Regulation of Mitochondrial Biogenesis and Function

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David Murdock Dole Professor
Distinguished Investigator
Endocrinology, Diabetes and Nutrition

Disclosure

No Relevant Financial Relationships

Off-Label/Investigational Uses

None

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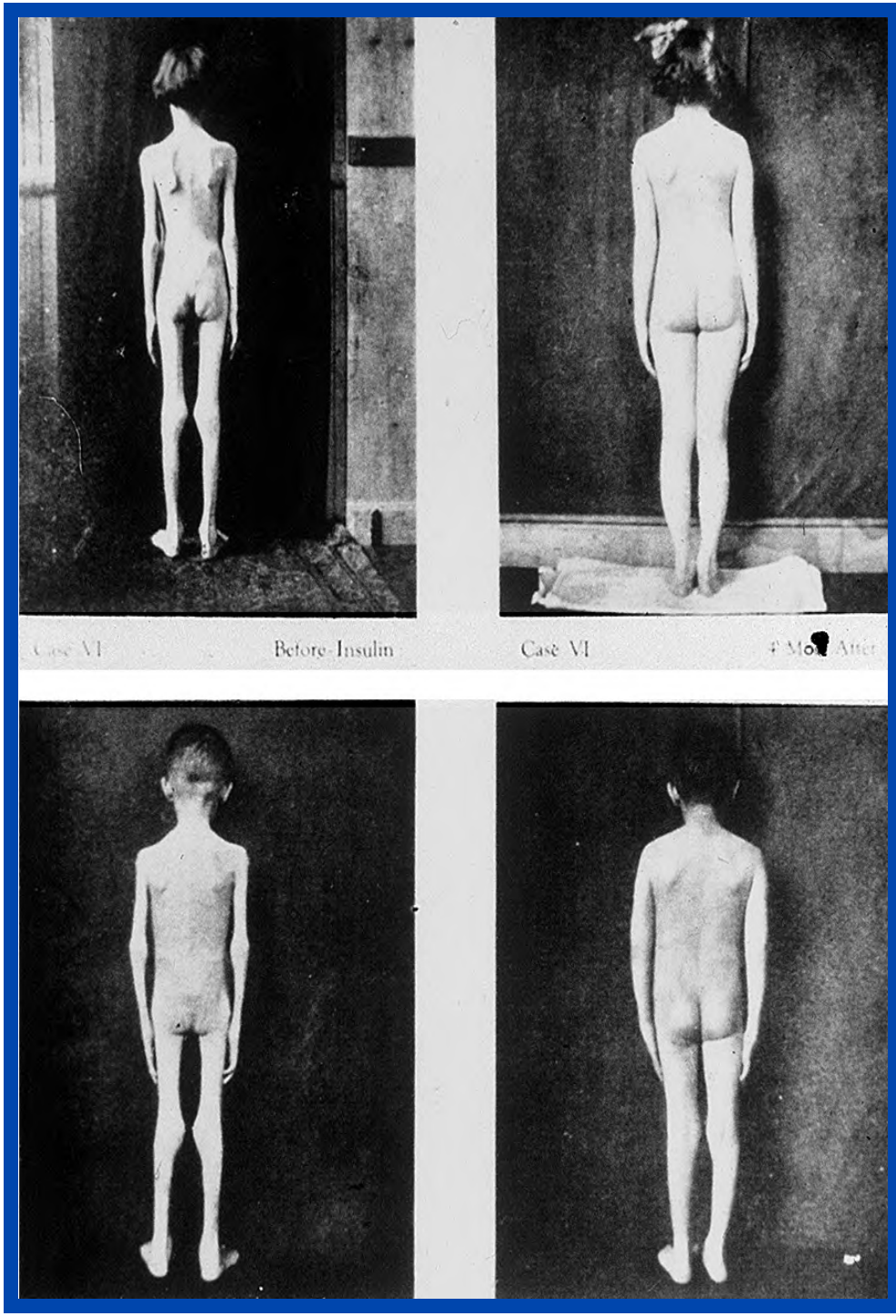
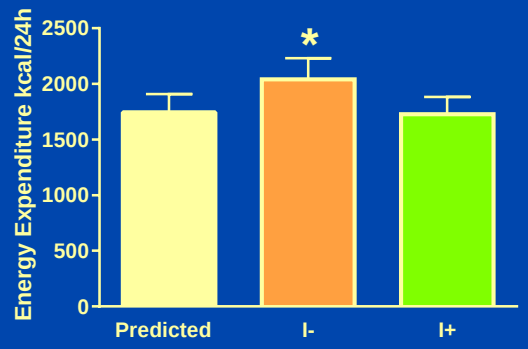
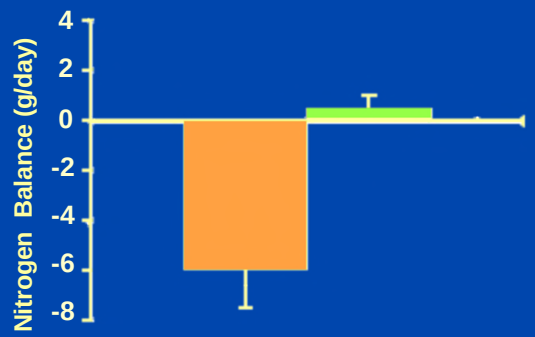


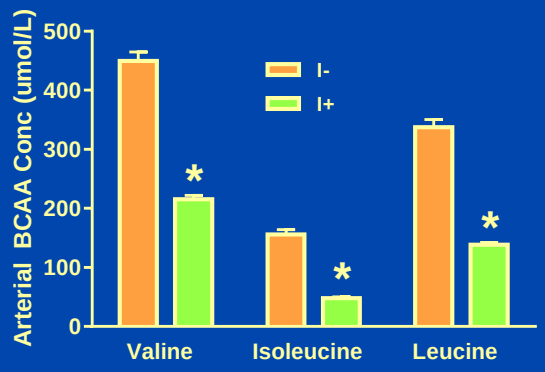
Type 1 Diabetics



Nair *et. al.*, 1984, Diabetologia. 27:13-16.



Atchley *et. al.*, 1933, J. Clin Invest. 12:297.



Nair *et. al.*, 1995, J. Clin Invest. 95(6):2926-37.

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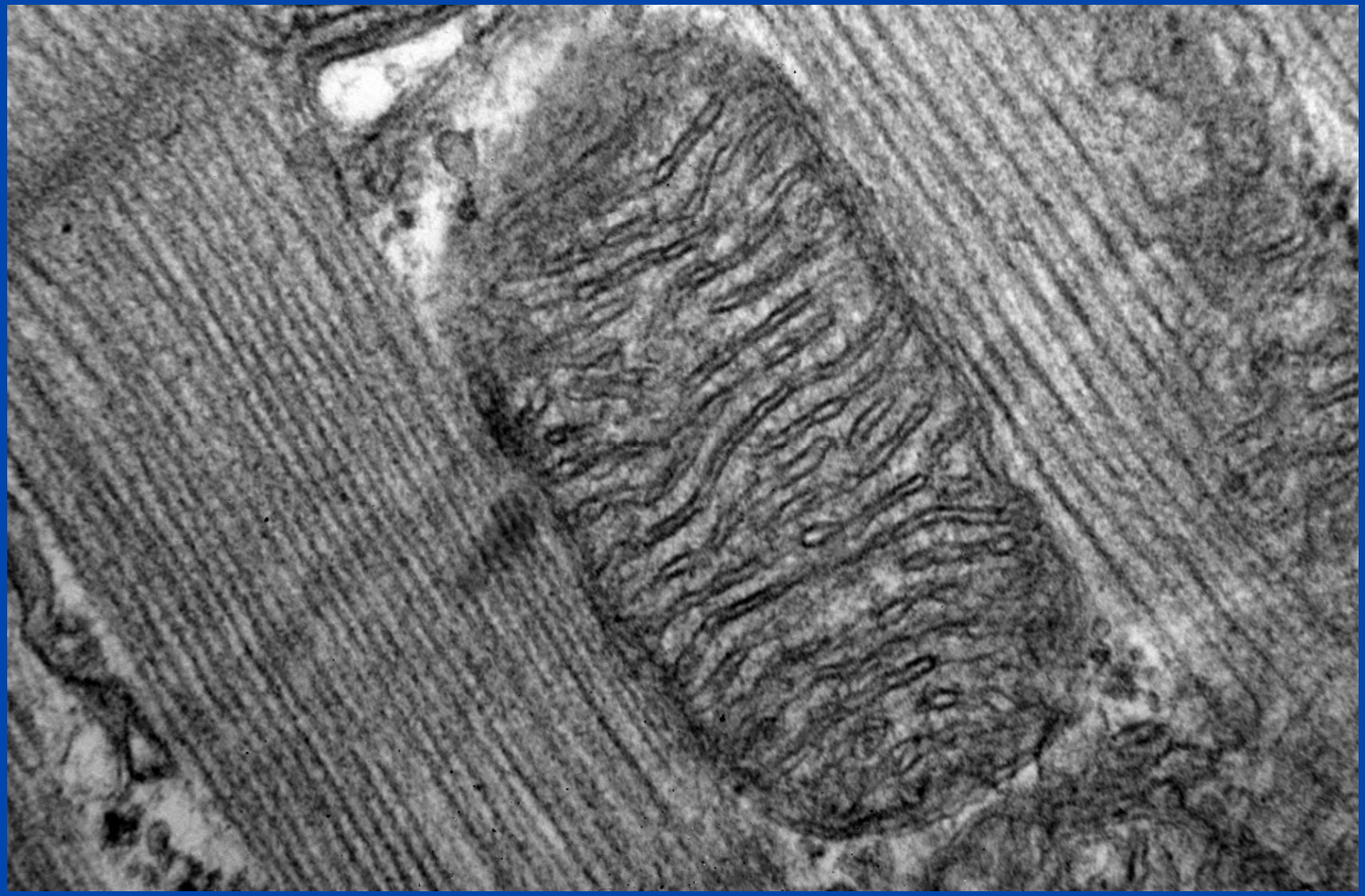

