“Primary” Carnitine Deficiencies in Children

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History of Concepts about Carnitine Deficiency & Fatty Acid Oxidation

- 1960-70: hypoglycin A & 4-pentanoate toxicity
  - postulated that these non-metabolizable fatty acids blocked ß-oxidation by binding up free CoA
  - carnitine proposed as a means to reverse this:
    - restore free CoA
    - remove toxic fatty acids
    - restore ß-oxidation
- 1975: mechanism of hypoglycin A & 4-pentanoate
  - Direct inhibition of ß-oxidation enzymes by acyl-CoAs
  - Hypoglycin A product (methylenecyclopropaneacetyl-CoA) is a suicide substrate for acyl-CoA dehydrogenases

Carnitine Deficiency Disorders (1970-80)

- "Systemic Carnitine Deficiency" -- episodic life-threatening coma with low carnitine in plasma & tissue
  - Some later shown to have ß-oxidation enzyme defects (MCAD, vLCAD) with "secondary carnitine deficiency".
  - Some "carnitine responsive" cases probably had mutations of the OCTN2 carnitine transporter
- "Muscle Carnitine Deficiency" -- chronic weakness with low carnitine levels in muscle only
  - underlying disorders undefined…?possible mitochondrial disorders
  - response to carnitine treatment unclear

"Primary carnitine deficiency" criteria

1. Tissue carnitine levels low enough to impair mitochondrial fatty acid oxidation
2. Evidence that fatty acid oxidation is impaired
3. Evidence that carnitine treatment normalizes fatty acid oxidation
4. Identify the mechanism of carnitine deficiency

Carnitine Deficiency Disorders in Children

- **Good evidence that carnitine deficiency causes pathology**
  - **Muscle-Kidney plasma membrane carnitine transporter deficiency (recessive OCTN2 mutations)**
  - **Chronic pivalate-conjugated antibiotic administration**

- **No evidence that carnitine deficiency causes pathology**
  - **“Secondary carnitine deficiency” (genetic defects in acyl-CoA oxidation)**
  - **Nutritional carnitine deficiency (vegetarian diet, hyperalimentation, soy formula)**

Muscle-Kidney Plasma Membrane Carnitine Transporter Deficiency
("primary carnitine deficiency")

Recessive genetic defect

- **~30-40 reported cases**
- **OCTN2 Na-dependent transporter (SLC22A5, 5q)**

Clinical presentations:

1. **Cardiac: progressive cardiomyopathy**
2. **Liver: acute life-threatening hypoglycemic coma**
3. **Muscle: progressive weakness**

<u>Profound</u> tissue & plasma carnitine deficiency (<< 5% of normal)

Carnitine supplements effective (but tissue levels remain 5-10% of normal)

TABLE 1.
Clinical Findings in 20 Patients With the Muscle/ Kidney Plasma Membrane Carnitine Transporter Defect*

	Number	Median Age, yr
Hypoglycemia	9	1.5
Cardiomyopathy	8	4.0
Muscle weakness	3	1.4

*From Stanley CA, DeLeeuw S, Coates PM, et al: *Ann Neurol* 30:709–716, 1991. Used by permission.

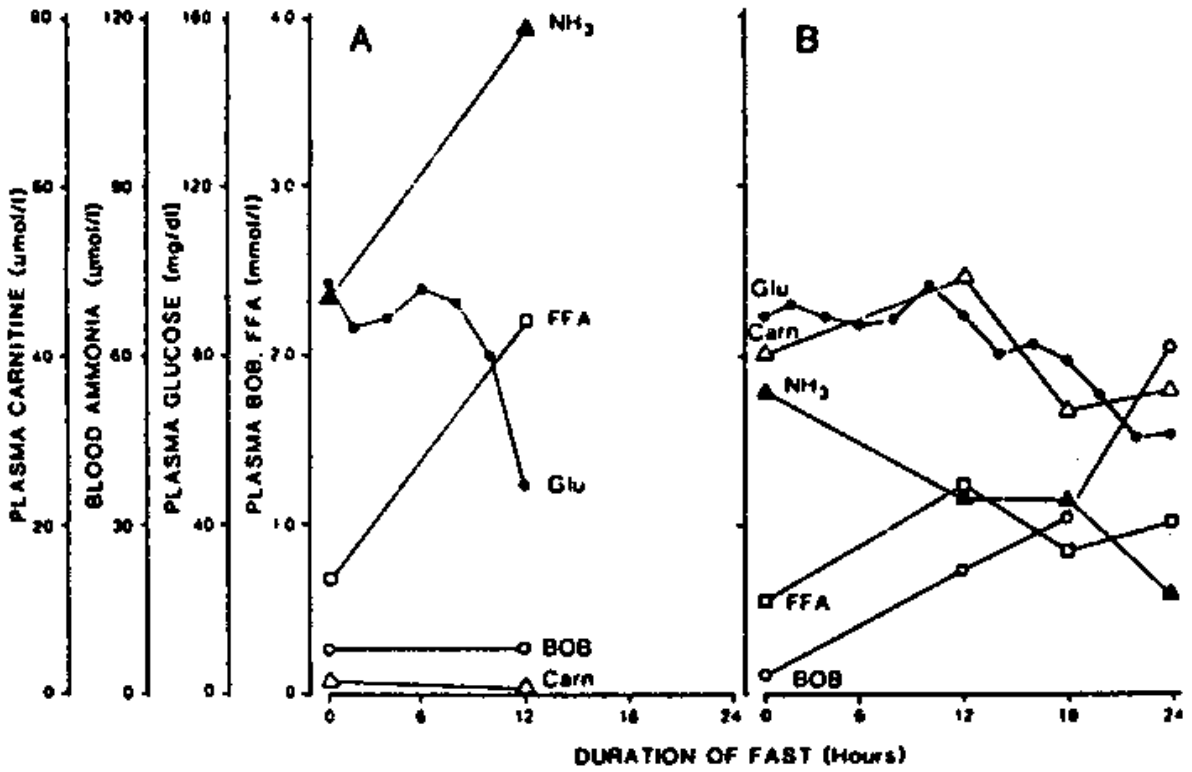


FIGURE 10.

Response to fasting in a patient with carnitine transporter deficiency before **(A)** and after **(B)** treatment with oral carnitine. Shown are plasma levels of glucose *(Glu)*, β-hydroxybutyrate *(BOB)*, free fatty acids *(FFA)*, carnitine *(Carn)*, and ammonia *(NH_3)*. The response after treatment **(B)** is identical to that of normal children. (From Treem WR, Stanley CA, Finegold DN, et al: *N Engl J Med* 319:1331–1336, 1988. Used by permission.)

