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Disclosure

Charles Hoppel, M.D.

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<u>Collaborators</u>

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- Oxidation of palmitoylcarnitine
- Mitochondrial outer membrane (MOM) Carnitine palmitoyltransferase pathway
- Mitochondrial contact sites (CS) Carnitine palmitoyltransferase pathway

Stoichiometry of oxygen uptake during palmitoylcarnitine oxidation

Reaction Sequence	Electron Acceptor	ΔΟ/ΔΡ	$\Delta O_2 / \Delta P$
Palmitoylcarnitine → palmitoyl-Co	A	0	0
Palmitoyl-CoA → acetyl-CoA 7 X acyl-CoA DH 7 X 3-OHacyl-CoA DH	ETF NAD+	7 <u>7</u> 14	3.5 <u>3.5</u> 7
8 Acetyl-CoA \rightarrow 4 acetoacetate		0	0
8 Acetyl-CoA + 8 malate \rightarrow 8 cit 8 X malate DH	trate NAD⁺	8	4
8 Citrate → 8 malate 8 X isocitrate DH 8 X 2-ketoglutarate DH 8 X succinate DH Sum of citric acid cycle	NAD⁺ NAD⁺ fps	8 8 <u>8</u> 24	4 4 <u>4</u> 12
<u>Totals</u>			
Palmitoylcarnitine \rightarrow 4 acPalmitoylcarnitine \rightarrow 8 citPalmitoylcarnitine \rightarrow 16 CO	etoacetate rate	14 22 46	7 11 23

ADP vs. DNP oxidation of palmitoylcarnitine rat liver mitochondria



Malate concentration on rate of oxygen uptake and $\Delta O/\Delta P$

L-Malate Concentration mM	Rate of Oxygen Uptake (natoms 0-min-1 •mg-l)	Δ0/ΔΡ	
0	51.9	13.4	
0.5	87.1	17.9	
1.0	812	20.2	
2.0	81.0	20.1	
3.0	81.0	21.4	
5.0	81.0	20.5	

Oxidation of (carboxy-14C)-palmitoylcarnitine rat liver mitochondria

dpm x 10-3 % of Added Radioactivity

•	Total Recovered	403.2	94 .7
•	Recovered in Combined Acid Extracts	386.0	90 .8
•	Recovered in Acid Washed Pellet	12 .6	2.9
•	Recovered as CO ₂	4.6	1.0
•	Added I-14c-palmitoyl carnitine	429 .0	100

Fluorocitrate oxidation palmitoylcarnitine + malate by rat liver mitochondria





Source of Mitochondria on $\Delta O/\Delta P$ Palmitoylcarnitine + malate

Source of mitochondria	Δ0/ΔΡ			
Rat Liver	22			
Rat Heart	34			
Rat Skeletal Muscle	45			

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- Protein-protein interactions within the MOM for fatty acyl group transfer
- Percol purified rat liver mitochondria
- Swell-shrink to release outer membrane
- Harvest crude outer membrane
- Resolve into purified outer membrane
- and contact sites
- Use purified MOM
- Treat with detergent and separate by mass using BNE



Table 1. LC-MS/MS analysis of protein complexes isolated by BNE. Sonicated mitochondrial outermembrane (S-MOM, 300 µg) was extracted and subjected to BNE under "Experimental Procedures". Each band was excised, reduced, alkylated, and followed by in-gel digestion analyzed by LC-MS/MS.

BNE Band	Protein name	Accession No.	Protein M.W. (Da)	Coverage (%)
	Carnitine palmitovltransferase 1a (CPT1a)	P32198	88069	29
	Long-chain-fatty-acid-CoA ligase 1 (ACSL1)	P18163	78128	57
A, 980 kDa	Voltage-dependent anion-selective channel protein 1 (VDAC1)	Q9Z2L0	30737	52
	Voltage-dependent anion-selective channel protein 2 (VDAC2)	P81155	31726	30
	Voltage-dependent anion-selective channel protein 3 (VDAC3)	Q9R1Z0	30778	15
	Carnitine palmitoyltransferase 1a (CPT1a)	P32198	88069	20
	Long-chain-fatty-acid-CoA ligase 1 (ACSL1)	P18163	78128	59
B, 780 kDa	Voltage-dependent anion-selective channel protein 1 (VDAC1)	Q9Z2L0	30737	37
	Voltage-dependent anion-selective channel protein 2 (VDAC2)	P81155	31726	41
	Voltage-dependent anion-selective channel protein 3 (VDAC3)	Q9R1Z0	30778	26
	Carnitine palmitoyltransferase 1a (CPT1a)	P32198	88069	23
	Long-chain-fatty-acid-CoA ligase 1 (ACSL1)	P18163	78128	60
C, 535 kDa	Voltage-dependent anion-selective channel protein 1 (VDAC1)	Q9Z2L0	30737	46
	Voltage-dependent anion-selective channel protein 2 (VDAC2)	P81155	31726	30
	Voltage-dependent anion-selective channel protein 3 (VDAC3)	Q9R1Z0	30778	30
	Carnitine palmitoyltransferase 1a (CPT1a)	P32198	88069	1
	Long-chain-fatty-acid-CoA ligase 1 (ACSL1)	P18163	78128	60
D, 230 kDa	Voltage-dependent anion-selective channel protein 1 (VDAC1)	Q9Z2L0	30737	76
	Voltage-dependent anion-selective channel protein 2 (VDAC2)	P81155	31726	18
	Voltage-dependent anion-selective channel protein 3 (VDAC3)	Q9R1Z0	30778	14
	Long-chain-fatty-acid-CoA ligase 1 (ACSL1)	P18163	78128	60
	Long-chain-fatty-acid-CoA ligase 5 (ACSL5)	O88813	76405	5
E 210 kDa	Long-chain-fatty-acid-CoA ligase 6 (ACSL6)	P33124	78180	2
E, 210 kDa	Voltage-dependent anion-selective channel protein 1 (VDAC1)	Q9Z2L0	30737	76
	Voltage-dependent anion-selective channel protein 2 (VDAC2)	P81155	31726	41
	Voltage-dependent anion-selective channel protein 3 (VDAC3)	Q9R1Z0	30778	22