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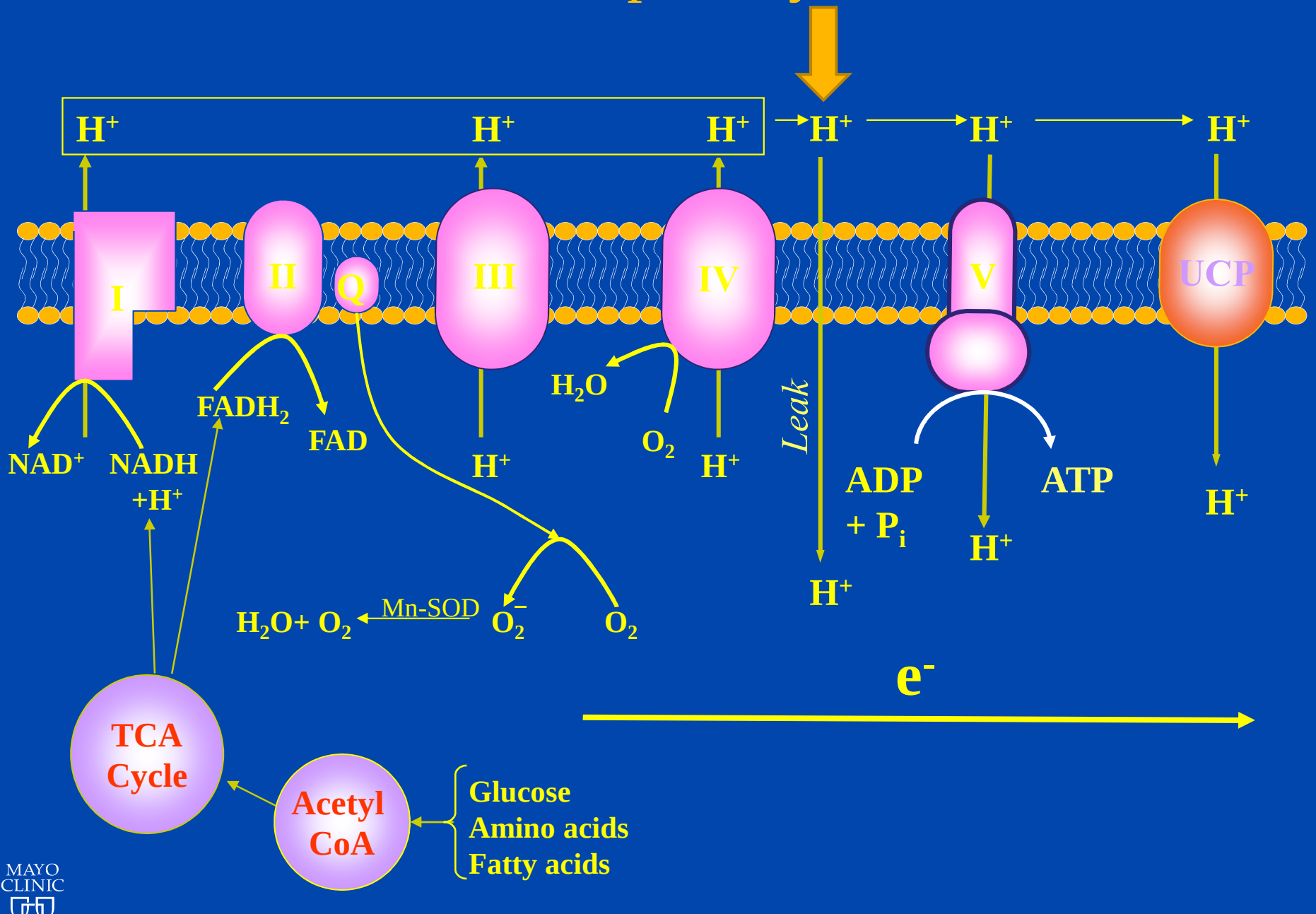
Mitochondrial Respiratory Chain



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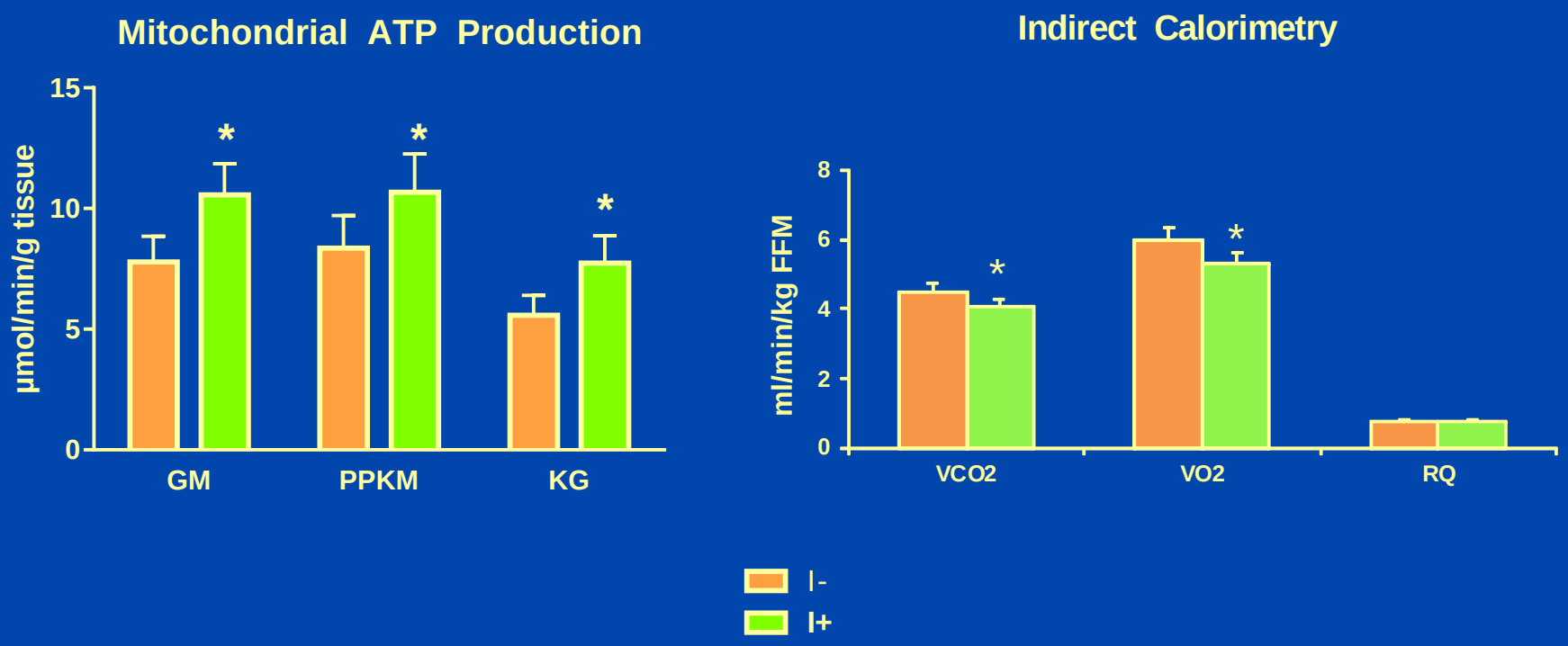
Insulin Deprivation In T1DM Leads to Reduced Mitochondrial ATP Production with Increased VO_2

Inefficient Mitochondrial Coupling



Karakelides *et. al.*, 2017 Diabetes. 56(11): 2683-2689.

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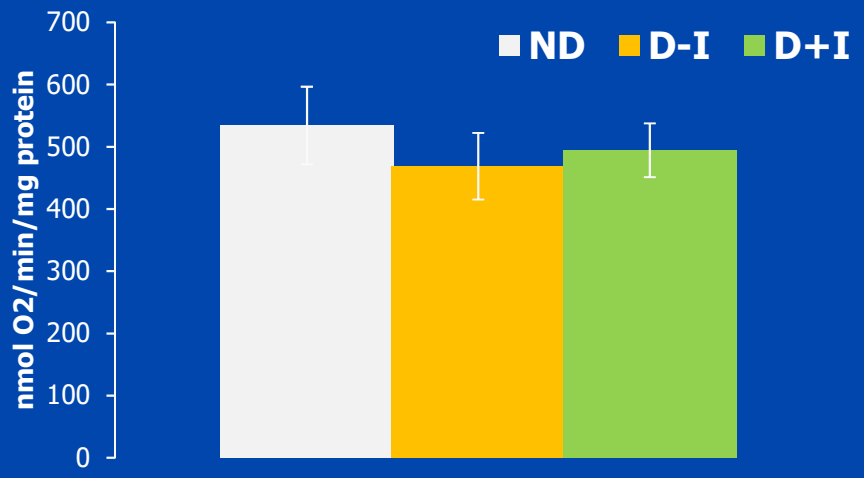
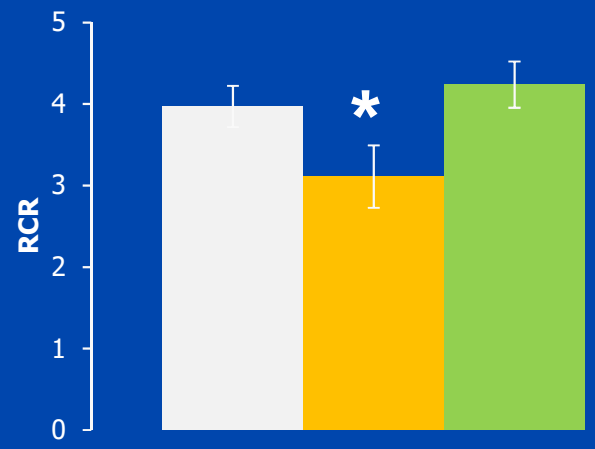
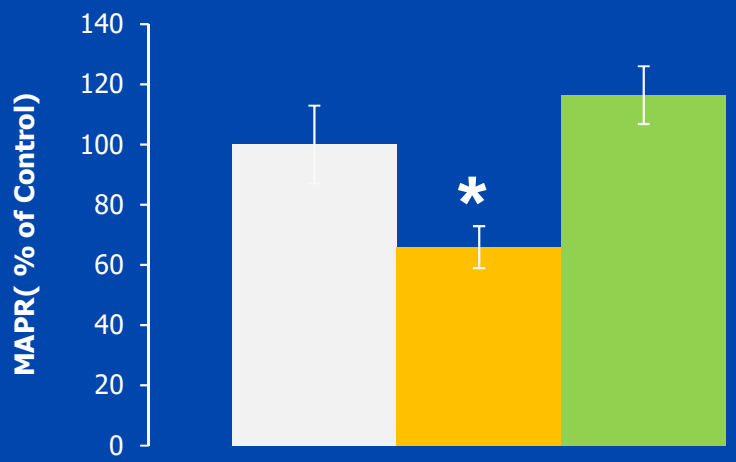
Muscle mitochondrial Function in Diabetic Mice



