Advances in understanding skeletal muscle insulin sensitivity across the lifespan

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No relationships to report

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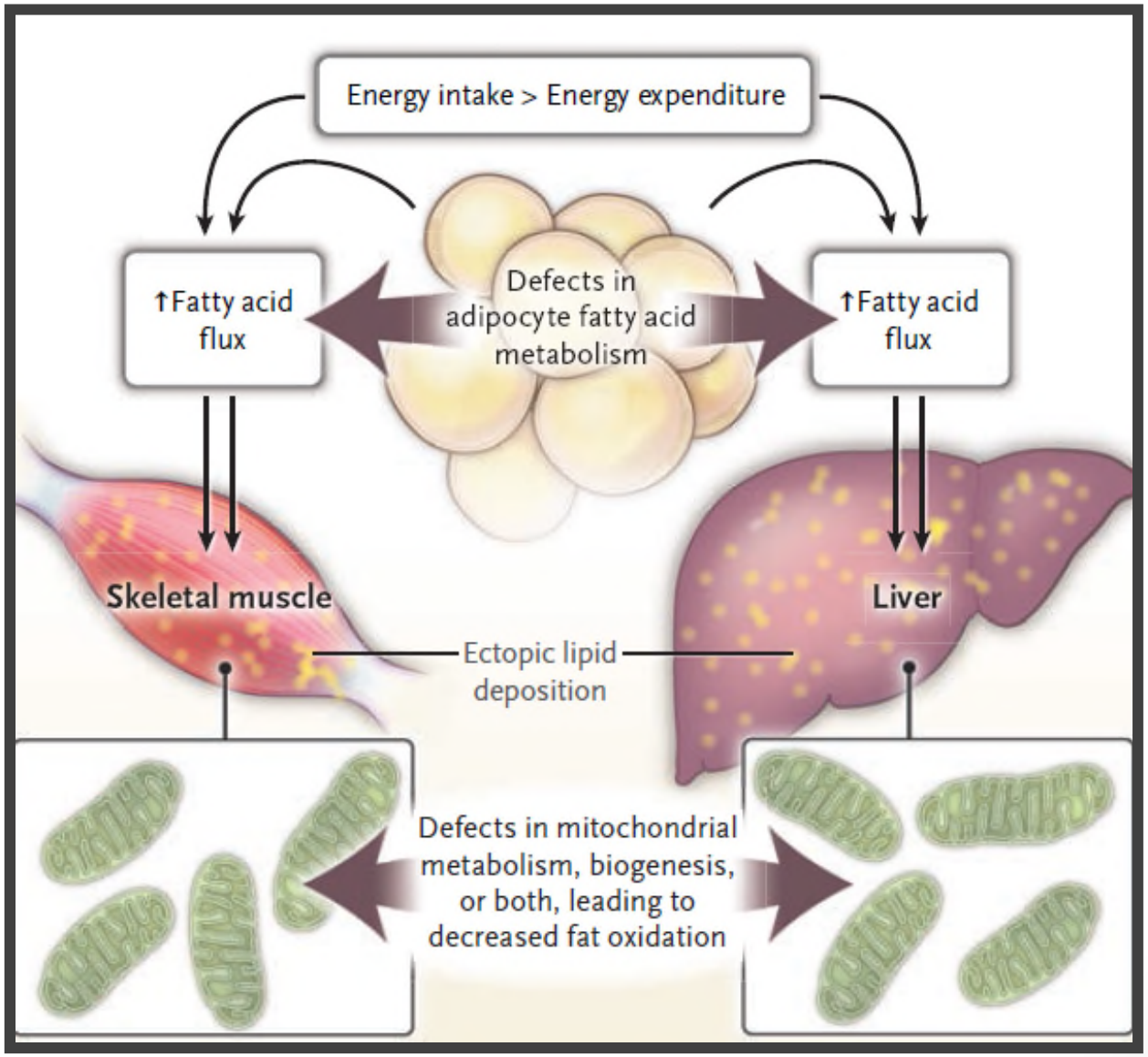
Goals

- Briefly review relationship between mitochondria, free fatty acids and insulin sensitivity and how to assess these endpoints in *in-vivo*

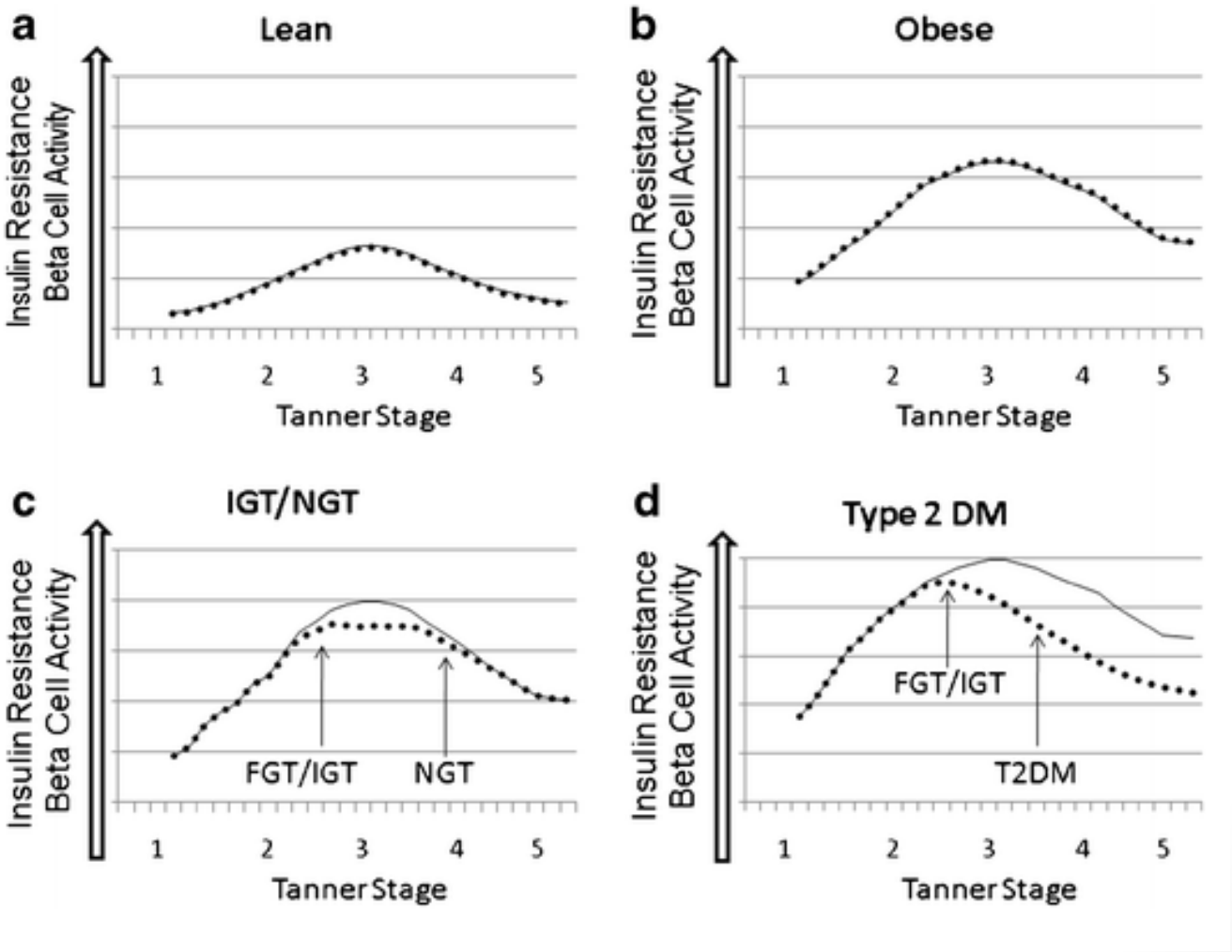
- Compare results from populations with different degrees of insulin sensitivity and with oxygen intervention

