Carnitine induces autophagy and restores high-fat diet-induced mitochondrial dysfunction

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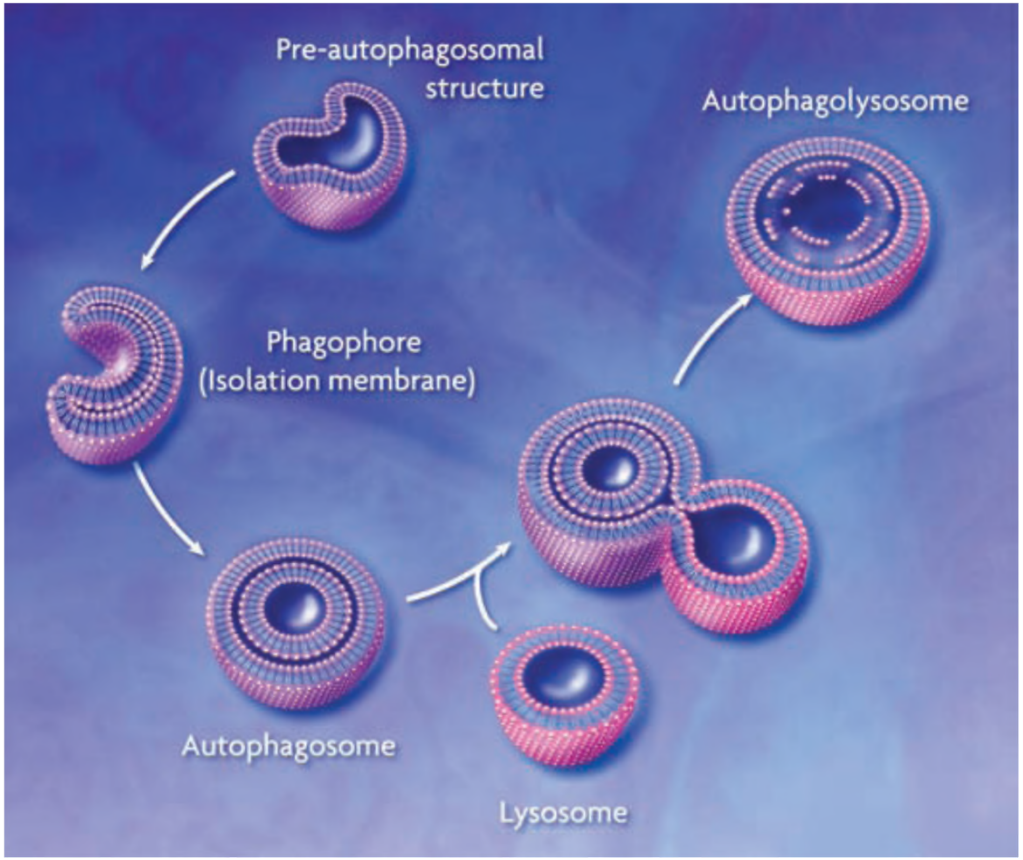
Conflict of interest disclosure

None

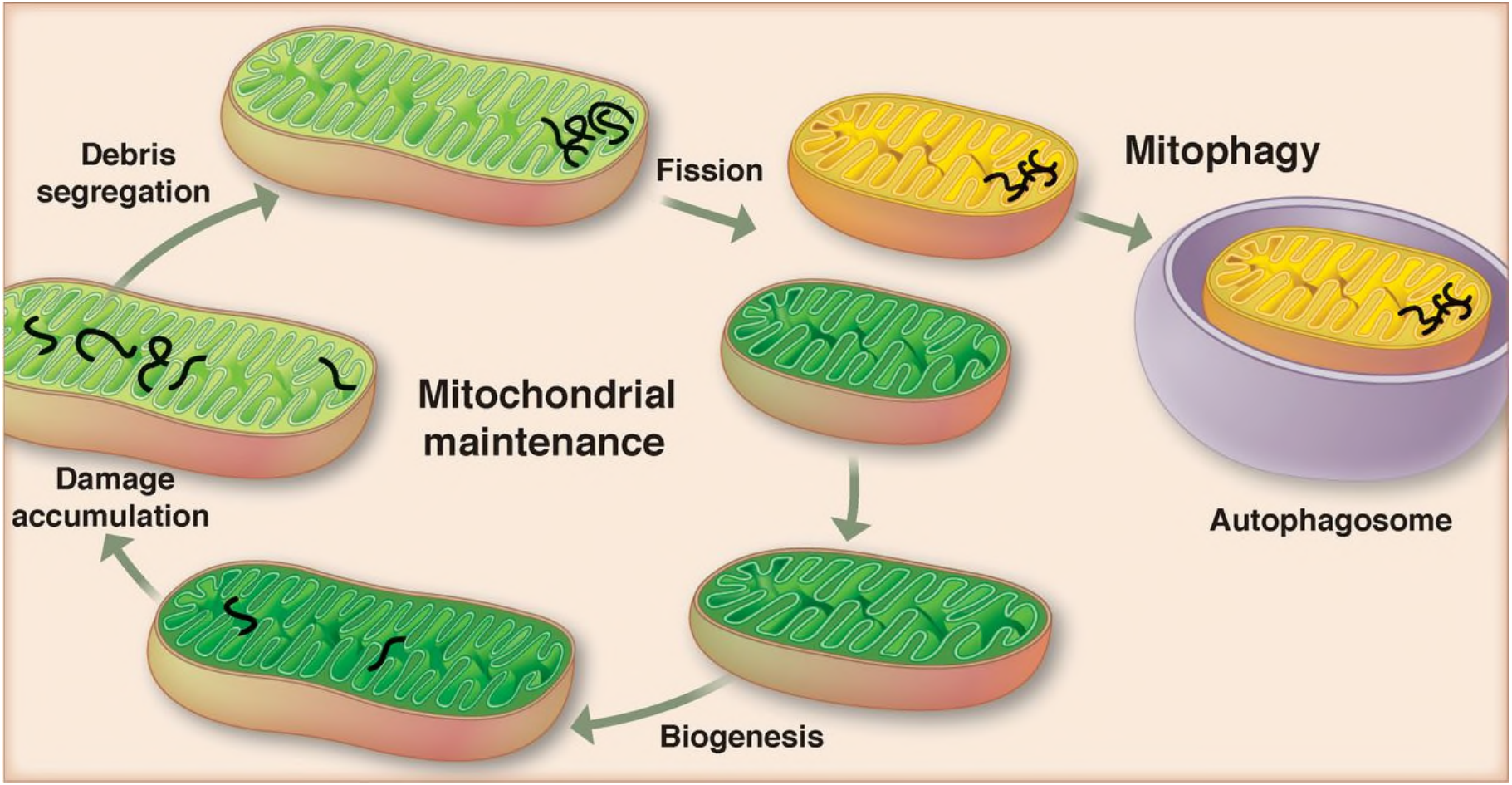
Autophagy

- Autophagy plays an essential quality-control function by promoting the basal turnover of long-lived proteins, as well as by removing damaged organelles (mitophagy, ribophagy, peroxphagy, reticulophagy, lipophagy...)



Shintani T, Klionsky DJ. Science 2004;306:990–5.

Autophagy purifies the cellular pool of mitochondria



Science. 2012 Aug 31;337(6098):1062-5

Autophagy deficiency in metabolic disease

- In high-fat diet-induced rodent models of obesity, autophagy activity is suppressed in the liver and promotes ER stress and insulin resistance.

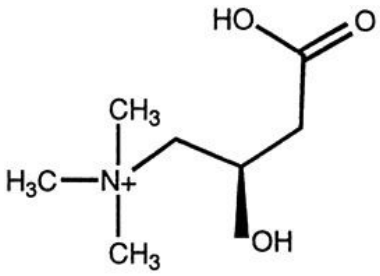
Yang L et al. Cell Metab 2010;11:467–78

Liu HY et al. J Biol Chem 2009;284:31484–92

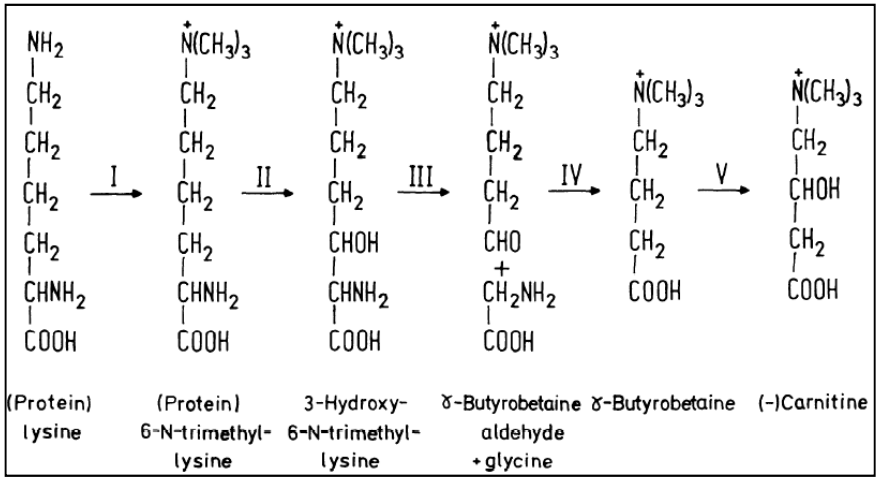
- In skeletal muscle, autophagy was suppressed by high-fat diet and relieved by exercise