Oxygen Flux



RCR –Coupling Efficiency



Maximal ATP production Rate

ROS Emission

Zabielski P et al, *Diabetes* 2016

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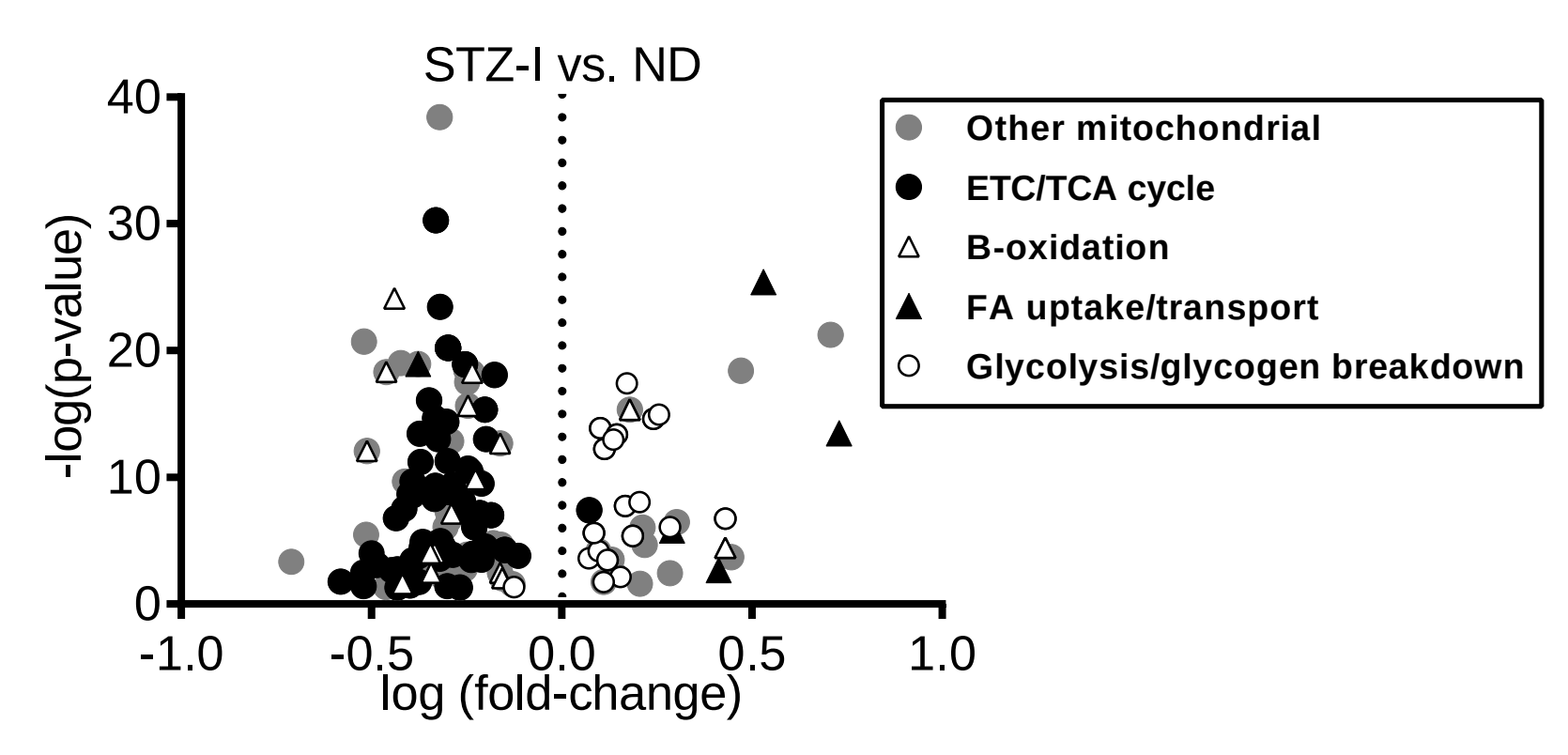
Reduction of Mitochondrial Proteins On insulin withdrawal in STZ Mice



- Zabielski P et al Diabetes 2016

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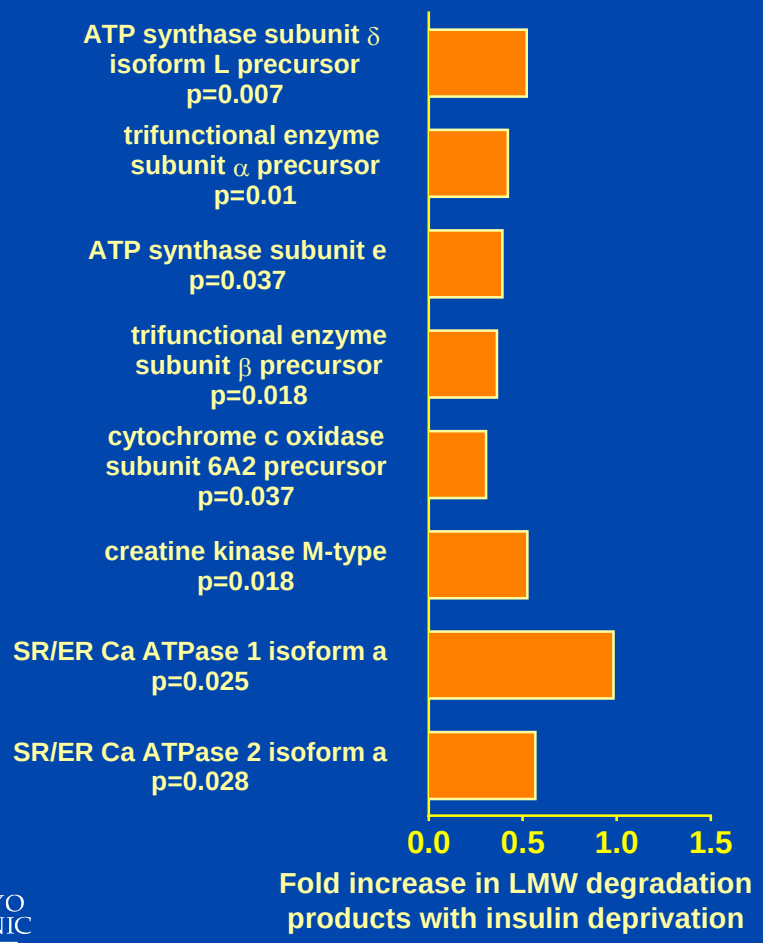
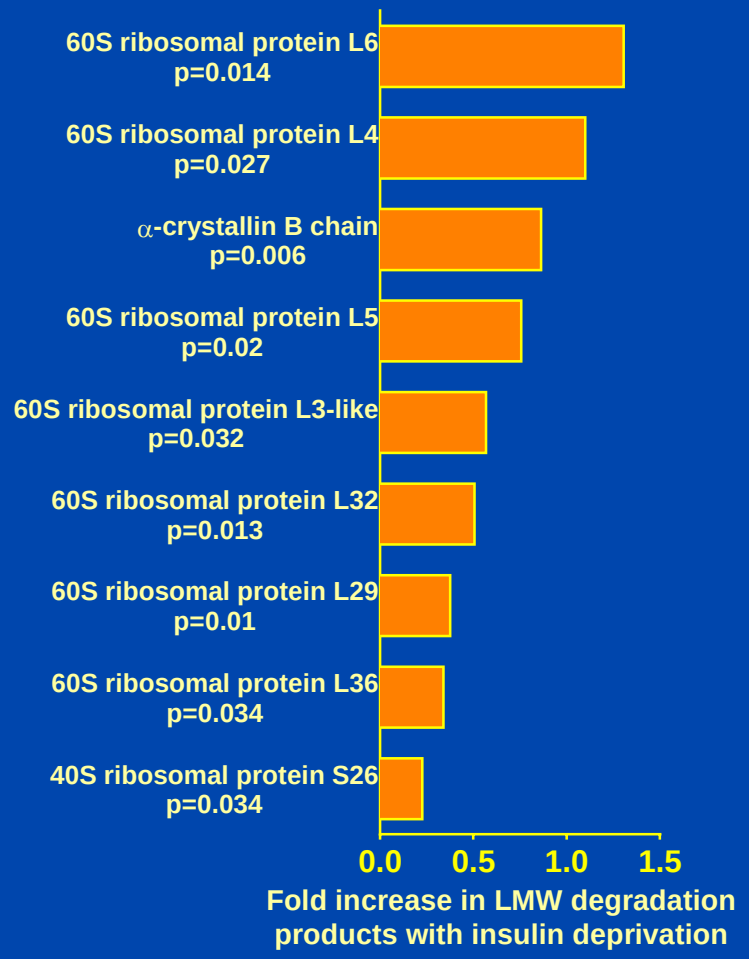
Proteins Involved In Mito Function and Protein Synthetic Machinery Are Degraded During Insulin Withdrawal in T1DM – Robinson ML et al 2016

