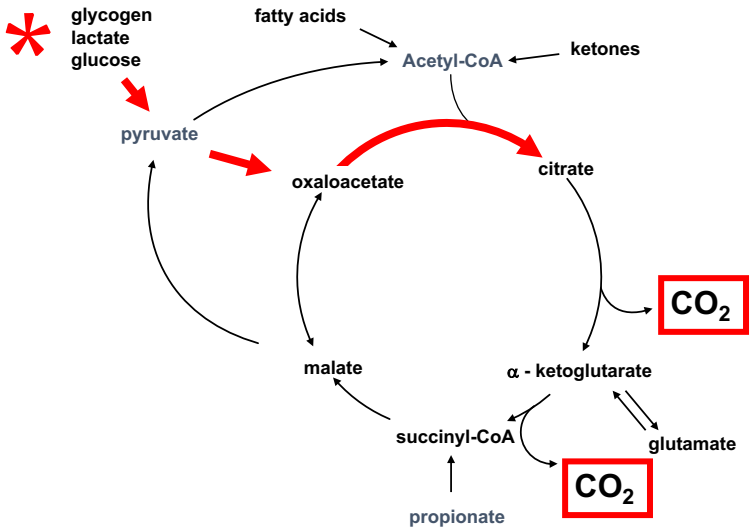
# Tracers are Required to Measure Fluxes by Detecting Enrichment in Product Molecules




# Analysis of Substrate Oxidation: $CO_2$ Release is Not Pathway - Specific



# Analysis of Substrate Oxidation: $CO_2$ Release is Not Pathway - Specific




# Properties of Carbon Isotopes

| Nucleus | Abundance | Half-life | |
|---|---|---|---|
| $^{11}C$ | - | 20 minutes | cyclotron & radiochemists |
| $^{12}C$ | 98.9% | - | |
| $^{13}C$ | 1.1% | - | catalogue & lab sink |
| $^{14}C$ | - | 5730 years | catalogue, paperwork & radiation containmet |

$^{11}C$, $^{13}C$ and $^{14}C$ may all be used as tracers

## Carbon-13: Natural Abundance

✓Natural Abundance of $^{13}C$ ~1.1%

✓$\gamma^{13}C$ ~1/4 $\gamma^{1}H$

✓Low Sensitivity ⇒ ↑[sample]

✓Consecutively Labeled $^{13}C$ Nuclei ≤ 0.011*0.011
(~1:8300)

## Carbon-13: Enriched Metabolites

✓Abundance of $^{13}C$ > 1.1%

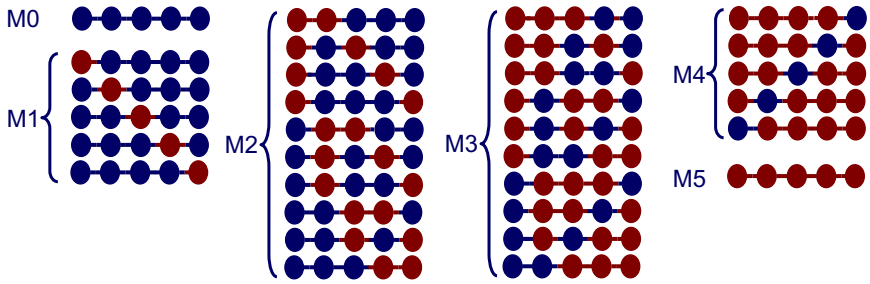
✓Consecutively Labelled $^{13}C$ Nuclei ⇒ $^{1}J_{CC}$ Couplings

✓Multiplets in Each Carbon Resonance

Isotopomer Information

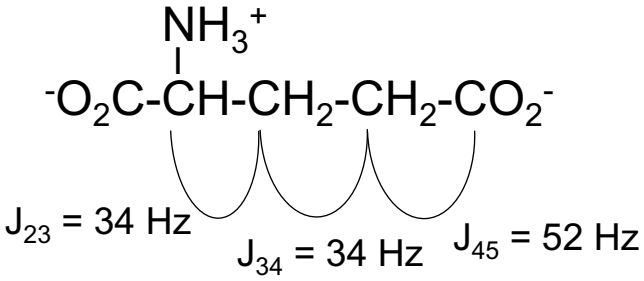
## Glutamate Isotopomers

- ✓ Isotopomer ⇔ Isotope + Isomer
- ✓ Metabolite with n Carbons has $2^n$ Isotopomers
  - glutamate → $2^5$ = 32 Isotopomers



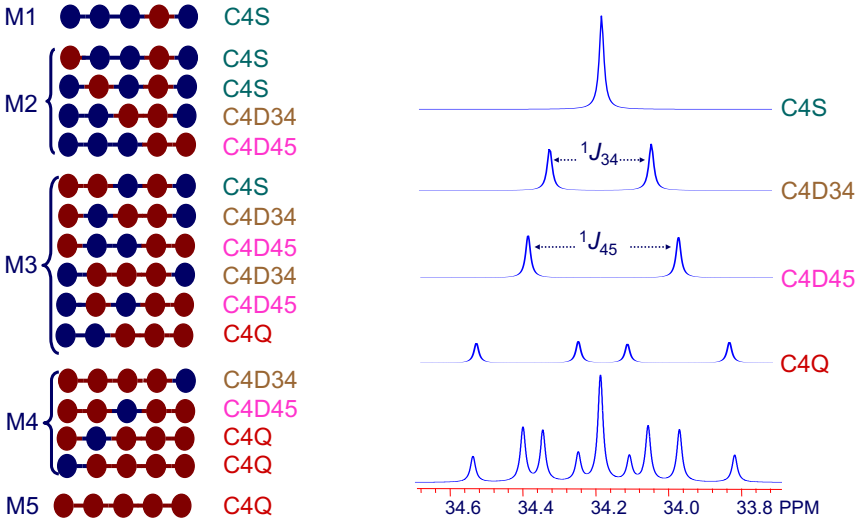

## The Overwhelming Advantage of $^{13}C$ NMR for Analysis of Substrate Oxidation

### Spin - Spin Coupling

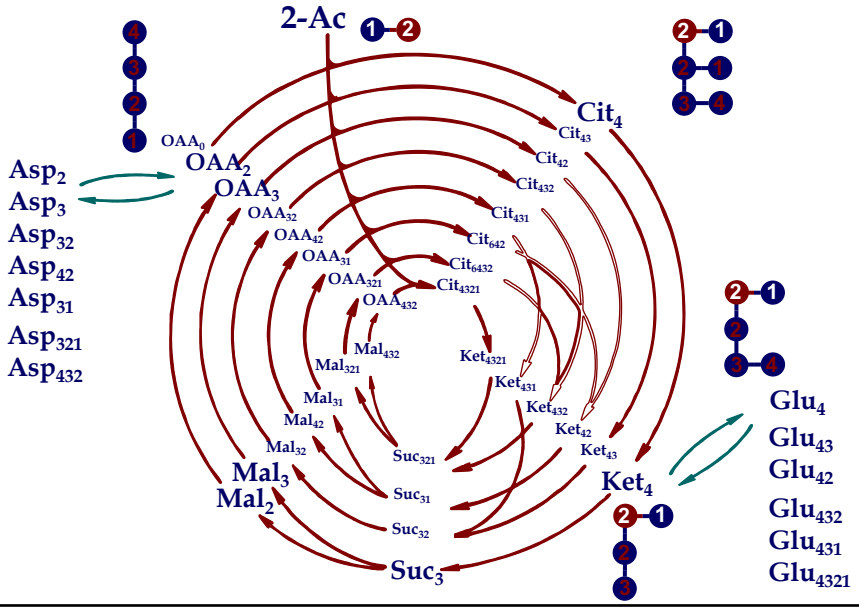


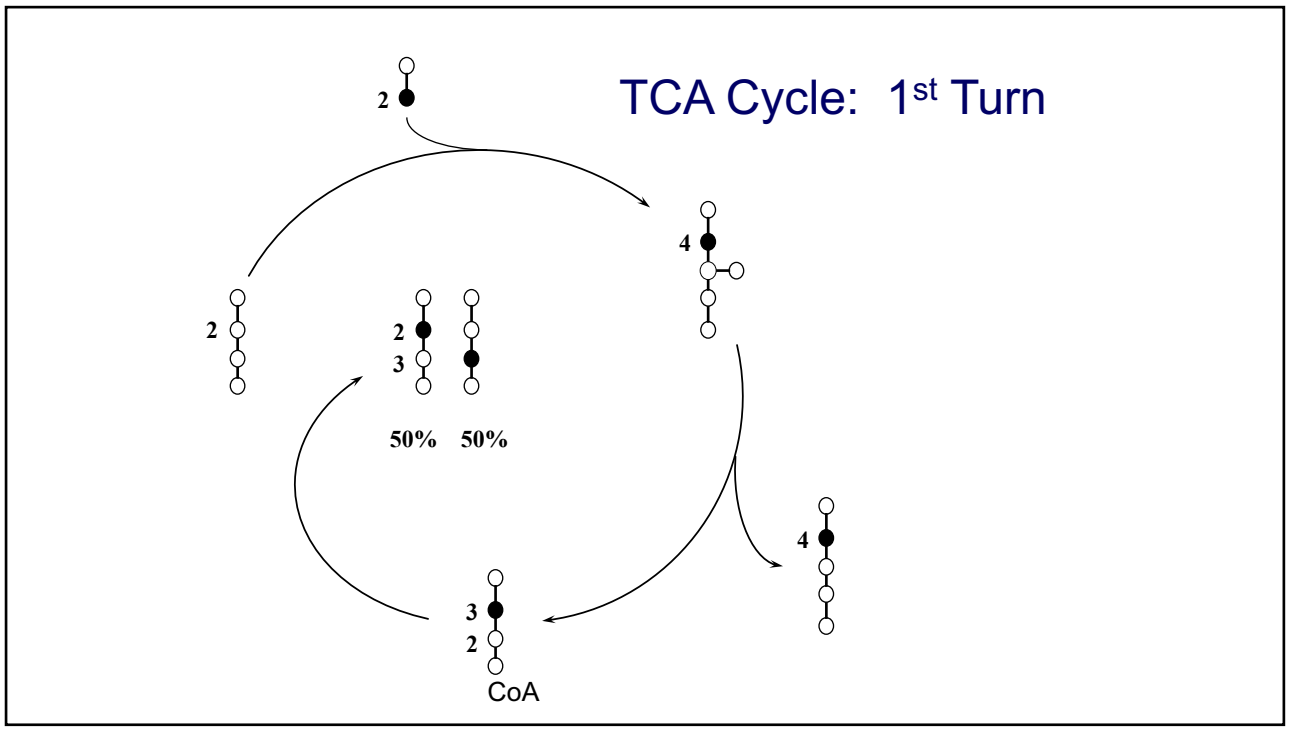
# Distinction of Glutamate $^{13}C$ Isotopomers

