

**Manipulation of the “Nutrient Sensors”(AMPK/TOR)  
with Anaplerotic Diet Therapy (Triheptanoin)**

***An Alternative to Diet Restriction***

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# Evolution of Therapy for Inborn Errors

1960's: Identification of inherited enzyme deficiencies

1970's: Dietary restriction of "precursor" for therapy  
(*Based on PKU*)

1980's - 1990's: Many more defects characterized;  
Molecular identification of disease-causing mutations

Current Therapy:

Diet Restriction,  
MCT oil & supplements (*vitamins & co-factors*),  
Enzyme & Gene replacement

Recently: Increasing concern about 2° energy compromise...

## **Rationale for Diet Restriction:**

**The belief that metabolites that accumulate behind the block account for the toxicity in these diseases.**

**Reduction of the dietary precursor that enhances abnormal metabolite production should reduce clinical consequences.**

**If the defect prevents oxidation of a major nutrient, what is the impact on cellular energy metabolism ?**

**Increasing concern about 2<sup>o</sup> energy compromise...**

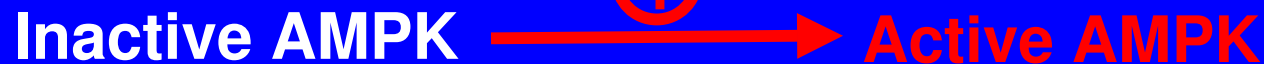
# **CYTOSOLIC NUTRIENT SENSORS**

(serine/threonine *kinases*)

**AMP-Activated Protein Kinase (AMPK)**

**Mammalian Target of Rapamycin (mTOR)**

# AMP-Activated Protein Kinase (*AMPK*) and its Effects on Intermediary Metabolism



**Result:** *ATP producing pathways (catabolic) are activated;*  
*Pathways consuming ATP (synthetic) are inhibited*

(REFS: Hardie,DG. *Endocrinology* 144:5179-5183, 2003  
Hardie,DG. *Current Opinions in Cell Biology* 17:167,2005)

# Mammalian “*Target Of Rapamycin*”: “*mTOR*”

**TOR** is a serine/threonine *kinase* that **regulates protein synthesis, cell growth, and cell proliferation**

It integrates signals from nutrients  
(*amino acids, energy, and growth factors*)

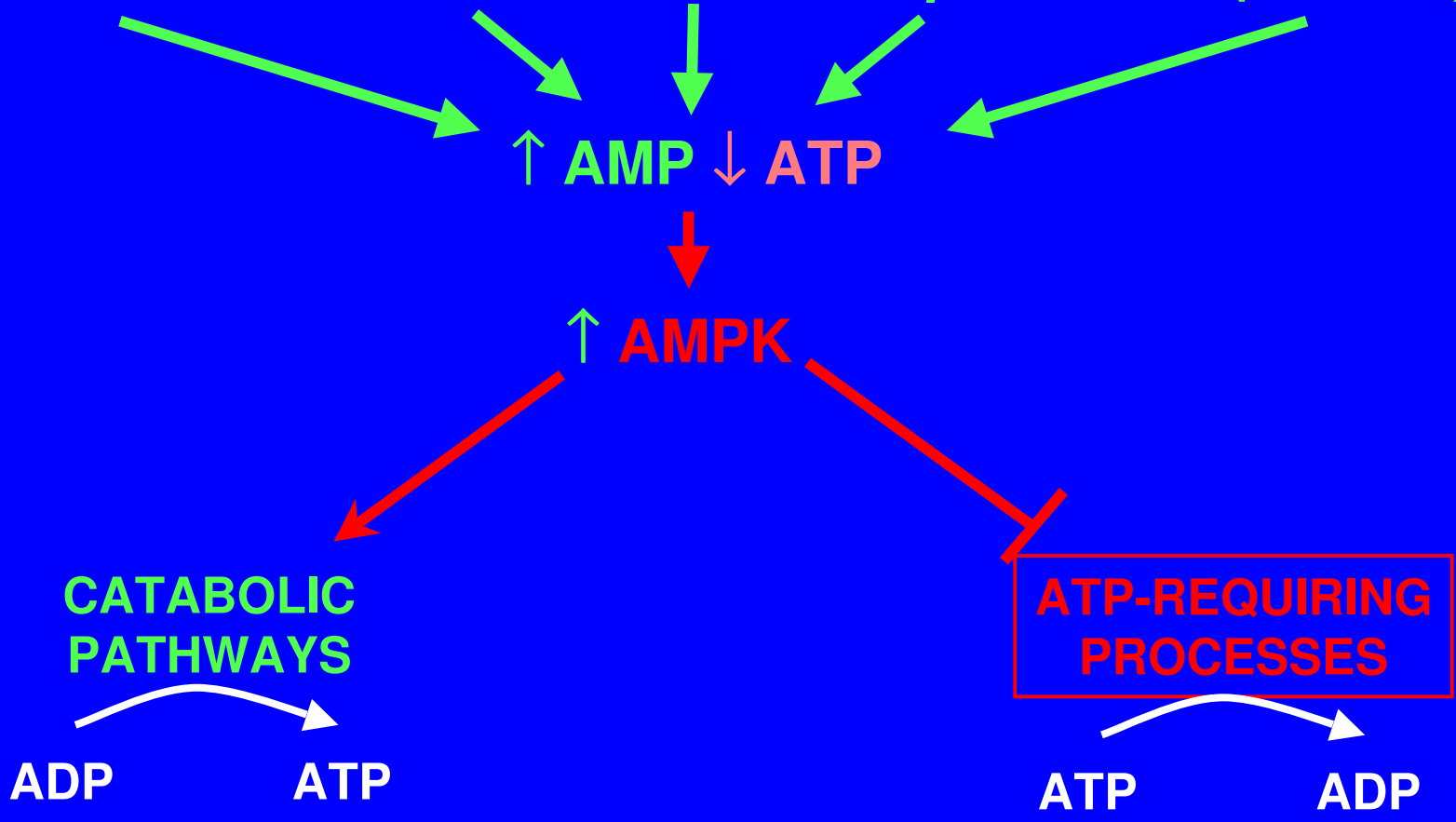
It regulates proteins that control protein translation  
(*ribosomal protein S6 Kinases & initiation factor binding Proteins [4E-BP's]*)