Lipids, Mitochondria and Insulin Resistance



Schulman, GI NEJM 2014

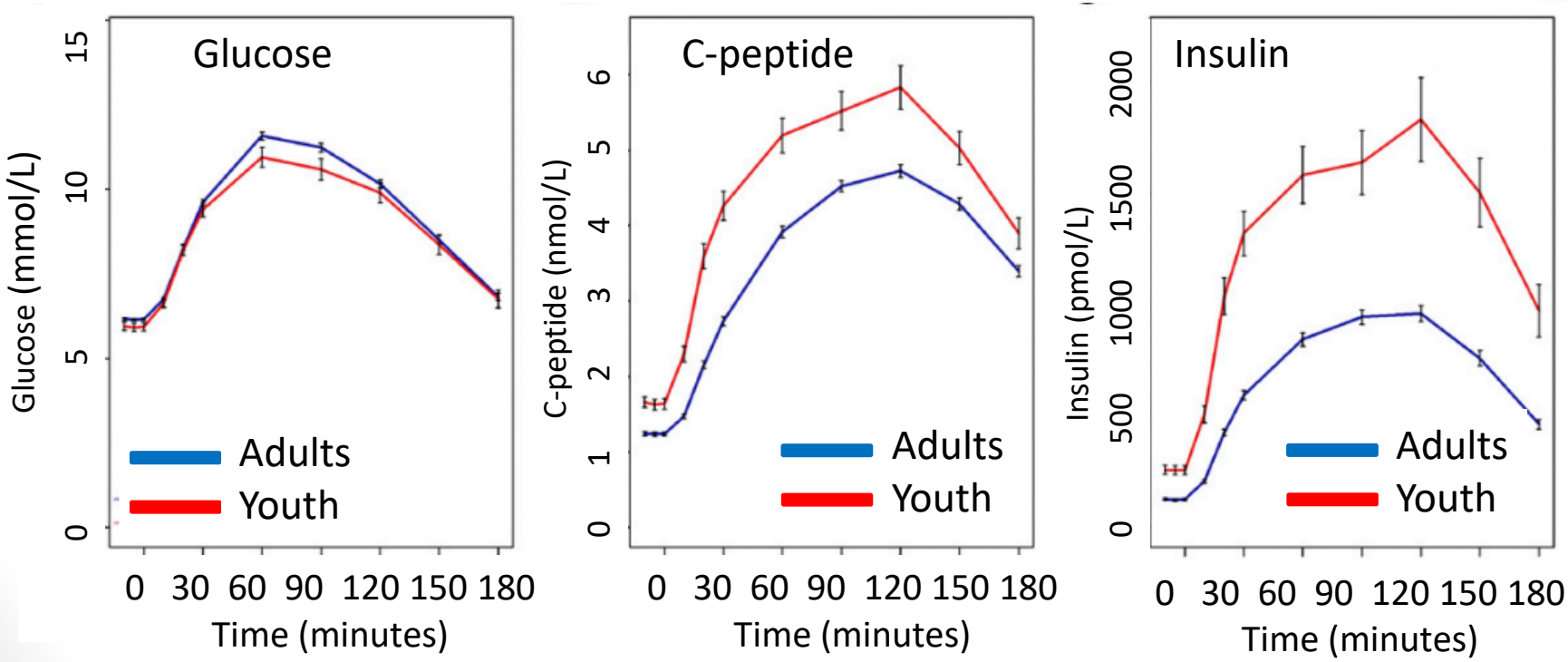
Effect of puberty on insulin action and secretion



Cree-Green, M. Curr Opin Endocrinol Diab Obes 2012 19(4):255-62

Insulin resistance in youth

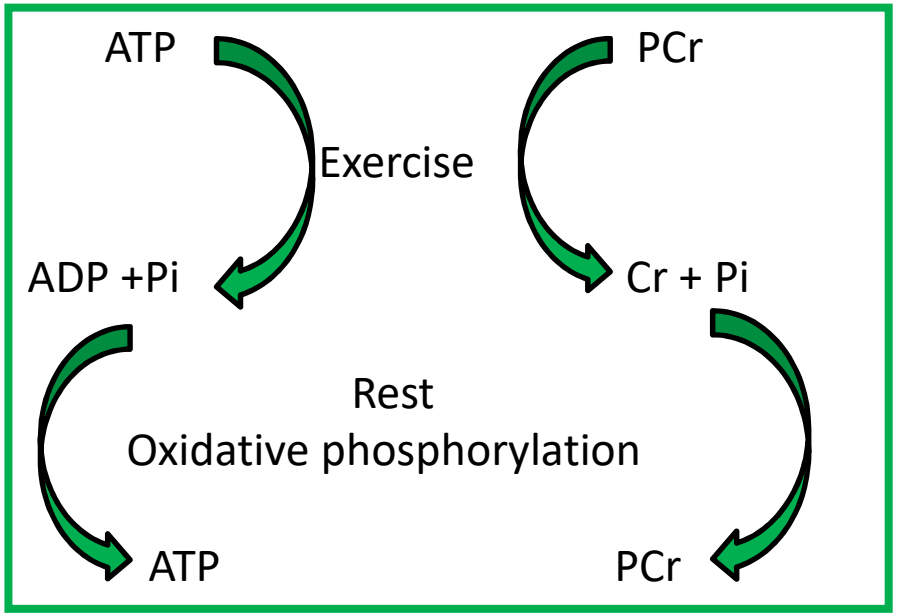
US wide study of prediabetes/diabetes
66 youth (80.3% with IGT) and 355 adults (70.7% with IGT)
3 hour oral glucose tolerance test



RISE Consortium Diabetes Care. 2018 Aug;41(8):1707-1716.