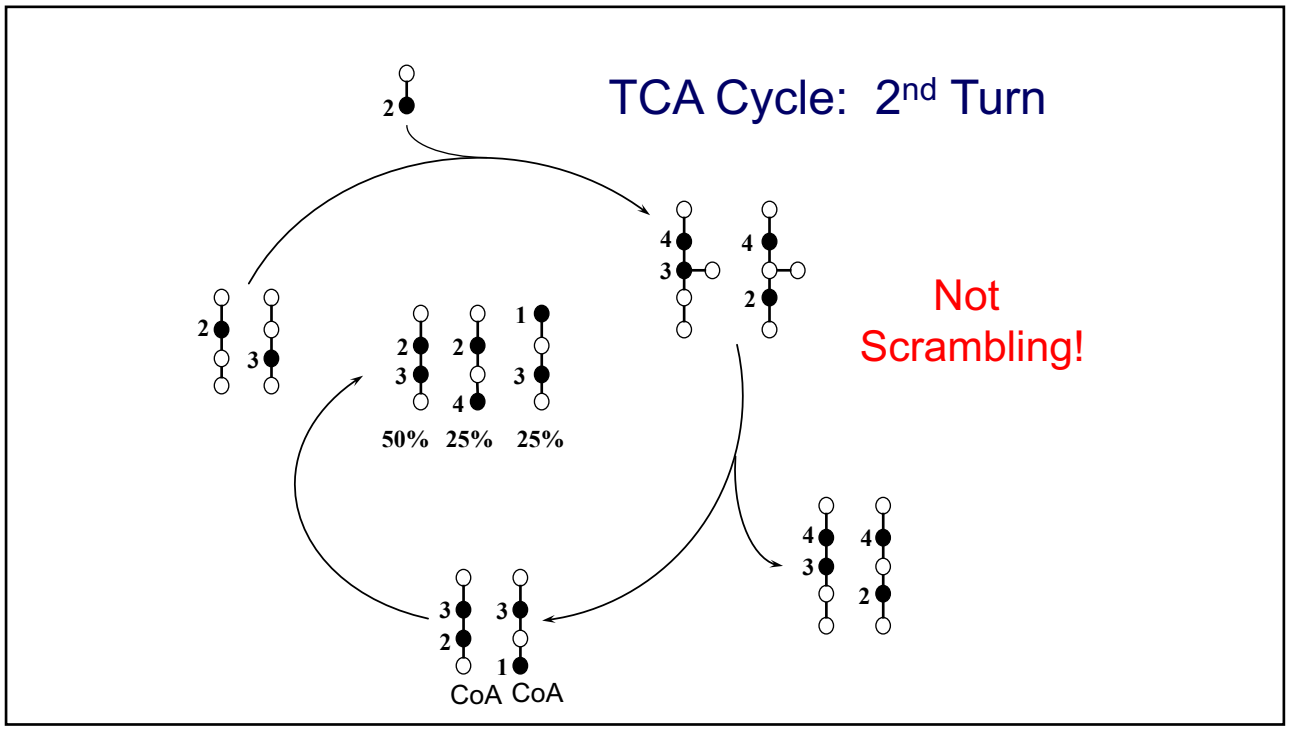
✓ Glutamate C4 Resonance ⇒ Labeling in C4