“**TOR**” is a “*rheostat*” that regulates protein biosynthesis

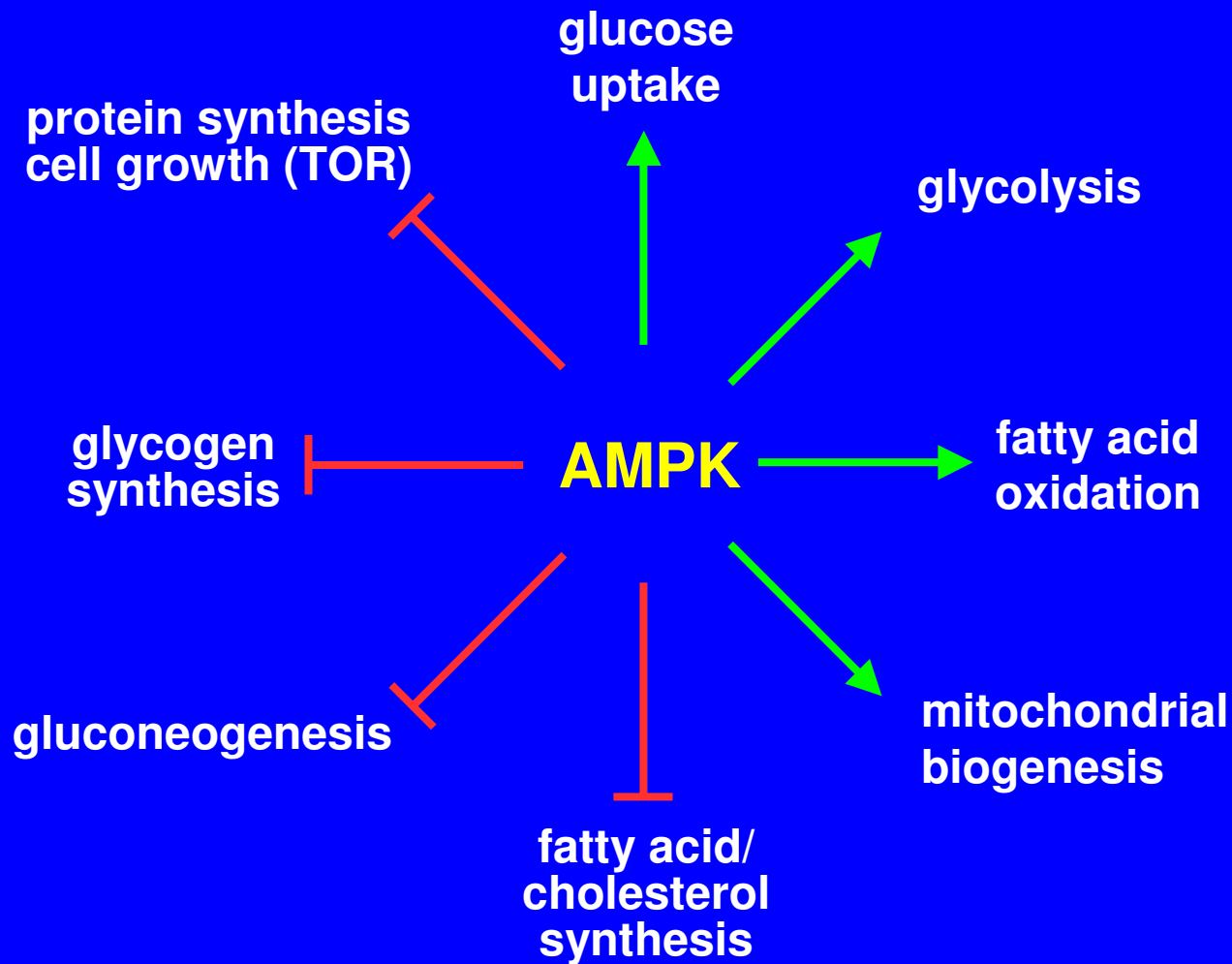
(Reviews: *Fingar & Blenis, Oncogene 23: 3151, 2004*  
*Martin & Hall, Current Opinions in Cell Biology 17:158,2005*)