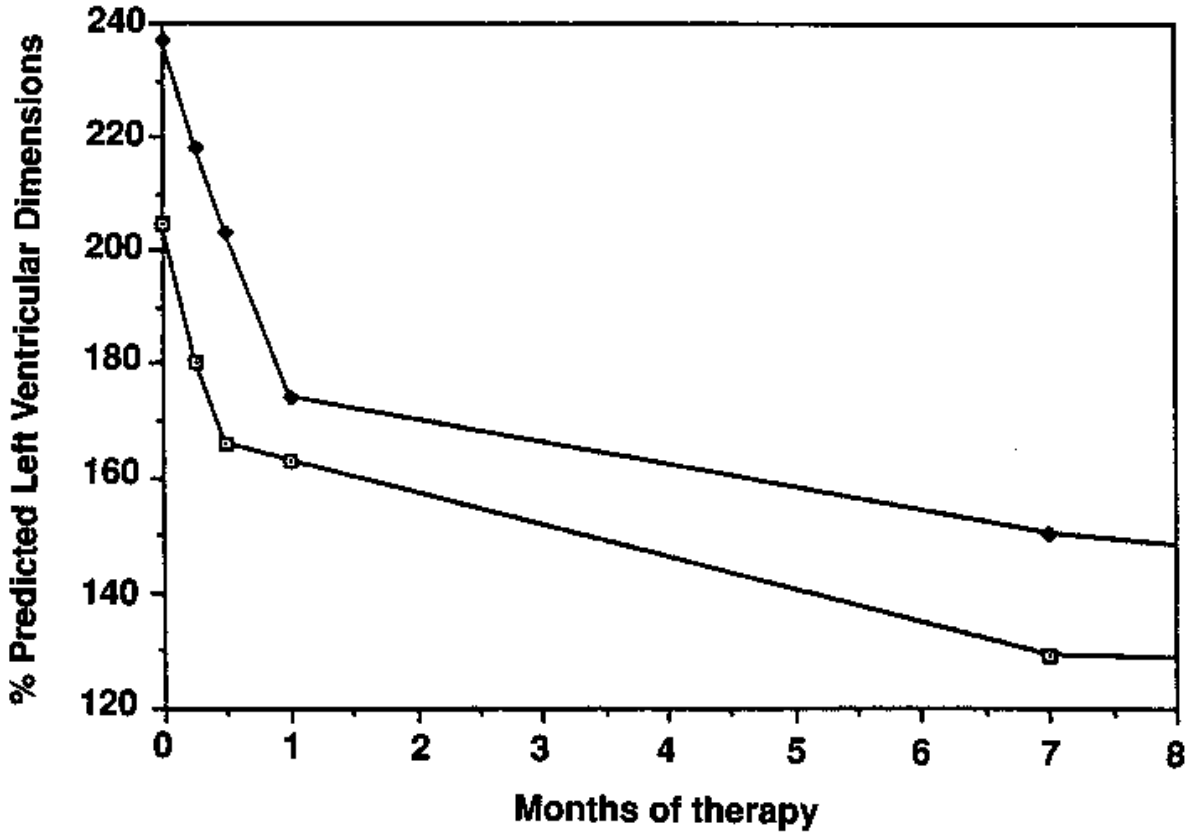


FIGURE 9.

Effect of oral L-carnitine on left ventricular end-diastolic (*open symbols*) and systolic (*closed symbols*) dimensions in a child with cardiomyopathy caused by plasma membrane carnitine transport deficiency. (From Tein I, DeVivo DC, Bierman F, et al: *Pediatr Res* 28:247–255, 1990. Used by permission.)

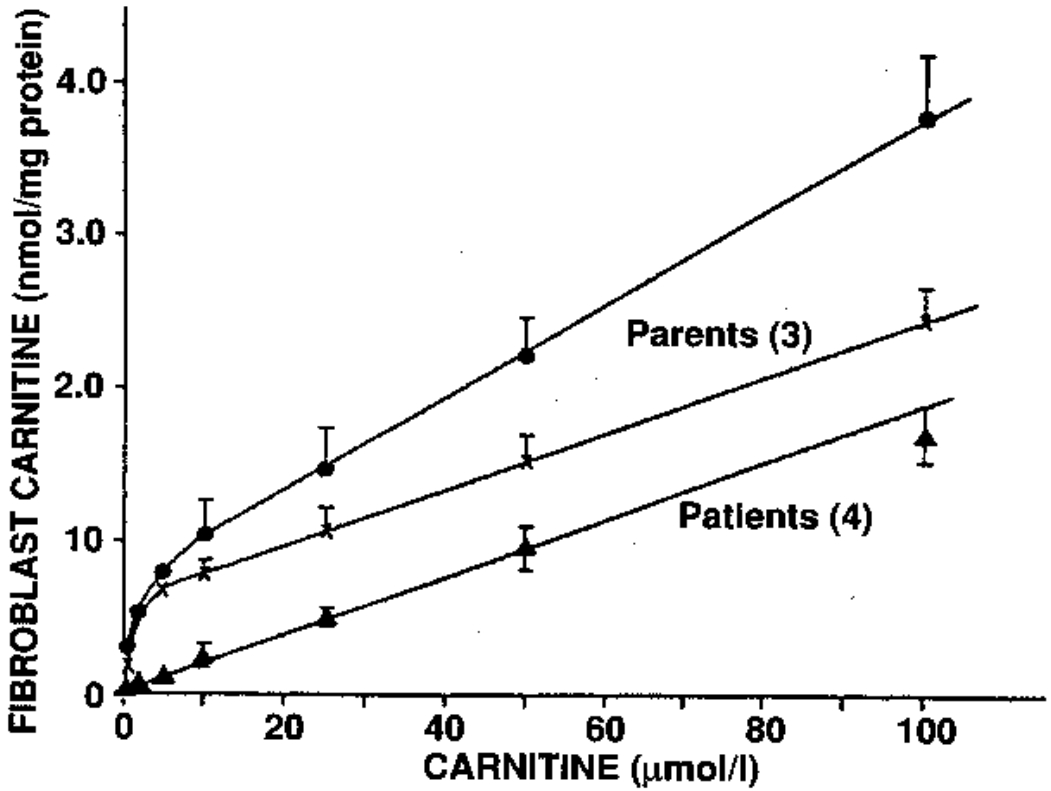


FIGURE 6.