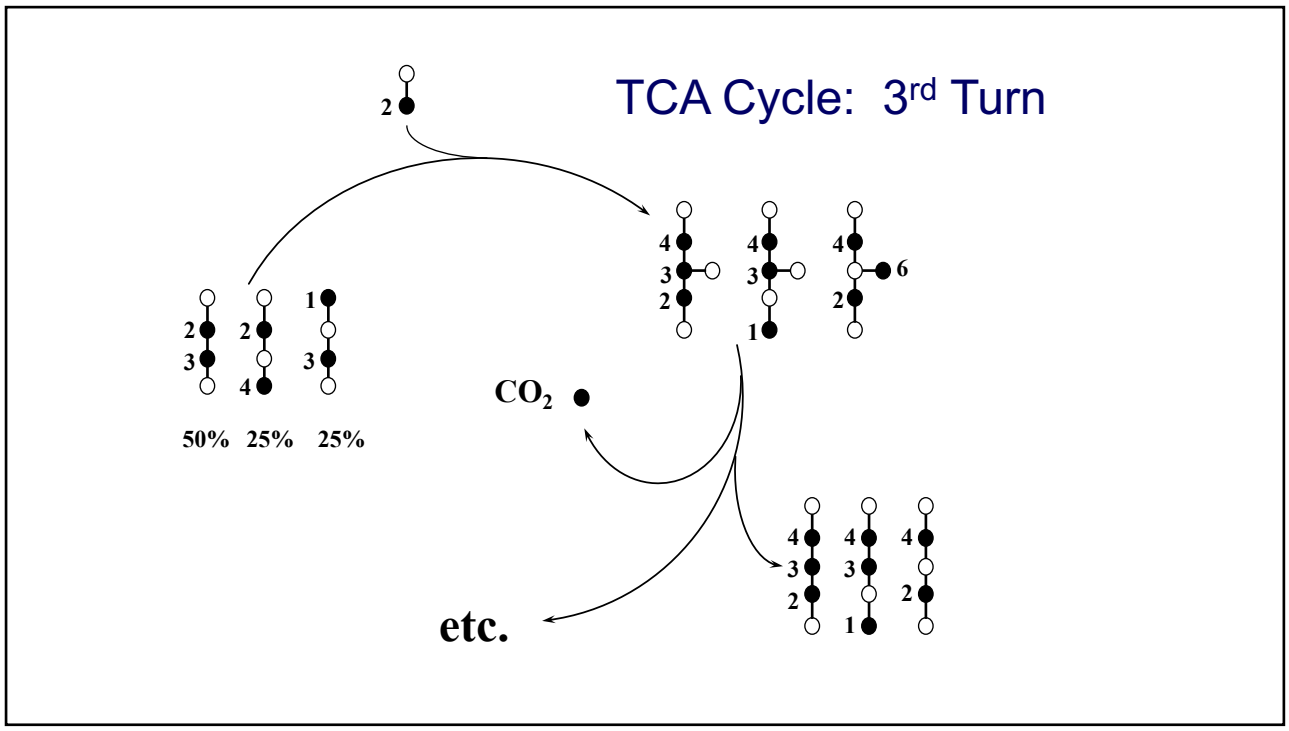


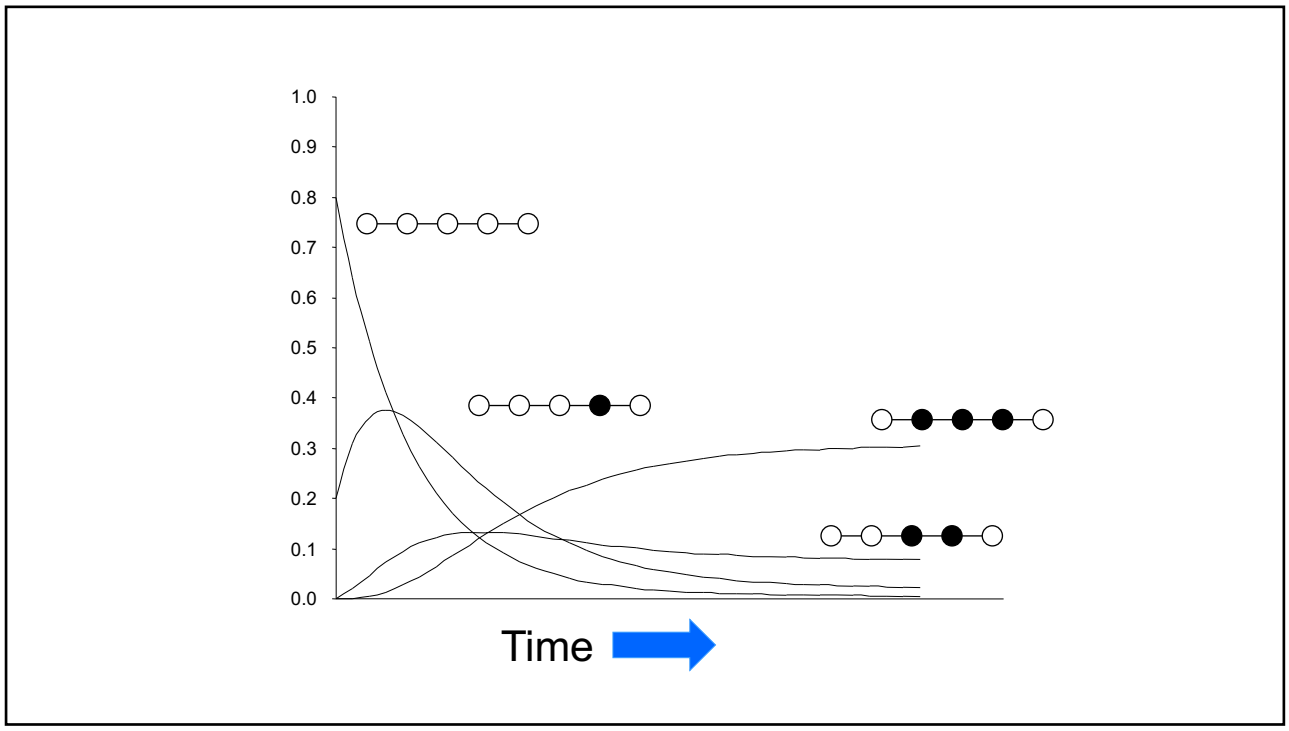

# $^{13}C$ Enrichment of Metabolites

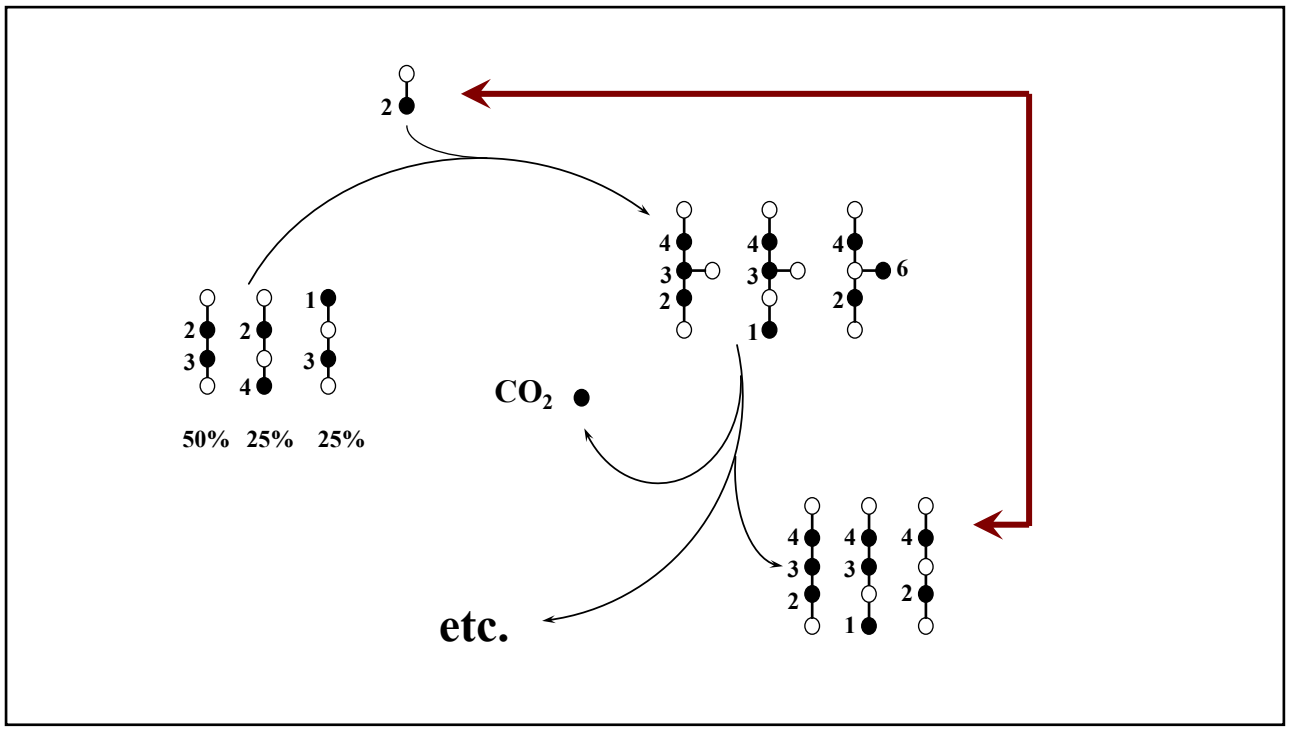



TCA Cycle: 1st Turn
2
4
2
2
3
50% 50%
4
3
2
CoA


TCA Cycle: 2nd Turn
2
4
3
4
2
2
3
2
3
1
4
50% 25% 25%
Not
Scrambling!
4
3
4
2
3
2
3
1
CoA CoA

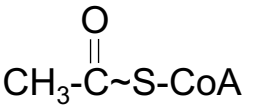

TCA Cycle: 3rd Turn
50% 25% 25%
CO2
etc.

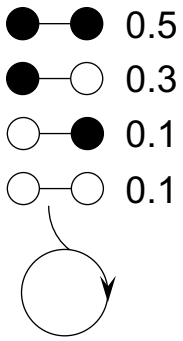

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Time

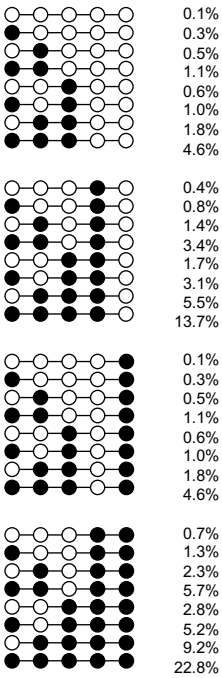



At steady state, a heart oxidizing:

| | |
|---|---|
| [U-$^{13}$C]LCFA | 50% |
| [3-$^{13}$C]LP | 30% |
| [1,3-$^{13}$C]KB | 10% |
| $^{12}$C-Glucose | 10% |

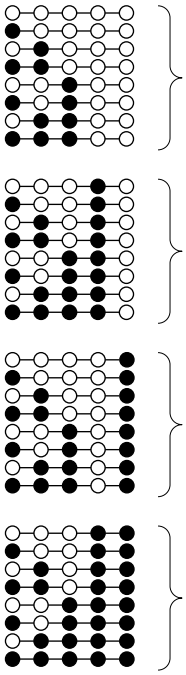


This metabolic situation generates 32 isotopomers

Since Ac-CoA label corresponds to glutamate C4 and C5 enrichment, it is useful to cluster 4 groups of isotopomers based on label in positions 4 & 5.

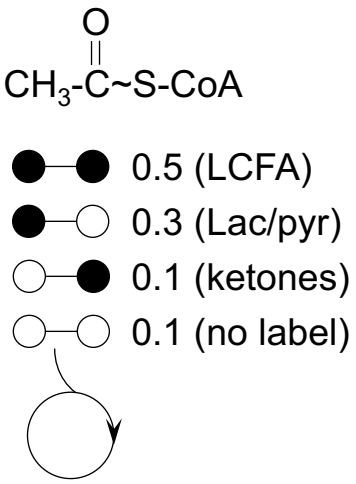


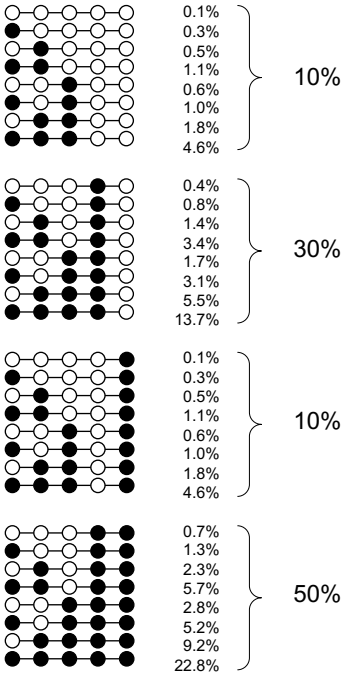
8 isotopomers from unlabeled sources

8 isotopomers from [3-$^{13}$C] lactate & pyr

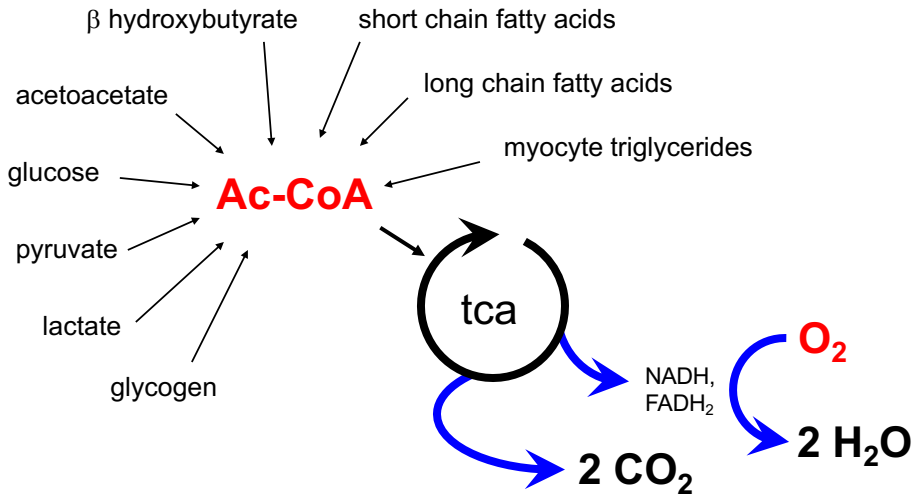
8 isotopomers from [1,3-$^{13}$C] ketones