# AMP-ACTIVATED PROTEIN KINASE IS SWITCHED ON BY METABOLIC STRESSES, INCLUDING EXERCISE

*heat shock*    *metabolic disorders*    *hypoxia ischemia*    *glucose deprivation*    *exercise (muscle)*



# PROCESSES REGULATED BY AMPK ACTIVATION



## Energy Compromise & Active AMPK in Metabolic Disorders

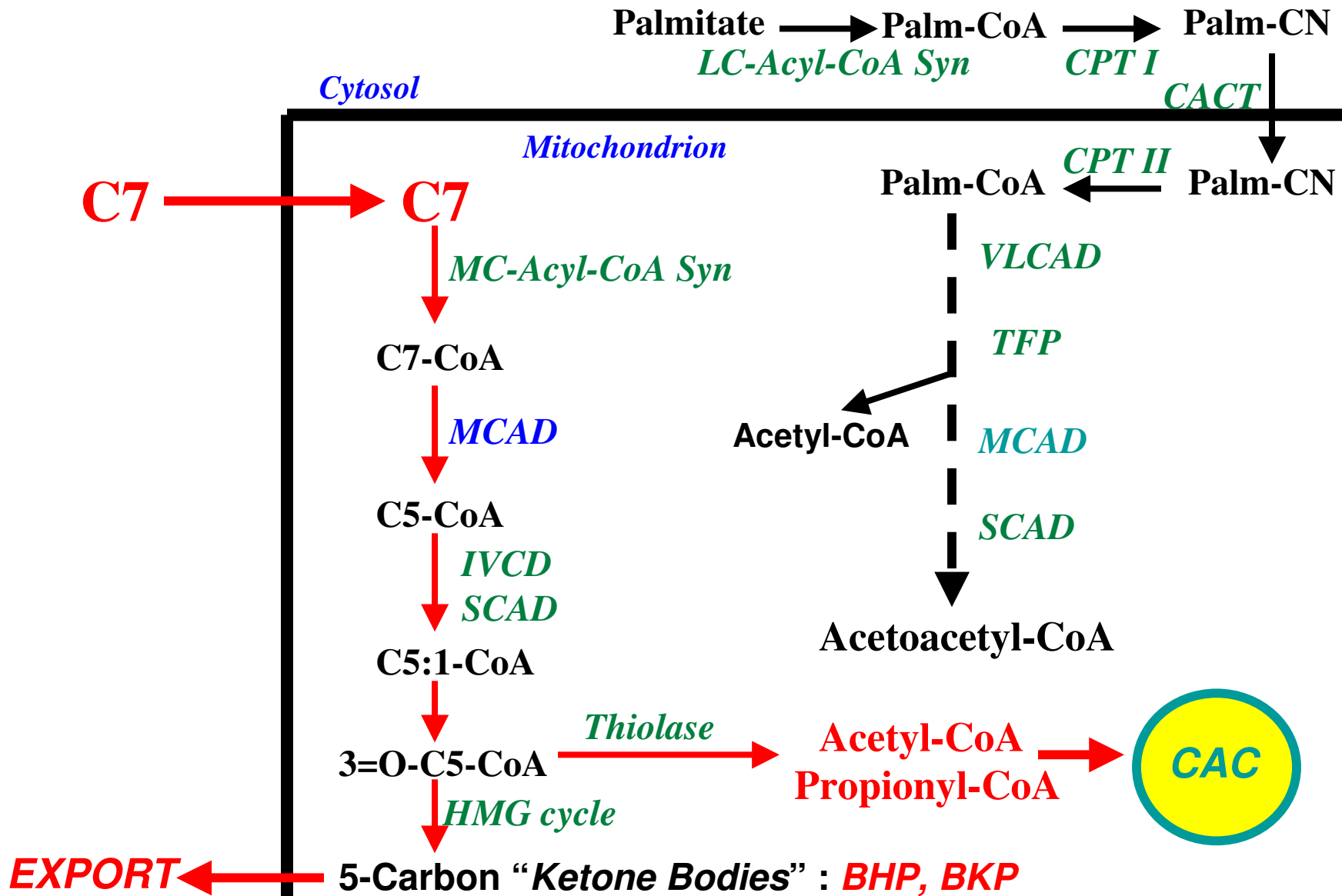
Since AMPK is activated by a high **AMP**:ATP ratio.....

