

A Randomized, Double-Blind Study of the Physiologic and Molecular Effects of Resveratrol in Mildly-Obese, Sedentary, Elderly Volunteers

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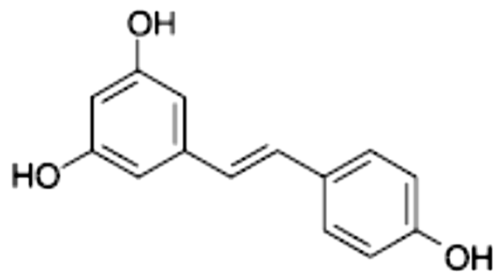
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Hypothesis and Objectives

Hypothesis

Oral administration of resveratrol at a dose of 2 grams per day for 3 months will lead to enhanced exercise endurance as well as physiologic and molecular correlates demonstrating changes in signal transduction, gene expression, and other markers of improved oxidative capacity and muscle function.

Objectives

Primary Objective

To determine if oral resveratrol administered for 3 months will improve exercise endurance and physiologic functions by measurement of peak VO_2 and submaximal aerobic capacity as compared to a placebo control group.

Secondary Objectives

To determine if administration of resveratrol induces molecular alterations of metabolic pathways important for improved muscle cell energetics, lipid metabolism, insulin sensitivity/glucose control, etc.

- For each experiment, comparisons will be made on tissue obtained from each patient prior to beginning resveratrol and at the end of the study, and between the resveratrol- treated and placebo groups.

To determine if resveratrol alters gene expression signatures of molecular pathways in muscle that regulate mitochondrial biogenesis, TCA cycle and ETC function, and fatty acid oxidation.

To determine if resveratrol improves oxidative phosphorylation in muscle as assayed by MR spectroscopy during exercise and the post-exercise recovery period.

To evaluate changes in levels of circulating inflammatory cytokines, growth factors, and hormones, to include (but not limited to) TNF- α , IL-6, IGF-1, and adiponectin.

To determine if resveratrol increases mitochondria content in muscle by performing quantitative PCR of mitochondrial DNA in biopsy samples.

To evaluate alterations in systemic lipid metabolism by measuring fasting levels of serum cholesterol, LDL, HDL, triglycerides.

To determine effects of RSV on fasting glucose levels, insulin resistance, and glucose challenge.

Pathways and Genes of Interest

- PGC-1 α and PGC-1 β , SIRT1 (Civitarese et al, 2007), activators and downstream pathways (i.e. eNOS)
- AMPK downstream targets: Nampt (Fulco et al., 2008), Akt/mTOR inhibition
- Mitochondrial biogenesis: Nrf-1, Nrf-2, ERR α , TFAM (Lagouge et al., 2006)
- TCA: citrate synthase (Civitarese et al., 2007)
- ETC: cytochrome c oxidase II (COX II) (Civitarese et al., 2007), Ucp3 (Barger et al., 2008), cytochrome c (Lagouge et al., 2006)
- FA metabolism: PPAR α/δ , β -hydroxyacyl-CoA dehydrogenase (Civitarese et al., 2007), Pdk4, CPT1 (Barger et al., 2008), adiponectin receptors1, 2 (Feige et al., 2008), MCAD (Lagouge et al., 2006)
- Fiber type: myoglobin, troponin 1 (Lagouge et al., 2006)

Stored Samples for Possible Future Analyses

RNA will be isolated from peripheral blood leukocytes for analysis of peripheral blood lymphocytes isolated pre- and post-resveratrol.

- Although lymphocytes are not thought to be a primary target of resveratrol's metabolic effects, they are accessible in all patients and may provide a signal of PD activity of this drug.

Muscle tissue obtained pre- and post-resveratrol (or placebo) will be stored for:

- Histologic and immunohistochemical analysis for mitochondrial content and structure, muscle fiber type, fat deposition
- Protein lysates for assays of AMPK and PGC-1 α levels and post-translational modifications, as well as modifications of downstream targets (pAMPK, pACC, acetylated-PGC-1 α)

Subject Selection and Eligibility

Inclusion criteria

- Age \geq 60-75
- BMI 30-35 kg/m²
- Normal laboratory tests of organ function:
- Ability to perform peak VO₂ and submaximal aerobic exercise tests.

Exclusion criteria

- No history of myopathy or known disease of muscle.
- No chronic steroid use
- No history of coronary artery disease, stroke, HIV, hepatitis B or C, active peripheral vascular disease
- No contraindication to performing peak VO₂ or submaximal aerobic exercise tests including a history of coronary artery disease, severe COPD, significant hip or knee arthritis, etc.
- Contraindication to vastus lateralis muscle biopsy is not an exclusion criteria (need 10 biopsies/group).
- Patients are eligible even if they have contraindications to MR spectroscopy.
- Individuals who exercise regularly (\geq 30 minutes more than once a week, including vigorous walking) are excluded. During the course of the study, participants should not alter their sedentary life style.

Study Design



Double blind, randomized study

Resveratrol (RevGenetics, IND) 2g/d versus equal number of placebo capsules for 12 weeks

20 subjects will be enrolled into each arm

10 patients in each arm will receive muscle biopsies prior to beginning and after 12 weeks of RSV.

Pre-treatment evaluation:

Eligibility and randomization

- Peak VO_2
- SubMax exercise
- Muscle biopsy (GEP)
- Blood: leukocytes, cytokines, lipids
- MR spectroscopy
- OGTT



**Day 1:
Begin treatment**



**Day 14:
Evaluate for
AE, tolerability**



**Day 29:
2nd peak VO_2
2nd SubMax exercise
Blood: leukocytes,
cytokines, lipids**



**Day 57 (8 weeks):
3rd peak VO_2
3rd SubMax exercise
Blood: leukocytes,
cytokines, lipids**



**Day 85 (12th week):
4th peak VO_2
4th SubMax exercise
2nd muscle biopsy
Blood: leukocytes,
cytokines, lipids
2nd MR spectroscopy
2nd OGTT**

Each visit will include: history (evaluation of AE), physical exam, routine labs (CBC, comprehensive metabolic panel, pill counts)

Statistical Considerations

The main objective of the study is to assess whether orally administered resveratrol can induce specific physiologic and/or molecular alterations to improve muscle function of elderly patients. Eligible patients will be randomized into two groups, treated with resveratrol or placebo.

The corresponding end-points will be change in peak VO_2 and the submaximal exercise capacity. Measurements will be taken for each study participant at baseline, i.e., prior to resveratrol, at 4, 8 weeks, and at the completion of the study at 12 weeks.