Energy Homeostasis



Translational Machinery



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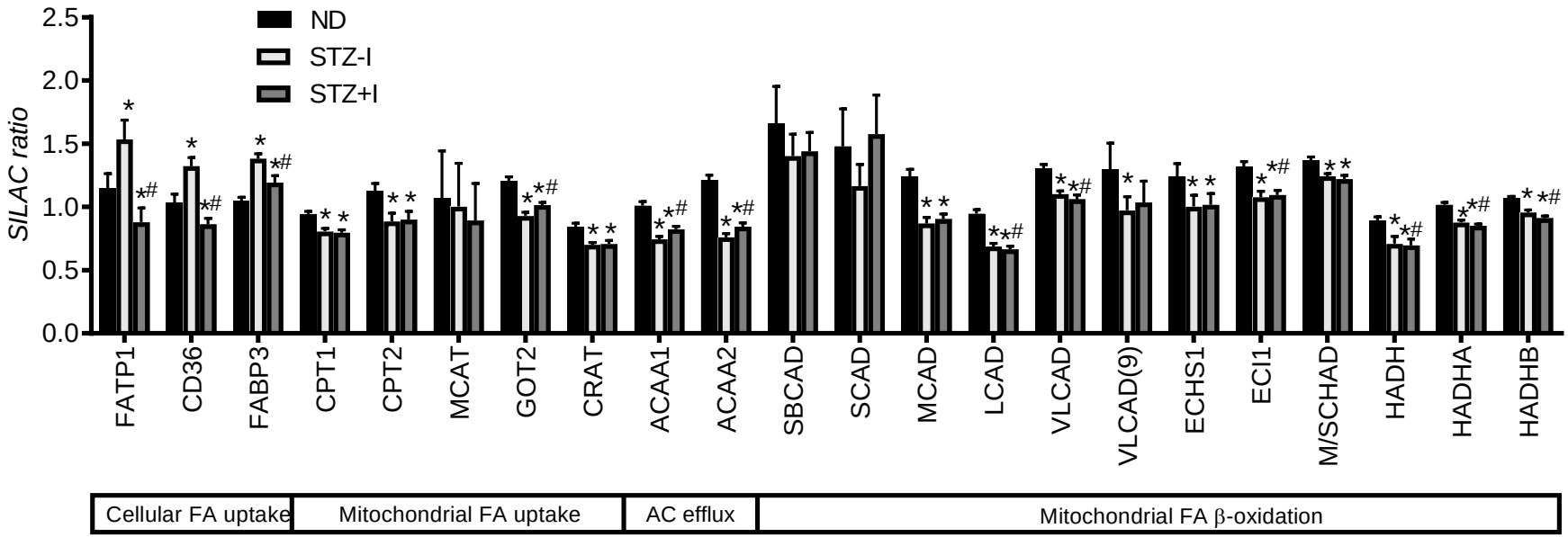
Fatty acid uptake proteins increase and β-oxidation proteins decrease with insulin withdrawl

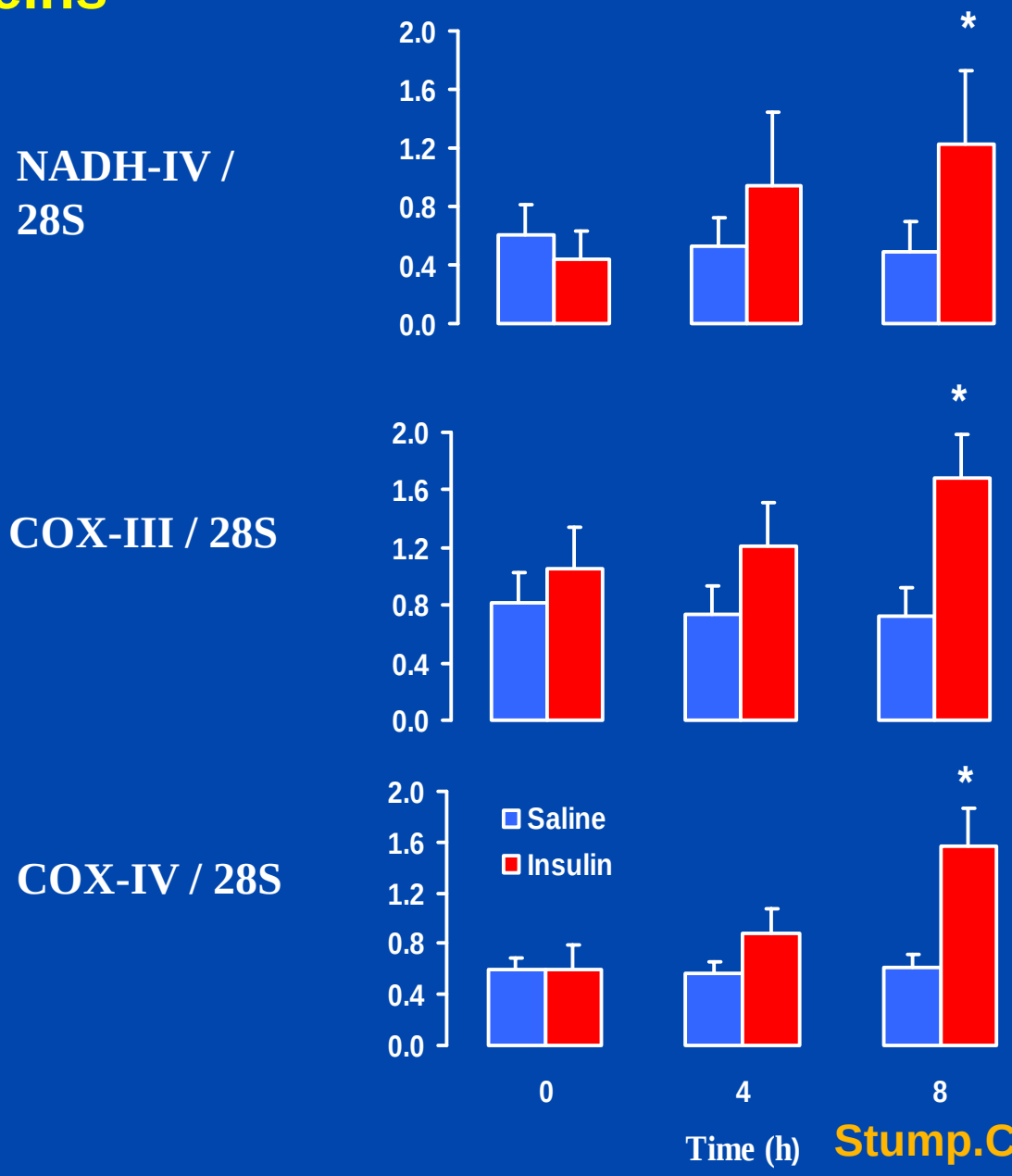


- **FABP1&3, CD36 increased with insulin with drawl and proteins involved in FA uptake and β-oxidation pathway decreased, causing a mismatch between fuel intake and oxidation**
- **Insulin supplementation reversed increased in FA uptake proteins; However, not all proteins in β-oxidation pathway were normalized**

- Zabielski P et al Diabetes 2016

- **Insulin and Amino Acids As Regulators of Mitochondrial Biogenesis and Function**

Insulin + AA Effect on mRNAs Encoding Mitochondrial Proteins



Stump.C et al PNAS 100:7996, 2003

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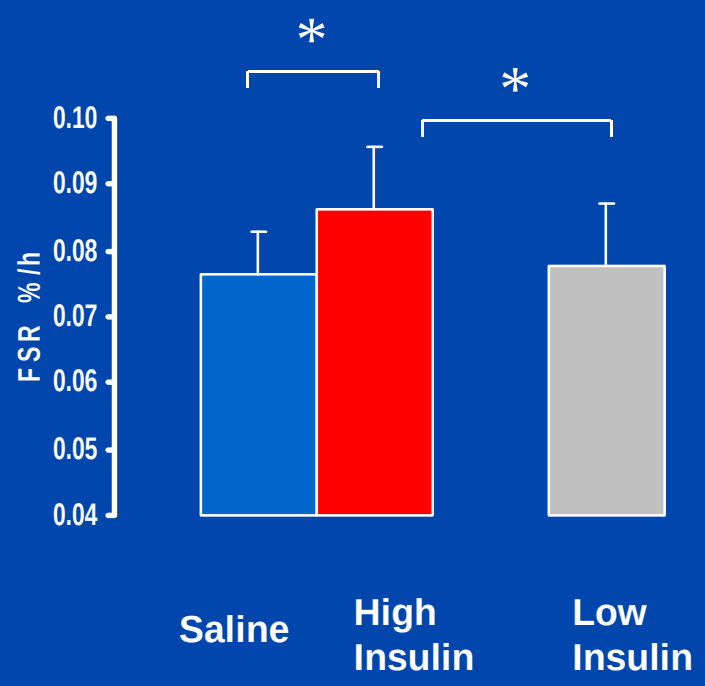
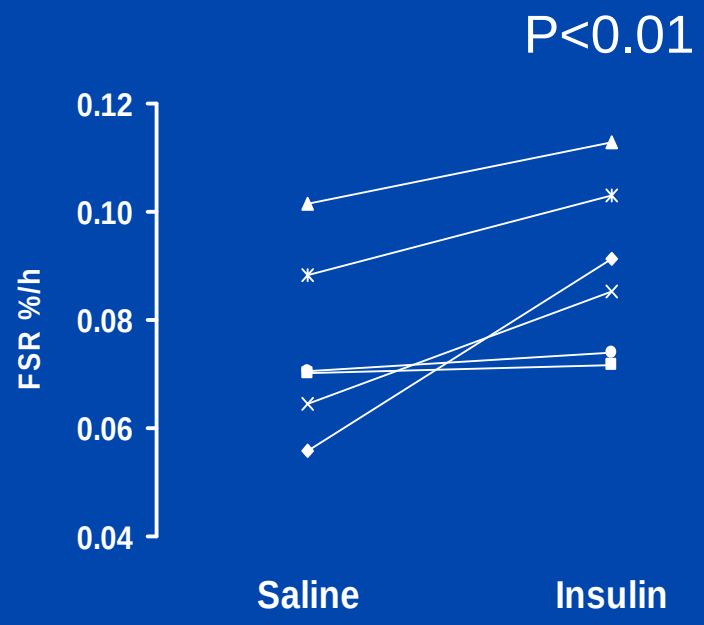
Mitochondrial Protein Synthesis Rate