An *Alternative* Therapy designed to enhance ATP Production would be required for correction.

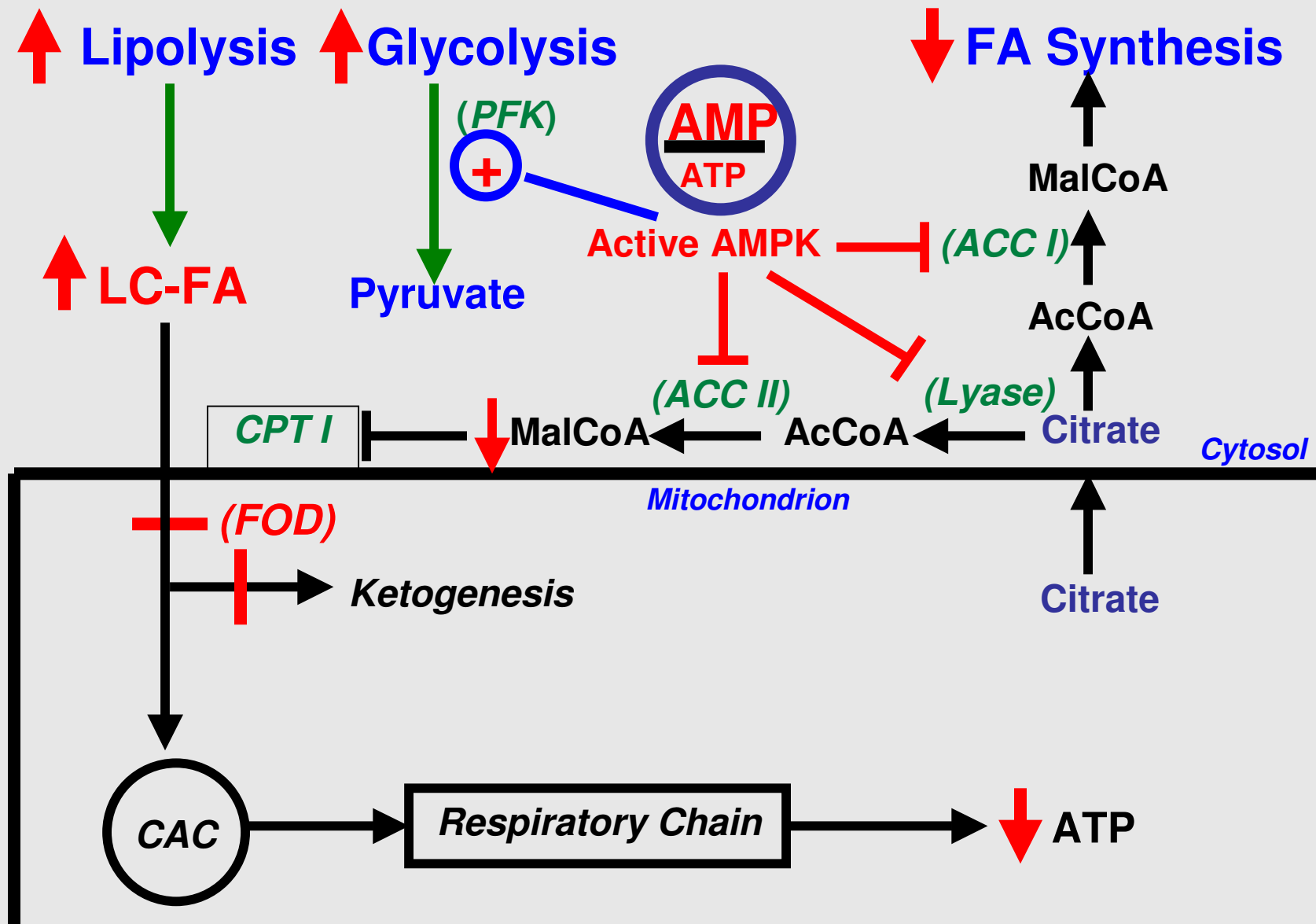
*Anaplerotic* therapy would require an **exogenous source of substrate to directly fuel the Citric Acid Cycle and produce sufficient ATP to *inactivate* AMPK.**