METHODOLOGIES

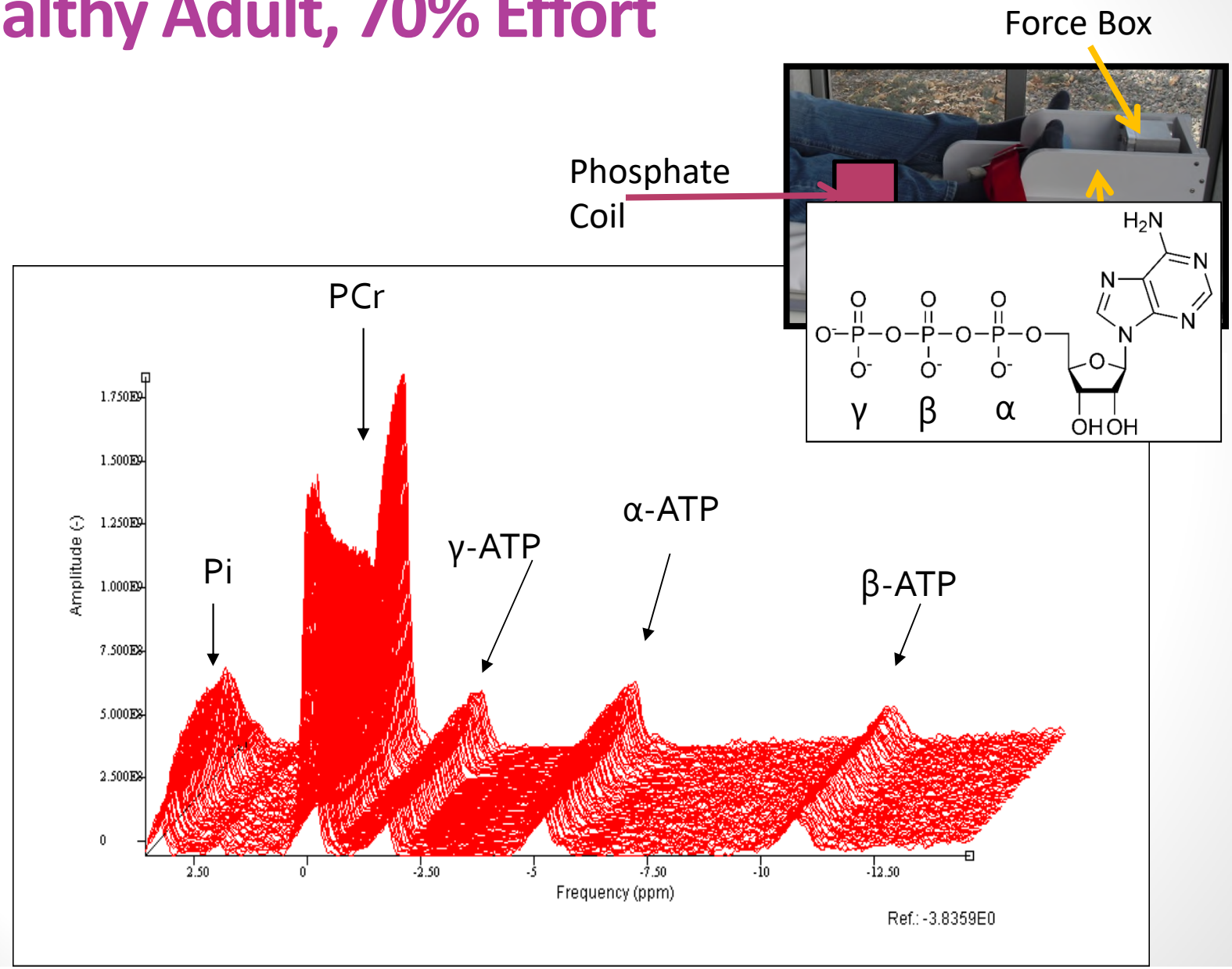
^{31}Phosphorus MR Spectroscopy



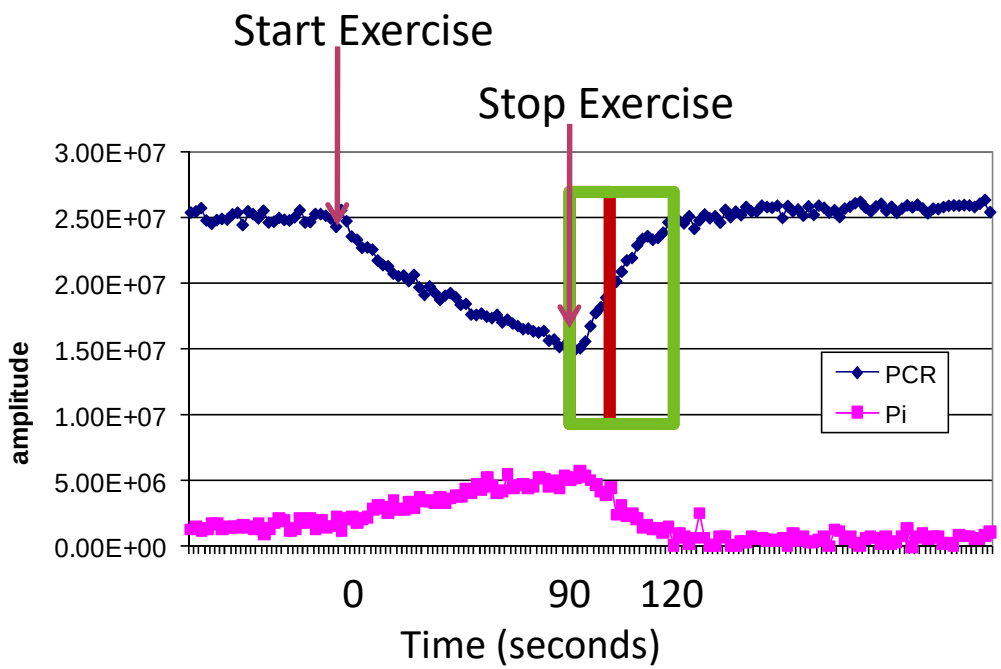
- ATP & PCr pools are full prior to exercise.
- During initial exercise, [ATP] decreases as it breaks down to ADP and Phosphate
- Once exercise stopped, oxidative metabolism starts, and ATP pools are repleated as phosphate is added back to ADP
- The rate of ATP recovery is effected by number and efficiency of mitochondria

Raw Spectra

Healthy Adult, 70% Effort



^{31}P-MRS Analysis Methods



- Calculated mitochondrial endpoints:
  - Oxidative phosphorylation (substrate dependent)
  - ADP time constant (substrate, vascular, and pH dependant)
  - QMAX (maximum mitochondrial function, vascular)

Overall Study Designs

Populations:

Youth-Age 12-21 years -Lean, Obese, PCOS T2D, T1D

Adults- Age 30-60 – Lean, Obese, T2D, T1D

Assessment of Insulin Sensitivity

Fasting Samples – HOMA-IR

2 hour Oral Glucose Tolerance Test

4 phase hyperinsulinemic-euglycemic clamp with tracers

Mitochondrial Measures

Post-exercise ^{31}P-MRS of the calf

^{1}H-MRS of the calf to measure IMCL

Muscle Biopsies of calf

RESULTS

Does IR relate to mitochondria in active pubertal, healthy, normoglycemic youth

Characteristics	Males (n = 119)	Females (n = 156)
Age (years)	17.1 (16.52,17.63)	17.2 (16.43,17.67)
BMI-Z Score	0.36±1.20	0.72±1.07 +
Tanner 4/5(%5)	39/77(65%)	28/123(79%)
Laboratory Measures		
Fasting Glucose (mg/dL)	90 (86, 93)	86 (82, 91) **
2 hour Glucose (mg/dL)	88 (77, 103)	100 (85, 119) **
Fasting Insulin (IU/mL)	12 (9 , 17)	14 (11, 19) +
2 hour Insulin (IU/mL)	36 (20, 55)	72 (45, 117) **
TG (mg/dL)	76 (55, 99)	85 (61, 122) +
HDL (mg/dL)	44 (39, 49)	45 (38, 53)
TG:HDL	1.69 (1.21, 2.23)	1.95 (1.30, 2.79) *
FFA Baseline (mmol/L)	364 (264, 526)	458 (343, 617) +
Mean total METS	67.3 (57.5, 82.0)	62.7 (56.7, 71.0) +
Mitochondrial Controls		
Muscle area (mm^2)	4238 (3575,5100)	3644 (3205,41965) **
Average force (kg)	27.7 (24.1, 31.4)	28.4 (24.9, 31.5)