Steady-state intracellular carnitine concentrations in cultured skin fibroblasts from patients homozygous for carnitine transporter deficiency, their heterozygous parents, and normal controls. Controls and heterozygotes actively accumulate carnitine at extracellular concentrations below the K_m for uptake (3 to 4 μmol/L), whereas carnitine enters patient cells only by passive diffusion. (From Stanley CA, DeLeeuw S, Coates PM, et al: *Ann Neurol* 30:709–716, 1991. Used by permission.)

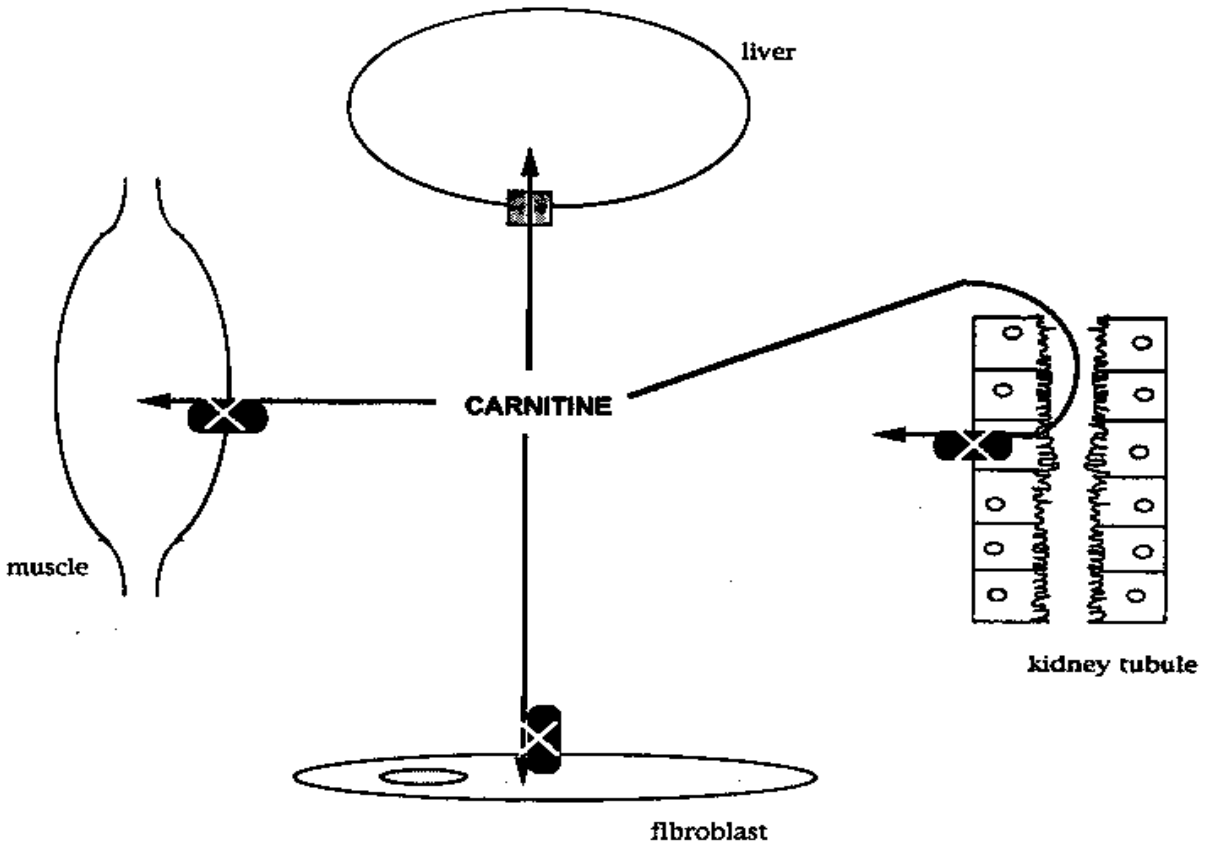
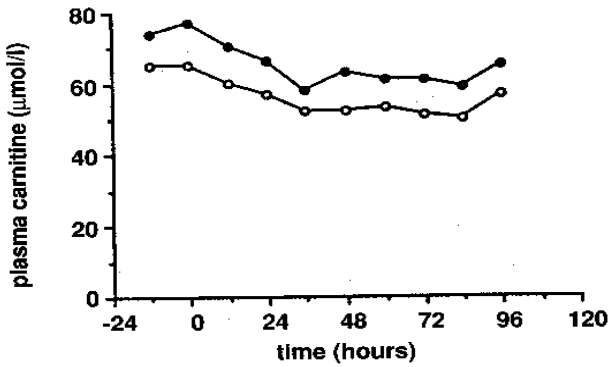


FIGURE 5.
The genetic defect in muscle-kidney plasma membrane carnitine transport. The defect appears to be expressed in muscle, kidney, and fibroblasts, but not in liver.

TABLE 2.
Plasma and Tissue Total Carnitine Concentrations in Patients With the Plasma Membrane Carnitine Transporter Defect (Means ± SD)*

	Plasma μmol/L	Muscle μmol/kg	Liver μmol/kg
Patients			
Pretreatment	2 ± 2 (n = 19)	120 ± 100 (n = 7)	50 (n = 1)
Carnitine therapy	42 ± 12 (n = 12)	30 ± 40 (n = 3)	720 (n = 1)
Parents			
Mothers	26 ± 6 (n = 6)	?	?
Fathers	35 ± 9 (n = 9)	?	?
Normal range	40–60	2,500–3,500	900–1,500

*From Stanley CA, DeLeeuw S, Coates PM, et al: Ann Neurol 30:709–716, 1991. Used by permission.