The odd-carbon triglyceride, **Triheptanoin**, is an **exogenous anaplerotic substrate.**

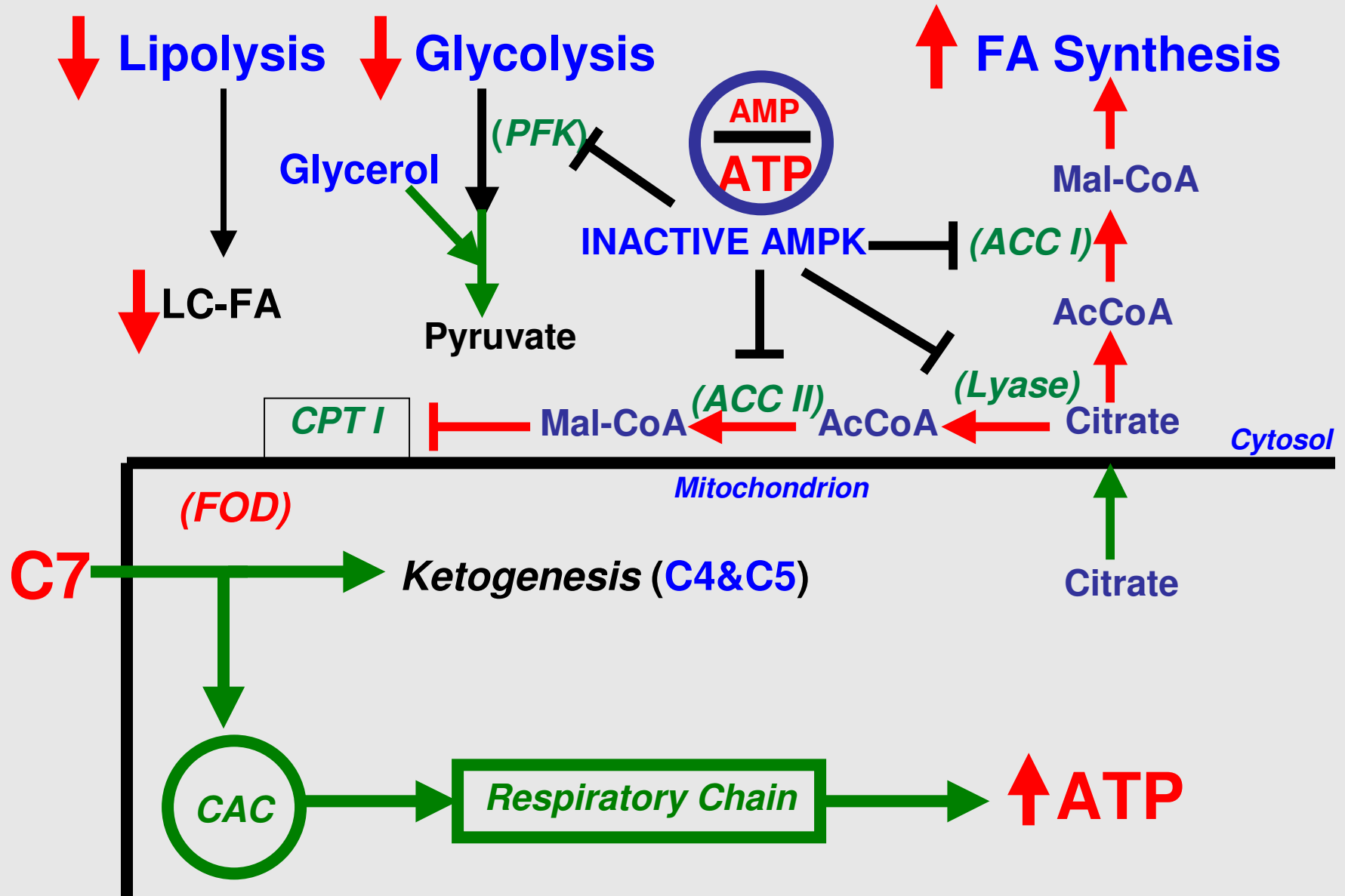
# OXIDATIVE SEQUENCE FOR HEPTANOATE (*Liver*)