8 isotopomers from [U-$^{13}$C] fatty acids



## Cardiac metabolism transfers energy from many sources to the contractile apparatus



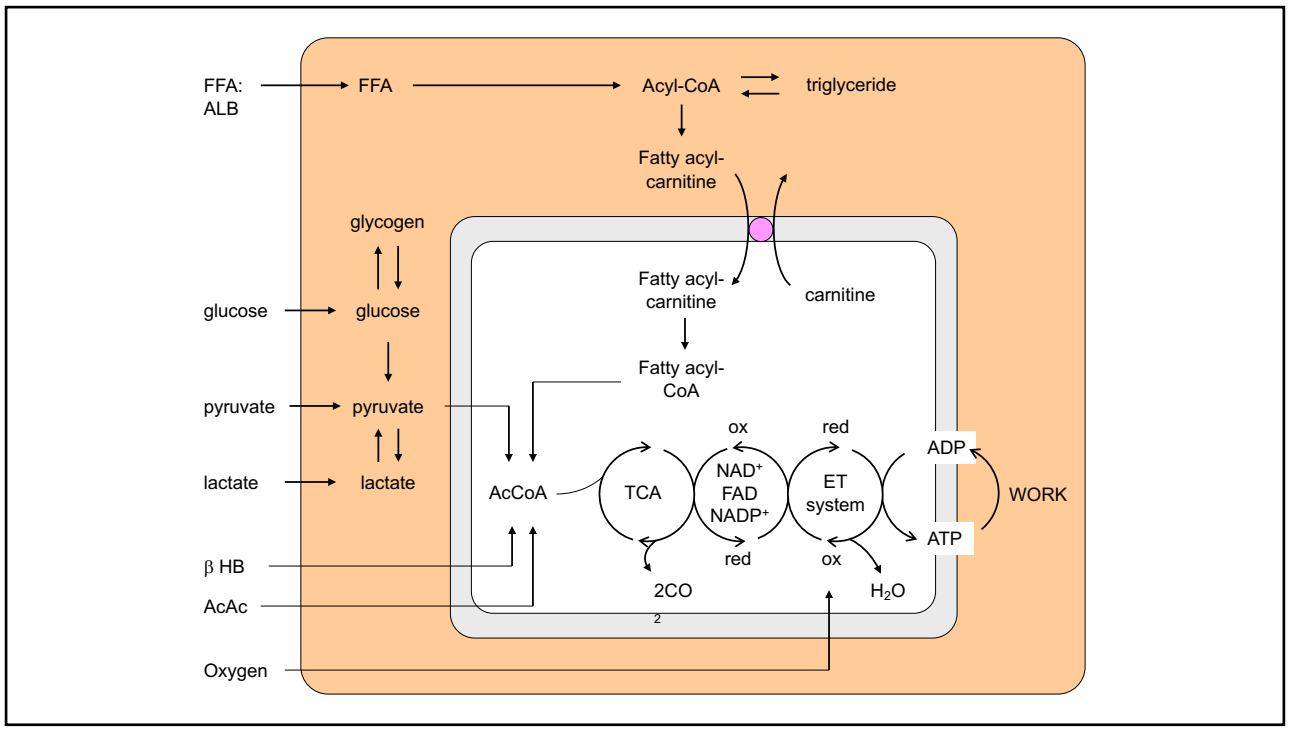

## The enzymatic machinery to oxidize is available to oxidize each substrate.

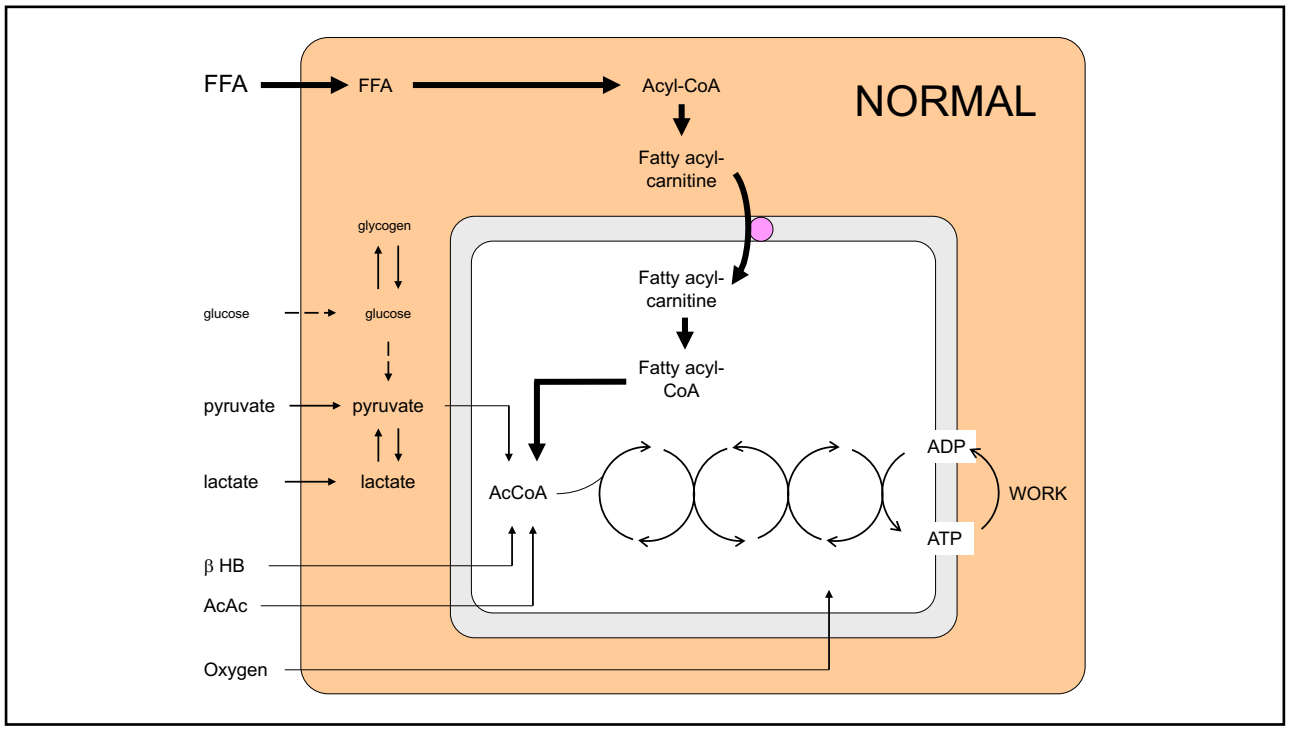
By definition, sources of Ac-CoA in vivo are complementary:

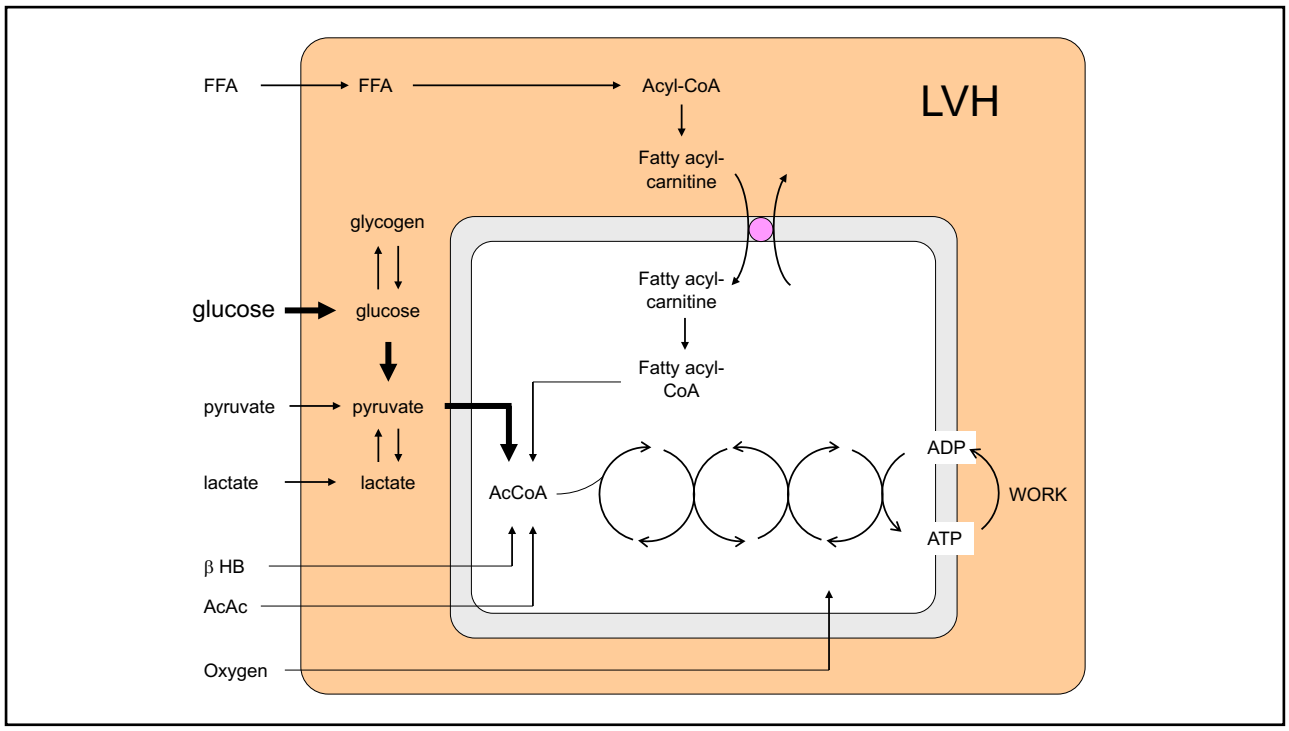
$F_{trig} + F_{LCFA} + F_{glucose} + \ldots = 1$