The time-averaged difference in peak VO_2 between the two groups will be estimated. A power analysis is based on a meaningful difference (d) between the average response for two groups to be detected. According to Diggle et al. (1996), the appropriate statistical model is

$$(\text{Peak } VO_2)_{ij} = \beta_0 + \beta_1 x + \varepsilon_{ij}, j = 1, \dots, n; i = 1, \dots, 2m,$$

where x is the group/treatment indicator variable. With type I error $\alpha = 0.05$, $n=4$ repeated measurements, and $m=20$ subjects per group, the table below gives the smallest difference that can be detected with an adequate power of at least 0.80 for several plausible values of correlation (ρ). The values for meaningful differences in time-average peak VO_2 and *correlation between repeated observations* $\text{Corr}(Y_{ij}, Y_{ik}) = \rho$ for all $j \neq k$, are chosen based on our preliminary data.

ρ	Smallest difference in the time-averaged peak VO_2 , %
0.2	42.5%
0.5	52.5%
0.6	56.5%

Important Questions for the Clinical Trial

Does RSV have biological activity in humans?

- Insulin/glucose homeostasis
- Fat metabolism
- Mitochondrial biogenesis and energetics
- Muscle efficiency (strength, endurance)
- Sarcopenia and frailty

What is the PK/PD relationship?

- Do RSV metabolites have biologic activity?
- Is the low PK profile of the parent compound sufficient to activate the PGC-1 α program in muscle?

What is the optimal biologic dose, schedule, and duration of treatment?

Does RSV activate both AMPK and SIRT1 in vivo and is this necessary for beneficial effects on mitochondria and muscle?

Table 2. Pharmacokinetics of resveratrol and three metabolites in the plasma of volunteers after a single oral dose

Variable	Dose level (g)			
	0.5	1.0	2.5	5.0
Resveratrol				
AUC _{inf} (ng h/mL)	223.7*	544.8 (57.2)	786.5 (36.2)	1,319 (59.1)
C _{max} (ng/mL)	72.6 (48.9)	117.0 (73.1)	268.0 (55.3)	538.8 (72.5)
T _{max} (h)	0.833 (0.5-1.5)	0.759 (0.5-4.0)	1.375 (0.5-4.0)	1.500 (0.67-5.0)
C _{av} (ng/mL)	8.36 (57.8)	18.04 (51.6)	32.25 (43.0)	51.90 (80.7)
Half-life (h)	2.85*	8.87 (91.1)	4.22 (51.6)	8.52 (95.8)
CL/F (L/h)	2,235*	2,593 (65.1)	3,471 (29.9)	4,930 (50.0)
CL _R (L/h)	1.177 (102.5)	0.696 (71.5)	0.656 (53.1)	1.443 (139.2)
V/F (liters)	9,198*	19,298 (54.3)	22,226 (67.3)	66,991 (112)
Glucuronide 1				
AUC _{inf} (ng h/mL)	1,919 (33.6)	3,059 (60.9)	5,664 (27.7)	9,923 (40.9)
C _{max} (ng/mL)	404.6 (35.3)	473.6 (76.8)	874.4 (37.5)	1,285 (55.4)
T _{max} (h)	2.00 (1.0-6.0)	2.250 (1.0-6.0)	2.375 (1.0-8.0)	2.00 (1.5-5.0)
C _{av} (ng/mL)	76.9 (37.2)	110.3 (56.1)	215.5 (43.5)	344.1 (51.5)
Half-life (h)	2.85 (48.6)	7.27 (93.9)	10.6 (92.9)	7.90 (39.1)
CL/F (L/h)	282.7 (27.3)	493.5 (74.7)	469.5 (25.7)	590.6 (45.2)
Glucuronide 2				
AUC _{inf} (ng h/mL)	1,287 (21.7)	2,589 (66.4)	4,320 (32.9)	8,546 (62.3)
C _{max} (ng/mL)	369.5 (39.6)	672.6 (81.1)	1,626 (71.5)	1,735 (66.4)
T _{max} (h)	1.500 (1.0-5.0)	1.750 (1.0-5.1)	2.000 (1.0-6.0)	2.520 (1.5-8.0)
C _{av} (ng/mL)	51.0 (27.6)	99.9 (66.2)	193.8 (39.3)	317.8 (65.6)
Half-life (h)	3.09 (69.8)	6.64 (92.1)	8.42 (88.9)	5.83 (51.2)
CL/F (L/h)	408.8 (26.7)	642.5 (83.0)	636.9 (32.6)	1,017 (94.6)
3-Sulfate				
AUC _{inf} (ng h/mL)	4,049 (26.6)	10,053 (73.2)	16,984 (41.7)	30,898 (46.1)
C _{max} (ng/mL)	1,135 (25.7)	2,102 (81.3)	2,786 (27.2)	4,294 (48.0)
T _{max} (h)	1.500 (1.0-5.0)	2.000 (1.0-5.0)	2.000 (1.0-5.2)	2.050 (1.0-6.0)
C _{av} (ng/mL)	172.0 (23.2)	402.6 (70.5)	597.0 (27.0)	1,089 (49.4)
Half-life (h)	3.21 (56.6)	4.51 (82.8)	11.5 (95.5)	7.71 (42.3)
CL/F (L/h)	131.2 (25.8)	151.8 (62.7)	171.2 (40.0)	207.8 (63.9)

NOTE: Values are the mean of $n = 10$ with coefficient of variation (in percent) or range in brackets.

Abbreviations: AUC_{inf}, area under the concentration versus time curve to time infinity; C_{max}, maximal plasma concentration; T_{max}, median time of maximal plasma concentration; C_{av}, average plasma concentration; CL/F, apparent total body clearance (calculated as dose/AUC_{inf}); CL_R, apparent renal clearance approximated by amount excreted with urine within 24 h over AUC₀₋₂₄; V/F, apparent volume of distribution.

* $n = 1$, value for AUC_{inf} at the lowest dose could be established in only one participant.