# Effects of AMPK in “LC”-Fat [O] Disorders



# Effect of C7 on AMPK in “LC”-Fat [O] Disorders



**Experience with Inborn Errors:  
Triheptanoin Diet Rx (30-35% of total Kcal/day)**

## CLINICAL SYMPTOMS AND RESULTS OF DIET THERAPY FOR FAT OXIDATION DISORDERS

DISORDER (#)	CARDIAC		RHABDOMY		WEAK/FATIG		HYPOGLYCE		HEPATOMEG		RETINOPAT	
	Conv.	*C7	Conv.	C7	Conv.	C7	Conv.	C7	Conv.	C7	Conv.	C7
CPT I (2)	0	0	0	0	2	0	2	0	2	0	0	0
CACT (1)	<i>intervened @ birth - asymptomatic x 7 months- died with rotavirus</i>											
CPT II (7)	1	0	6	1	7	0	4	0	2	0	0	0
VLCAD (19)	8	1	18	10	18	3	11	1	13	1	0	0
LCHAD (9)	0	0	7	1	8	1	4	0	5	1	3	3
TFP (5)	1	0	5	3	5	4	1	0	1	0	0	0
"SCAD" (5)	0	0	0	0	4	2	2	0	3	0	0	0
<b>TOTAL: 48</b>	<b>10</b>	<b>1</b>	<b>36</b>	<b>15</b>	<b>44</b>	<b>10</b>	<b>24</b>	<b>1</b>	<b>26</b>	<b>2</b>	<b>3</b>	<b>3</b>

\* **CONV**= CONVENTIONAL DIET (MCT AND/OR LOW FAT-HIGH CARBOHYDRATE);

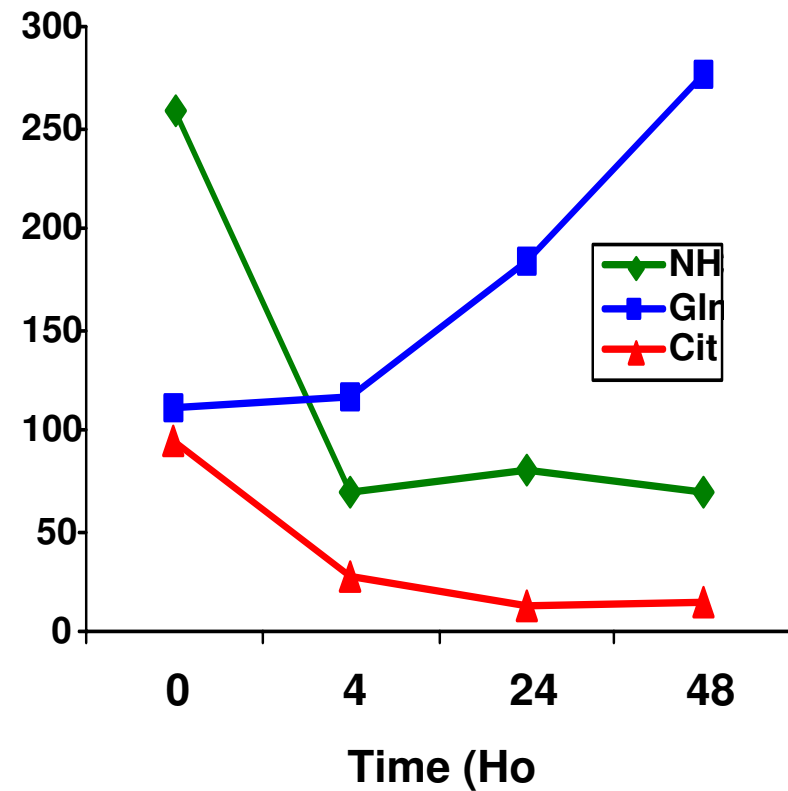
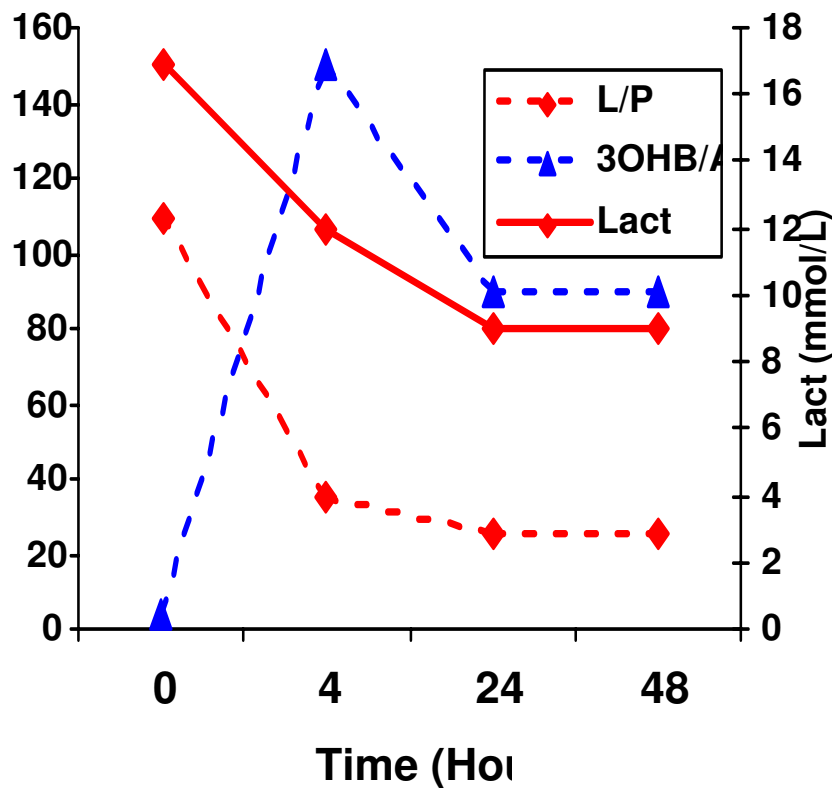
*Roe & Mochel, JIMD in press 2006*