He C et al. Nature 2012;481:511–5

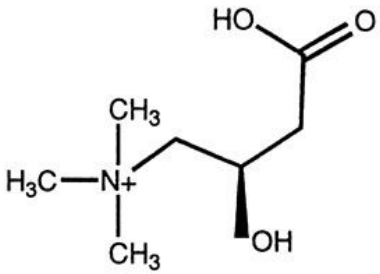
L-Carnitine

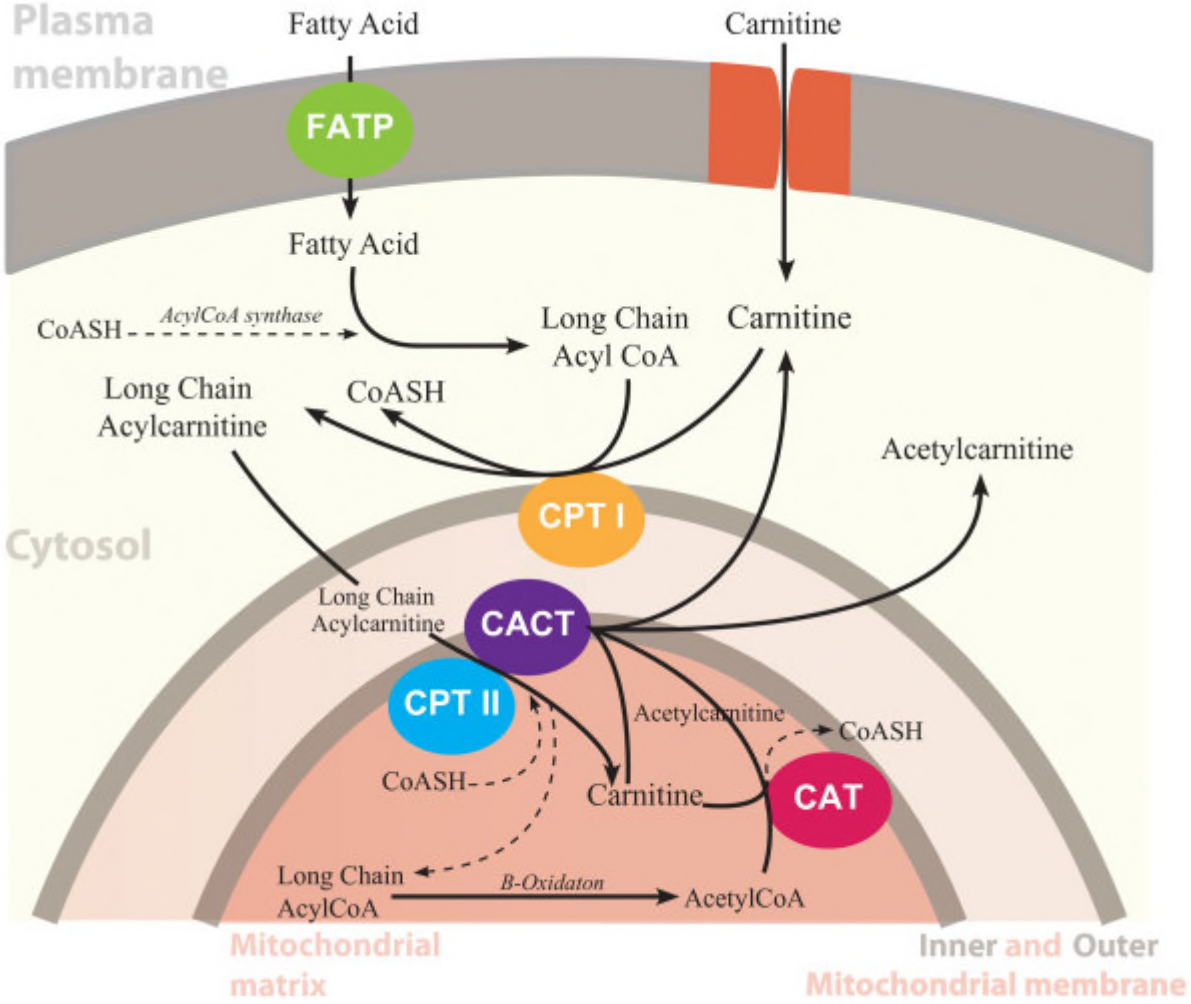


- Discovered in muscle tissue in 1905.
- Chemical structure determined in 1927.
- Present in all animals, in many microorganisms, and in many plants.
- Highest concentrations in heart and skeletal muscle (~3mM).
- Obtained primarily from meat or dairy products or Synthesized in human body from lysine and methionine in the liver or kidney.



L-Carnitine





- Carnitine is involved in transporting fatty acids across the mitochondrial membrane.

L-Carnitine deficiency in insulin resistance

- Diabetic subjects have reduced plasma free carnitine concentrations in 6 out of 8 studies.

Design	Carnitine status (plasma free carnitine)	Refs.
Diabetic patients versus healthy volunteers (control)	Control: 35 µmol/L	[85]
	Diabetic patients: 21 µmol/L ($P < 0.05$ vs. control)	
Type 1 diabetic children versus control	Reduced ($P < 0.05$ vs. control)	[86]
Type 2 diabetic women		[87]
Without (control; $n = 18$)	Control: 53.4 µmol/L	
With retinopathy	Retinopathy: 39.0 µmol/L ($P < 0.05$ vs. control)	
With hyperlipidemia	Hyperlipidemia: 39.6 µmol/L ($P < 0.05$ vs. control)	
With neuropathy	Neuropathy: 40.4 µmol/L ($P < 0.05$ vs. control)	
Type 2 diabetic patients		[88]
Without complications (control, $n = 15$)	Control: 58.1 µmol/L	
With retinopathy ($n = 20$)	Retinopathy: 34.2 µmol/L ($P < 0.05$ vs. control)	
With hyperlipidemia ($n = 13$)	Hyperlipidemia: 34.9 µmol/L ($P < 0.05$ vs. control)	
With polyneuropathy ($n = 20$)	Polyneuropathy: 35.7 µmol/L ($P < 0.05$ vs. control)	
Type 1 diabetic patients versus healthy controls	Reduced ($P < 0.05$ vs. control)	[89]
Type 2 diabetic patients	Not different from control	[83]
Type 1 diabetic subjects versus normal subjects (controls)	Not different from control (48.2 vs. 48.7 µmol/L)	[84]
Diabetic children		[90]
Without ketosis (control)	Control: 50.0 µmol/L	
With ketosis	With ketosis: 29.7 µmol/L	
With ketoacidosis	With ketoacidosis; 24.6 µmol/L	

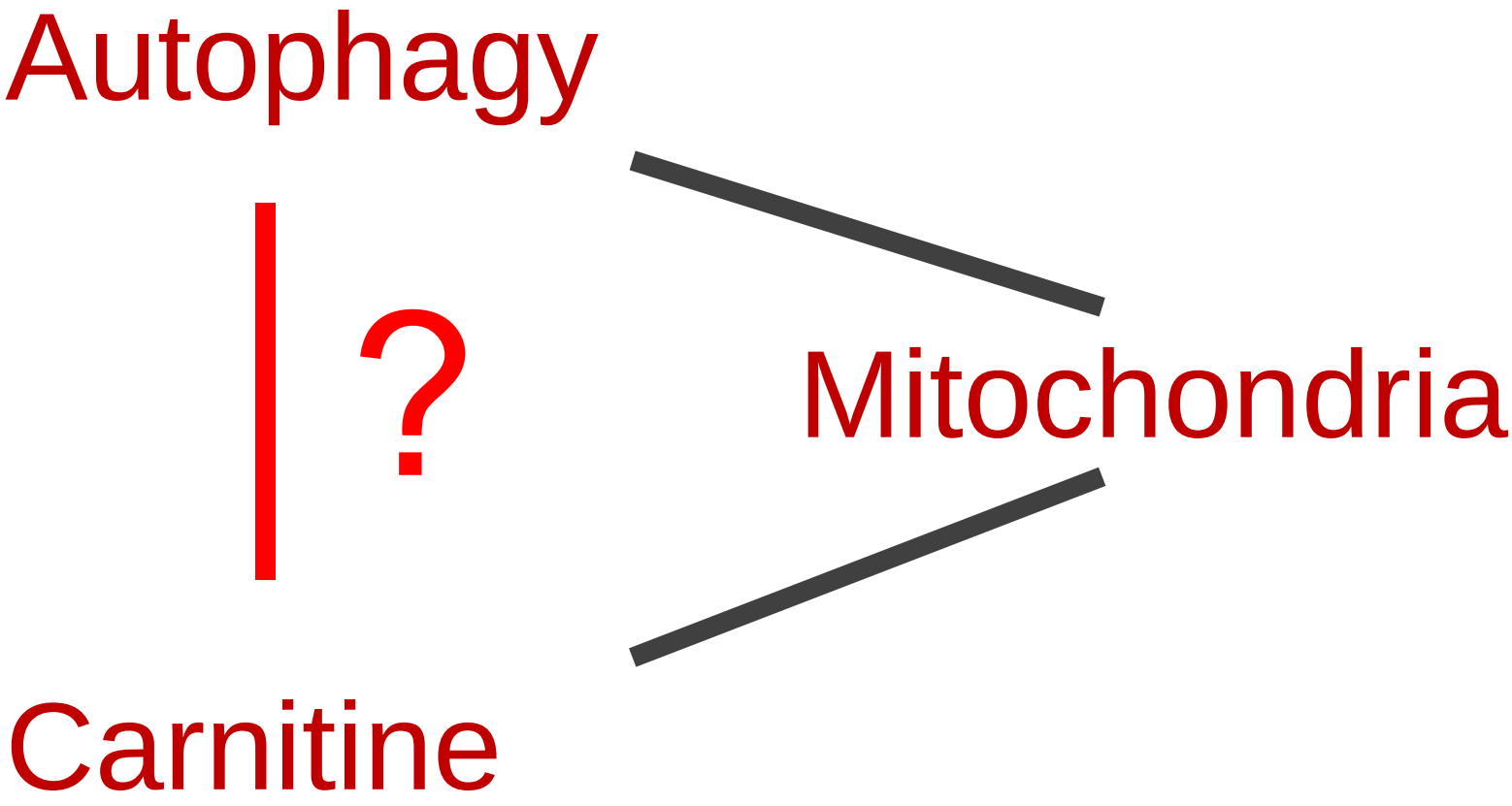
Ringseis R, Eur J Nutr 2012;51:1–18.

Carnitine treatment (2-4g L-carnitine/day) improves insulin sensitivity in patients with obesity and diabetes in clinical trials

- The majority of the studies (11 out of 16) revealed an improvement of parameters of glucose tolerance.

- Suggested mechanism
  - Promoting the mitochondrial oxidation of long-chain acyl-CoAs as accumulation of long-chain acyl-CoAs and other fatty acid metabolites impairs insulin signaling.
  - Carnitine strongly reduces intramitochondrial acetyl-CoA levels resulting in a 10- to 20-fold decrease in the acetyl-CoA/CoA ratio and stimulates PDHC for glucose utilization.
  - …

Ringseis R, Eur J Nutr 2012;51:1–18.

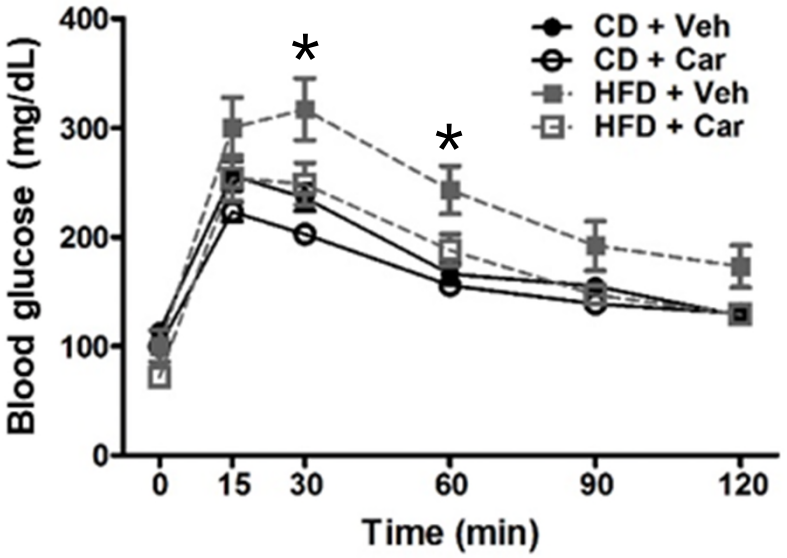

Autophagy
?
Mitochondria
Carnitine

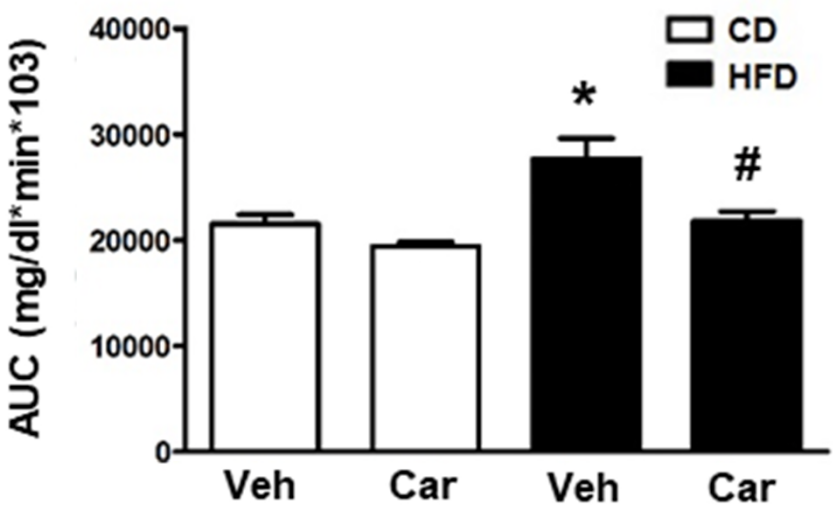
Study design

Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
CD+ Veh															
CD+ Carnitine														Carnitine 100mg/kg/day	
HFD+ Veh								HFD(48 Kcal%)							
HFD+ Carnitine								HFD(48 Kcal%)						Carnitine 100mg/kg/day	