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Background Information

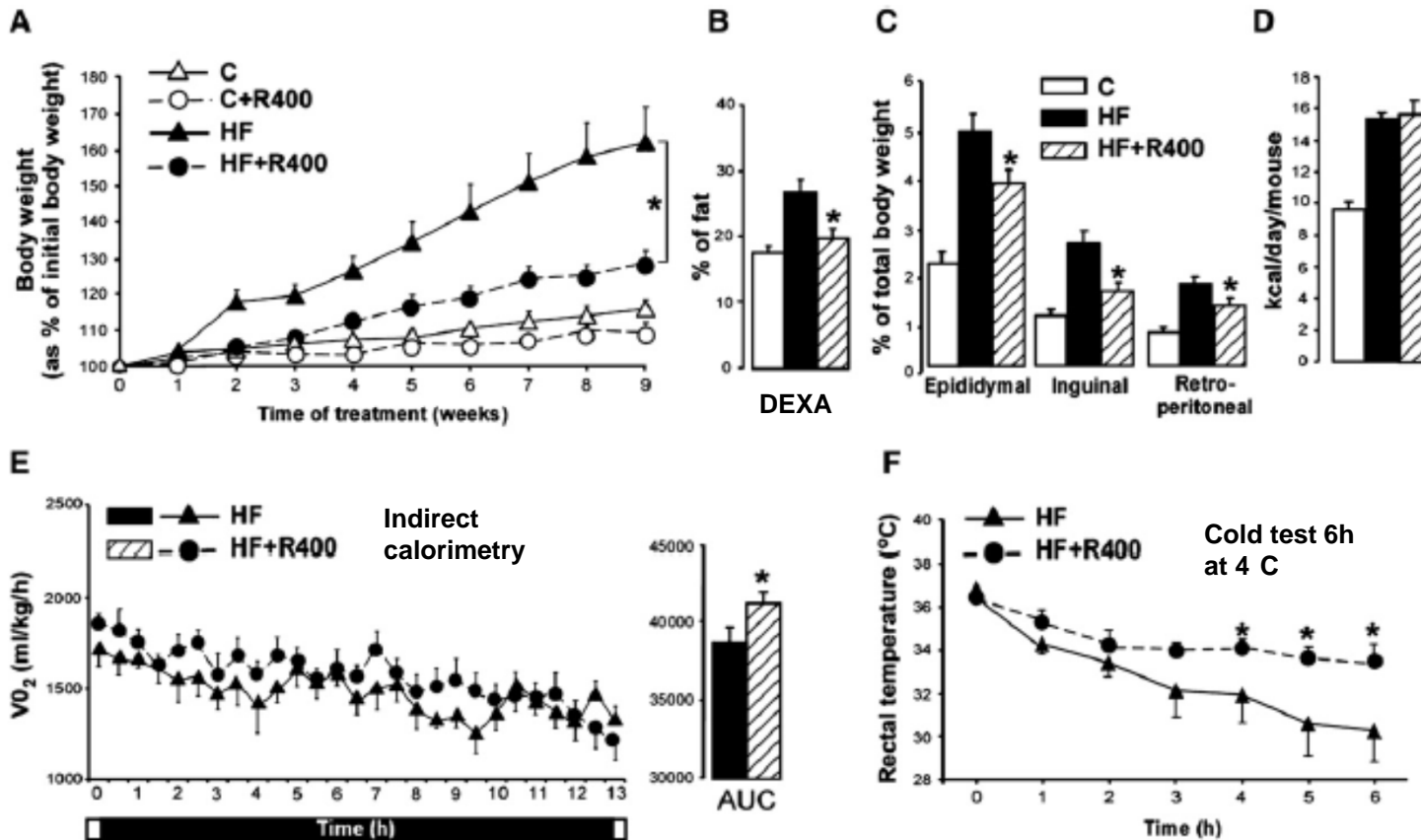
- 1. Resveratrol in animal models**
- 2. Resveratrol studies in man**
- 3. Calorie restriction in man**
- 4. Resveratrol targets**

Sirtuins
AMPK
PGC-1 α

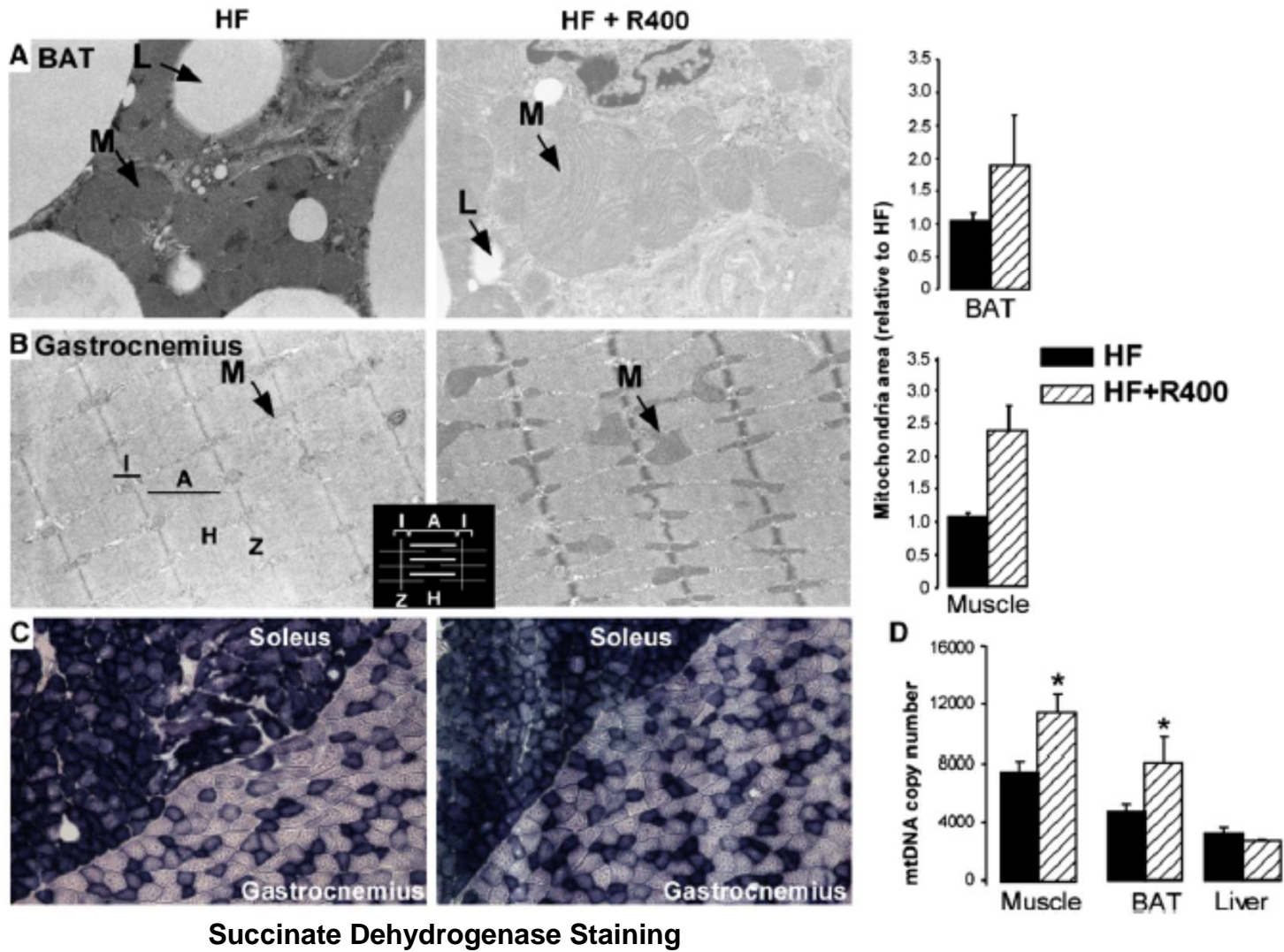
RSV Improves Mitochondrial Function and Protects against Metabolic Syndrome by Activating SIRT1 and PGC-1 α

1. Treatment of mice with RSV (15 weeks, serum levels 10-120 ng/ml) increased aerobic capacity (running time, O₂ consumption in muscle fibers)
2. RSV was associated with induction of genes for OxPhos, mitochondrial biogenesis, conversion to oxidative type I muscle fibers, and cold tolerance (BAT) that were associated with PGC-1 α deacetylation (by SIRT1).
3. RSV treatment protected mice against diet-induced obesity and insulin resistance.