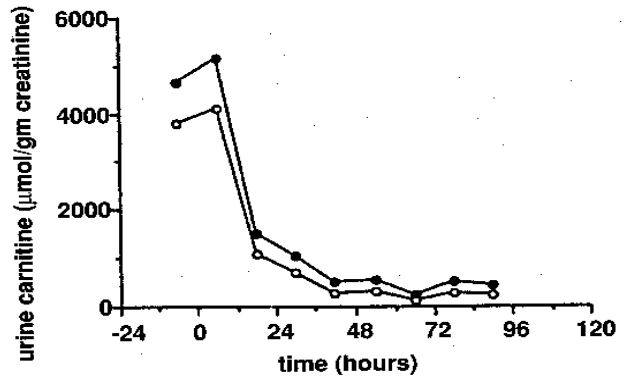
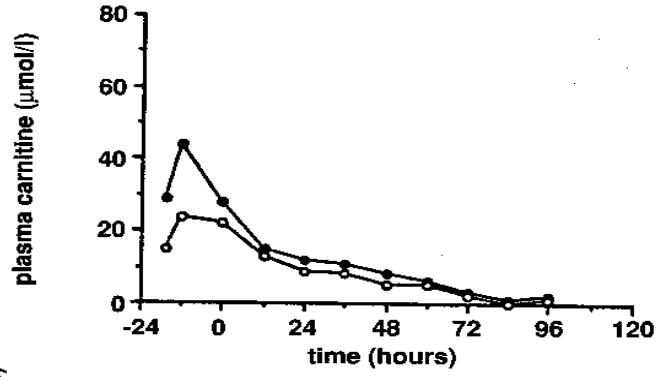


FIGURE 7.
Response to withdrawal of oral carnitine therapy in a control child. Carnitine was discontinued at 0 hours. Free *(open circles)* and total *(closed circles)* carnitine values are shown. For comparison, total carnitine values in untreated controls for plasma are 40 to 60 μmol/L and for urine, 225 to 390 μmol/g creatinine. (From Stanley CA, Berry GT, Bennett MJ, et al: *Pediatr Res* 34:89–97, 1993. Used by permission.)



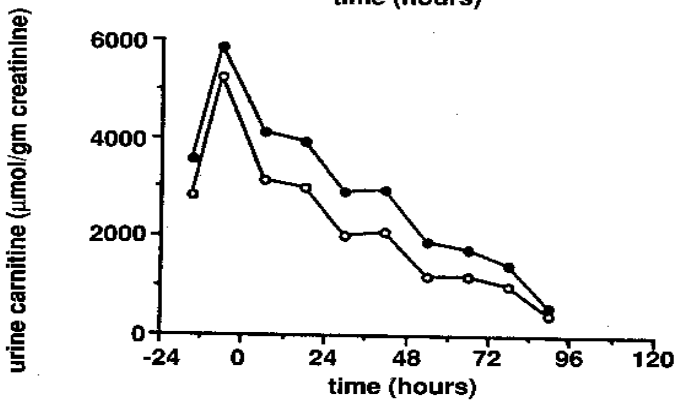


FIGURE 8.
Response to withdrawal of oral carnitine therapy in a patient with carnitine transporter deficiency. Carnitine was discontinued at 0 hours. Free *(open circles)* and total *(closed circles)* carnitine values are shown. (From Stanley CA, Berry GT, Bennett MJ, et al: *Pediatr Res* 34:89–97, 1993. Used by permission.)

Pivalate-induced Carnitine Deficiency

- Pivalate esters used to enhance drug absorption and duration (used in Sweden for UTI prophylaxis)
- Pivalate excreted solely as pivaloyl-carnitine
- Chronically-treated patients develop very low plasma and tissue carnitine levels
- Pivalate-induced carnitine deficiency appears to be well-tolerated, but potential threat (no documented symptomatic cases)

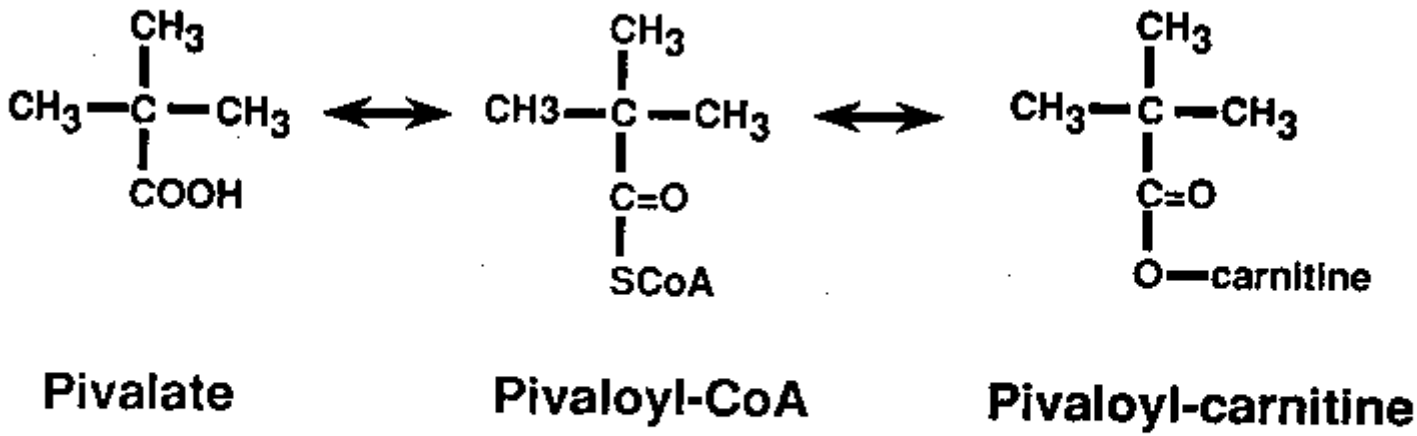


FIGURE 11.
Pivalate metabolism to its coenzyme A and carnitine esters.

TABLE 3.
Effect of Pivalate Administration for 14 to 36 Months on Plasma and Tissue Total Carnitine Concentrations*

	Plasma, μmol/L	Muscle, nmol/mg Protein
Patients	2.3–4.7	1.6–2.2
Normal range	40–60	7.4–26

*From Holme E, Jacobson CE, Nordin I, et al: Lancet 2:469–472, 1989. Used by permission.