CARNITINE TREATMENT AND GLUCOSE TOLERANCE

Carnitine treatment improves glucose tolerance - IPGTT

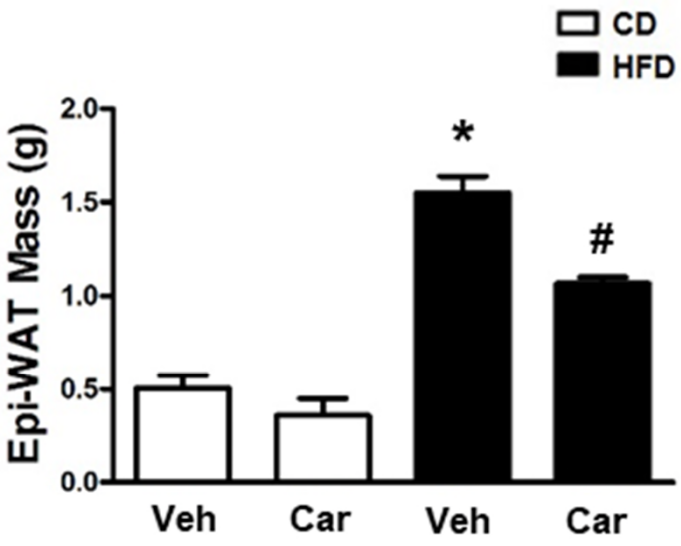


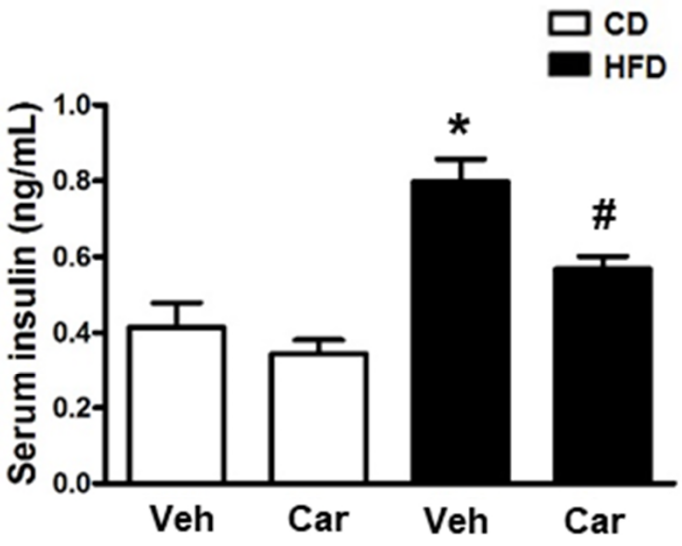


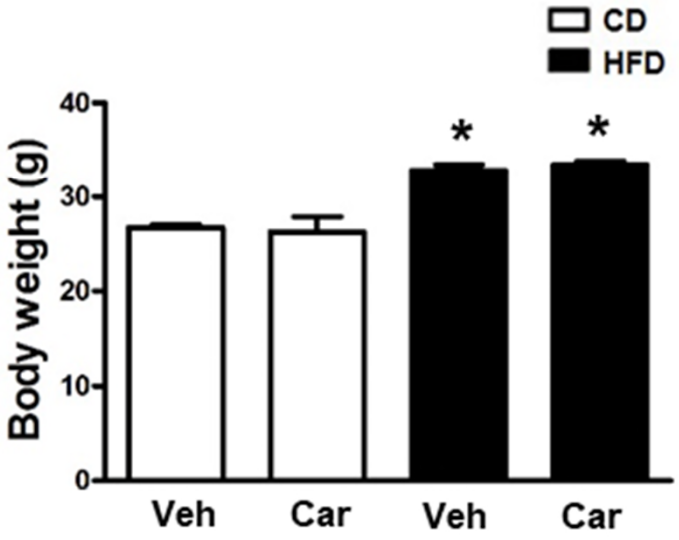
* p < 0.05 vs. CD+Veh

# p < 0.05 vs. HFD+Veh

Carnitine treatment improves glucose tolerance



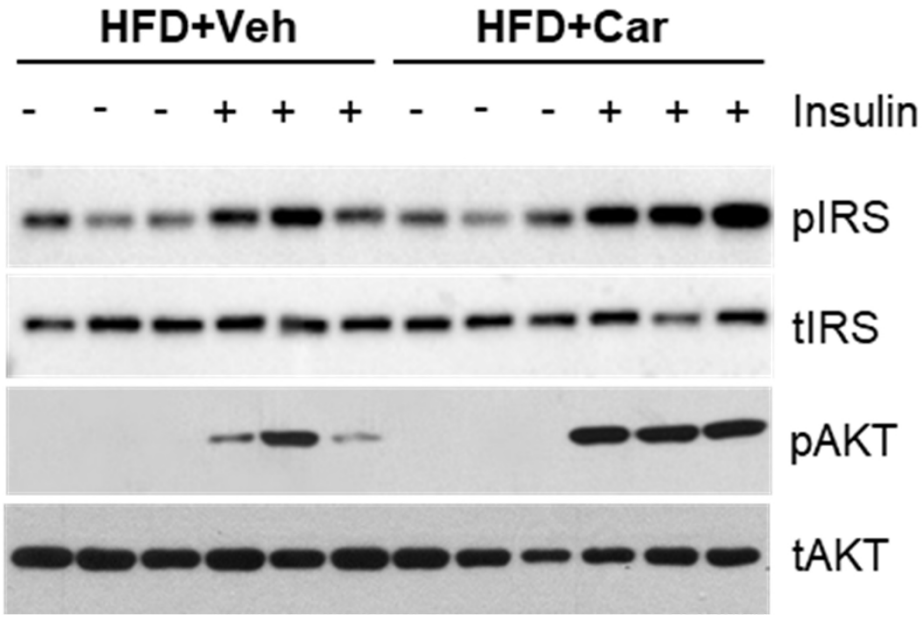


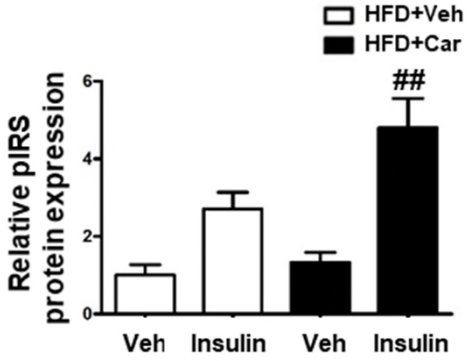


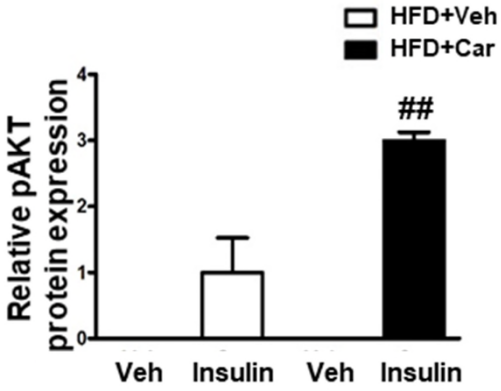
* $p < 0.05$ vs. CD+Veh

# $p < 0.05$ vs. HFD+Veh

Carnitine treatment improves skeletal muscle insulin signaling