## Current Comparison of Diets for Fat Oxidation Disorders

<u>Disorder</u>	<u>DIET:</u> <u>Phenotype</u>	*Conventional			Triheptanoin		
		<u>Total</u>	<u>Dead</u>	<u>Alive</u>	<u>Total</u>	<u>Dead</u>	<u>Alive</u>
CPT I	Hepatic	4	0	4	2	0	2
CPT II	infant	5	4	1	0	0	0
	adult	5	0	5	5	0	5
Translocase	infant	5	5	0	1	1	0
VLCAD	Cardiac	7	6	1	8	0	8
	Muscle	1	0	1	8	0	8
MTP		4	4	0	4	1	3
LCHAD		10	2	8	8	0	8
<b>Total:</b>		<b>41</b>	<b>21</b>	<b>20</b>	<b>36</b>	<b>2</b>	<b>34</b>

\*J.Inherit.Metab Dis. 22: 488-502, 1999.

# Pyruvate Carboxylase: Odd-Carbon Therapy Acute Hepatic Failure



*Mol Gen & Metab 84: 305-312, 2005*

# **Clinical Outcome of the Triheptanoin Diet: (Fat Oxidation Disorders)**

*Mortality is reduced (6%)*

*Cardiomyopathy Resolved*

*Attenuation of Rhabdomyolysis*

*Normal Glucose Homeostasis*

*Elimination of Hepatomegaly*

*Improved muscle Strength & endurance*

*Peripheral Neuropathy is unchanged in TFP*

*Retinopathy is unchanged in LCHAD*

## Conclusions re. Nutrient Sensors & IMD's

- Inborn errors due to oxidative defects are treated with diet formulations that restrict the offending precursor and provide a variety of supplements.
- An energy deficit of ATP occurs due to incomplete [O] of these nutrients.
- AMPK is activated due to an increased AMP:ATP ratio causing increased catabolism & decreased synthesis.
- An exogenous *anaplerotic* substrate (C7) can provide fuel to the CAC, inactivate AMPK, prevent catabolism, and stimulate synthesis and growth.
- The Goal: provide energy and spare endogenous turnover as an alternative (adjunct) to dietary restriction.
- Defects involving enzymes critical for C7 [O] can not be treated with C7 (*e.g. MCAD*)