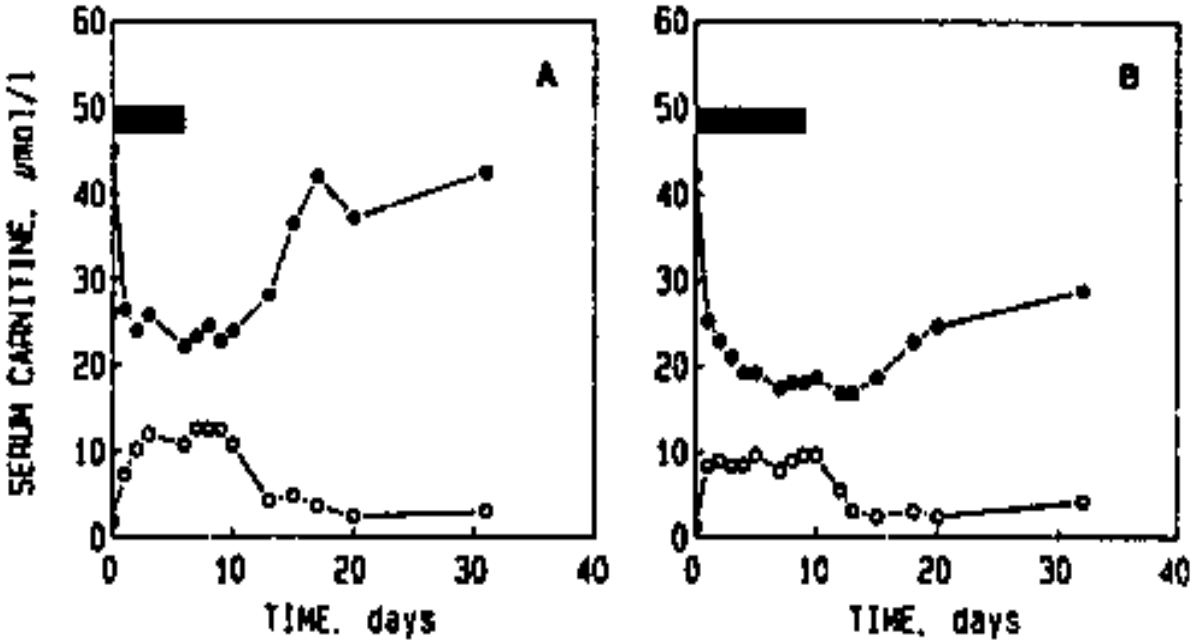


FIGURE 12.

Serum carnitine levels in two normal adults during and after treatment with pivalate-conjugated antibiotics for 6 to 10 days (*solid bars*). Shown are concentrations of serum total carnitine (*closed symbols*) and acylcarnitine (*open symbols*). (From Holme E, Jacobson CE, Nordin I, et al: *Lancet* 2:469–472, 1989. Used by permission.)

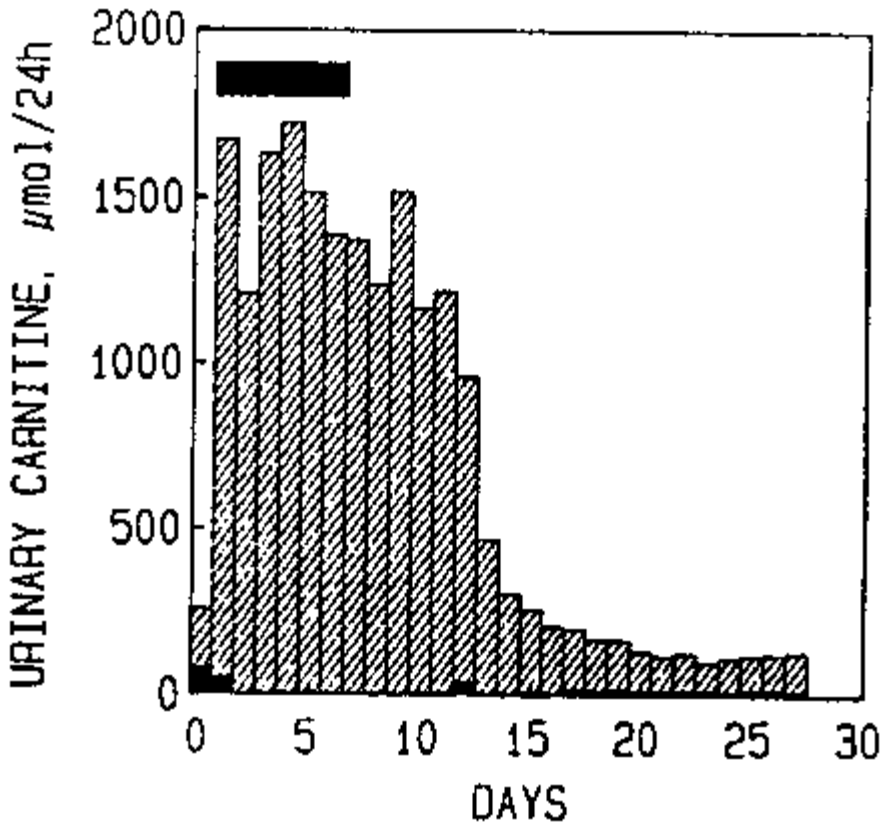


FIGURE 13.

Effect of pivalate-conjugated ampicillin on urinary carnitine in the control adult shown in Figure 12, A. The *solid bar* shows free carnitine: *hatched bars* show acylcarnitine (separately shown to be 100% pivaloylcarnitine during pivalate administration). (From Holme E, Jacobson CE, Nordin I, et al: *Lancet* 2:469–472, 1989. Used by permission.)

Is Pivalate-induced Carnitine Deficiency Sufficient to Impair Fatty Acid Oxidation?

- Fasting tests reveal impaired ketogenesis in 2 of 6 patients on chronic pivalate therapy.
- No obvious symptoms in chronically-treated patients
- Some parents described improved energy and well-being in children after carnitine repletion

"Secondary Carnitine Deficiency" Disorders

- Associated with acyl-CoA oxidation disorders (vLCAD, LCHAD, MCAD, isovaleric acidemia)
- Decreased plasma and tissue total carnitine (50% of normal)
- Increased plasma acyl-carnitines (e.g., isovaleryl-carnitine)
- Mechanism of deficiency? … competitive inhibition of the plasma membrane carnitine transporter by acyl-carnitines
- Benefits of oral carnitine not shown (fasting ketogenesis, cardiac/skeletal muscle function)

TABLE 4.
Plasma Carnitine Alterations in Mitochondrial Fatty Acid Oxidation Genetic Defects in Infants and Children*

Defect	Abbreviation	Total Plasma Carnitine μmol/L	Plasma Acylcarnitine, % of Total
Carnitine cycle			
Plasma membrane transporter	CTD	<5	<30
Carnitine palmitoyl-transferase 1	CPT-1	60–100	<20
Carnitine/acylcarnitine translocase	TRANS	5–30	80–100
Carnitine palmitoyl-transferase 2	CPT-2	10–20	40–80
β-Oxidation cycle			
Acyl-CoA dehydrogenases			
Long chain/very long chain	LCAD/ VLCAD	10–30	30–60
Medium chain	MCAD	10–30	30–60
Short chain	SCAD	10–30	30–60
3-Hydroxyacyl-CoA dehydrogenases			
Long chain	LCHAD	10–30	30–60
Short chain	SCHAD		
Electron transfer cycle			
Electron transfer flavoprotein	ETF	10–30	30–60
ETF dehydrogenase	ETF-DH	10–30	30–60
Ketone synthesis			
Hydroxymethylglutaryl-CoA synthase	HMG-synthase	40–60	<30
Hydroxymethylglutaryl-CoA lyase	HMG-lyase	10–30	30–60
Normal values		40–60	<30

*From Stanley CA, Hale DE, Berry GT, et al: *N Engl J Med* 327:19–23, 1992. Used by permission.