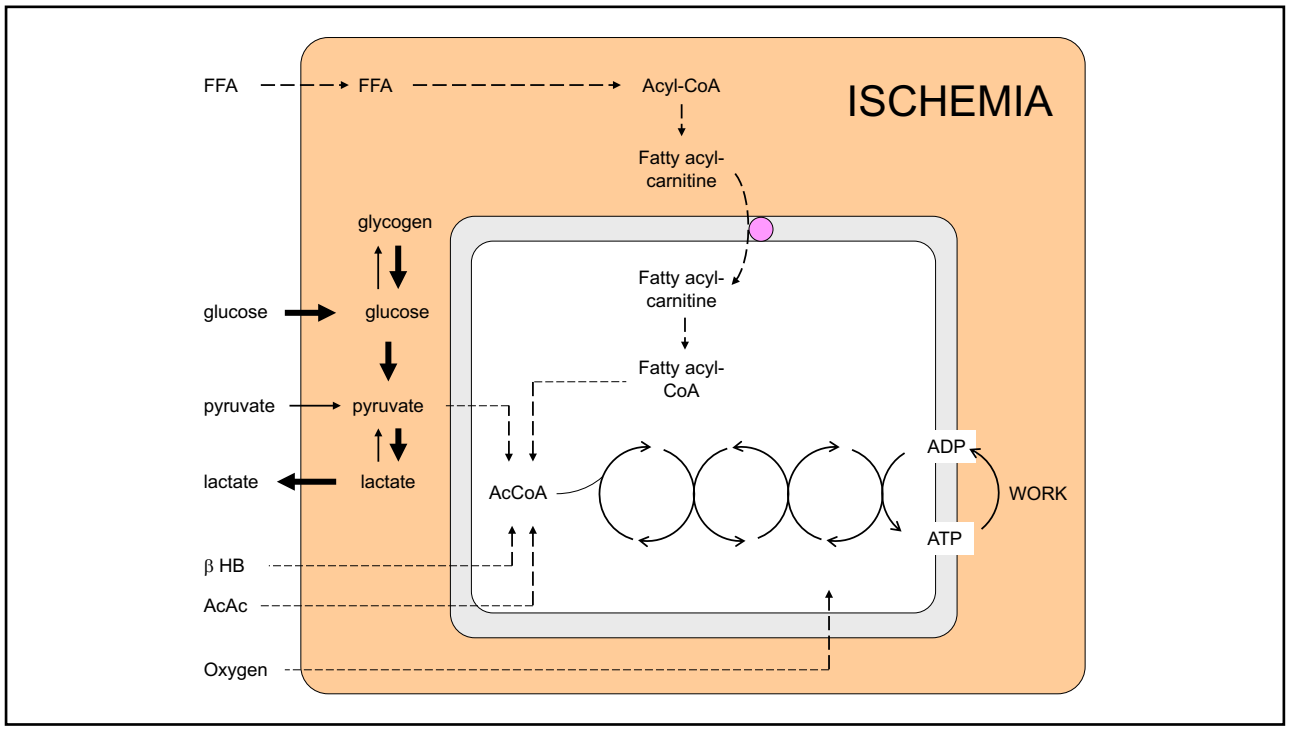
myocyte triglycerides
long chain fatty acids
short chain fatty acids
β hydroxybutyrate
acetoacetate
glucose
pyruvate
lactate
glycogen

} **Ac-CoA**


FFA:
ALB
FFA
Acyl-CoA
triglyceride
Fatty acyl-
carnitine
glycogen
glucose
glucose
Fatty acyl-
carnitine
carnitine
Fatty acyl-
CoA
pyruvate
pyruvate
lactate
lactate
ox
red
ADP
AcCoA
TCA
NAD+
FAD
NADP+
ET
system
WORK
ATP
β HB
red
ox
AcAc
2CO
2
H2O
Oxygen


FFA
FFA
Acyl-CoA
NORMAL
Fatty acyl-
carnitine
glycogen
Fatty acyl-
carnitine
glucose
glucose
Fatty acyl-
CoA
pyruvate
pyruvate
lactate
lactate
ADP
AcCoA
WORK
ATP
β HB
AcAc
Oxygen


LVH
FFA
FFA
Acyl-CoA
Fatty acyl-carnitine
glycogen
glucose
glucose
Fatty acyl-carnitine
Fatty acyl-CoA
pyruvate
pyruvate
lactate
lactate
AcCoA
ADP
WORK
ATP
β HB
AcAc
Oxygen


ISCHEMIA
FFA
FFA
Acyl-CoA
Fatty acyl-carnitine
glycogen
glucose
glucose
Fatty acyl-carnitine
Fatty acyl-CoA
pyruvate
pyruvate
lactate
lactate
AcCoA
ADP
WORK
ATP
β HB
AcAc
Oxygen

## Conditions Associated with Altered FA Oxidation or Altered Gene Expression

High fatty acid oxidation

- Transition from fetal environment
- Fasting
- Diabetes
- Exercise

High carbohydrate oxidation

- Fetal environment
- Hypertrophy
- Myocardial ischemia
- Drugs

## Why is it important to measure substrate oxidation in the heart?

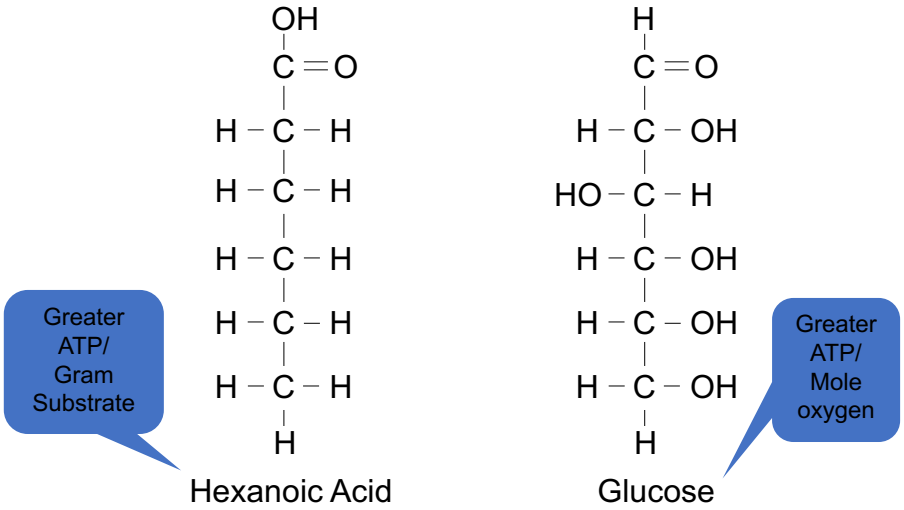
Many diseases or physiological states are associated with altered substrate oxidation or metabolism, which implies:

Understanding metabolism provides insight into pathogenesis

Measurement of metabolism yields diagnostic information

Therapeutic control of metabolism may improve clinical outcome

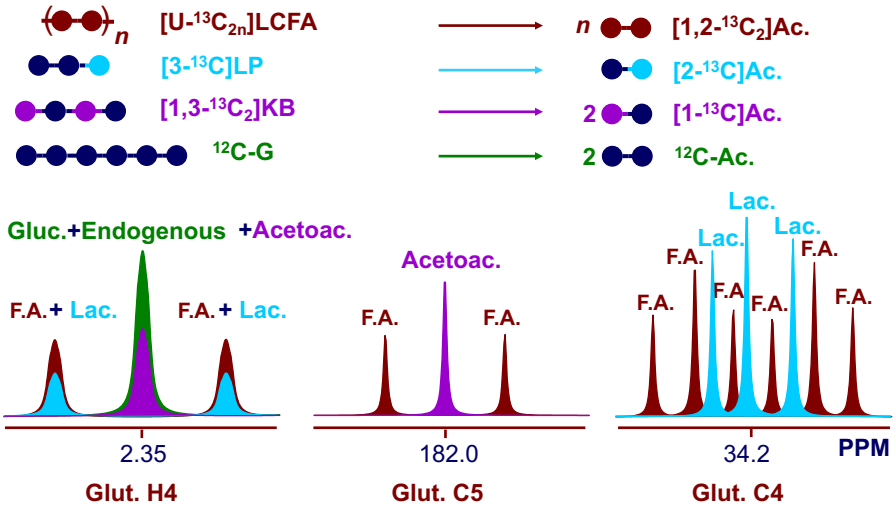
- Improve ATP yield / mole $O_2$ – critical for ischemic syndromes
- Reduce TG storage
- Reduce rhythm disturbances