# **Contribution of Dietary Fats to Abnormal Metabolites in LC-FOD's in Cultured Skin Cells**

**Charles R. Roe, MD  
Mary Wallace, RD  
Brenda Garritson, RD**

## Comparison of Fatty Acid Composition (gm/100 gm)

<u>OILS</u>	<u>C16 + C18</u>	<u>C18:1</u>	<u>C18:2</u>	<u>C18:3</u>	<u>C16+18:0+</u>	<u>C18:2+18</u>
Canola	7.0	59.0	20.4	9.4	66.0 gms	29.8 gms
Soy	13.9	22.6	49.4	6.6	33.6 gms	56.0 gms
Corn	12.0	23.0	55.6	6.0	35.0 gms	61.6 gms
Flaxseed	9.6	17.2	13.4	55.3	26.8 gms	68.7 gms
Walnut	9.0	22.2	52.9	10.4	31.2 gms	63.3 gms

## ***Contribution of 0.2 mM Fats to Abnormal Metabolites In Cultured Skin Cells***

<b><i>Disorder</i></b>	<b><i>Total Metabolites*</i></b>
<b>VLCAD</b>	<b>72.30</b>
<b>TFP</b>	<b>25.74</b>
<b>LCHAD-1</b>	<b>50.34</b>
<b>LCHAD-2</b>	<b>80.31</b>

***\* ng/mg Protein/72 Hrs***

# ***Abnormal Metabolites produced in Culture from Equal Amounts of Fatty Acids***

***(ng/mg Protein/72 Hrs incubation)***

## **VLCAD**

<b>Fat</b>	<b>ng/mgProt</b>	<b>% Total Met:</b>
C16	41.03	56.7%
C18:1	16.42	22.7%
C18:2	11.31	15.6%
C18.3	3.54	4.9%
<b>Total:</b>	<b>72.30</b>	

## **TRIFUNCTIONAL PROTEIN**

<b>Fat</b>	<b>ng/mgProt</b>	<b>% Total Mets</b>
C16	12.69	56.3%
C18:1	5.92	26.2%
C18:2	3.18	14.1%
C18.3	0.77	3.4%
<b>Total:</b>	<b>22.56</b>	

## **LCHAD-1**

<b>Fat</b>	<b>ng/ml/72hr</b>	<b>% Total Mets</b>
C16	17.85	46.2%
C18:1	16.76	43.4%
C18:2	2.98	7.7%
C18.3	1.03	2.7%
<b>Total:</b>	<b>38.62</b>	

## **LCHAD-2**

<b>Fat</b>	<b>ng/ml/72hr</b>	<b>% Total Mets</b>
C16	33.01	51.1%
C18:1	27.57	42.7%
C18:2	2.90	4.5%
C18.3	1.15	1.8%
<b>Total:</b>	<b>64.63</b>	

# ***Abnormal Hydroxy-Metabolites produced in Culture from Equal Amounts of Fatty Acids***

***(ng/mg Protein/72 Hrs incubation)***

**VLCAD**

<b>HYDROXYS</b>	<b>ng/ml/72hr</b>
C16-OH	0
C18:1-OH	0
C18:2-OH	0
C18:3-OH	0
<b>Total:</b>	<b>0</b>

**TRIFUNCTIONAL PROTEIN**

<b>HYDROXYS</b>	<b>ng/ml/72hr</b>	<b>% Total Mets</b>
C16-OH	1.09	34.3%
C18:1-OH	1.00	31.4%
C18:2-OH	0.75	23.6%
C18:3-OH	0.34	10.7%
<b>Total:</b>	<b>3.18</b>	

**LCHAD-1**

<b>HYDROXYS</b>	<b>ng/ml/72hr</b>	<b>% Total Mets</b>
C16-OH	4.01	34.2%
C18:1-OH	5.81	49.6%
C18:2-OH	1.47	12.5%
C18:3-OH	0.43	3.7%
<b>Total:</b>	<b>11.72</b>	

**LCHAD-2**

<b>HYDROXYS</b>	<b>ng/ml/72hr</b>	<b>% Total Mets</b>
C16-OH	7.23	46.1%
C18:1-OH	6.80	43.4%
C18:2-OH	1.03	6.6%
C18:3-OH	0.62	3.9%
<b>Total:</b>	<b>15.68</b>	

# Conclusions

- **Palmitate and Oleate (C16&C18:1) produce most of the abnormal metabolites in LC-FOD's**
- **The “essentials” are mainly used in synthesis**
- **Commercial oils contain too much Palmitate and Oleate for LC-FOD's**
- **A combination of Flax& Walnut Oils is better for providing Essential Fatty Acids**
- **A preparation of Linoleic and Linolenic (only) would be best**