TABLE 7.
Inhibitory Effects of Acylcarnitines on Free Carnitine Transport by Cultured Fibroblasts*

Acylcarnitine Ester	Concentration for Half-Maximal Inhibition, μmol/L
Acetyl (C2)	4.6 ± 0.5
Octanoyl (C8)	2.9 ± 0.4
Myristoyl (C12)	0.16 ± 0.02
Palmitoyl (C16)	0.37 ± 0.06
Free Carnitine K_m	2.7 ± 0.6

*From Stanley CA, DeLeeuw S, Coates PM, et al: *Ann Neurol* 30:709–716, 1991. Used by permission.

TABLE 6.

Apparent Renal Thresholds for Free Carnitine in Fatty Acid Oxidation Disorders and Organic Acidemias*

Defect†	Renal Free Carnitine Threshold, μmol/L
CTD (n = 2)	<2
CPT-1 (n = 1)	>90
TRANS (n = 1)	<10
LCAD/VLCAD (n = 2)	43–52
MCAD (n = 2)	13–25
Isovaleric acidemia (n = 3)	16–18
Propionic acidemia (n = 1)	14–23
Controls (n = 3)	50–60

*From Stanley CA, Berry GT, Bennett MJ, et al: Pediatr Res 34:69–97, 1993. Used by permission.

†See Table 4 for abbreviations.

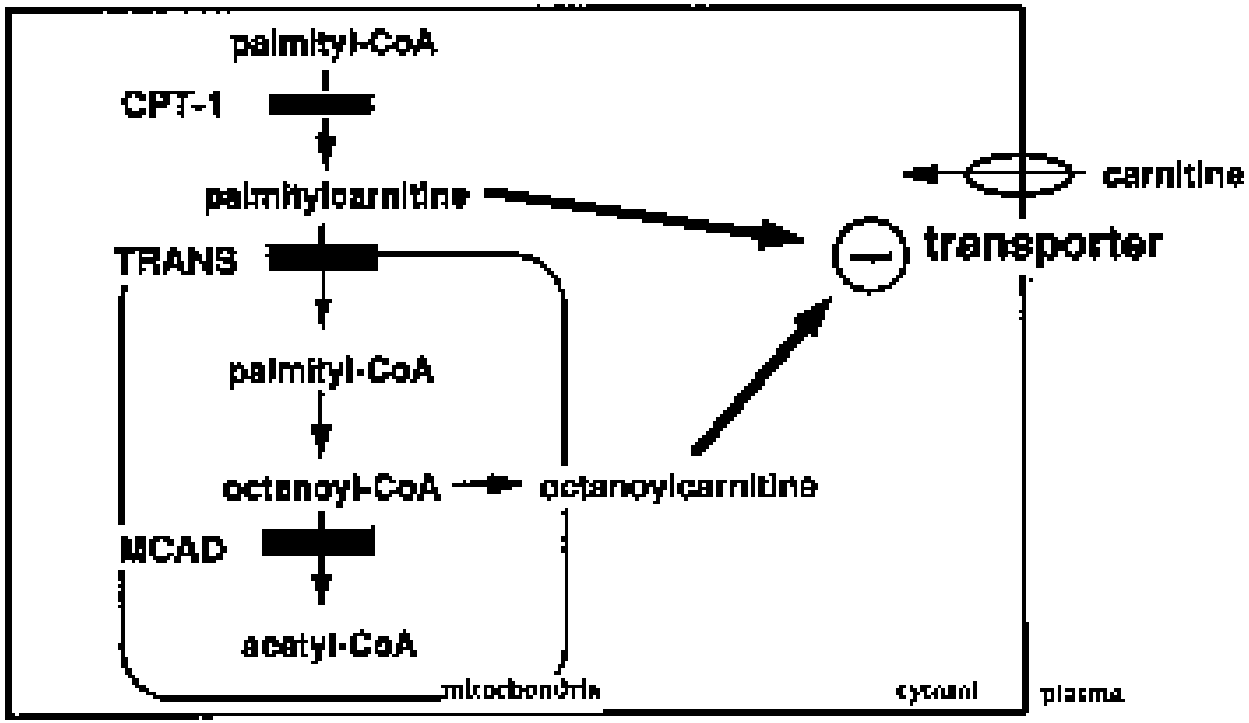


FIGURE 16.

Acylcarnitine inhibition of carnitine transport and secondary carnitine deficiency in fatty acid oxidation disorders. Via acylcarnitine accumulation, blocks at the carnitine/acylcarnitine translocase (TRANS) or medium-chain acyl-CoA dehydrogenase (MCAD) step inhibit the plasma membrane carnitine transporter and lead to tissue carnitine deficiency and reduced renal carnitine threshold. A carnitine palmetoyltransferase 1 (CPT-1) defect prevents acylcarnitine formation, which leads to less inhibition of transporter and hence an increased tissue carnitine and renal carnitine threshold.

Nutritional Carnitine Deficiency??

- Adults on vegetarian diet (no carnitine)
  - plasma carnitine normal
  - urinary carnitine excretion reduced (25% of controls)
- Neonates & Infants
  - Infants on soy-milk formulas before ~1985 (serum carnitine nearly normal)
  - Neonates on hyperalimentation (serum carnitine 10-50% of normal)

TABLE 6.
Criteria for Symptomatic Carnitine Deficiency and the Carnitine Disorders

Disorder	Mechanism	Carnitine Level % Normal	Pathway Impaired
Carnitine transporter defect	PM* transport and renal threshold	<5	FAO
Pivalate administration	Excessive acylcarnitine loss	<5	FAO
Acyl-CoA oxidation defects (includes FAO defects and organic acidemias)	PM transport and renal threshold	25–50	FAO Detoxification CoA buffering
Valproate administration	PM transport and renal threshold (?)	50–75	?
Renal Fanconi syndrome	Renal threshold	50	? FAO
Carnitine-free feedings in neonates	?	25–50	?

*PM = plasma membrane; FAO = fatty acid oxidation.