FAs are more reduced (contain more chemical energy) than glucose

FA oxidation requires activation to acyl-CoA which consumes ATP

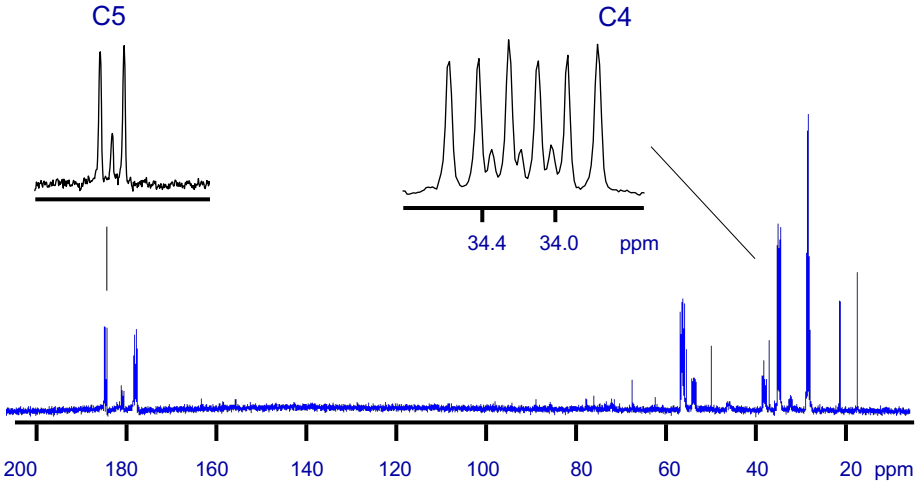
## Substrate Competition by $^{13}$C-NMR



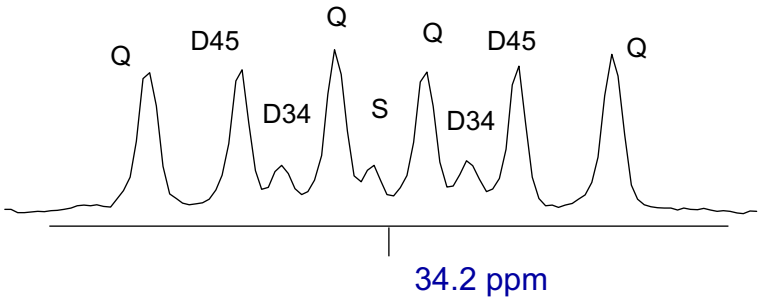
## Acetyl-CoA Enrichments ($F_{ci}$)

- ✓ $F_{C3}$ = %[U-$^{13}C_n$]LCFA = C4Q*(C4F/C3F)
- ✓ $F_{C2}$ = %[3-$^{13}$C]LP = C4D34*(C4F/C3F)
- ✓ $F_{C1}$ = %[1,3-$^{13}C_2$]KB = (C5S/C5D)*$F_{C3}$
- ✓ $F_{C0}$ = %Glucose + Endogenous= 1-($F_{C3}$+ $F_{C2}$+ $F_{C1}$)

## Analysis of a $^{13}$C NMR Spectrum from a Rat Heart Extract Supplied with [U-$^{13}$C] LCFA, [1,3-$^{13}$C] ketones and [3-$^{13}$C] lactate/pyruvate

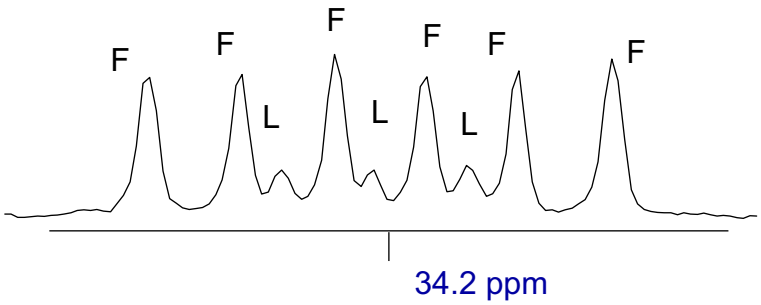


# Analysis of C4



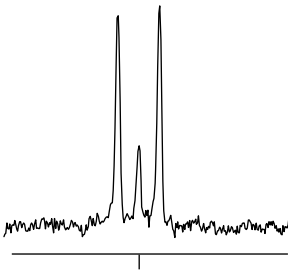

$$\frac{Q + D45}{S + D34} = \frac{62 + 29}{3 + 6} = \frac{\bullet\bullet}{\circ\bullet} = 10$$

# Analysis of C4




$$\frac{\text{LCFA oxidation}}{\text{Lactate oxidation}} = 10$$

# Analysis of C5

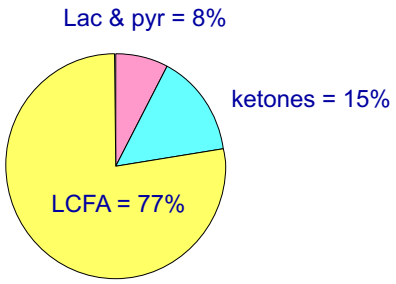


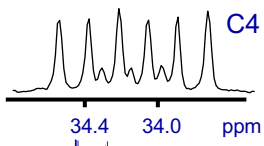
181.5 ppm

$$\frac{C5D45}{C5S} = \frac{84}{16} = \frac{\bullet\bullet}{\bullet\circ} = 5.2$$

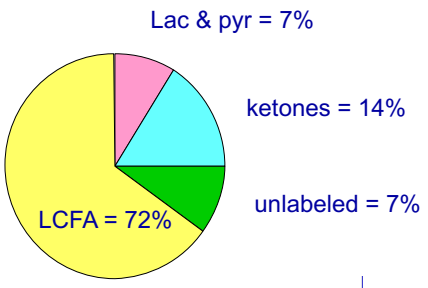
$$\frac{LCFA}{AcAc} = 5.2$$

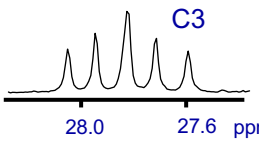
## Analysis of C4 & C5:



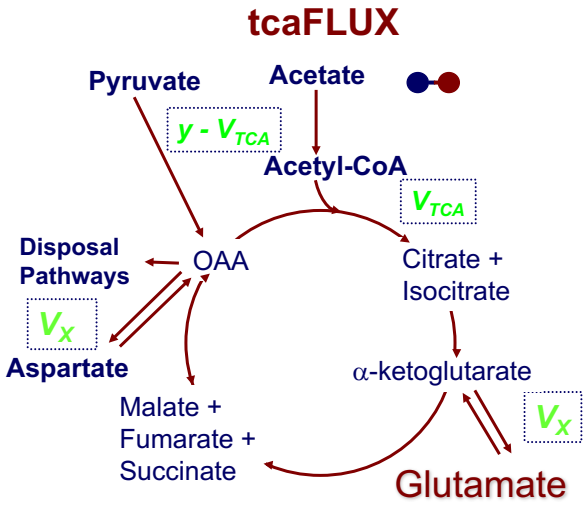




## Analysis of C3, C4 & C5:



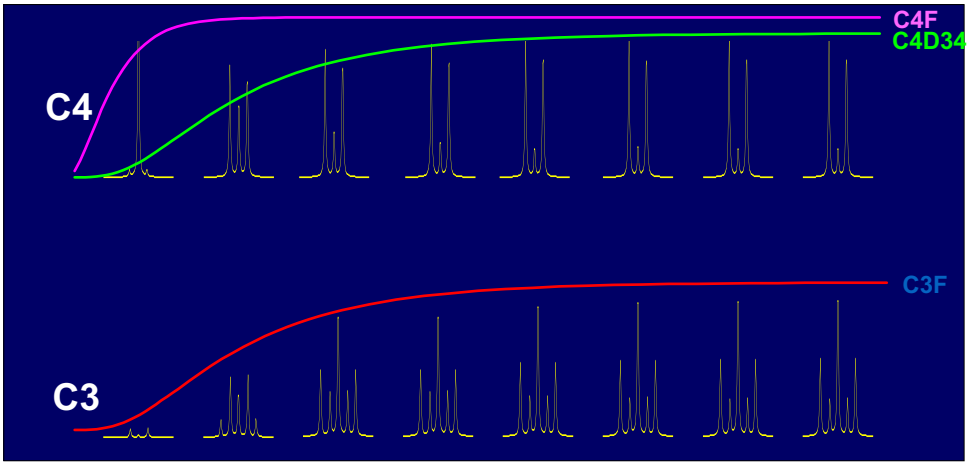



# Kinetic Model of the Krebs Cycle

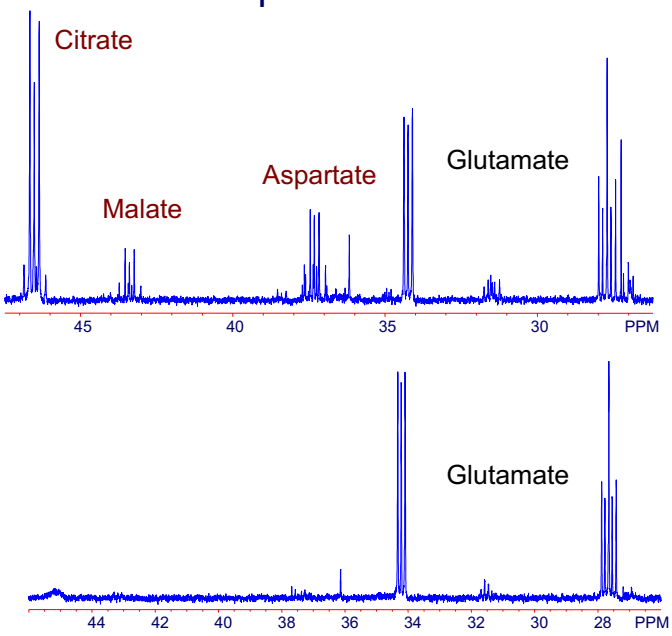



# TCA cycle kinetics

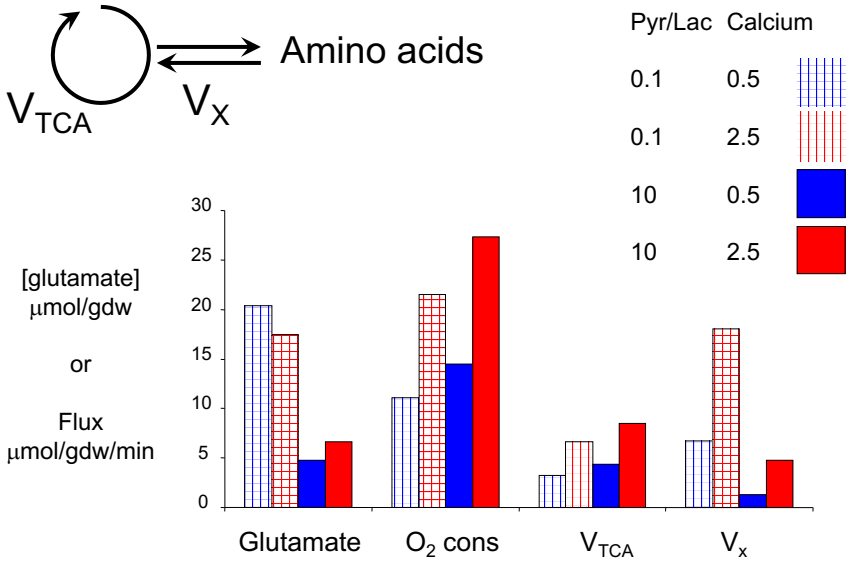
**for a singly enriched substrate, $F_{C2}$ = 90%**