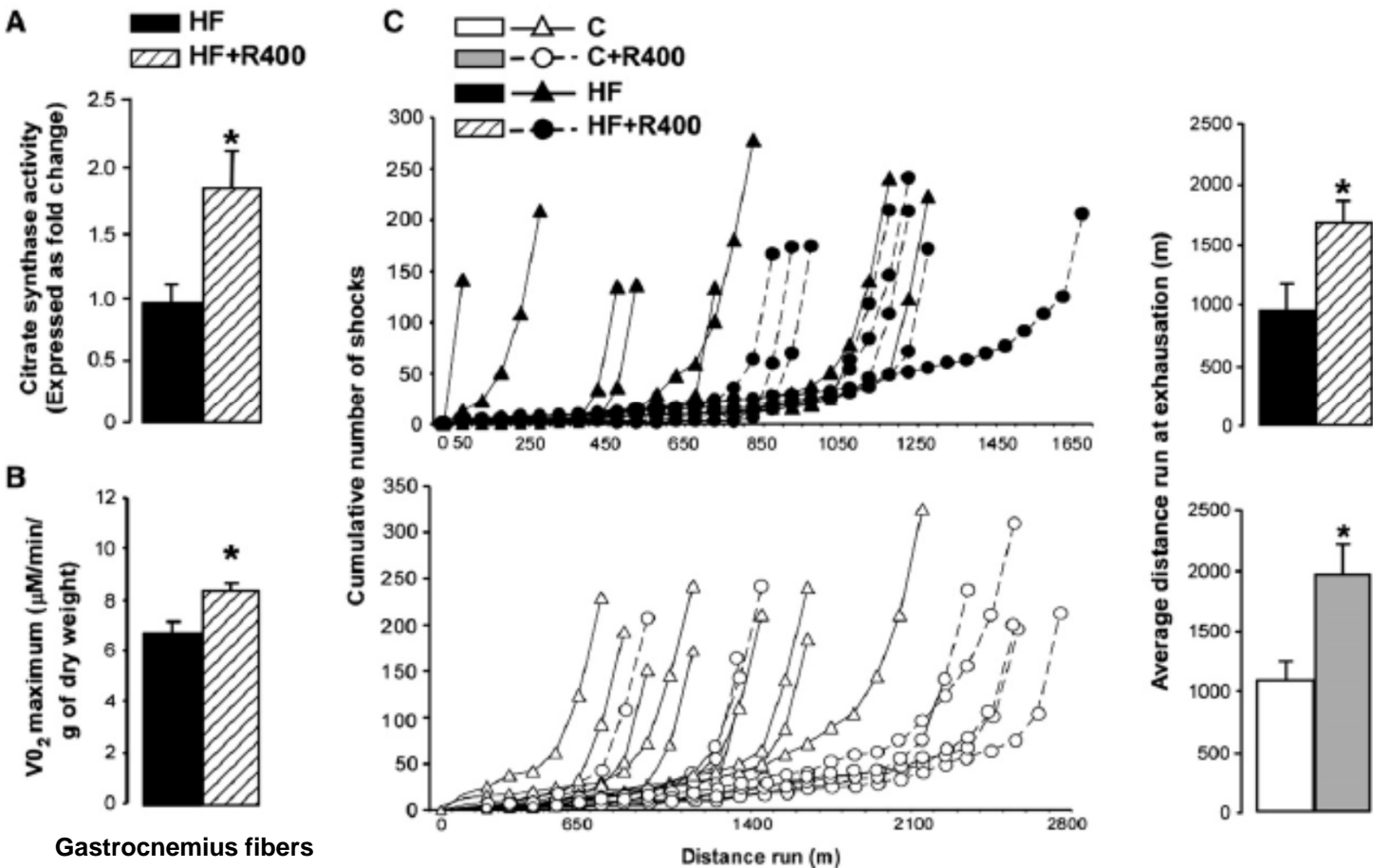
Resveratrol Prevents Diet-Induced Obesity



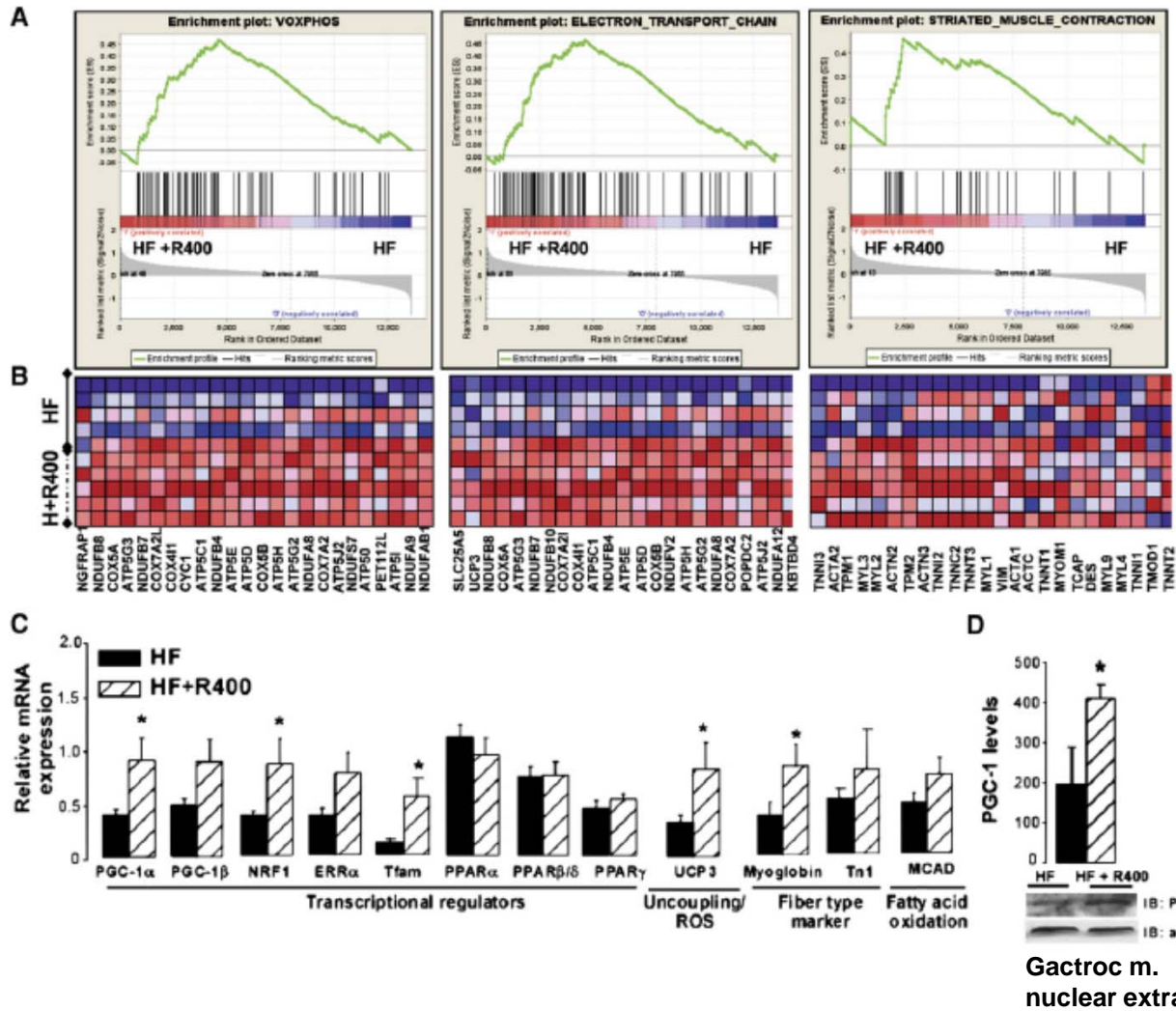
Resveratrol Increases Mitochondrial Activity in BAT and Muscle