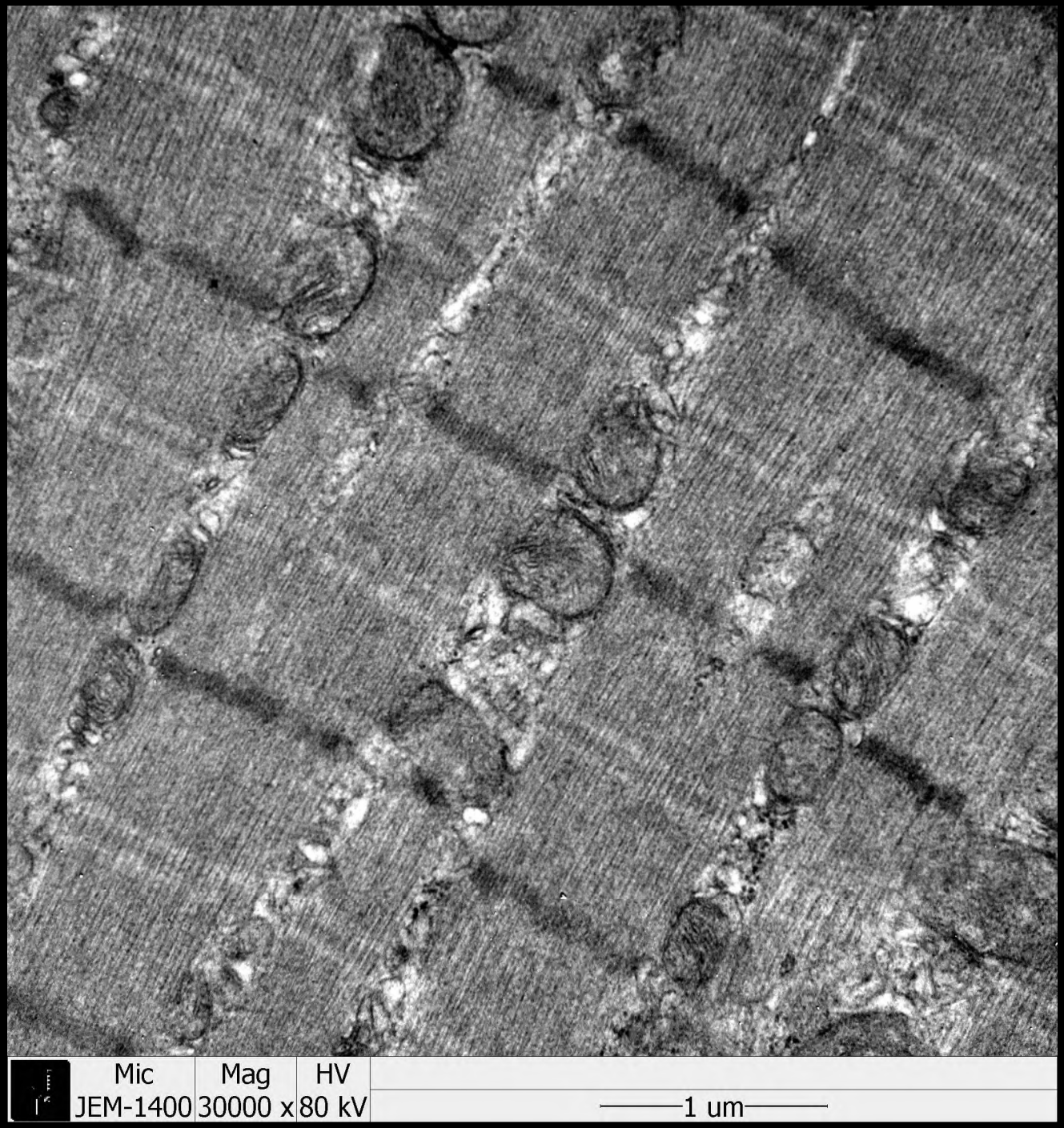




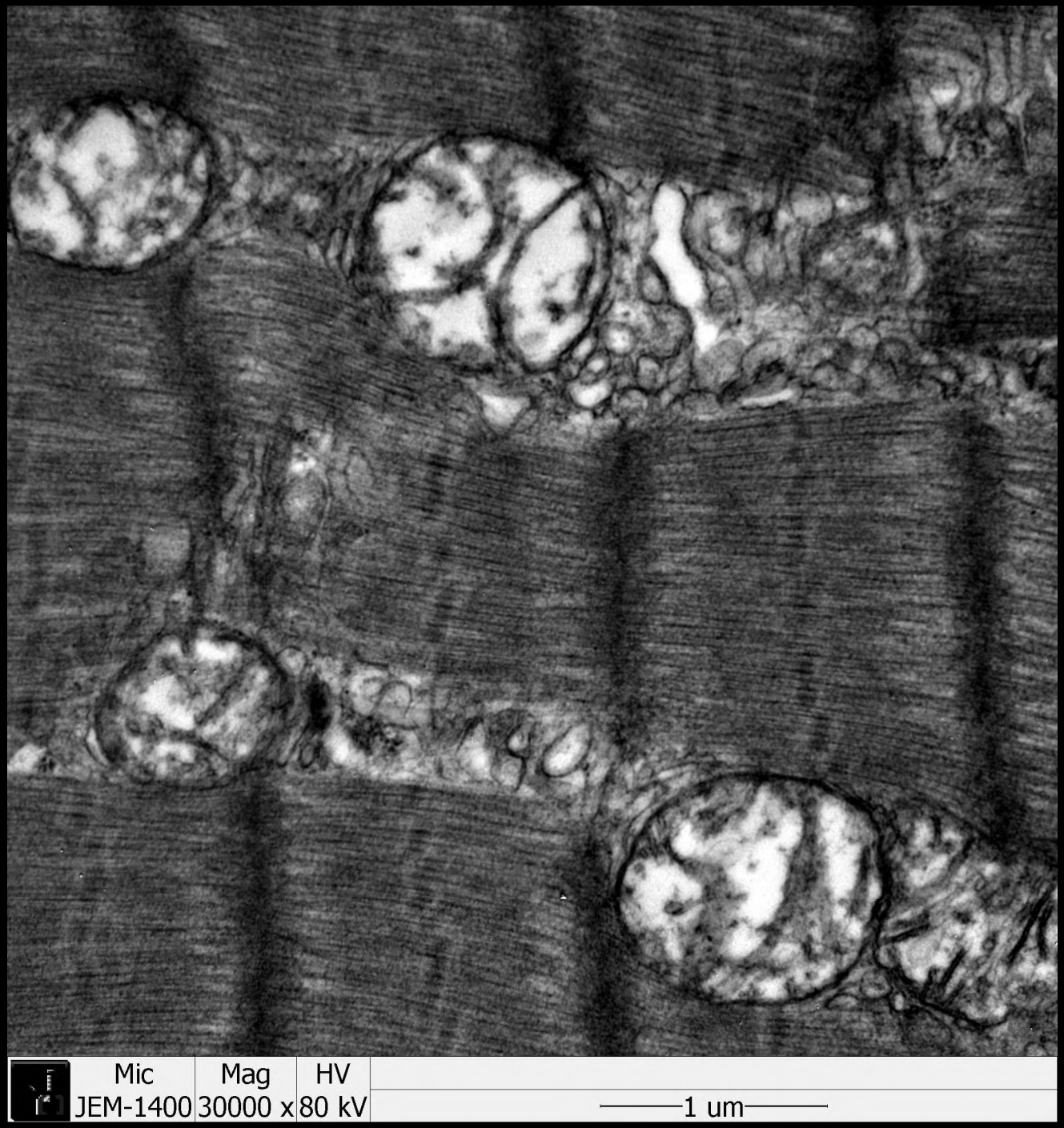


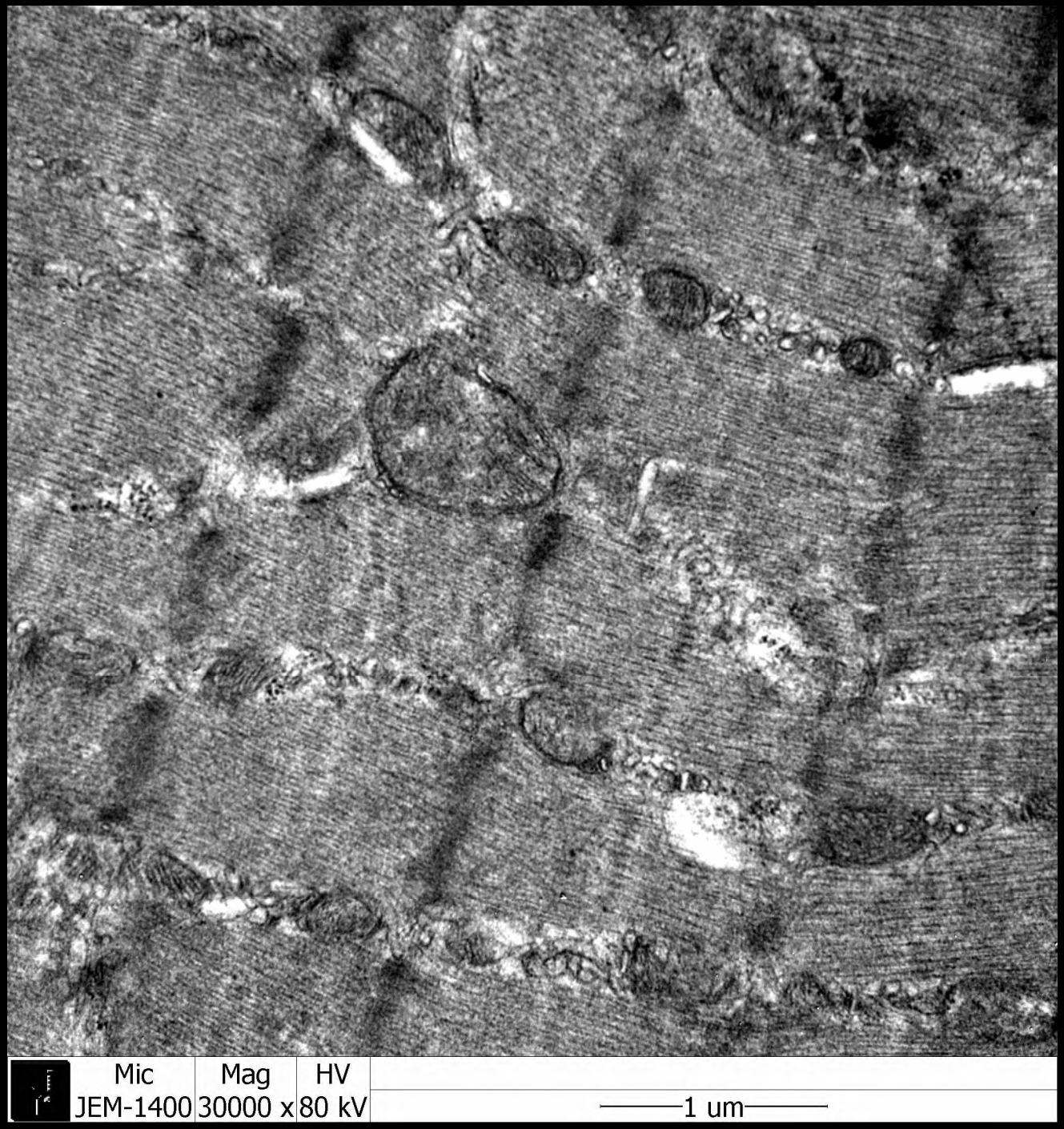
##p<0.05 vs. HFD+Veh+insulin

CARNITINE TREATMENT AND MITOCHONDRIAL DYSFUNCTION

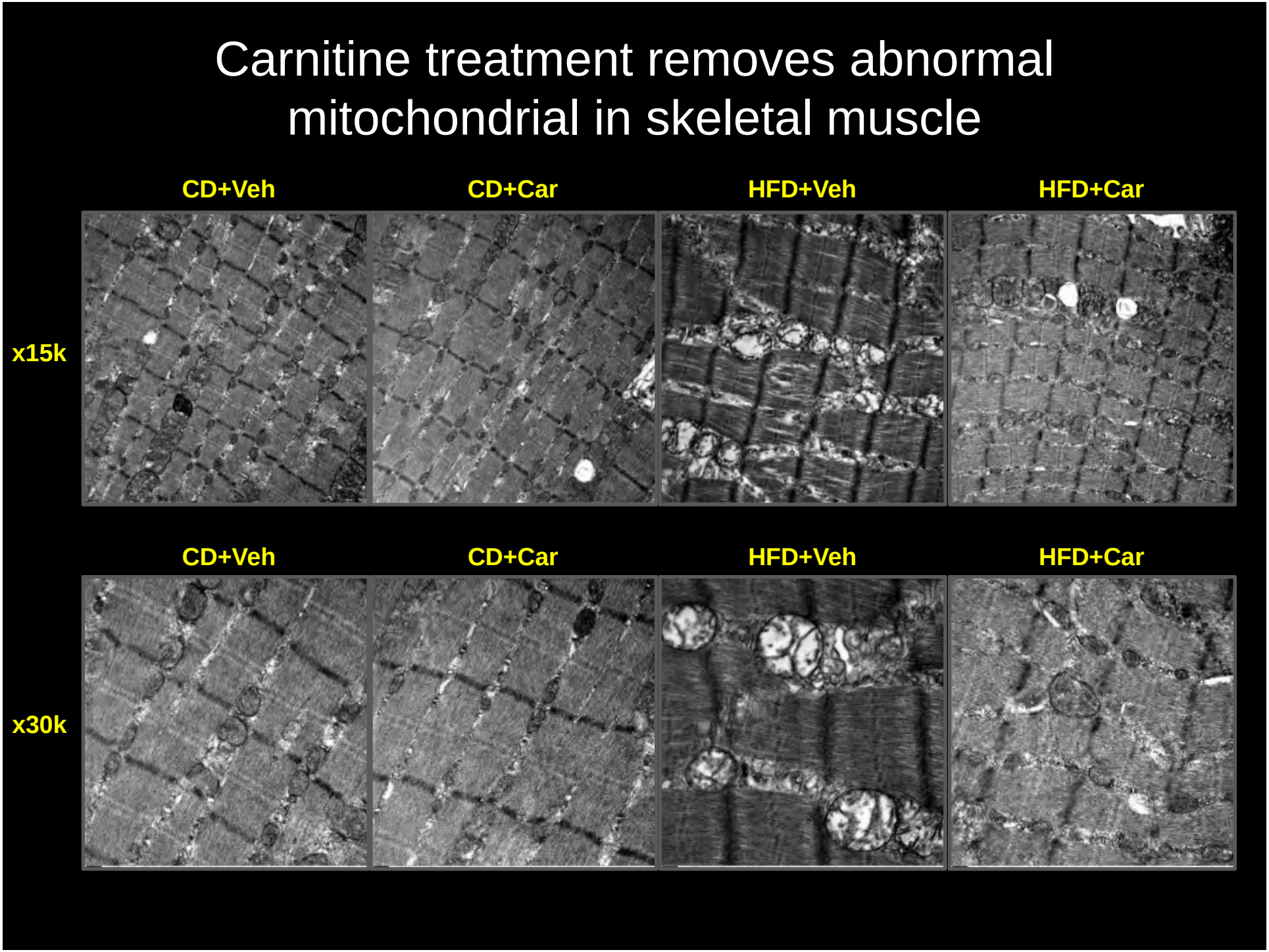


Chow Diet
x30K

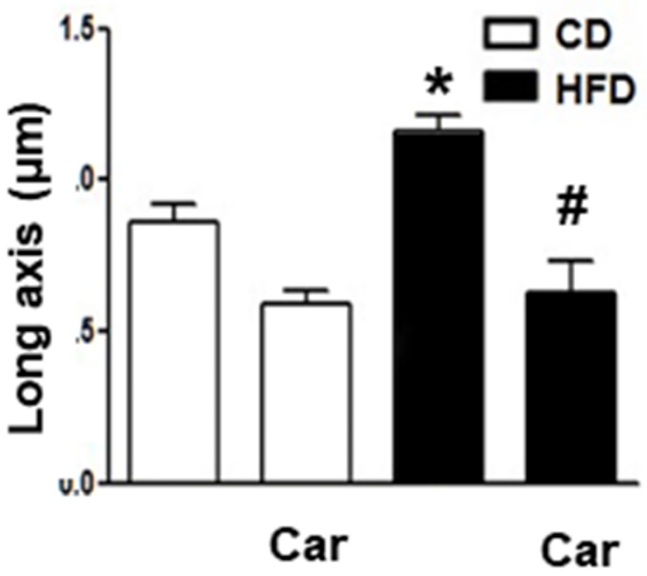

Mic
JEM-1400
Mag
30000 x
HV
80 kV
1 um
HFD
x30K

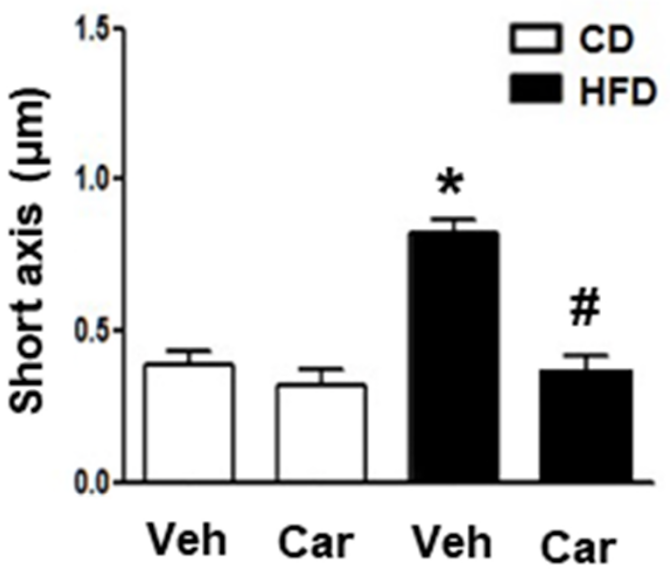

Mic
JEM-1400
Mag
30000 x
HV
80 kV
1 um
HFD+Car
x30K

Carnitine treatment removes abnormal mitochondrial in skeletal muscle