Stump et al PNAS 100:7996, 2003

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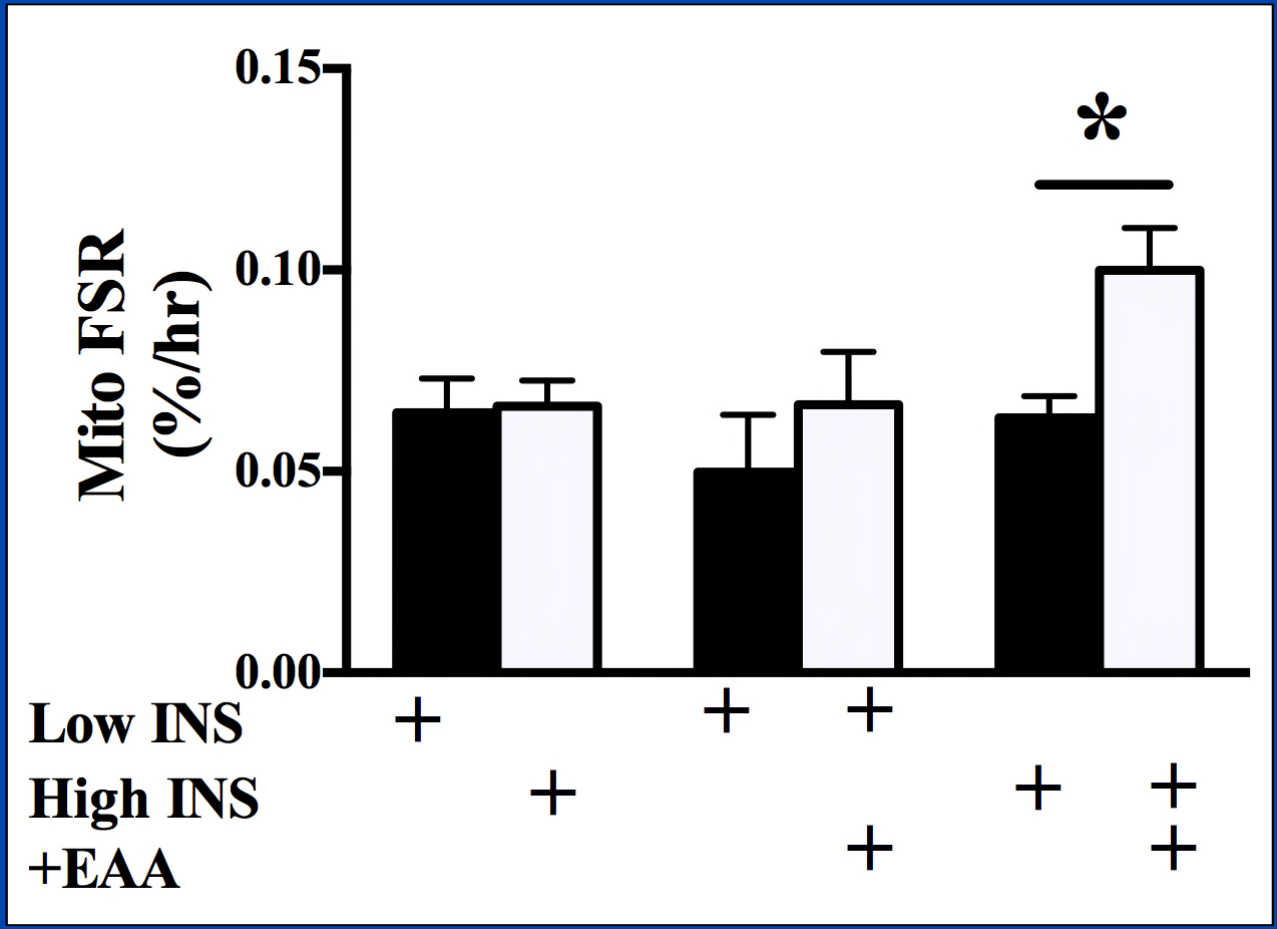
Mitochondrial Protein Synthesis



Robinson MM *et al*. JCEM 2014

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ENERGY METABOLISM IN Insulin Deficient State

Insulin deprivation in T1 D diabetic human and diabetic mice causes mismatch O_2 consumption and ATP Production in Sk. Muscle - uncoupling

Muscle ROS production is high during insulin deficiency

Muscle mito protein degradation increases

Insulin deficiency reduces mito protein synthesis

Insulin and AA together enhances mitochondrial biogenesis at transcriptional and translational level

Muscle mito protein content declines

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- **Insulin-Deficiency:**
- **Reduces Mitochondrial Biogenesis**
- **Increases Mitochondrial Protein Degradation**
- **Reduction of Mitochondrial Proteome**
- **Inefficient Fuel Metabolism with Increased Fuel Oxidation But Reduced ATP Production Mitochondrial Function and Increased ROS Emission**

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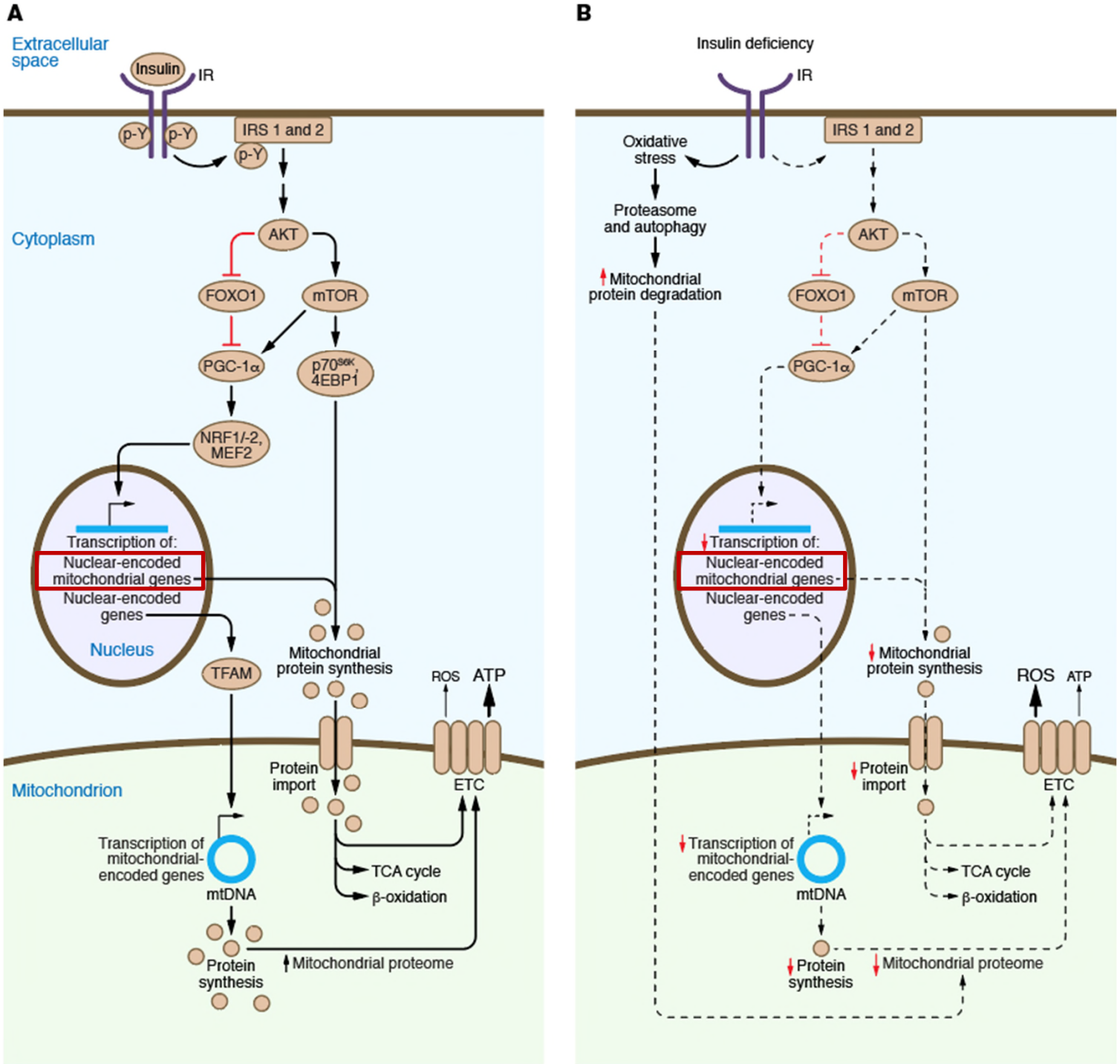
A
Extracellular space
Insulin
IR
p-Y
p-Y
IRS 1 and 2
p-Y
Cytoplasm
AKT
FOXO1
mTOR
PGC-1α
p70S6K, 4EBP1
NRF1/-2, MEF2
Transcription of:
Nuclear-encoded mitochondrial genes
Nuclear-encoded genes
Nucleus
TFAM
Mitochondrial protein synthesis
ROS
ATP
Protein import
ETC
Mitochondrion
Transcription of mitochondrial-encoded genes
mtDNA
TCA cycle
β-oxidation
Protein synthesis
Mitochondrial proteome
B
Insulin deficiency
IR
IRS 1 and 2
Oxidative stress
Proteasome and autophagy
Mitochondrial protein degradation
AKT
FOXO1
mTOR
PGC-1α
Transcription of:
Nuclear-encoded mitochondrial genes
Nuclear-encoded genes
Mitochondrial protein synthesis
ROS
ATP
Protein import
ETC
Transcription of mitochondrial-encoded genes
mtDNA
TCA cycle
β-oxidation
Protein synthesis
Mitochondrial proteome

- **Reduced Mitochondrial Capacity and Inefficient Mitochondrial Respiration have detrimental Effects on Human Health**

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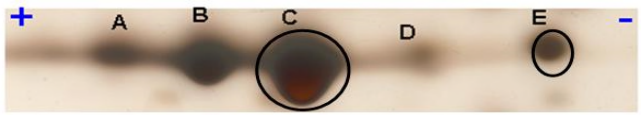
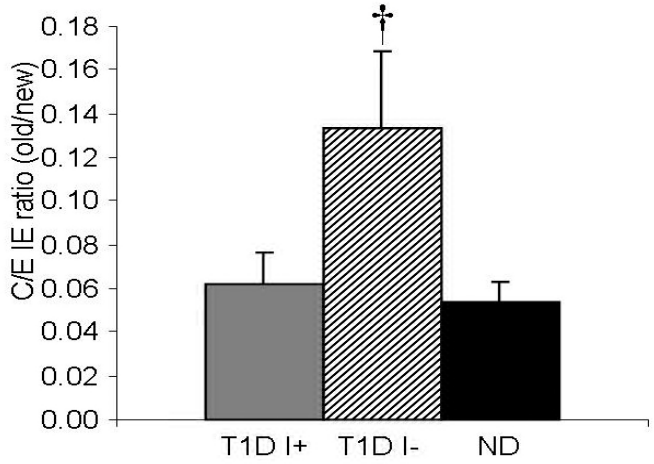
Identification of De Novo Synthesized and Relatively Older Proteins: Accelerated Oxidative Damage to De Novo Synthesized Apolipoprotein A-1 in Type 1 Diabetes During Insulin Deprivation