Conclusions

- **Carnitine supplementation clearly shown to correct impairments in hepatic, heart, muscle fatty acid oxidation in only 2 disorders:**
  1. **Recessive mutations of the muscle-kidney plasma membrane carnitine carrier (OCTN2).**
  2. **Carnitine depletion due to chronic use of pivalate conjugated drugs.**

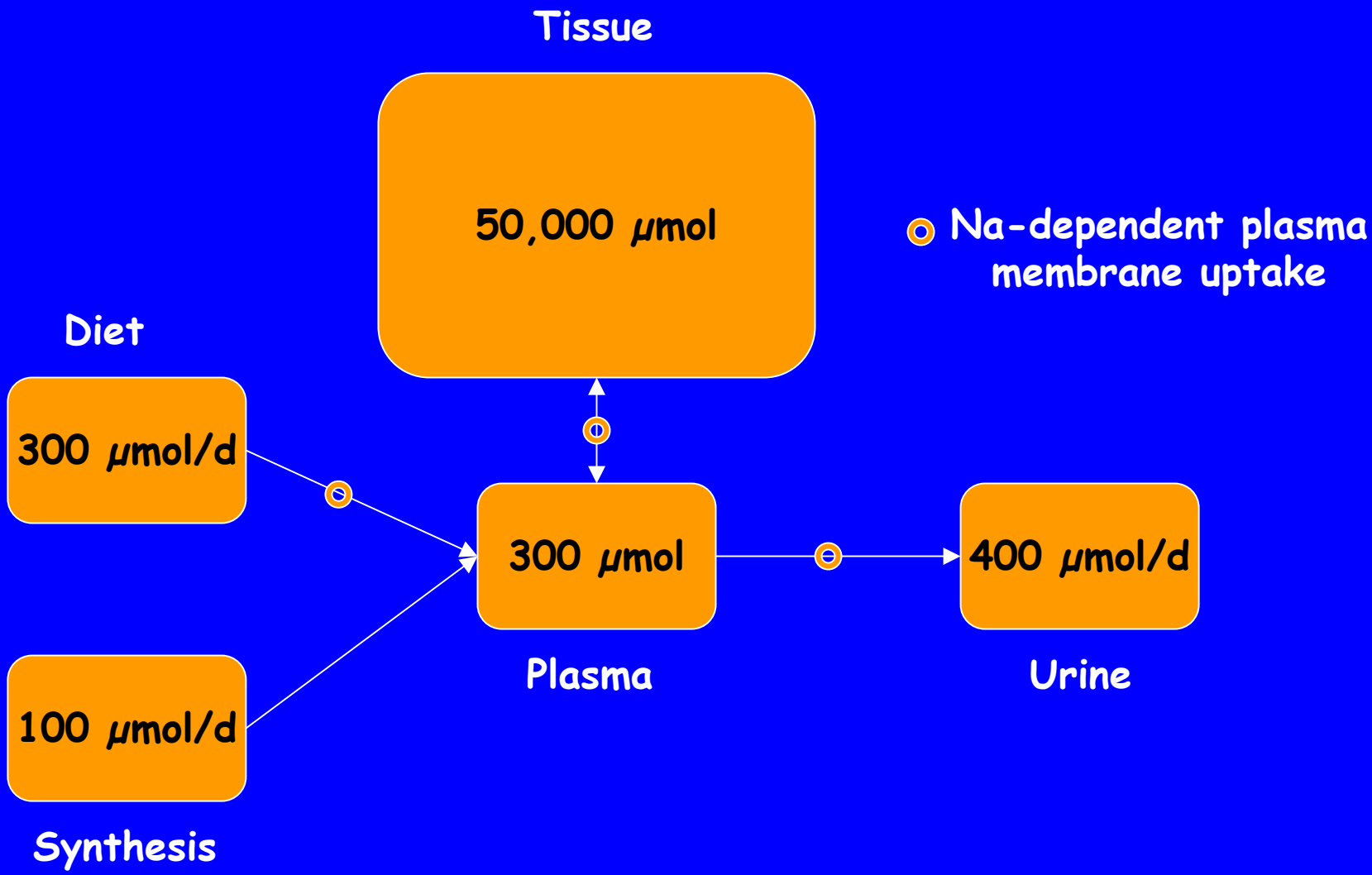
- **Benefits of carnitine in other forms of “carnitine deficiency” remain unproven.**

Perspectives for Futute Research

- Important to critically scrutinize studies of carnitine supplements for adequate “power” from pathophysiologic, as well as, statistical viewpoints.
- Any benefits of carnitine supplements are likely to be subtle, requiring improved methods to demonstrate effects, in vivo (e.g., improved capacity for cardiac fatty acid oxidation).
- May be important to consider potential positive (or negative) effects of carnitine or acyl-carnitine ester supplementation on pathways other than fatty acid oxidation (e.g., regulation of gene transcription).