Values are mean ± SD, median (25 %ile, 75 %ile), or N(%) as indicated.
Significance is noted as *<0.05-0.01, + = <0.01-0.001, ** = <0.001

M. Cree-Green, J Clin Endocrinol Metab. 2017, 102(5):1652-1660.

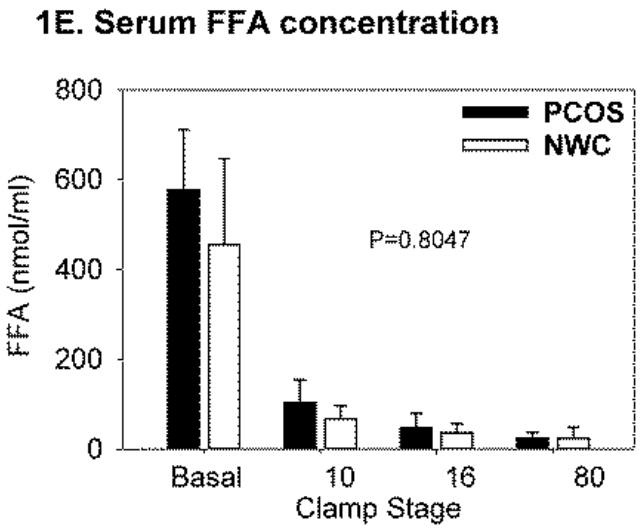
Mitochondria and IR measures

	Entire Cohort (n =275)	Females (n =156)	Males (n =119)
Variable	Regression Coefficient	Regression Coefficient	Regression Coefficient
Outcome: Oxidative Phosphorylation			
HOMA-IR	0.000	-0.004	0.005
Matsuda Index	-0.001	-0.001	-0.002
TG:HDL	-0.009	-0.020	0.009
Outcome: ADP Time Constant			
HOMA-IR	-0.115	-0.139	0.146
Matsuda Index	-0.112	-0.031	-0.246
TG:HDL	0.760	0.916	1.410
Outcome: PCr Time Constant			
HOMA-IR	-0.226	-0.260	-0.071
Matsuda Index	-0.248	-0.204	-0.259
TG:HDL	1.186	0.167	2.276 *

Adjusted for age, BMI-Z and physical activity.

Mild IR: Normal weight girls with PCOS

	Control	PCOS	P
Number	20	18	n/a
Age (years)	15.0 ± 2.13	15.9 ± 1.83	0.15
BMI (kg/m^2)	21.3 ± 2.9	22.7 ± 2.3	0.13
BMI %ile	60 ±21	68 ±18	0.20
GIR (mg/kg/min)	13.5 ± 2.4	10.9 ± 2.7	**0.02**
PCr time constant (seconds)	28.1(24.9, 31.0)	36.0(28.7, 41.0)	**0.018**
ADP time constant (seconds)	17.7 ± 3.3	22.8 ± 4.7	**0.009**
Oxidative Phos (mmol/L/sec)	0.21 ± 0.10	0.16 ± 0.05	0.09
Qmax (mmol/seconds)	0.41 (0.19, 0.57)	0.45 (0.38, 0.54)	0.72



Correlations with GIR (mg/kg/min)
ADP TC R=-0.577, p=0.012
PCR TC R=-0.602, p=0.008

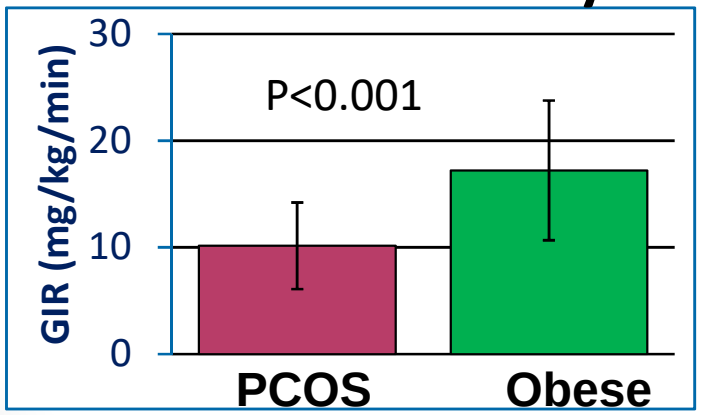
Cree-Green, Journal of the Endocrine Society. 2017 1(7):931–944.

Moderate IR: obese girls with PCOS

	Obese	PCOS
Number	13	30
Age (years)	14.4±0.5	14.9±1.5
BMI percentile	96±1	98±1
Waist/Hip Ratio	0.88±0.09	0.88±0.07
Triglycerides (mg/dl)	78±8	129±7*
HbA1C (%)	5.1±0.2	5.4±0.3
Impaired Glucose Tolerance	20%	50%*