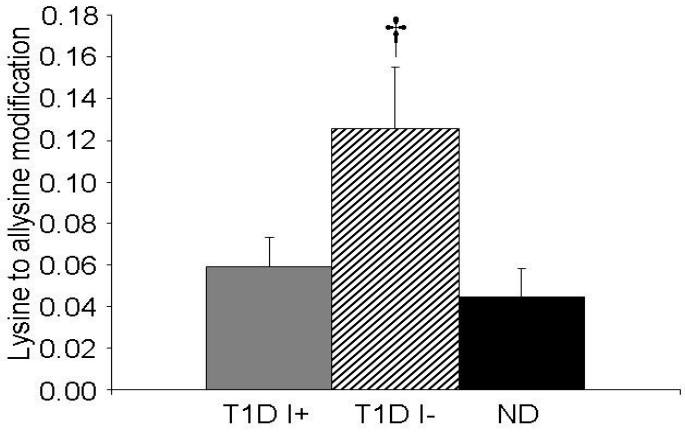
Jaleel A et al; Diabetes; 59(10), 2010, 2366–2374

Accelerated Oxidative Damage to De Novo Synthesized Apolipoprotein A-1 in Type 1 Diabetes During Insulin Deprivation





The ratio of isotopic enrichment in older versus newer (C/E) ApoA-1 spots



The ratio of allysine to lysine was higher in ApoA-1

-measure carbonylation (oxidation)

Altered High Density Lipoprotein Functions and Proteomic Modifications in Type 1 Diabetes Mellitus

Manjunatha Shankarappa, Klaus Distelmaier, Surendra Dasari
Yogish C Kudva, & K Sreekumaran Nair

Division of Endocrinology, Mayo Clinic College of Medicine,
Rochester MN 55905, USA

Hypothesis

- HDL of T1DM are dysfunctional because of irreversible modifications of protein constituents despite normal "quantity"

- Important question in view of normal or high HDL-C in T1D irrespective high CV mortality

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High Density Lipoprotein Functions and Proteomic Modifications in T1D

Subject Characteristics

	T1D-I	Controls	T1D+I	Controls
Age (Years)	33.6±3.34	35.2±3.40	34.5±3.22	34.0±3.14
BMI (Kg/m^2)	24.7±0.87	25.3±0.79	25.8±0.81	25.6±0.91
HbA1C (%)	**10.0±0.31***	5.3±0.07	**6.3±0.12***	5.3±0.08
HDL-C (mg/dl)	44.4±1.71	44.4±3.28	42.5±3.90	41.1±2.76

*** p<0.05 compared to respective matched control group**

Manjunatha S et al 2016

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HDL Functions and Proteomic Modifications in T1D

HDL Function Tests

	T1D-I vs Controls (Mean±SEM)			T1D+I vs Controls (Mean±SEM)		
	T1D-I	Controls	p value	T1D+I	Controls	p value
Cholesterol Efflux Capacity (%)	**16.9±0.20**	17.9±0.28	**0.011**	**17.1±0.26**	18.2±0.38	**0.038**
Antioxidative Capacity (RFU)	**209.6±10.0**	243.0±10.5	**0.025**	**192.9±23.9**	248.5±6.2	**0.040**
PON1 activity (U/ml)	5.45±0.34	6.11±0.74	0.39	5.42±0.29	5.04±0.28	0.38

Cholesterol Efflux Capacity and Anti-oxidative capacity of HDL from both poorly controlled and well controlled diabetic people were significantly lower than non-diabetic controls

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Manjunatha S et al 2016

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HDL Functions and Proteomic Modifications in T1D

	T1D-I vs Controls (Mean±SEM)			T1D+I vs Controls (Mean±SEM)		
	T1D-I	Controls	p value	T1D+I	Controls	p value
Apo A4 (Oxidation)	**21.26±0.1**	20.42±0.34	**0.041**	**21.11±0.49**	16.81±3.39	**0.025**
Apo D (Amadori)	**22.4±0.16**	17.3±3.47	**0.002**	**22.0±0.45**	17.7±3.54	**0.041**
Apo A4 (Deamidation)	**137.09±0.4**	133.7±1.29	**0.025**	134.94±1.62	120.9±10.0	**0.064**
Apo E (Oxidation)	263.5±23.1	245.2±23.8	0.58	**283.3±24.2**	223.3±15.8	**0.041**
Apo A1 (Amadori)	386.4±22.2	327.6±24.9	0.13	**329.4±19.2**	267.2±20.1	**0.064**
Apo J (Deamidation)	156.5±11.6	164.7±11.7	0.81	**122.8±3.11**	100.3±16.3	**0.092**

Evidence of Irreversible PTMs of the functional Apo proteins of HDL in T11diabetic people

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Manjunatha S et al 2016

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Conclusion

In both well controlled and poorly controlled T1DM have reduced HDL functions that could be attributed to irreversible damage to the HDL proteins rendering them dysfunctional – Potential explanation for increased CVD

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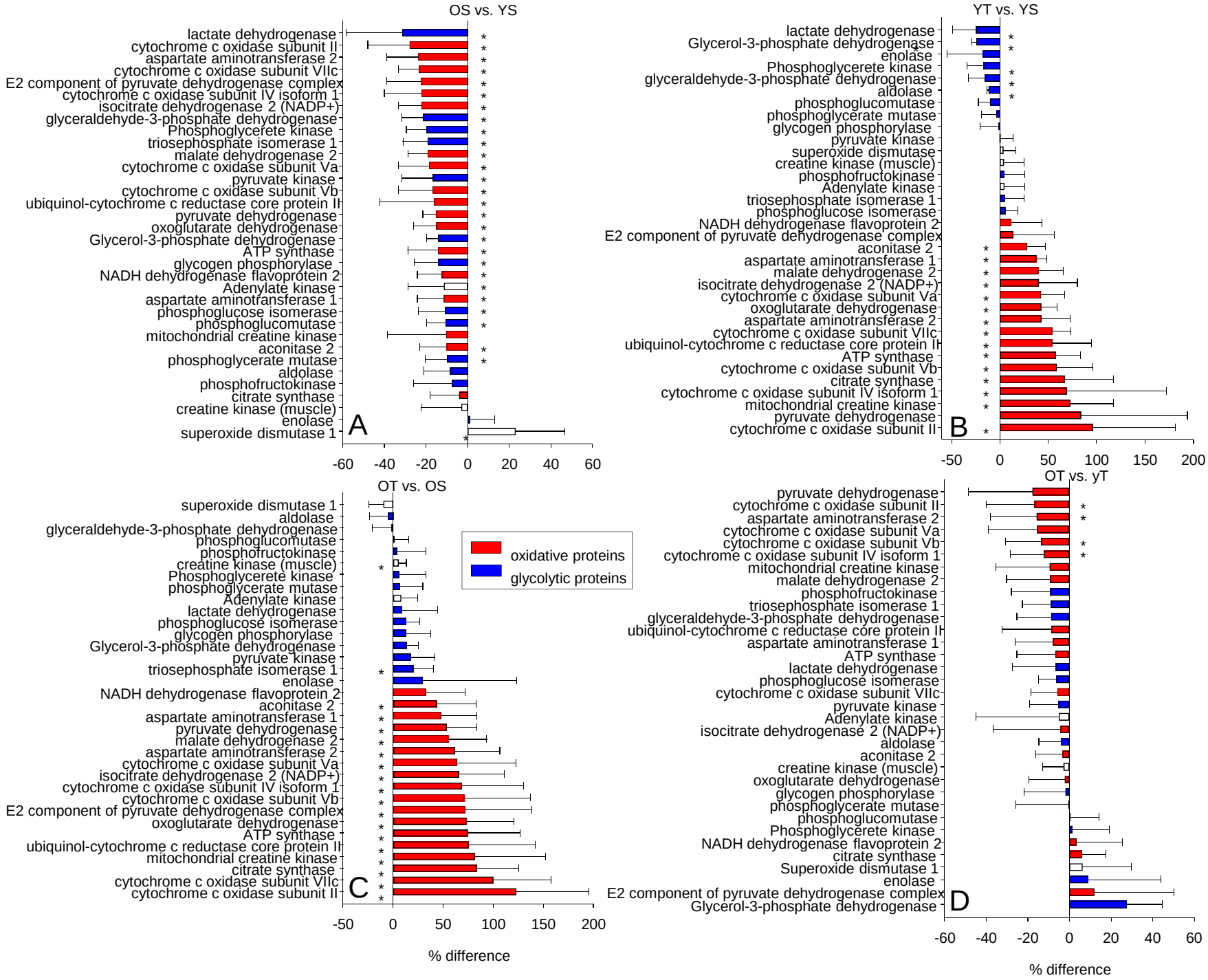
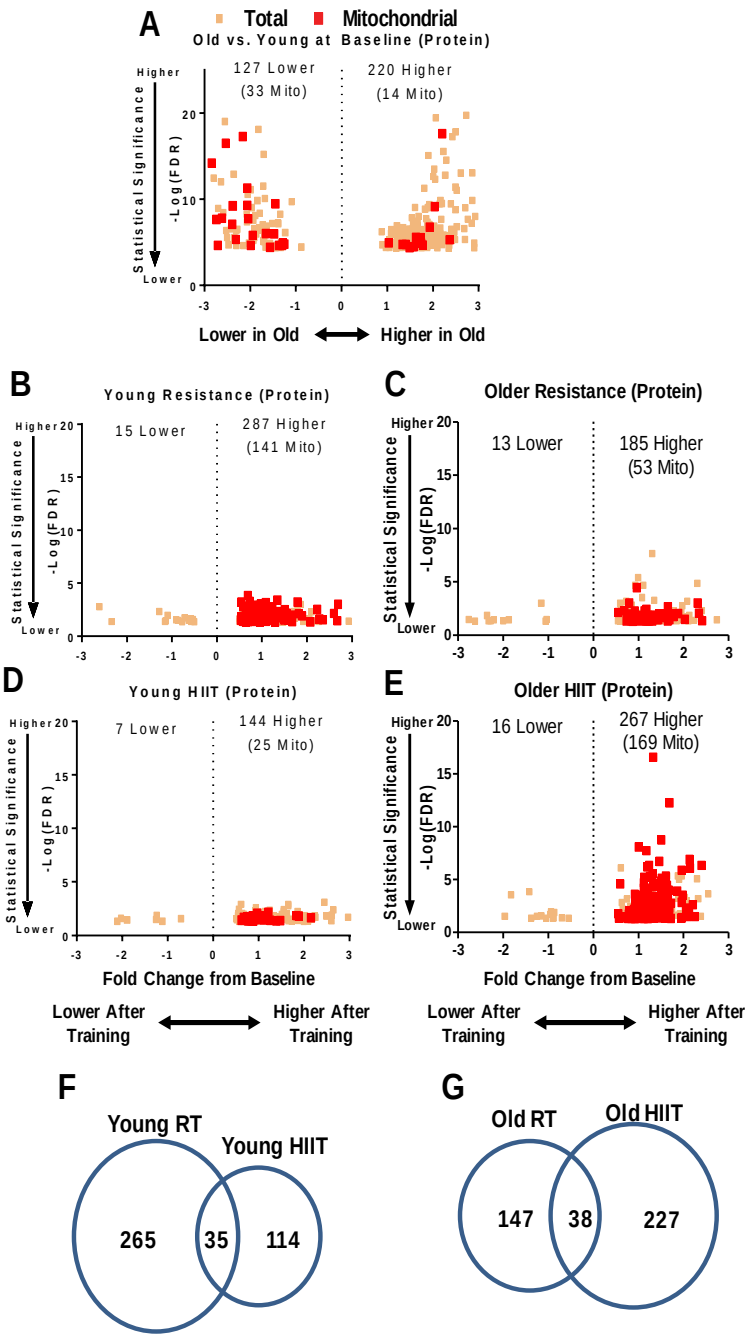