Table 2. Identification of proteins immunocaptured with CarboLink resin-coupled affinity-purified CPT1a antibody. CPT1a and associated proteins were immunocaptured with a mixture of five affinity purified CPT1a antibodies directly conjugated to CarboLink resin as described under "Experimental Procedures". Immunocaptured proteins were reduced, alkylated and then digested with trypsin before LC-MS/MS.

Protein	Score	MW (Da)	Coverage (%)	Accession	No. of unique peptides (hits)
Long chain fatty acid CoA ligase 1 (ACSL1)	779	78128	16	P18163	10
carnitine palmitoyltransferase 1a (CPT1a)	1605	88069	34	P32198	30
Voltage-dependent anion channel protein 1 (VDAC1)	248	30737	19	Q9Z2L0	3





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- Resolve into purified outer membrane
- and contact sites
- Use purified contact sites (BUT heterogenous)
- Treat with detergent and separate by mass using BNE



Running condition : 1200 Vhr



MW (kDa)	960	810	740	600	400	310	290	170	Localization
CPT1	12%	12%	5%	6%					MOM
ACSL1	35%	28%	33%	27%	34%	34%	37%	36%	МОМ
ACSL6	2%		2%	1%			1%	2%	MOM
VDAC1	10%	10%	14%	36%	48%	50%	50%	44%	MOM
VDAC2	24%	15%	21%	19%	27%	15%	15%	14%	MOM
VDAC3	10%	17%	10%	10%	7%	7%	7%	21%	MOM
CACT					3%	5%	14%	20%	MIM
VLCAD	12%	8%	4%	9%	13%	8%	12%		MIM
ΤFΡα	13%	14%	12%	19%					MIM
ΤΕΡβ	18%	4%	4%	9%					MIM
LCAD								30%	Matrix
MCAD								15%	Matrix
ACDSB								5%	Matrix
ECHM								26%	Matrix
ТНІМ					6%	5%	14%	67%	Matrix
VLCAD	12%	8%	4%	9%	13%	8%	12%		MIM
ΤFΡα	13%	14%	12%	19%					MIM
ΤΕΡβ	18%	4%	4%	9%					MIM
ANT2	40%	18%	38%	33%	19%		7%	10%	MIM
MPCP	1 0 %	21%	22%	14%	18%	7%	10%	6%	MIM
AIFM1	11%		5%	4%	3%	1%			Intermembrane

Table 1. Representative protein composition of MCS protein complexes and sequence coverage

The sequence coverage of a protein in a specific BNE band was shown as percentage. CPT1: carnitine palmitoyl transferase 1; ACSL1: acyl-CoA synthetase1; ACSL6: acyl-CoA synthetase 6;VDAC1: voltage-dependent anion channel1; VDAC2: voltage-dependent anion channel 2; VDAC3: voltage-dependent anion channel3; CACT: carnitine acylcarnitine translocasel; VLCAD: very-long chain acyl-CoA dehydrogenase; TFP α : trifuntional protein subunit α ; TFP β : trifuntional proteinsubunit β ; LCAD: very-long chain acyl-CoA dehydrogenase; ACDSB: short-chain/branched acyl-CoA dehydrogenase; ECHM: Enoyl-CoA hydratase; THIM: 3-ketoacyl-CoA thiolase; ANT2: adenine nucleotide transporter2; MPCP: mitochondrial phosphate carrier protein; AIFM1: apoptosis inducing factor 1.



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Summary

- FAO predictable
- Mitochondrial outer membrane (MOM) Carnitine palmitoyltransferase pathway
- Mitochondrial contact sites (CS) Carnitine palmitoyltransferase pathway more interesting and in progress
 - Mitoplasts with 90% MOM removed still oxidize palmitate at 100%
 - Contact sites are still present here and retain 50-60% of CPT and LCAS!!!