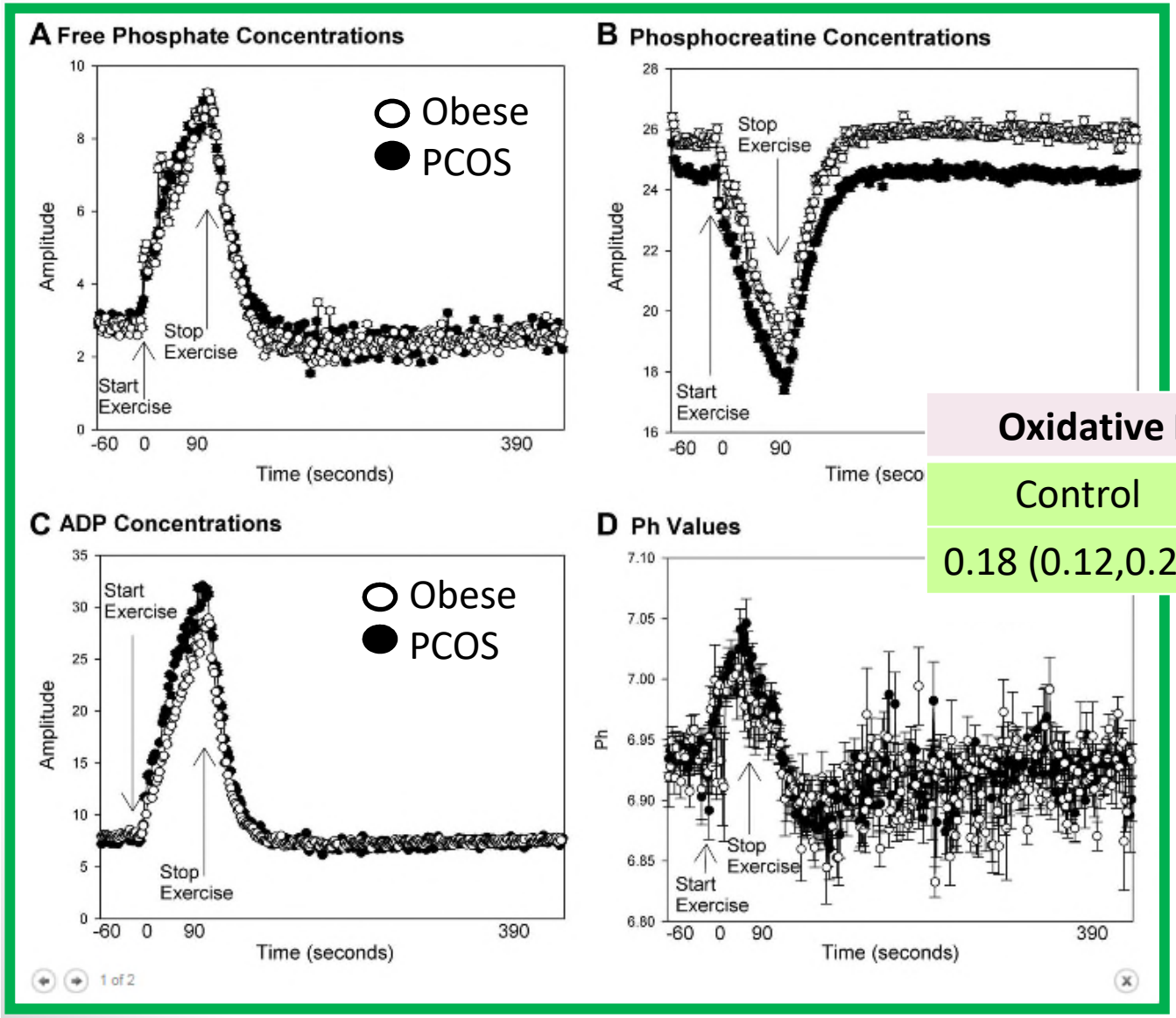
*P<0.05

Muscle Sensitivity



Cree-Green, M, Obesity 2016

Altered Mitochondrial Function



Oxidative Phosphorylation	
Control	PCOS
0.18 (0.12,0.23)	0.11 (0.06,0.19)

Cree-Green M, Am J Physiol Endocrinol Metab. 2015 May 1;308(9):E726-33

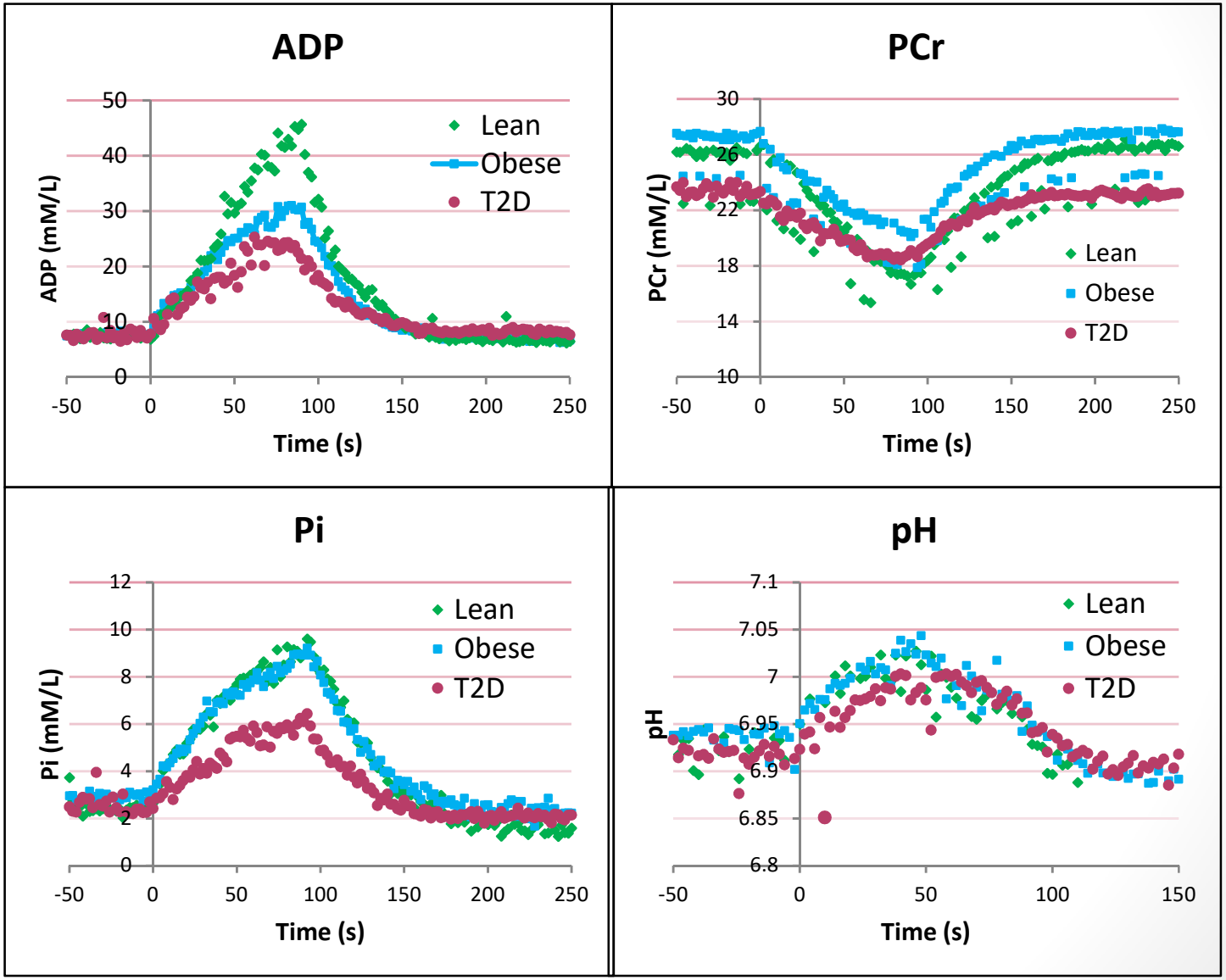
Type 2 diabetes in youth

	Sedentary Lean	Sedentary Obese	T2D
Number	23	26	17
Gender (M/F)	6/17	4/22	3/14
Age (years)	15.1 (12.3,17.0)	14.2 (13.0,16.0)	15.1 (13,16.5)
Ethnicity (C/H/B/NA)	13/4/6/0	9/11/7/1	3/8/6/1
BMI (kg/m2)	20.1 (18.9,22.4)	31.8 (29.7,34.4)	33.1 (28.3,38.9)*
Waist/Hip Ratio	0.80±0.01	0.92±0.02	0.96±0.02*
IMCL (fat/water ratio)	1897±340	3211±457	3649±553*
HbA1c (%)	5.3 (5.1,5.4)	5.4 (5.2,5.5)	6.9 (5.7,8.4) *