the end

Carnitine Pools & Turnover



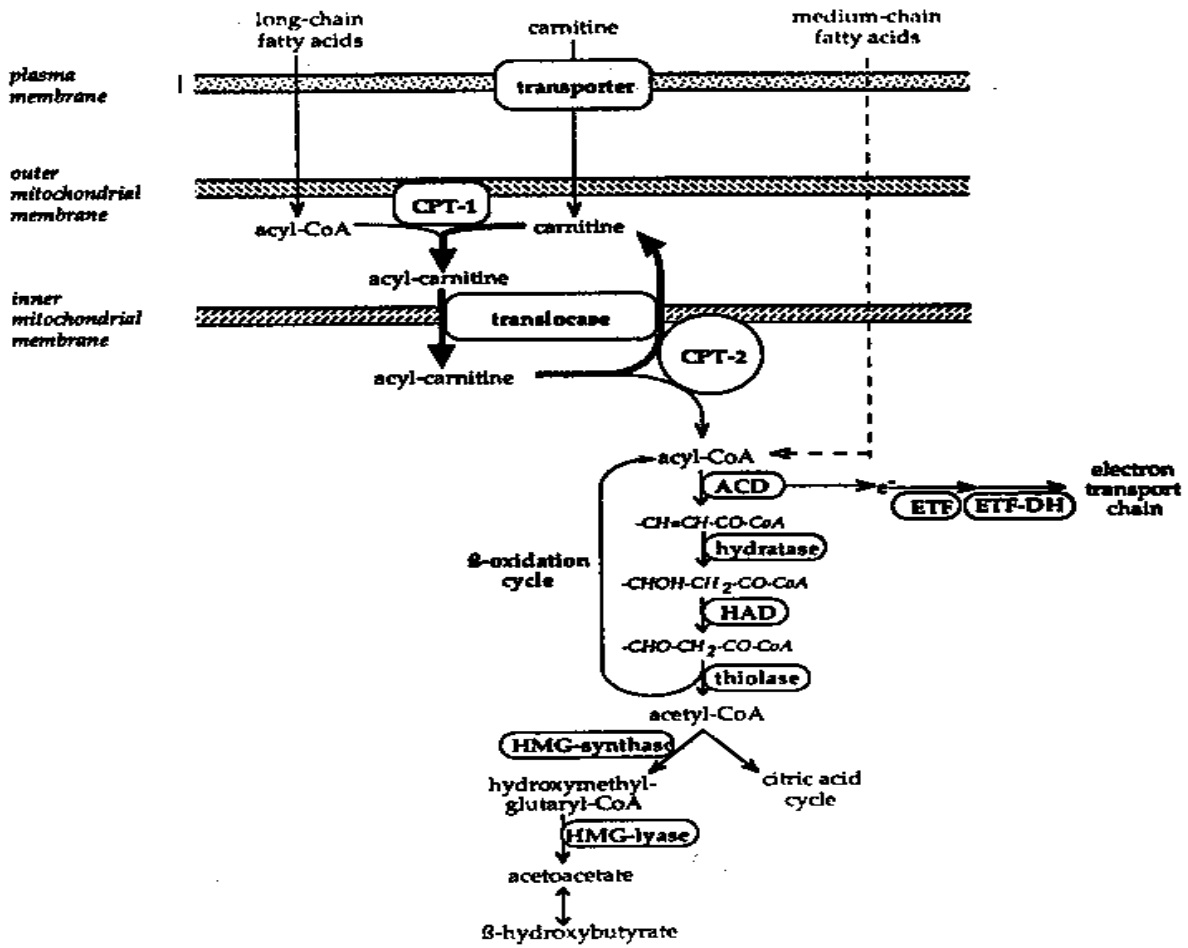


FIGURE 2.

Carnitine and the mitochondrial pathway of fatty acid oxidation and ketone synthesis. CPT-1 and CPT-2-carnitine palmitoyltransferase 1 and 2; *ACD* = acyl-CoA dehydrogenase; *HAD* = OH-acyl-CoA dehydrogenase; *ETF* = electron transfer flavoprotein; *ETF-DH* = ETF dehydrogenase.

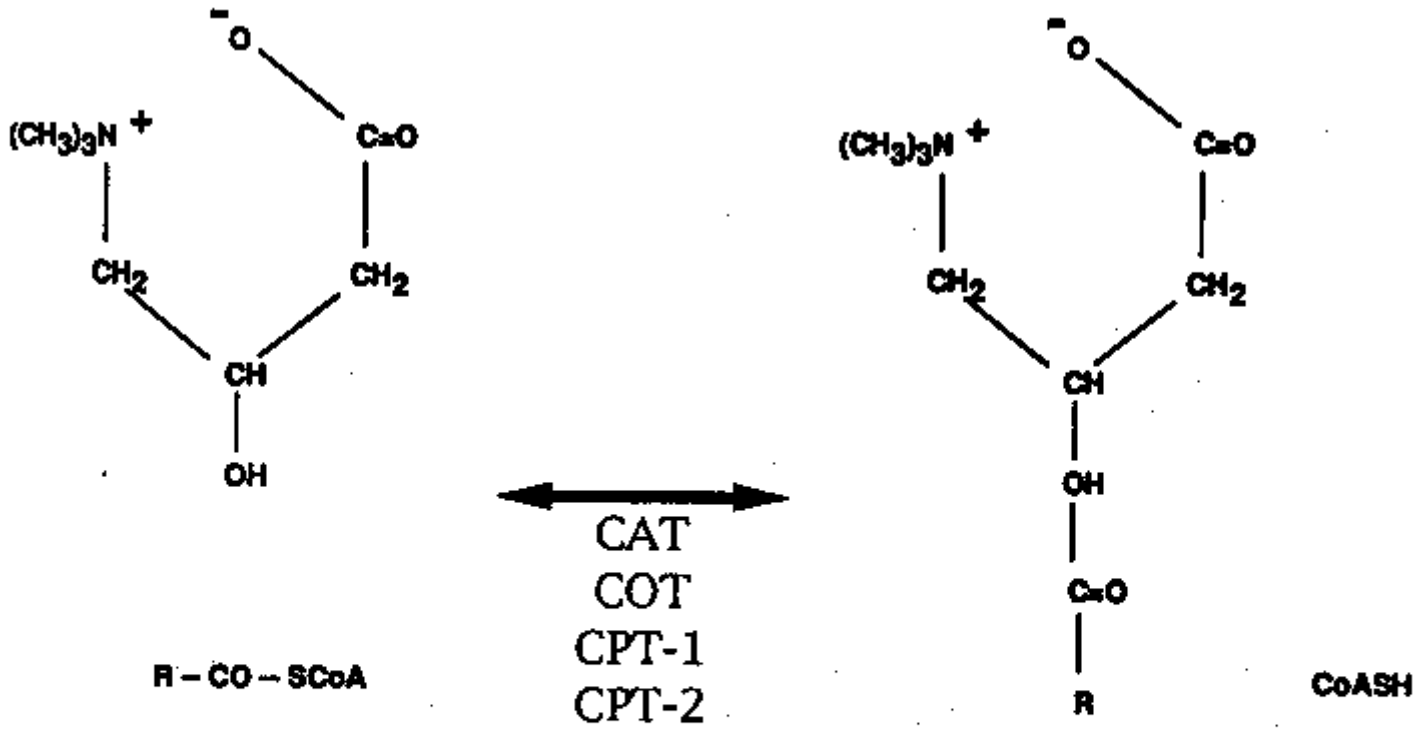


FIGURE 1.

Carnitine acyltransferase (*CAT*) reaction. Acyl groups are transferred from coenzyme A to carnitine by one of four chain-length specific enzymes: CAT, carnitine octanoyltransferase (*COT*), and outer and inner mitochondrial carnitine palmitoyltransferases (*CPT-1* and *CPT-2*).

Hopes for Carnitine Rx

- Correct primary carnitine deficiency (yes)
- Remove toxic fatty acyl-CoAs (no)
- Restore free-carnitine:acyl-carnitine ratio (no)
- Replete carnitine deficiency due to acyl-carnitine wastage (yes & no)