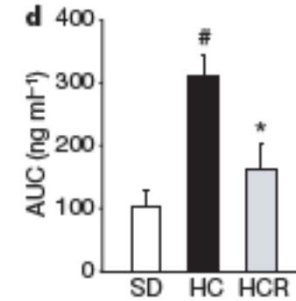
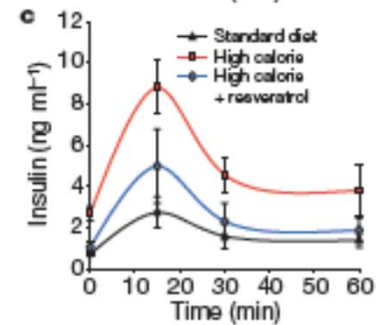
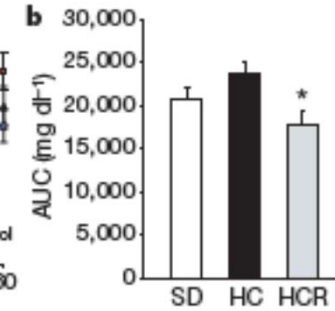
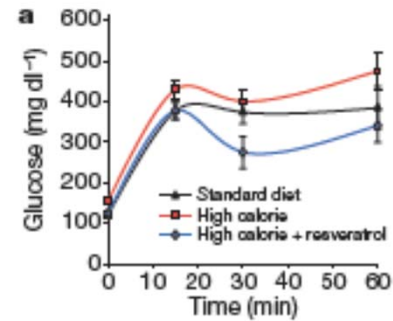
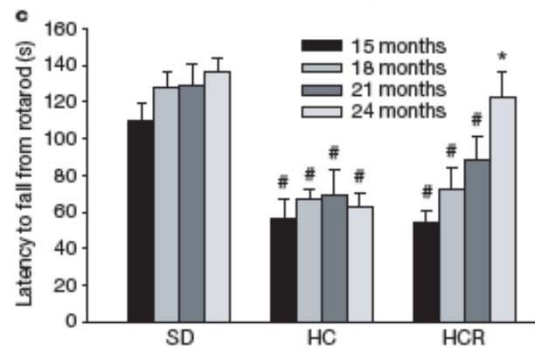
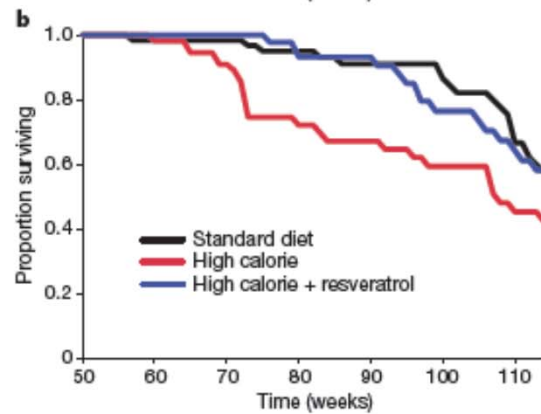
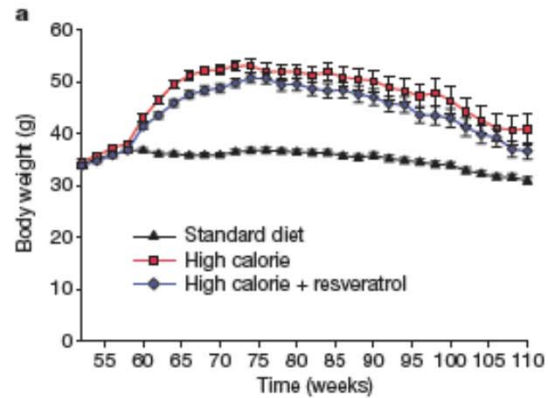
Enhanced Oxidative Capacity and Endurance in RSV-Treated Mice



Gene-Expression Profile of Skeletal Muscle from RSV-Treated Mice is Enriched in Pathways Related to Mitochondrial Biogenesis and Function