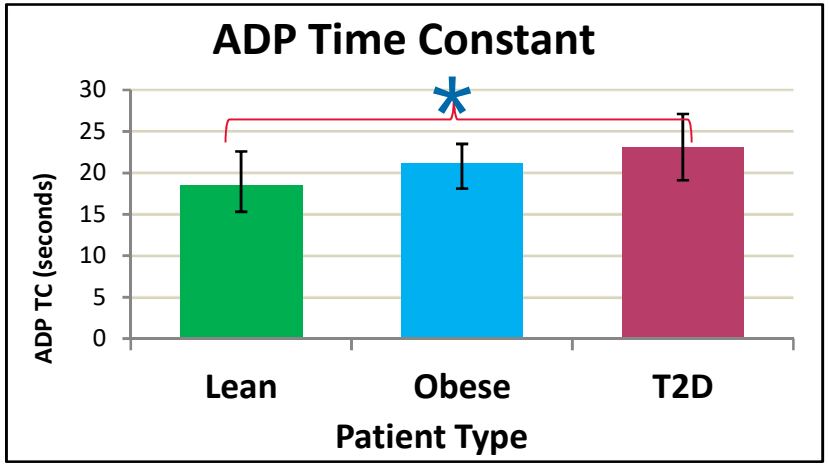
*P<0.05

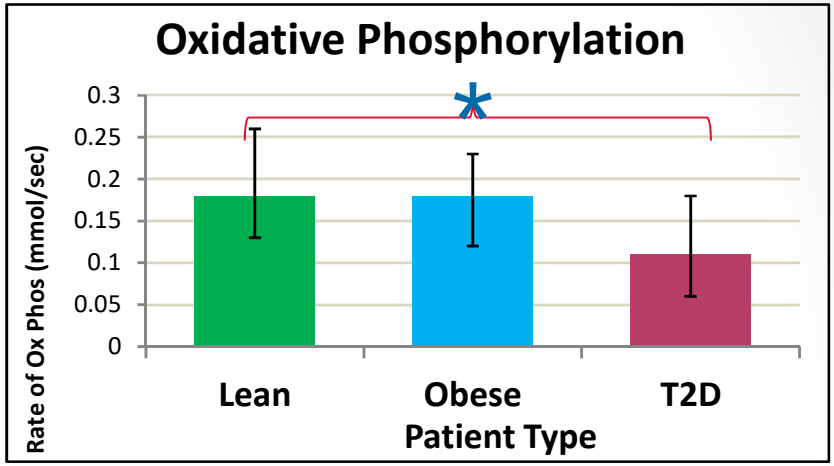
Cree-Green M, J Diabetes Complications. 2017 Jan;31(1):141-148.

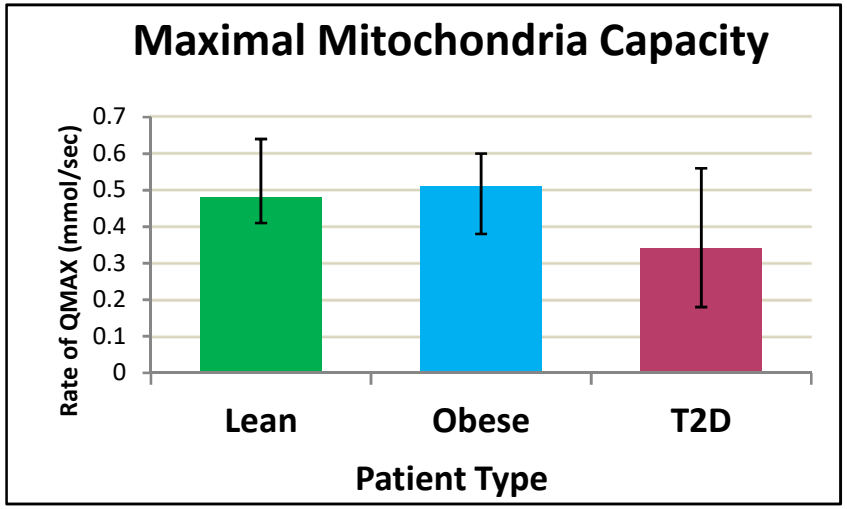
Metabolic changes with exercise



Calculated mitochondrial function

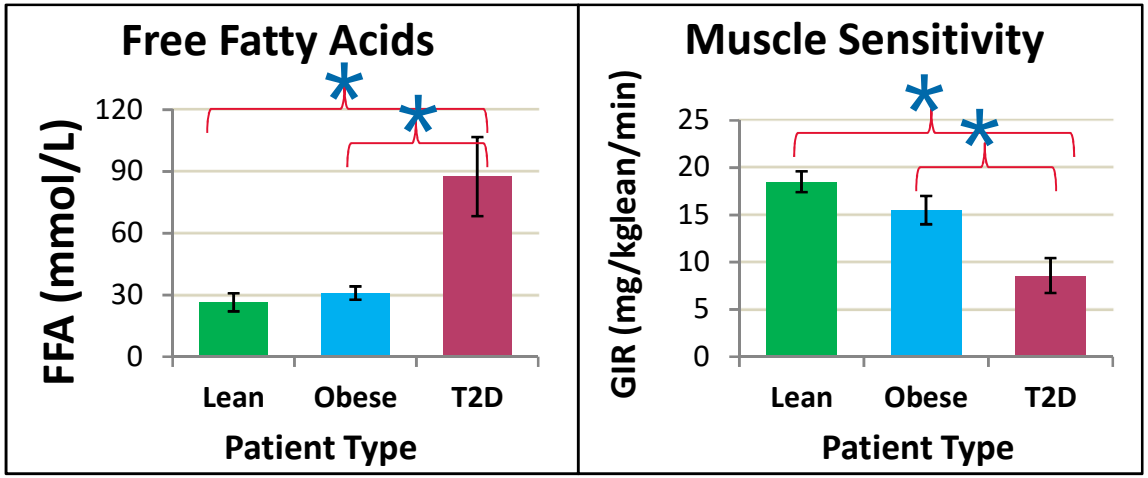






*P<0.05 between T2D and lean, and T2D and obese

Clamp results and correlations



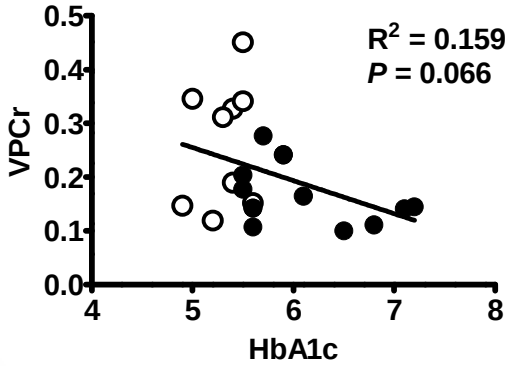
- Initial Spearman's Analysis:
  - GIR related to FFA, HbA1c, ADP TC, OxPhos, QMAX
  - No relationship with IMCL: (R=-0.226, P=0.330)

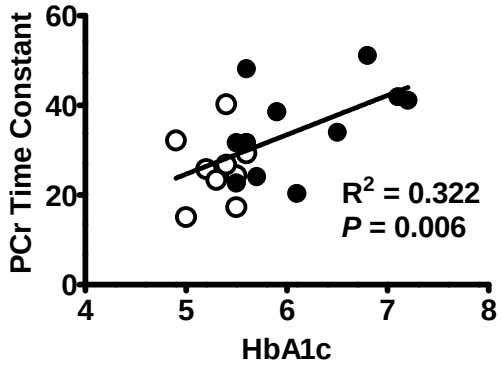
- Linear regression models:

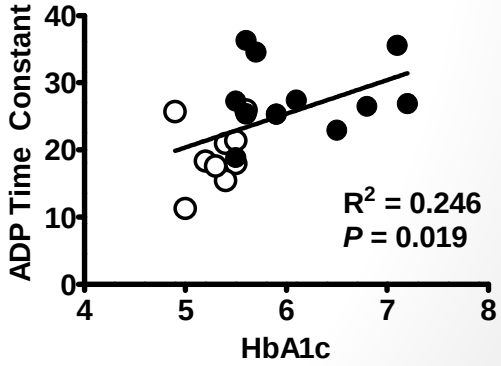
Model	Overall R^2	FFA (P-value)	GIR (P-value)	HbA1c (P-value)
1 ADP TC	0.593	0.002	0.014	0.103
2 QMAX	0.715	0.002	<0.001	0.197
3 OXPhos	0.527	<0.001	0.981	0.106