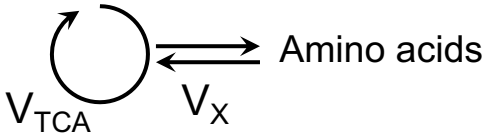
## Metabolite pools are sensitive to cell redox




## $^{13}$C Isotopomer Analysis of TCA Cycle Kinetics: Redox & Calcium

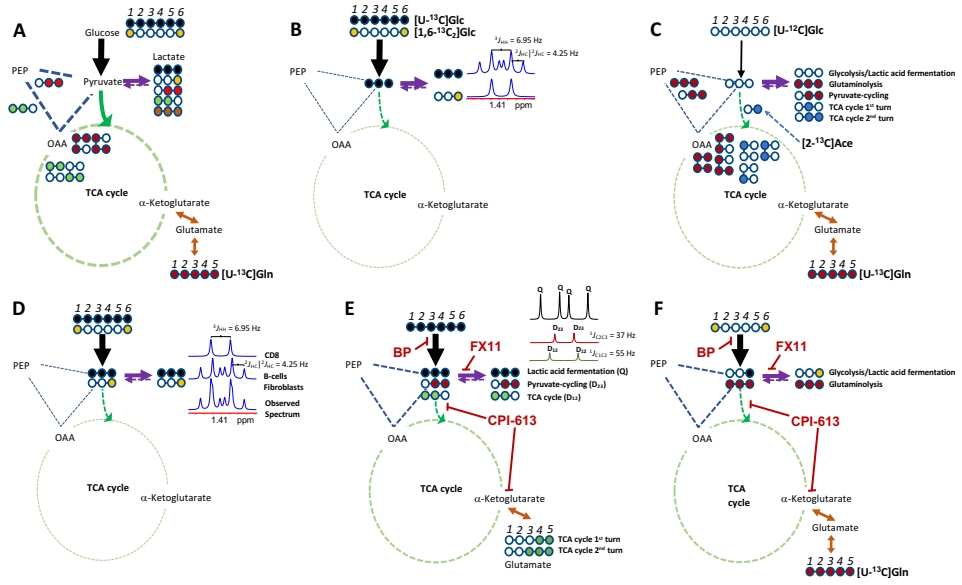


## $^{13}$C Isotopomer Analysis of TCA Cycle Kinetics: Redox & Calcium

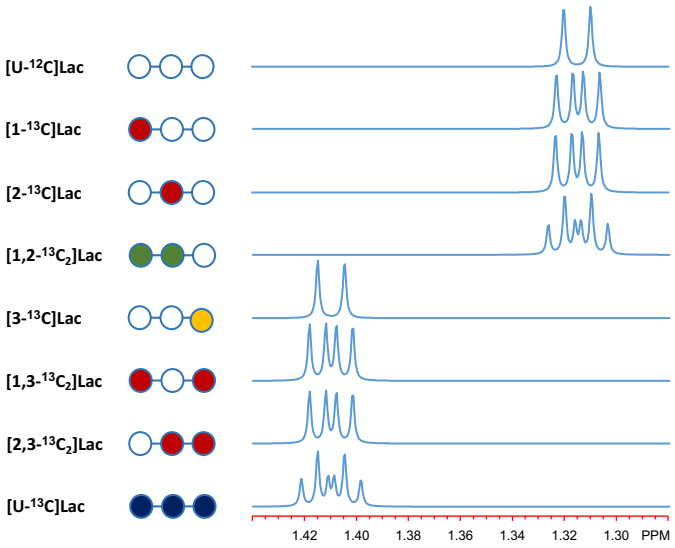


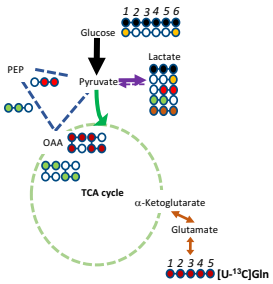

| | Pyr/Lac | Calcium | $V_x$ / $V_{TCA}$ |
|---|---|---|---|
| | 0.1 | 0.5 | 2.0 |
| | 0.1 | 2.5 | 2.7 |
| | 10 | 0.5 | 0.3 |
| | 10 | 2.5 | 0.6 |

## $^{13}$C Isotopomer Analysis of Intermediary Metabolism

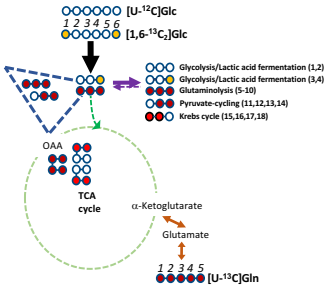


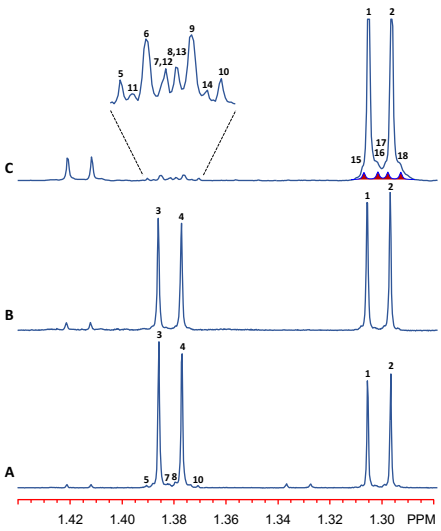
# $^{13}$C Isotopomer Analysis of Lactate