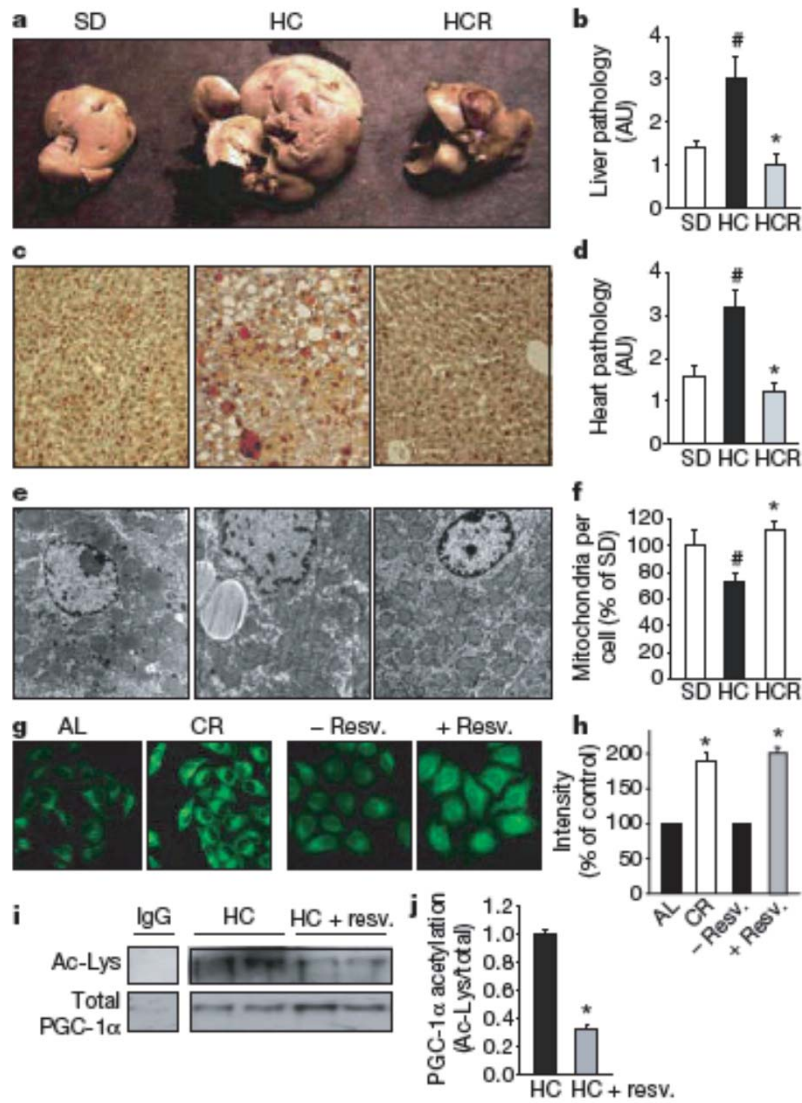


RSV Improves Health and Survival of Mice on a High-Calorie Diet



HCR = resveratrol at 22.4 mg/kg/d

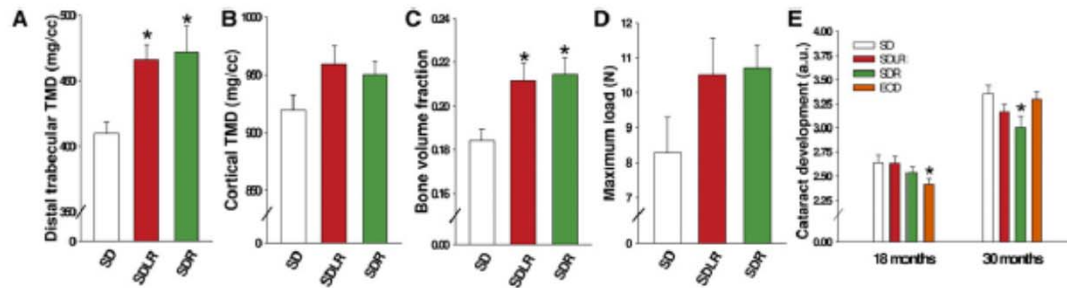
RSV Decreases Hepatic Steatosis and Increases Mitochondrial Number



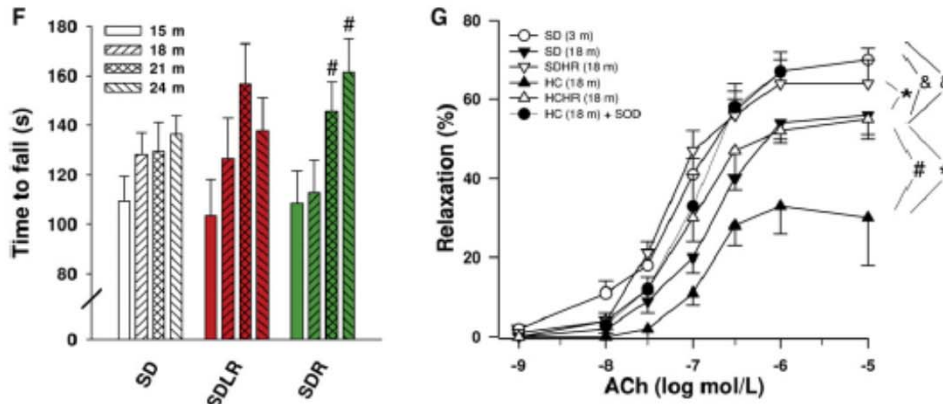
EM of liver cells

Mitochondrial counts in HeLa cells.

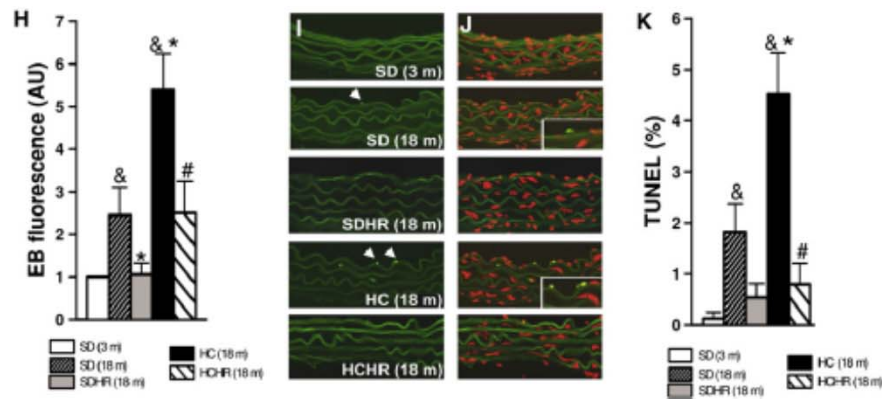
RSV Delays Age-Related Deterioration without Extending Lifespan



Effects on bone, cataract formation



Acetylcholine relaxation in aortic ring preps.
HDR shown.



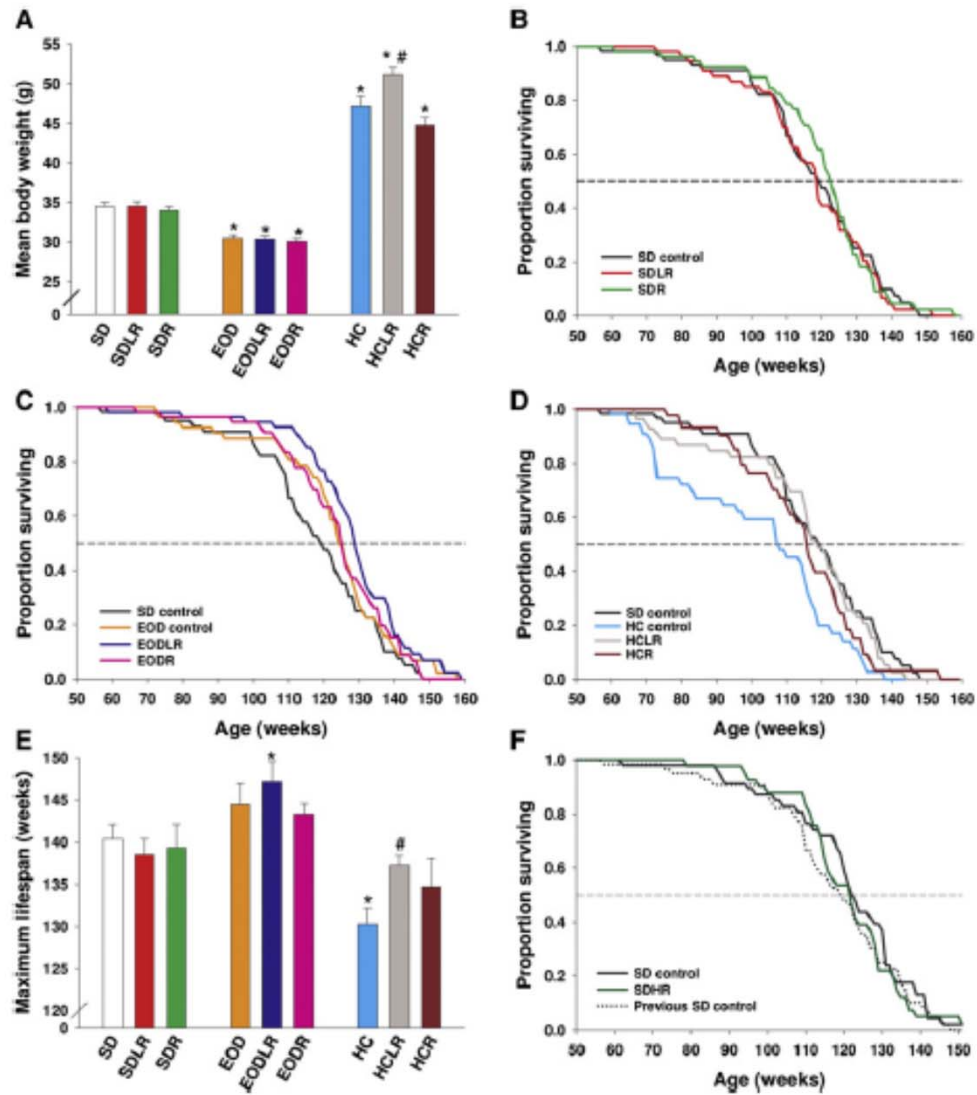
Dihydroethidine reacts with ROS to become EB.