OS vs. YS
lactate dehydrogenase
cytochrome c oxidase subunit II
aspartate aminotransferase 2
cytochrome c oxidase subunit VIIc
E2 component of pyruvate dehydrogenase complex
cytochrome c oxidase subunit IV isoform 1
isocitrate dehydrogenase 2 (NADP+)
glyceraldehyde-3-phosphate dehydrogenase
Phosphoglycerete kinase
triosephosphate isomerase 1
malate dehydrogenase 2
cytochrome c oxidase subunit Va
pyruvate kinase
cytochrome c oxidase subunit Vb
ubiquinol-cytochrome c reductase core protein II
pyruvate dehydrogenase
oxoglutarate dehydrogenase
Glycerol-3-phosphate dehydrogenase
ATP synthase
glycogen phosphorylase
NADH dehydrogenase flavoprotein 2
Adenylate kinase
aspartate aminotransferase 1
phosphoglucose isomerase
phosphoglucomutase
mitochondrial creatine kinase
aconitase 2
phosphoglycerate mutase
aldolase
phosphofructokinase
citrate synthase
creatine kinase (muscle)
enolase
superoxide dismutase 1
A
-60 -40 -20 0 20 40 60
YT vs. YS
lactate dehydrogenase
Glycerol-3-phosphate dehydrogenase
enolase
Phosphoglycerete kinase
glyceraldehyde-3-phosphate dehydrogenase
aldolase
phosphoglucomutase
phosphoglycerate mutase
glycogen phosphorylase
pyruvate kinase
superoxide dismutase
creatine kinase (muscle)
phosphofructokinase
Adenylate kinase
triosephosphate isomerase 1
phosphoglucose isomerase
NADH dehydrogenase flavoprotein 2
E2 component of pyruvate dehydrogenase complex
aconitase 2
aspartate aminotransferase 1
malate dehydrogenase 2
isocitrate dehydrogenase 2 (NADP+)
cytochrome c oxidase subunit Va
oxoglutarate dehydrogenase
aspartate aminotransferase 2
cytochrome c oxidase subunit VIIc
ubiquinol-cytochrome c reductase core protein II
ATP synthase
cytochrome c oxidase subunit Vb
citrate synthase
cytochrome c oxidase subunit IV isoform 1
mitochondrial creatine kinase
pyruvate dehydrogenase
cytochrome c oxidase subunit II
B
-50 0 50 100 150 200
OT vs. OS
superoxide dismutase 1
aldolase
glyceraldehyde-3-phosphate dehydrogenase
phosphoglucomutase
phosphofructokinase
creatine kinase (muscle)
Phosphoglycerete kinase
phosphoglycerate mutase
Adenylate kinase
lactate dehydrogenase
phosphoglucose isomerase
glycogen phosphorylase
Glycerol-3-phosphate dehydrogenase
pyruvate kinase
triosephosphate isomerase 1
enolase
NADH dehydrogenase flavoprotein 2
aconitase 2
aspartate aminotransferase 1
pyruvate dehydrogenase
malate dehydrogenase 2
aspartate aminotransferase 2
cytochrome c oxidase subunit Va
isocitrate dehydrogenase 2 (NADP+)
cytochrome c oxidase subunit IV isoform 1
cytochrome c oxidase subunit Vb
E2 component of pyruvate dehydrogenase complex
oxoglutarate dehydrogenase
ATP synthase
ubiquinol-cytochrome c reductase core protein II
mitochondrial creatine kinase
citrate synthase
cytochrome c oxidase subunit VIIc
cytochrome c oxidase subunit II
C
oxidative proteins
glycolytic proteins
-50 0 50 100 150 200
% difference
OT vs. yT
pyruvate dehydrogenase
cytochrome c oxidase subunit II
aspartate aminotransferase 2
cytochrome c oxidase subunit Va
cytochrome c oxidase subunit Vb
cytochrome c oxidase subunit IV isoform 1
mitochondrial creatine kinase
malate dehydrogenase 2
phosphofructokinase
triosephosphate isomerase 1
glyceraldehyde-3-phosphate dehydrogenase
ubiquinol-cytochrome c reductase core protein II
aspartate aminotransferase 1
ATP synthase
lactate dehydrogenase
phosphoglucose isomerase
cytochrome c oxidase subunit VIIc
pyruvate kinase
Adenylate kinase
isocitrate dehydrogenase 2 (NADP+)
aldolase
aconitase 2
creatine kinase (muscle)
oxoglutarate dehydrogenase
glycogen phosphorylase
phosphoglycerate mutase
phosphoglucomutase
Phosphoglycerete kinase
NADH dehydrogenase flavoprotein 2
citrate synthase
Superoxide dismutase 1
enolase
E2 component of pyruvate dehydrogenase complex
Glycerol-3-phosphate dehydrogenase
D
-60 -40 -20 0 20 40 60
% difference



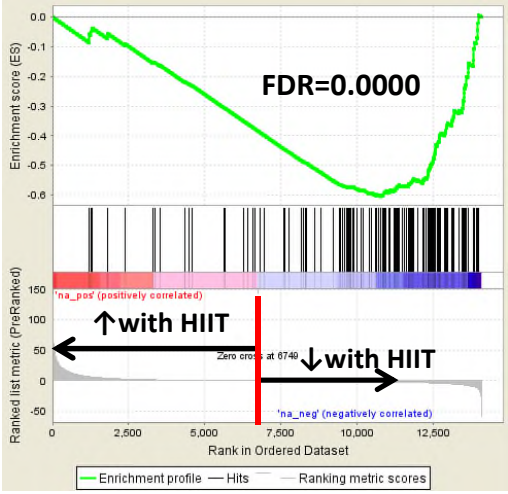
- **CHANGES IN MUSCLE PROTEOME IN RESPONSE TO EXERCISE TRAINING**

Older People have Reduced Muscle Protein Content Especially Those Involved In Mitochondrial Function. HIIT Normalized Most of These Proteins. Resistance Training Increased More Muscle Proteins in Younger Than In the Older

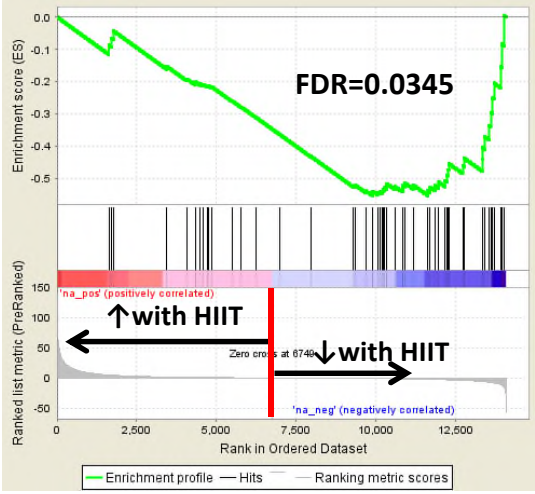
Robertson ML et al Cell Metabolism 2017

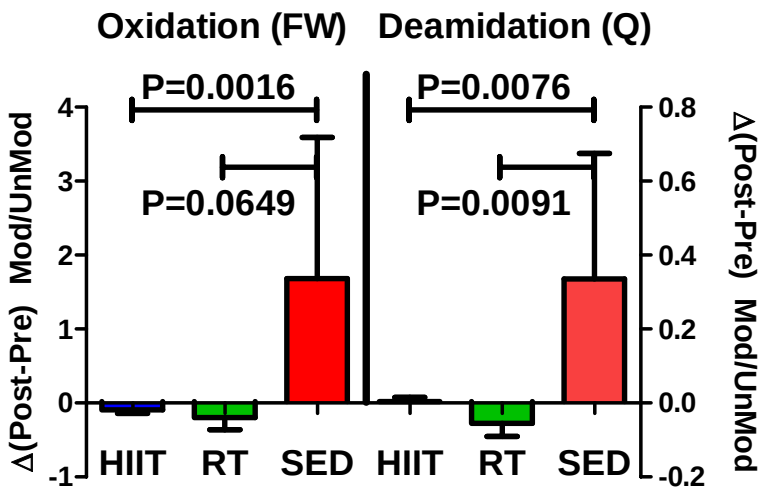
Down regulation of mRNA while Protein Abundance Increased for Ribosomal Protein with HIIT in Older Adults. mRNAs of protein catabolism decreased
Lesser Damages In Muscle Proteins In the Exercised than In the Sedentary