Carnitine treatment removes abnormal mitochondrial in skeletal muscle

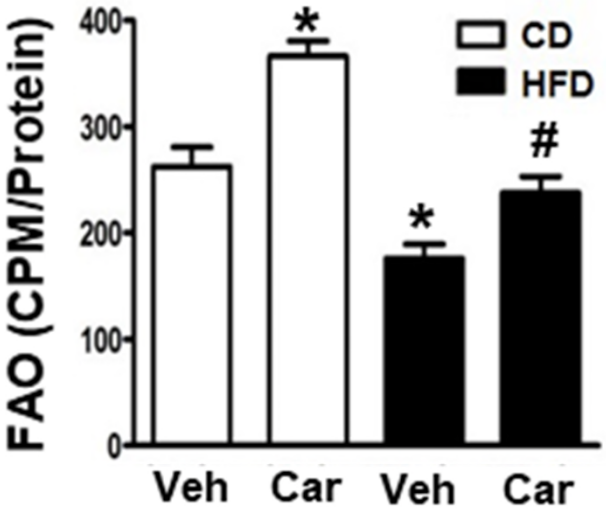


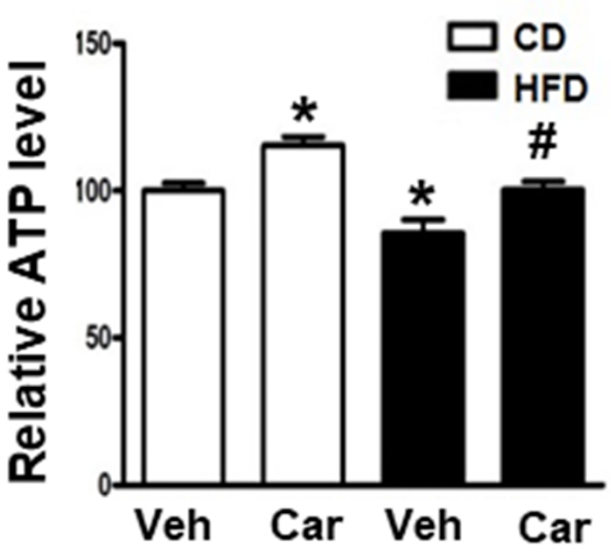


* $p < 0.05$ vs. CD+Veh

# $p < 0.05$ vs. HFD+Veh

Carnitine treatment recovers skeletal muscle mitochondrial dysfunction

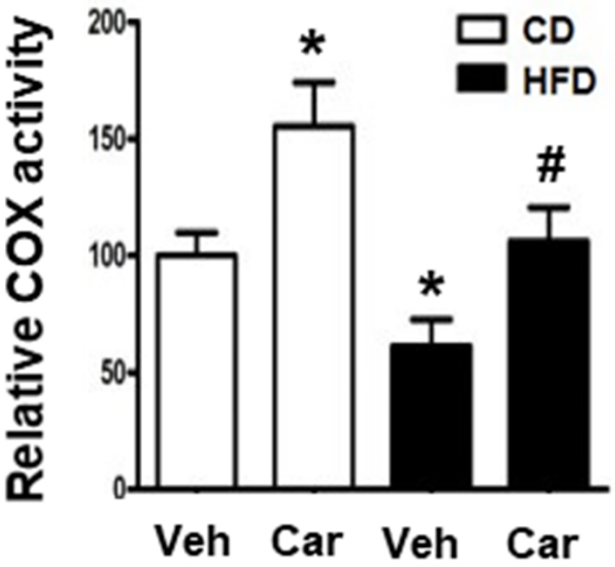


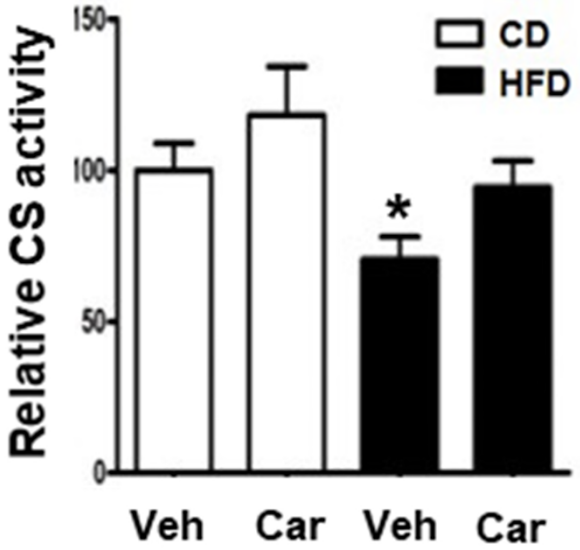


* $p < 0.05$ vs. CD+Veh

# $p < 0.05$ vs. HFD+Veh

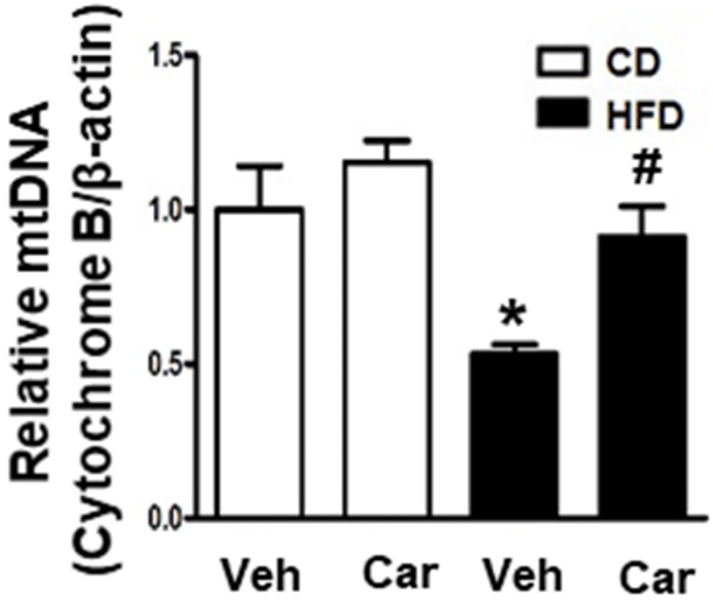
Carnitine treatment recovers skeletal muscle mitochondrial enzyme activity