TUNEL staining of aortas.

Low dose resveratrol: 5.4-7.9 mg/kg/day
Resveratrol: 24-30 mg/kg/day
High dose resveratrol: 167-204 mg/kg/day

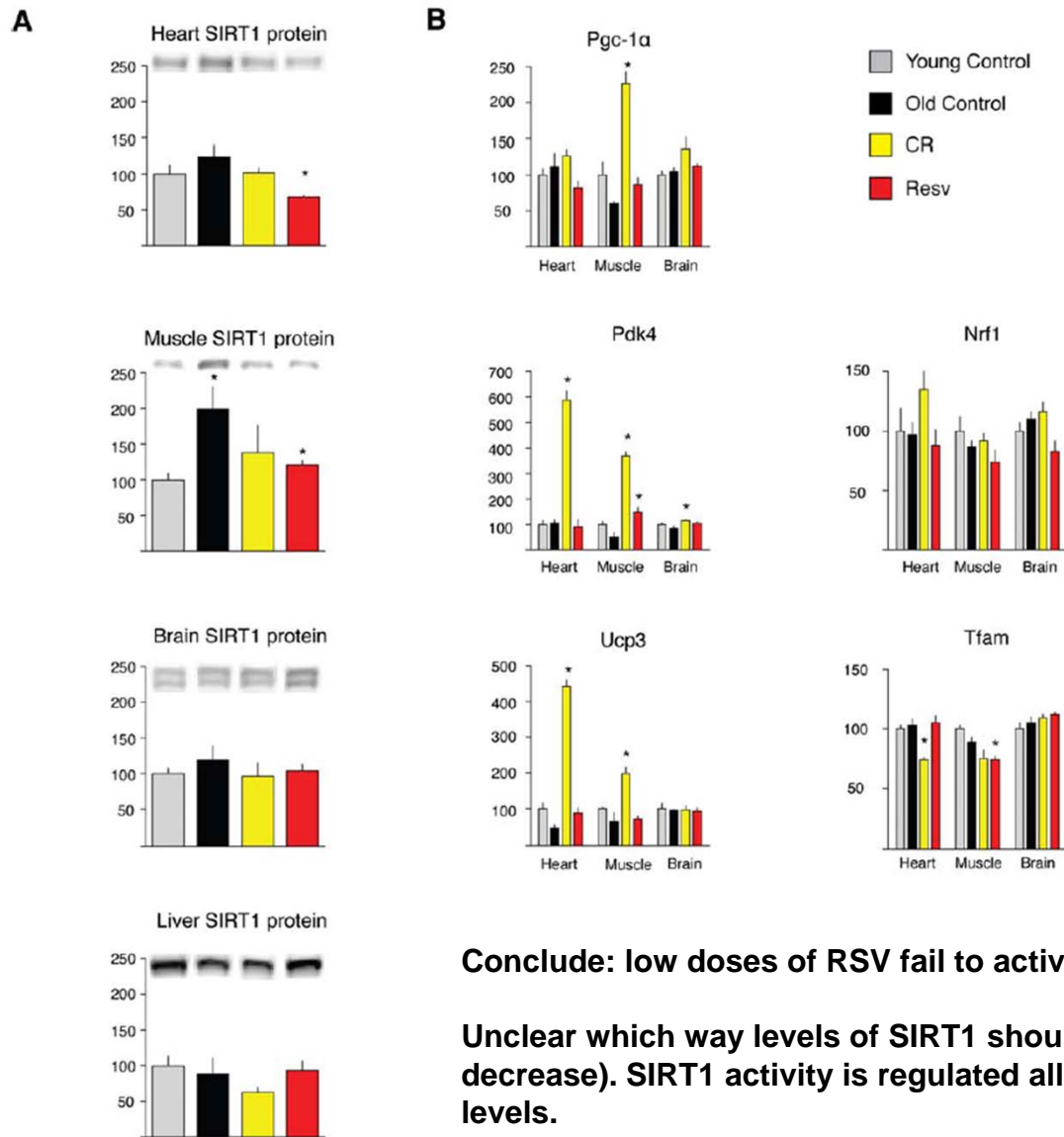
Note: key vascular studies all performed using mice fed HDR.

Effects of Resveratrol Treatment on Longevity in Mice Fed Standard or High-Calorie Diets



Low dose RSV partially mimics CR and retards aging parameters in mice.

SIRT1 levels and PGC-1 α transcriptional activity in response to CR and resveratrol (4.9 mg/kg/d, 14-30 mo).



Conclude: low doses of RSV fail to activate PGC-1 α pathway.

Unclear which way levels of SIRT1 should go (elderly, they are purported to decrease). SIRT1 activity is regulated allosterically, not necessarily by protein levels.