Older adults with type 2 diabetes

	Obese (N=17)	T2D (N=18)	p-value
Sex (F/ M)	(9/8)	(11/7)	1.000
Age (years)	56.7 (43.6, 64.6)	59.4 (53.2, 67.0)	0.276
BMI (kg/m2)	29.9 ± 3.5	31.5 ± 4.8	0.288
HbA1c (%)	5.4 (5.3, 5.5)	6.1 (5.6, 6.7)	<0.001*
Fasting FFA (mmol/L)	388 (246,542)	563 (467, 638)	0.038*

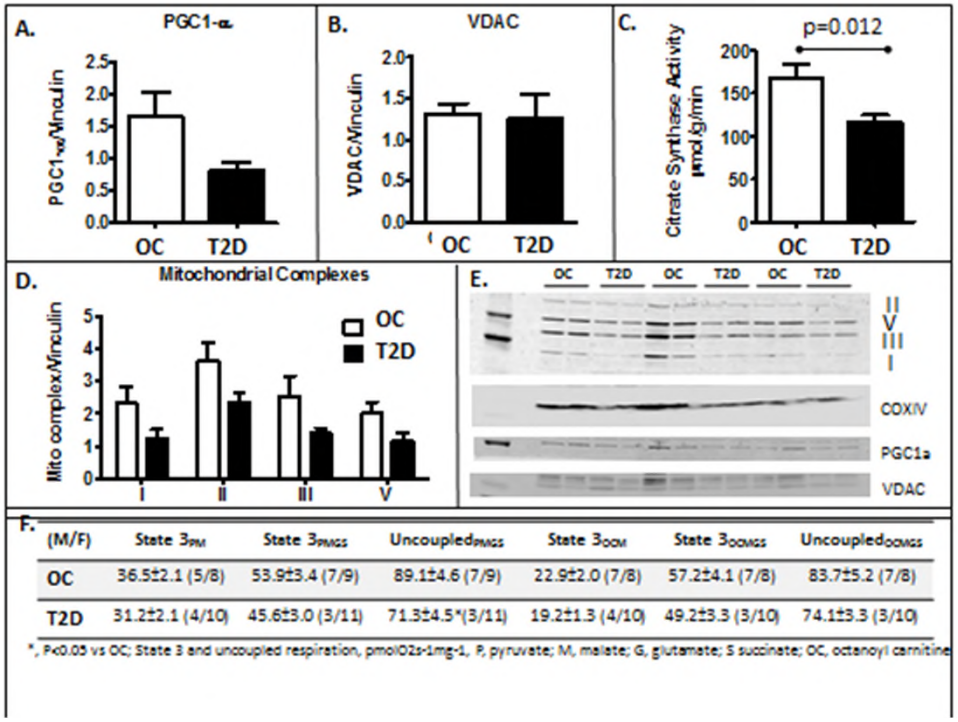






Cree-Green M, Diabetes. 2018 Jul;67(7):1369-1379.

Biopsy vs MRS, supplemental O_2



F.

(M/F)	State 3_{PM}	State 3_{PMGS}	Uncoupled$_{PMGS}$	State 3_{OCM}	State 3_{OCMGS}	Uncoupled$_{OCMGS}$
OC	36.5±2.1 (5/8)	53.9±3.4 (7/9)	89.1±4.6 (7/9)	22.9±2.0 (7/8)	57.2±4.1 (7/8)	83.7±5.2 (7/8)
T2D	31.2±2.1 (4/10)	45.6±3.0 (3/11)	71.3±4.5*(3/11)	19.2±1.3 (4/10)	49.2±3.3 (3/10)	74.1±3.3 (3/10)

*, P<0.05 vs OC; State 3 and uncoupled respiration, pmolO2s-1mg-1, P, pyruvate; M, malate; G, glutamate; S succinate; OC, octanoyl carnitine

Baseline ^{31}Phosphorus MRS 70% max

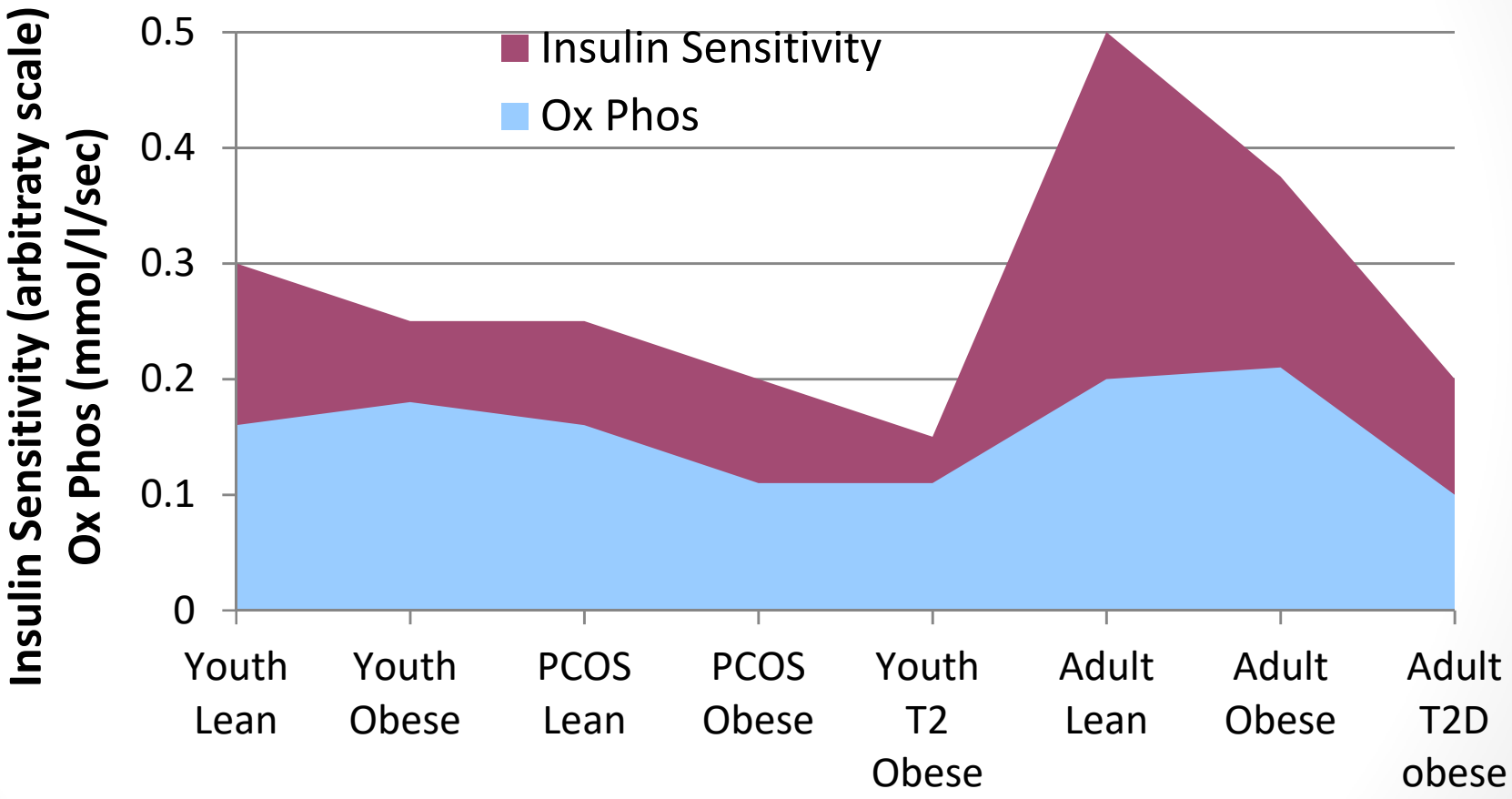
	Obese	T2D	P-value
PCr TC (sec)	29 ± 3	40 (27,45)	0.142
ADP TC (sec)	21 ± 3	28 ± 2	0.011
Ox Phos (mM/sec)	0.21 ± 0.09	0.10 ± 0.03	0.005
Qmax	0.56 ± 0.14	0.34 ± 0.04	0.003