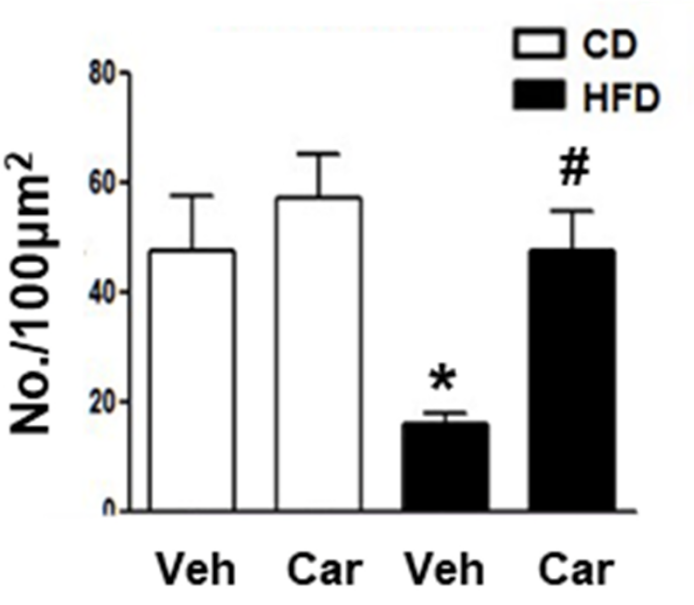




* $p < 0.05$ vs. CD+Veh
# $p < 0.05$ vs. HFD+Veh

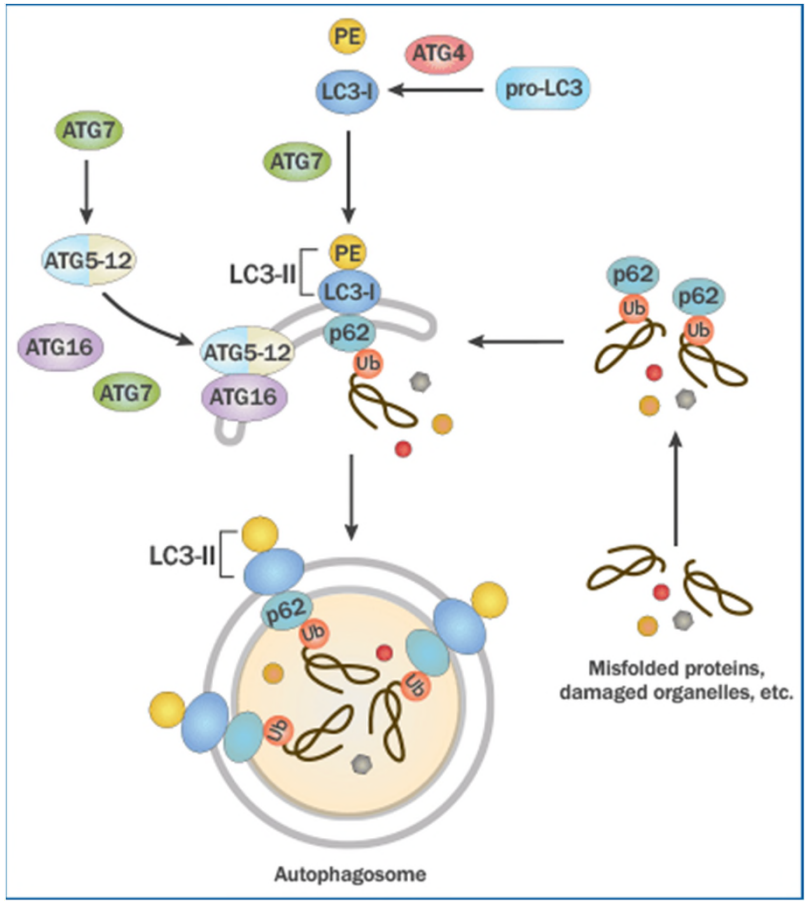
Carnitine treatment recovers skeletal muscle mitochondrial content





* $p < 0.05$ vs. CD+Veh
# $p < 0.05$ vs. HFD+Veh

CARNITINE TREATMENT AND AUTOPHAGY



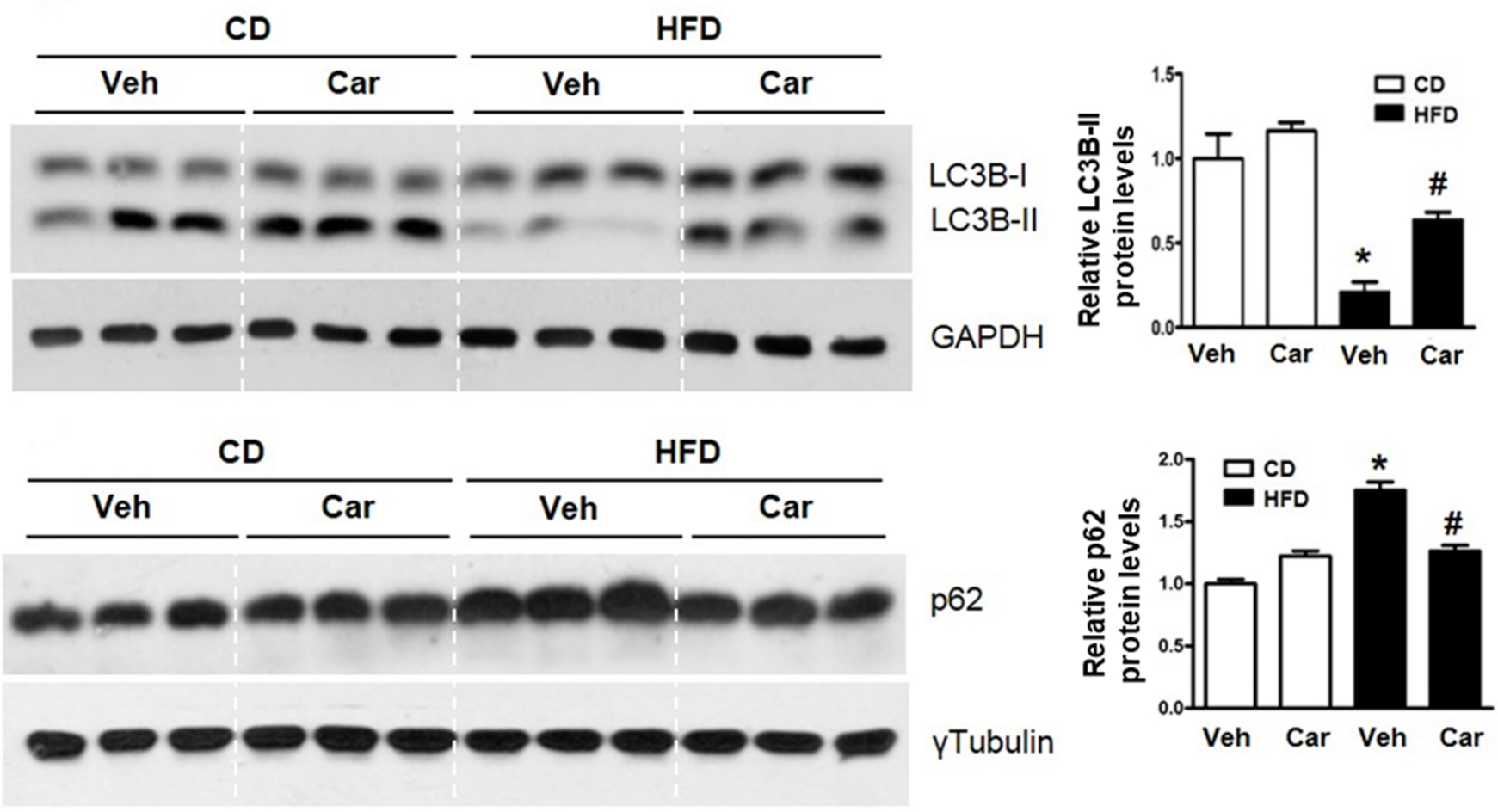
Marker of **autophagosome formation**

The conversion of **LC3-I** to **LC3-II** via phosphatidylethanolamine (PE) conjugation

Marker of **autophagic flux**

Measure **p62**/SQSTM1 degradation because p62/SQSTM1 binds LC3, thus serving as a selective **substrate of autophagy**.

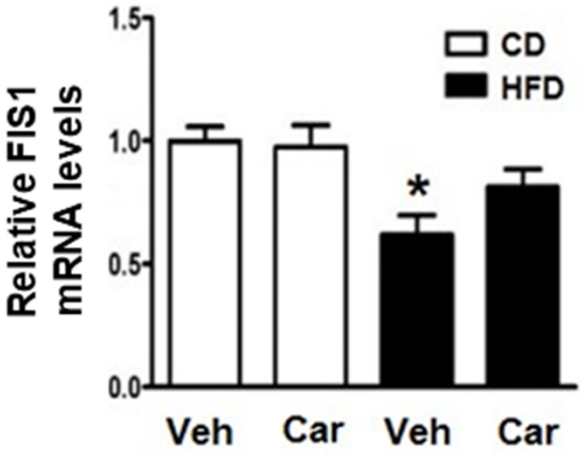
Carnitine activates autophagy suppressed by high-fat diet

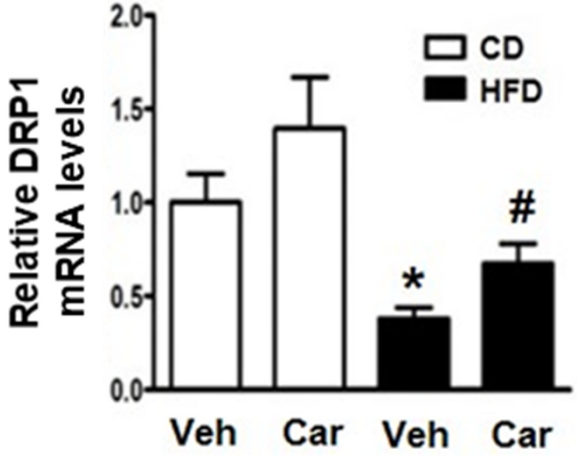


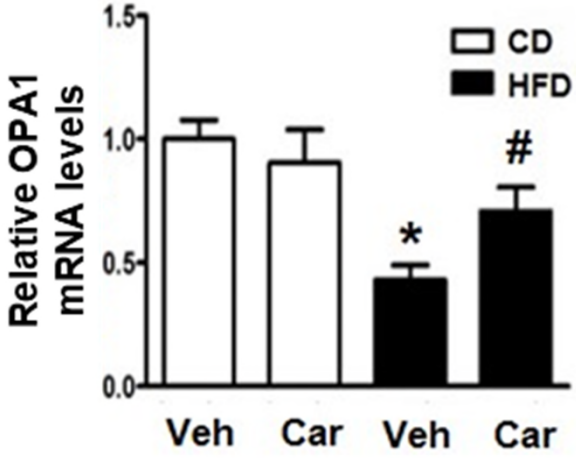
* $p < 0.05$ vs. CD+Veh

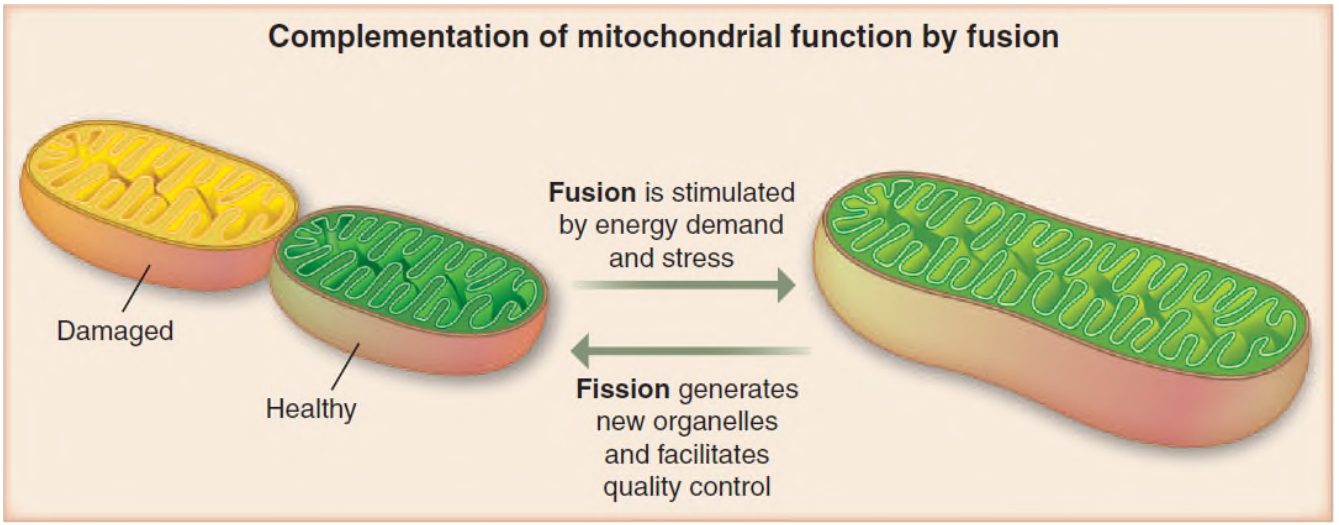
# $p < 0.05$ vs. HFD+Veh

Carnitine induces mitochondrial fission or fusion related genes suppressed by high-fat diet