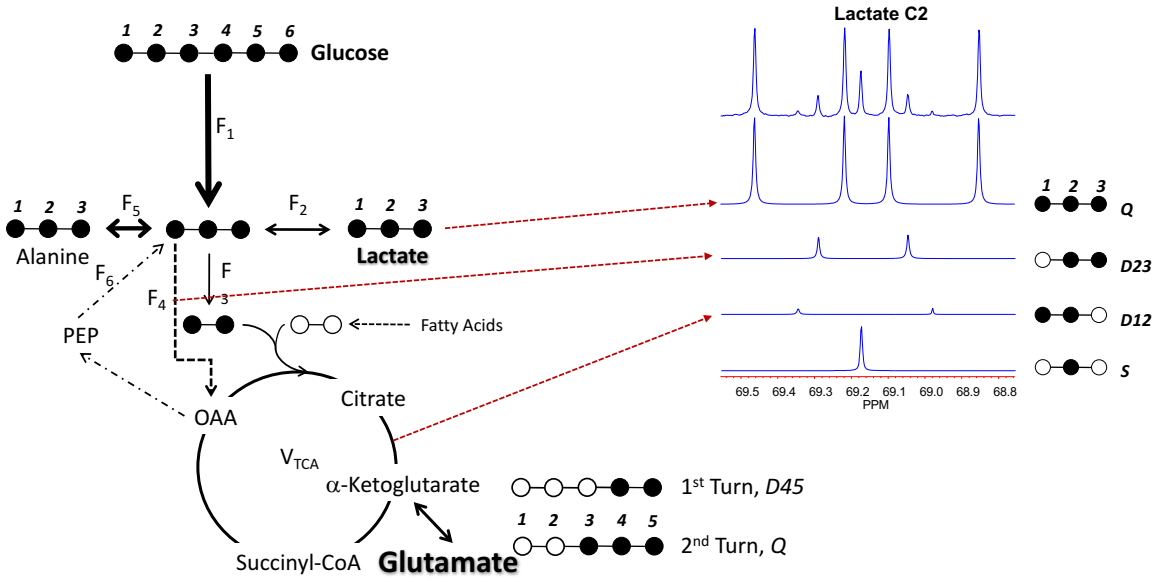




# $^{13}$C Isotopomer Analysis: Glycolysis vs Glutaminolysis

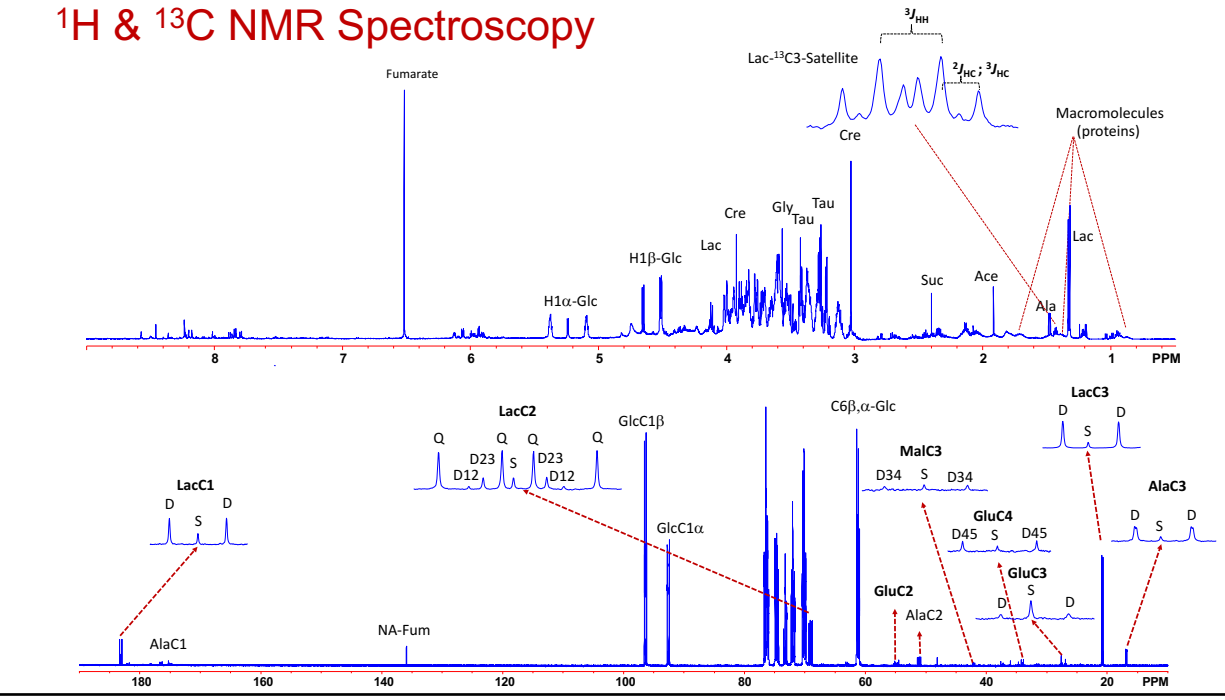


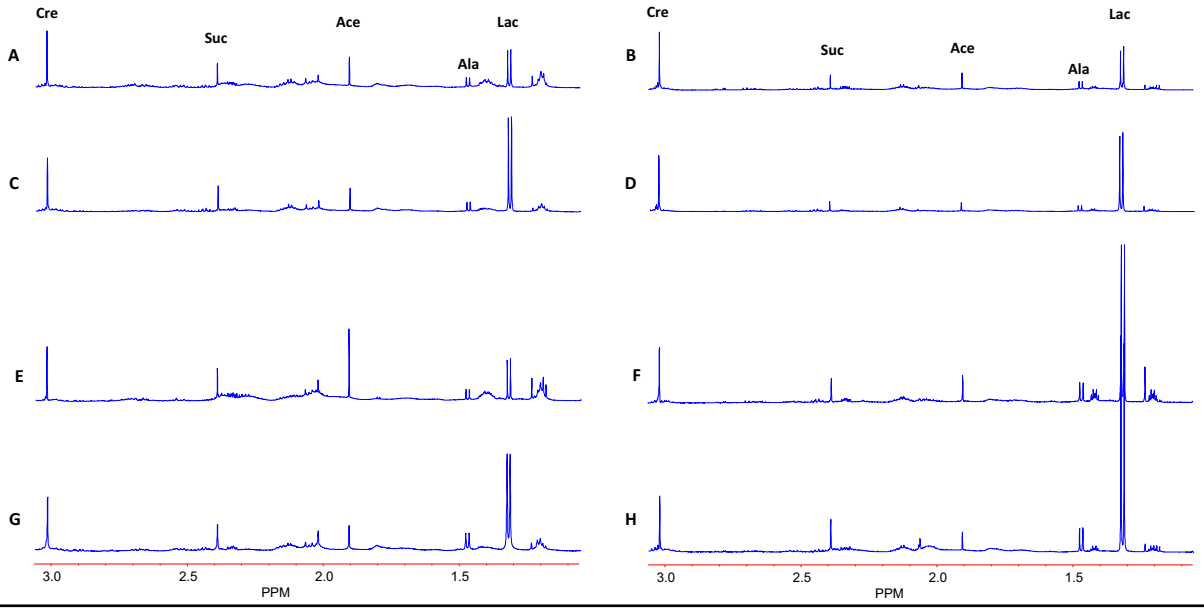
# $^{13}$C Isotopomer Analysis: Salivary Glands



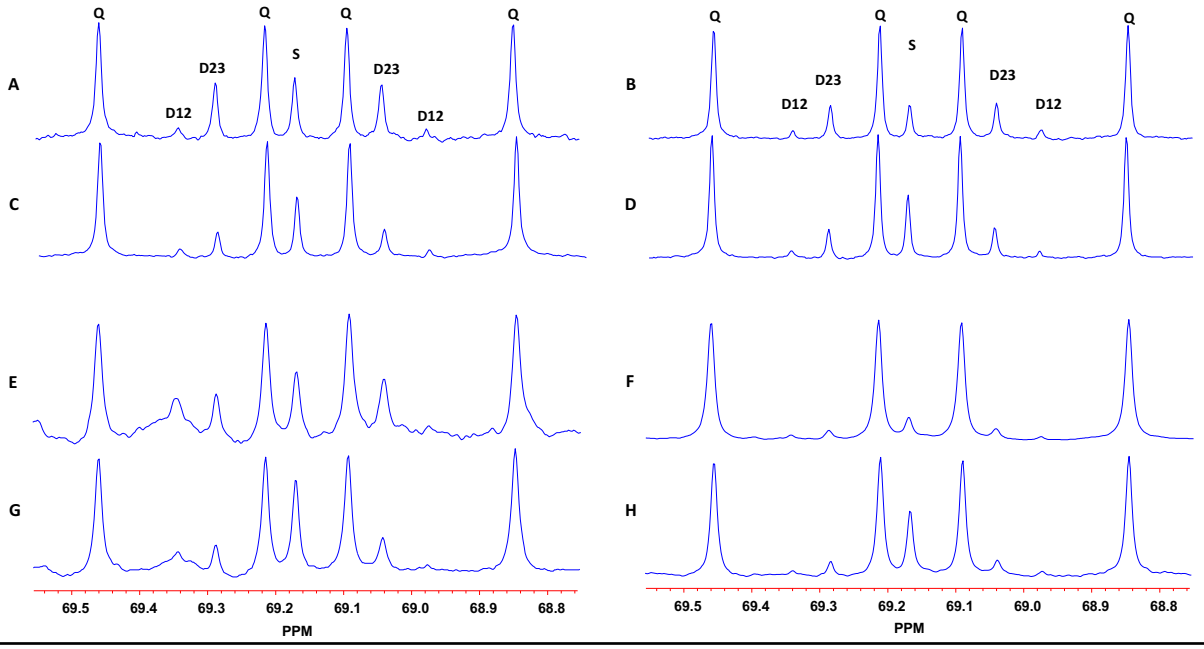

# $^{1}$H & $^{13}$C NMR Spectroscopy



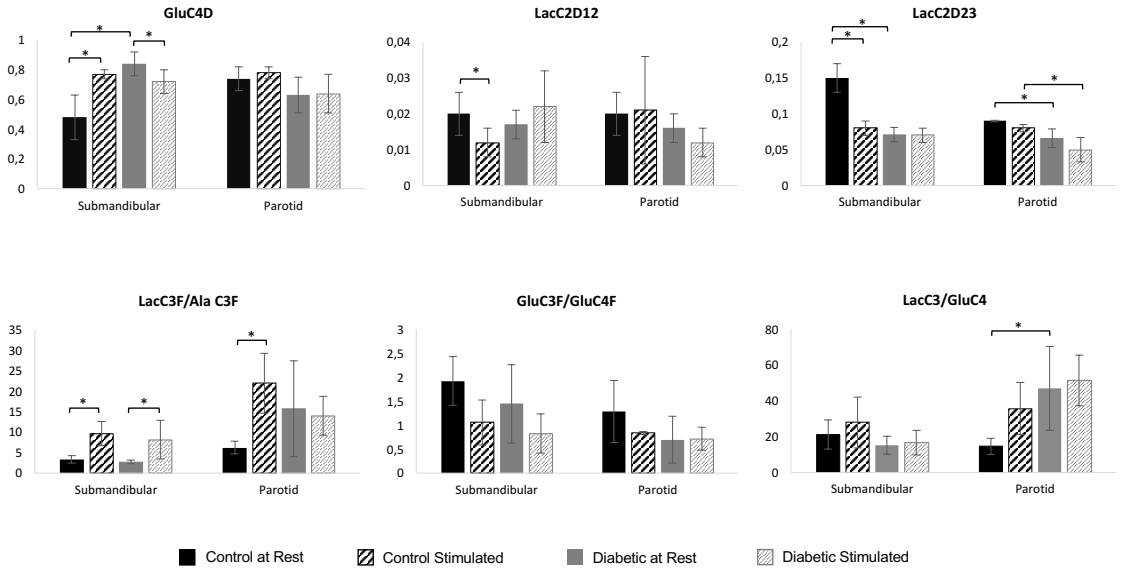
## $^{1}$H NMR Spectroscopy: Levels of Intermediary Metabolites




## $^{13}$C Isotopomer Analysis: Salivary Glands



# $^{13}$C Isotopomer Analysis: Salivary Glands




# $^{13}$C Isotopomer Analysis: Salivary Glands