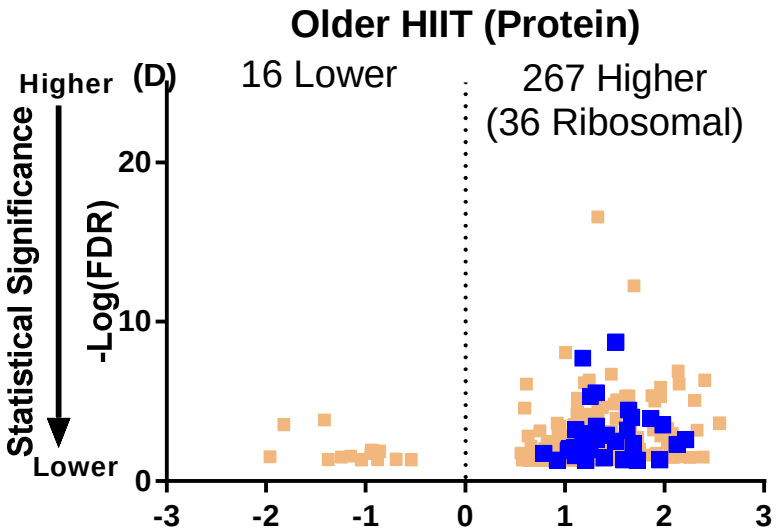
mRNA Translation (Transcript)



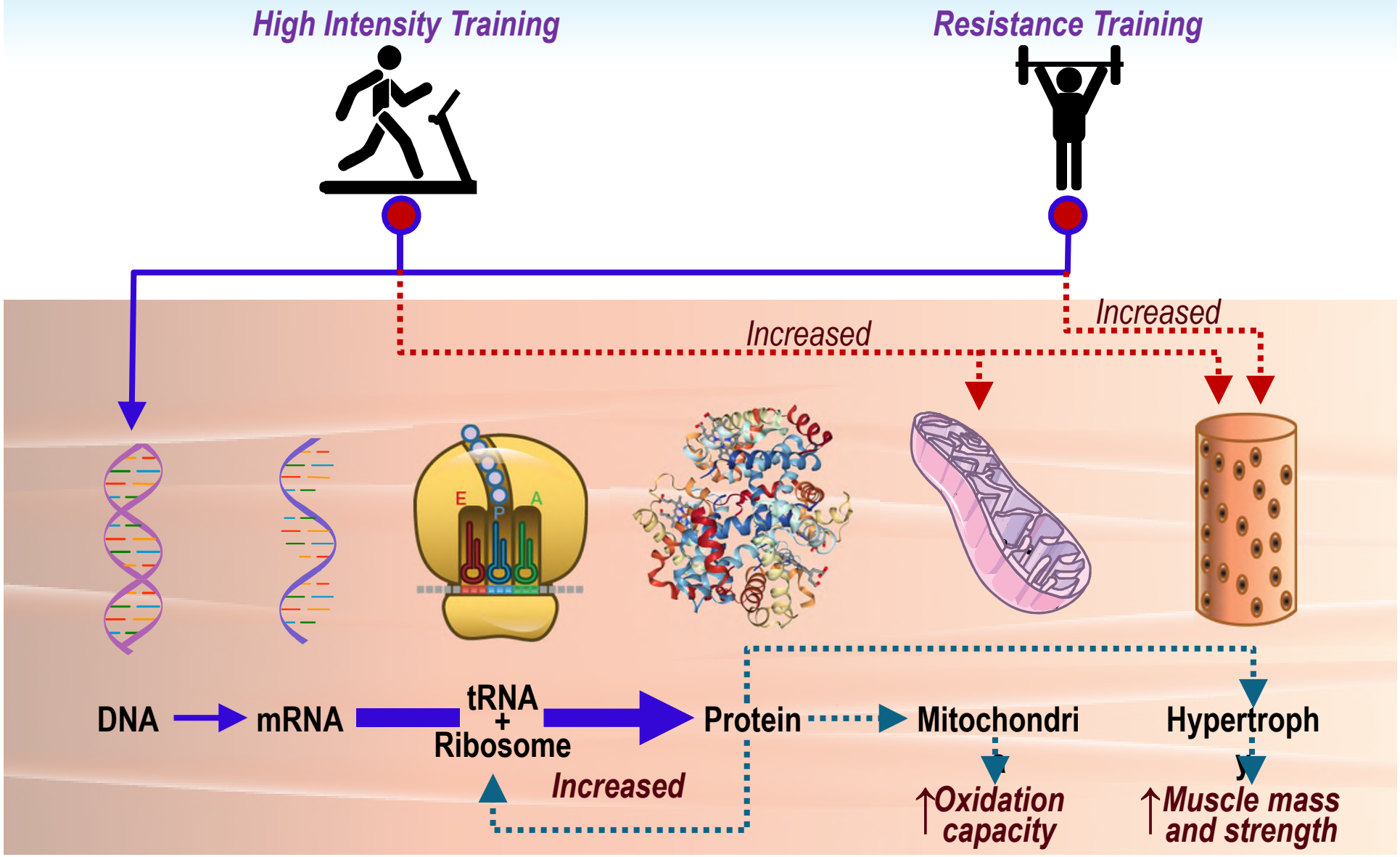
Protein Catabolism (Transcript)







12 Weeks Exercise Training in Younger and Older People



Skeletal Muscle Adaptation to Exercise Training

Acknowledgements

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- Adam Konopka
- Albert Asante
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- P. Balagopal
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- Daniel Jakaitis
- Jill Schimke
- Dawn Morse
- Bobbie Soderberg
- Charles Ford
- Mai Persson

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- RO1AG0953
- DK07325 (T32)
- Mayo Clinic CTSA grant UL1TR000135
- Metabolomics U24DK100469

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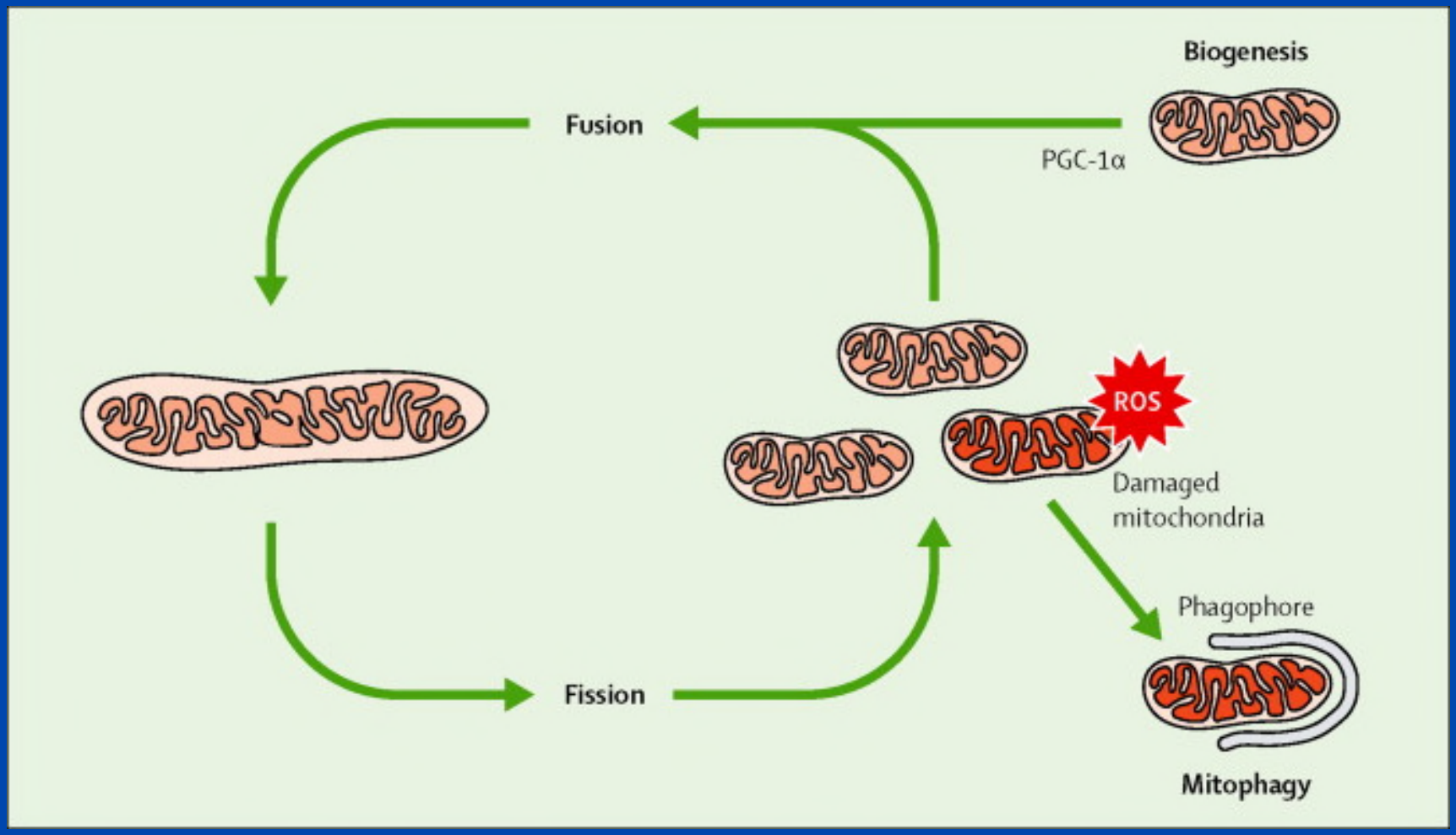
Biogenesis
Fusion
PGC-1α
ROS
Damaged
mitochondria
Phagophore
Fission
Mitophagy