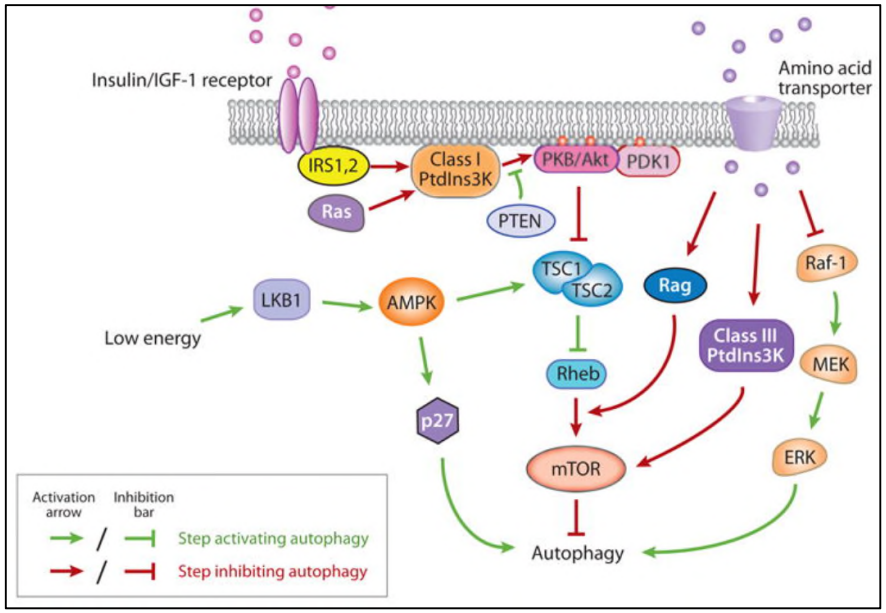


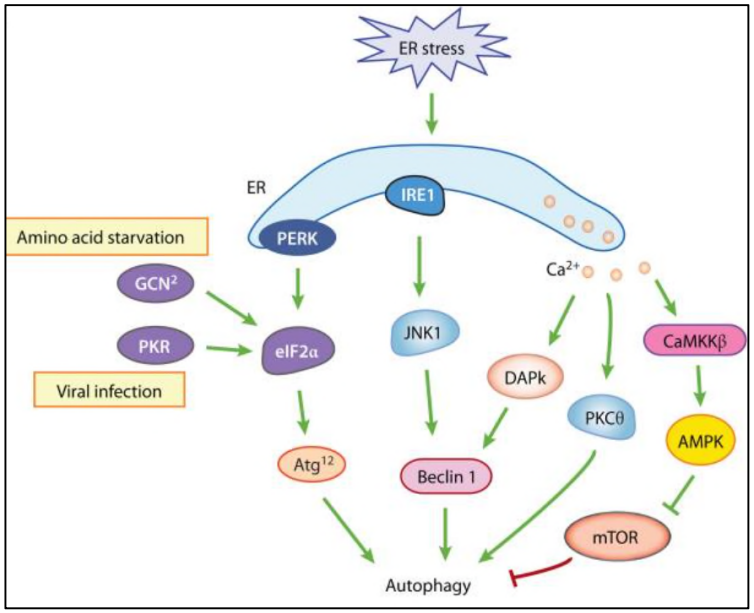


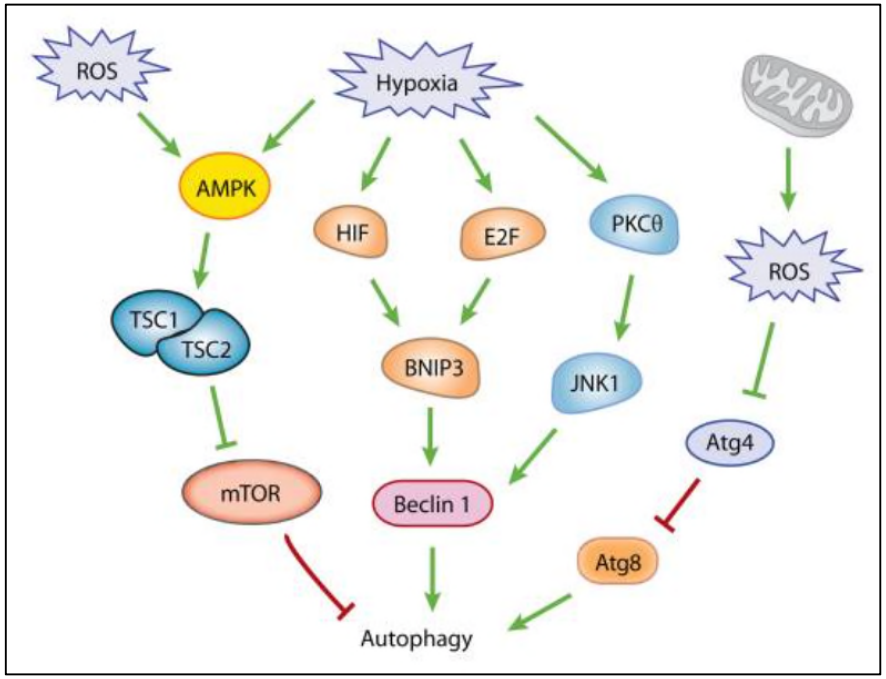
* $p < 0.05$ vs. CD+Veh

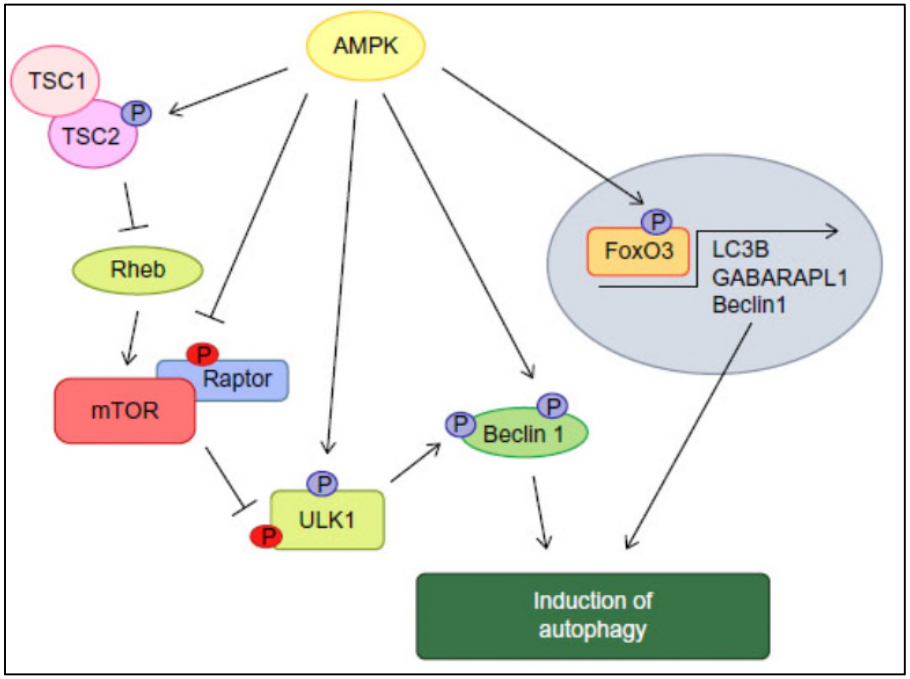
\# $p < 0.05$ vs. HFD+Veh

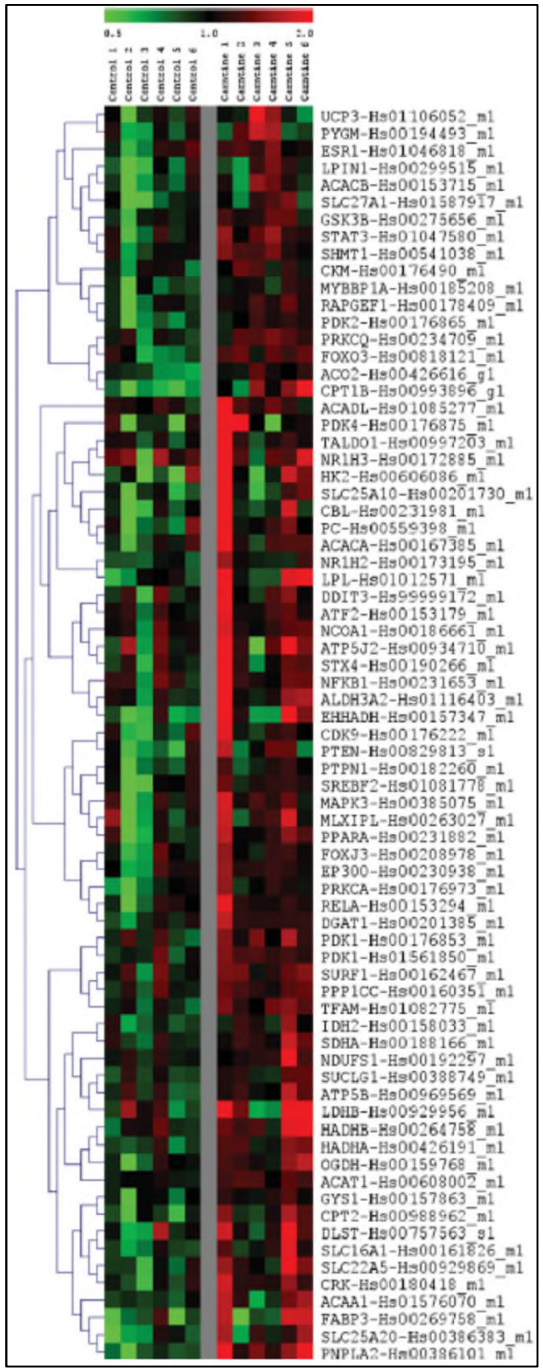
HOW DOES CARNITINE ACTIVATE AUTOPHAGY?


Insulin/IGF-1 receptor
Amino acid transporter
IRS1,2
Class I PtdIns3K
PKB/Akt
PDK1
Ras
PTEN
TSC1
TSC2
Rag
Raf-1
LKB1
AMPK
Low energy
Class III PtdIns3K
MEK
Rheb
p27
mTOR
ERK
Autophagy
Activation arrow
Inhibition bar
Step activating autophagy
Step inhibiting autophagy


ER stress
ER
IRE1
PERK
Amino acid starvation
GCN2
PKR
Viral infection
eIF2α
JNK1
Ca2+
DAPk
PKCθ
CaMKKβ
AMPK
Atg12
Beclin 1
mTOR
Autophagy


ROS
Hypoxia
AMPK
HIF
E2F
PKCθ
ROS
TSC1
TSC2
BNIP3
JNK1
Atg4
mTOR
Beclin 1
Atg8
Autophagy


AMPK
TSC1
TSC2
P
Rheb
FoxO3
LC3B
GABARAPL1
Beclin1
Raptor
mTOR
Beclin 1
ULK1
Induction of autophagy



12 weeks of twice daily oral ingestion in 12 males.

- 80 g of carbohydrate (Control)
- 80 g of carbohydrate containing 1.36 g L-carnitine (Carnitine).