Allowed participants to rest with supplemental oxygen, brought saturation from 95% to 99%, and repeated exercise bout

	Obese		T2D	
	Difference	p-value	Difference	p-value
VPCr (mmol/seconds)	-0.036 ± 0.108	0.185	-0.0330 ± 0.101	0.159
ADP TC (seconds)	0.150 ± 6.263	0.923	-5.021 ± 7.528	**0.012**
OxPhos (mmol/L/second)	-0.0104 (-0.0553, 0.0345)	0.190	0.059 ± 0.094	**0.016**
QMAX (mmol/second)	0.05 (-0.09, 0.20)	0.452	0.14 (-0.01, 0.28)	**0.068**

Cree-Green M, Diabetes. 2018 Jul;67(7):1369-1379.

Putting it all together



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U.S. Department of Veterans Affairs

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American Diabetes Association.

Center for Women's Health Research

American Heart Association. life is why™

Children's Hospital Colorado

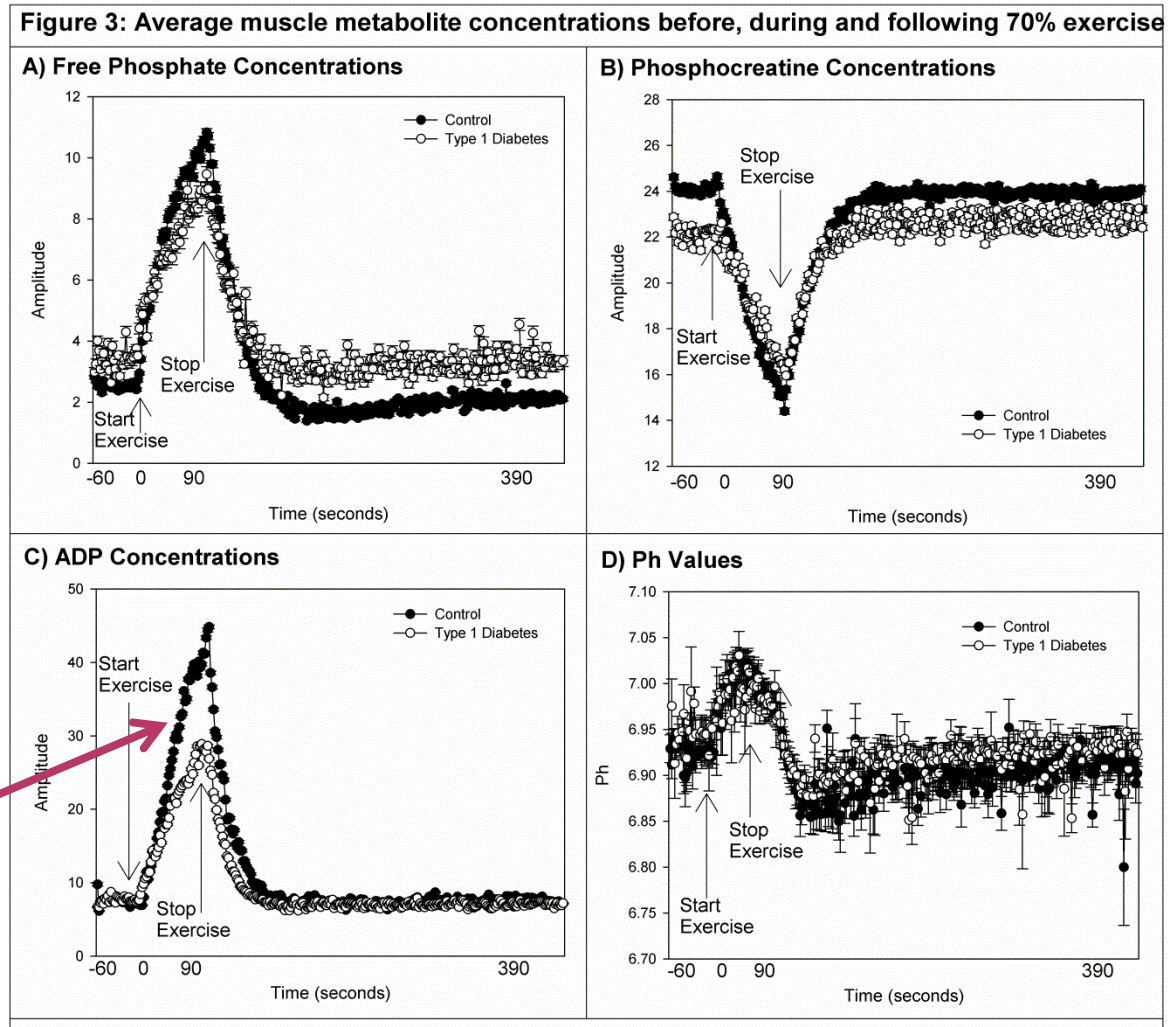
Thrasher Research Fund
Medical research grants to improve the lives of children

Youth with type 1 diabetes

	Type 1 diabetes (N=21)	Control (n=17)
Gender (M)	10 (47.6%)	5 (29.4%)
Age (years)	15.0 (13.0, 18.0)	14.0 (12.0, 18.0)
BMI (kg/m2)**	25.0 ± 4.2	21.4 ± 3.9
A1C (%)***	8.2 (6.7, 12.0)	5.3 (4.7, 5.8)
Clamp Measurements		
FFA end clamp (mmol/L)*	33.0 (7.5, 125.0)	20.5 (15.0, 35.0)
Glucose Infusion rate (mg/kglean/min)***	10.10 (3.01, 20.78)	18.92 (9.39, 28.09)

Cree-Green M, Diabetes. 2015 Feb;64(2):383-92.

Different phosphate patterns



Ox Phos and ADP ½ time related to GIR and QMAX to IMCL. Nothing to FFA or HbA1c

Adults with Type 1 Diabetes

	Room Air	Post Oxygen	Post-Metformin
PCr TC (sec)	32±4	37±4	33±3
ADP TC (sec)	23±3	25±4	18±1
Ox Phos (mM/sec)	0.13±0.02	0.13±0.02	0.18±0.03
Qmax	0.38±0.04	0.34±0.04	0.42±0.04

Data available that has not yet been analyzed:
Identical muscle biopsy to previous study
Relationships with
- insulin sensitivity (Clamp)
- FFA
- HbA1c

	T2D Adults	T1D Youth
PCr TC (sec)	40 (27,45)	29 (18,51)
ADP TC (sec)	28 ± 2	23 ± 5
Ox Phos (mM/sec)	0.10 ± 0.03	0.09 (0.01, 0.7)
Qmax	0.34 ± 0.04	0.43 (0.09,1.10)