Three most enriched pathways

- ✓ Insulin signaling
- ✓ peroxisome proliferator-activated receptor signaling
- ✓ fatty acid metabolism

The Journal of physiology. 2013;591:4655-66

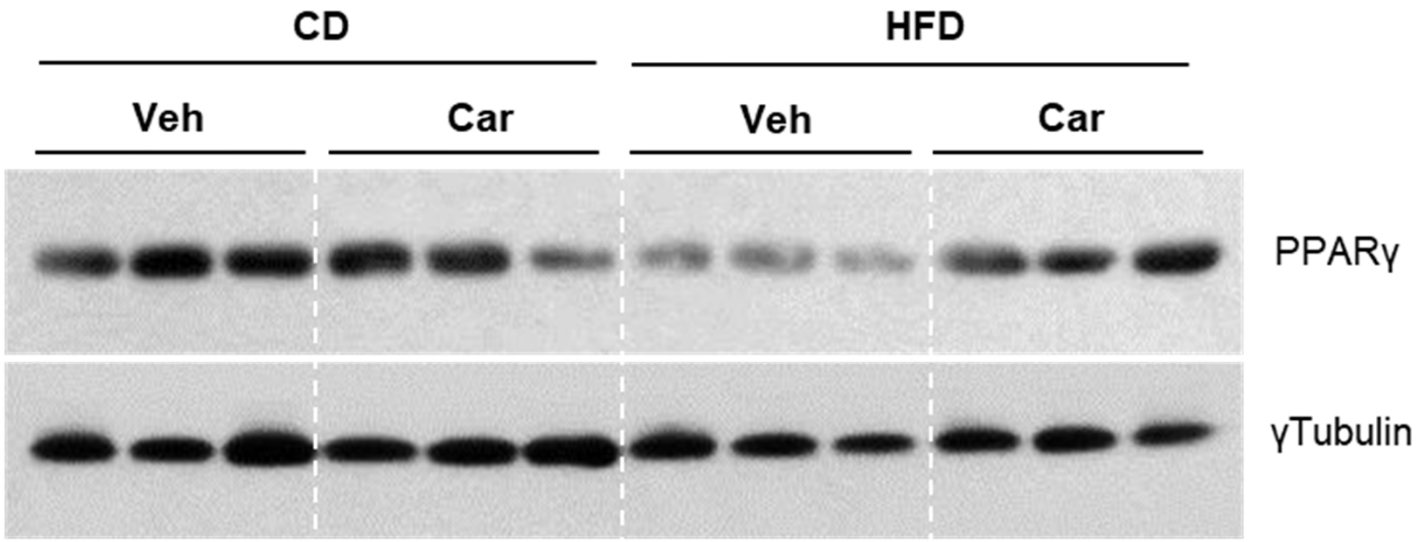
L-Carnitine Attenuates the Development of Kidney Fibrosis in Hypertensive Rats by Upregulating PPAR-γ

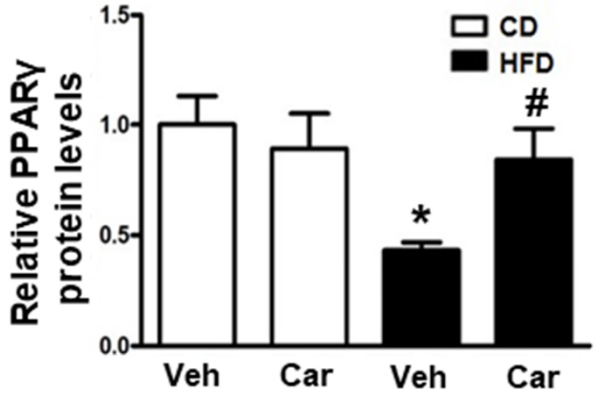
Sonia Zambrano,[1] Antonio J. Blanca,[1] María V. Ruiz-Armenta,[1] José L. Miguel-Carrasco,[1] Miguel Arévalo,[2] Alfonso Mate,[1] and Carmen M. Vázquez[1]

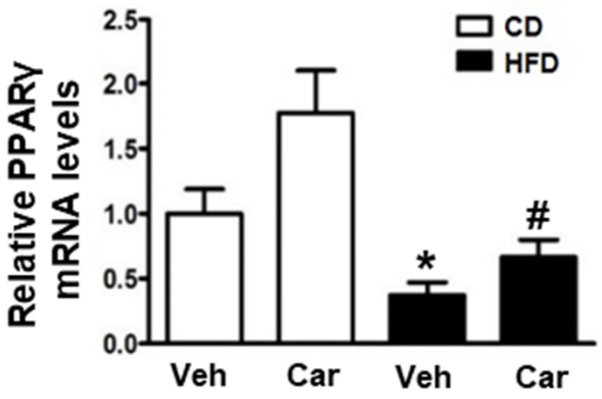
Am J Hypertens. 2014 Mar;27(3):460-70.

PPARγ agonists have been reported to activate autophagy in vascular smooth muscle (Molecular biology reports. 2015;42:179-86), neurons (PloS one. 2013;8:e55080), and cancer cells (Int J Biochem Cell Biol. 2009 Nov;41(11):2334-42; Exp Cell Res. 2011 Jun 10;317(10):1397-410).

Carnitine induces PPARγ suppressed by high-fat diet



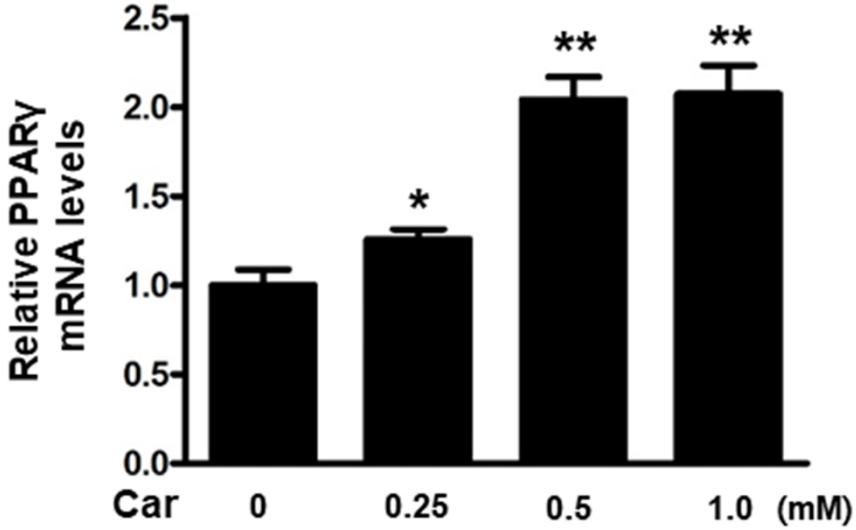




* $p < 0.05$ vs. CD+Veh

\# $p < 0.05$ vs. HFD+Veh

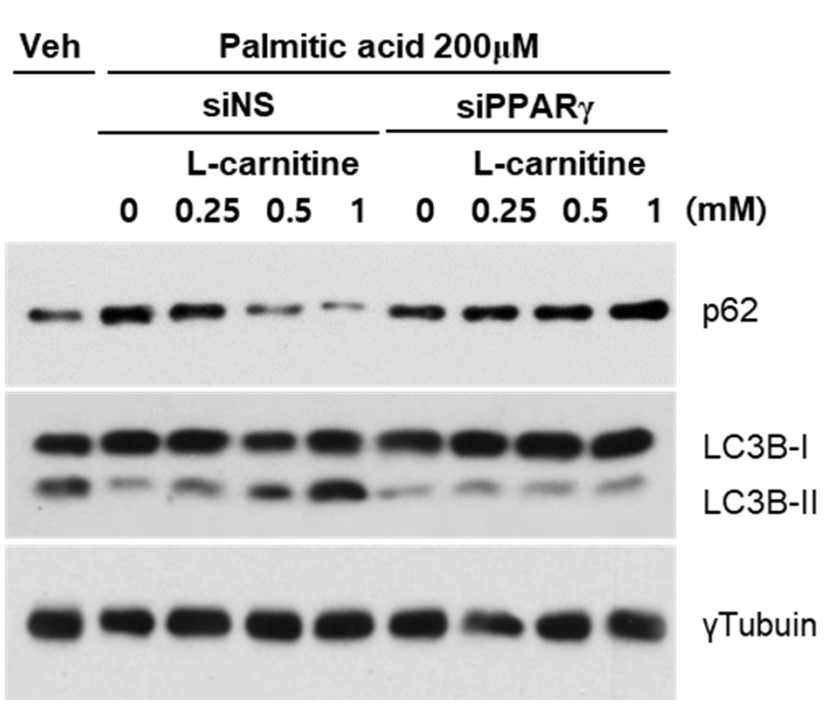
Carnitine treatment increases PPARγ mRNA expression in palmitate-treated C2C12 myotubes

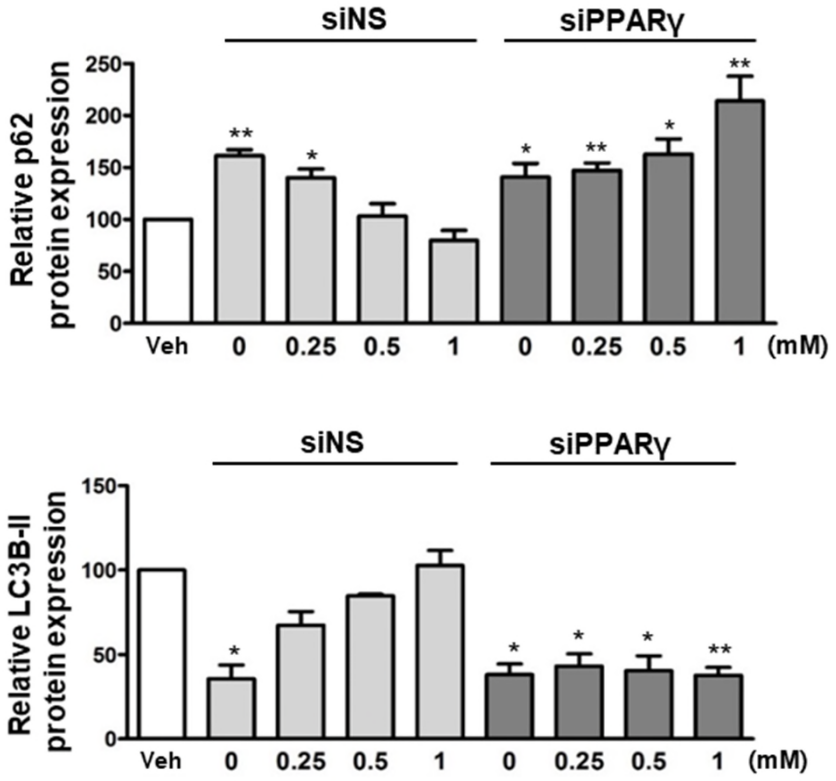


Preincubation with carnitine 2h before palmitate treatment (200 μM for 72 h) dose-dependently increased PPARγ mRNA expression in C2C12 myotubes.

*p < 0.05 vs control
**p < 0.01 vs control

Carnitine activates autophagy through PPARγ in palmitate-induced insulin resistance





*p < 0.05 vs control
**p < 0.01 vs control

Summary

- High-fat diet produces malfunctioning mitochondria in skeletal muscle of mice.
- The **activation of autophagy** may be a novel mechanism by which carnitine treatment **enhances mitochondrial function**.
- The autophagy activation by carnitine occurs through **PPARγ**.
- However, the molecular link between carnitine and PPARγ and how PPARγ activates autophagy in skeletal muscle require further investigations.

Acknowledgement

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