Calorie Restriction Increases Muscle Mitochondrial Biogenesis in Healthy Humans

Young (37 yo median), overweight (BMI 28)

6 mo intervention: Control (100% energy requirements)

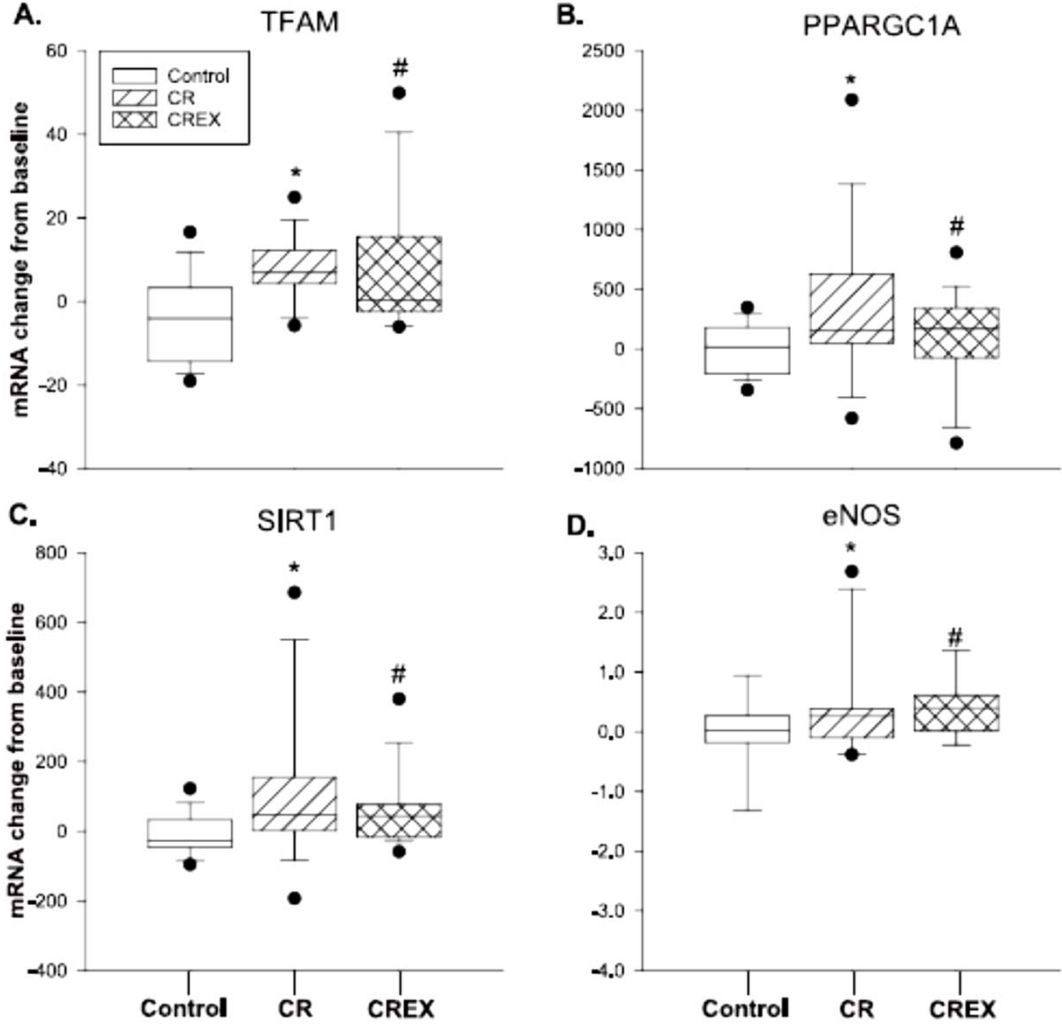
CR: 25% calorie restriction

CREX: 12.5% CR + 12.5% increased energy expenditure

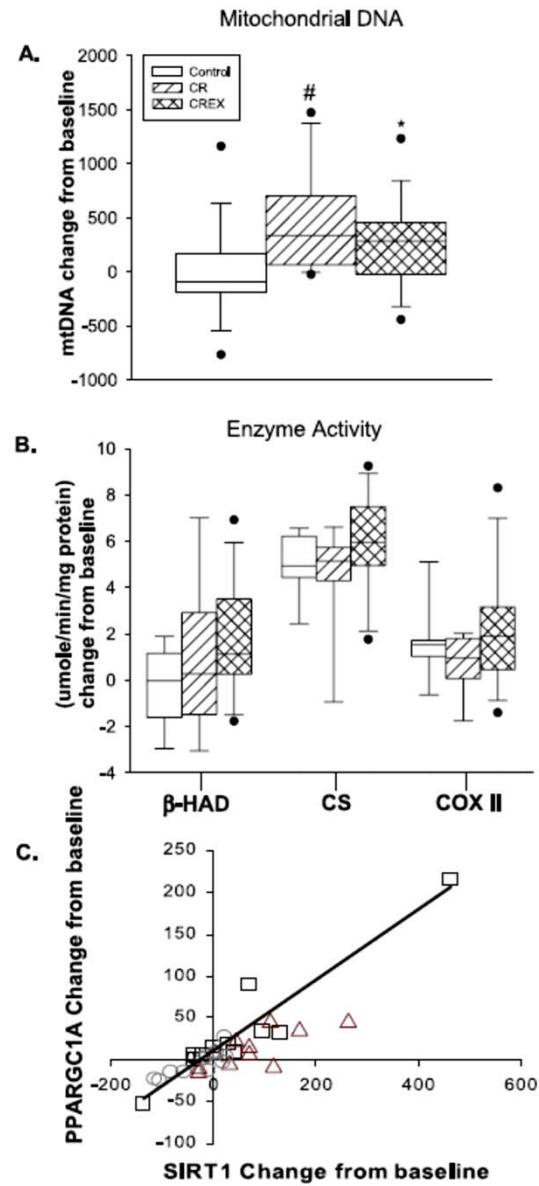
Table 1: Metabolic Characteristics of Subjects Completing the Study (*n* = 36)

Variable	Control		Calorie Restriction		Calorie Restriction + Exercise	
Sex (M/F)	5/7		6/6		5/7	
Age (y)	36.7 ± 2.1		38.9 ± 1.6		34.9 ± 1.6	
Weight (kg) ^a	82.3 ± 2.9	81.5 ± 3.0	81.1 ± 3.3	72.8 ± 3.1 ^b	82.1 ± 3.1	73.7 ± 2.8 ^b
Body Fat (%) ^a	32.3 ± 1.8	30.9 ± 2.1	31.0 ± 2.4	26.6 ± 2.4 ^b	32.6 ± 2.2	27.6 ± 2.4 ^b
Fat mass (kg) ^a	25.5 ± 1.2	25.0 ± 1.7	24.9 ± 1.8	19.1 ± 1.7 ^b	26.5 ± 1.7	20.1 ± 1.7 ^b
Fat-free mass (kg) ^a	56.8 ± 3.1	56.5 ± 3.2	56.3 ± 3.5	53.7 ± 3.3 ^b	55.6 ± 3.5	53.6 ± 3.2 ^b
24-h EE (kcal) ^a	2,129 ± 97	2,092 ± 97	2,079 ± 102	1,899 ± 101 ^b	2,106 ± 102	1,917 ± 91 ^b
Glucose (mg/dl) ^a	89.6 ± 1.2	92.3 ± 2.0	89.3 ± 1.8	88.2 ± 2.1	92.0 ± 1.8	91.5 ± 1.9
Insulin (μU/ml) ^a	12.3 ± 0.9	12.6 ± 1.7	9.4 ± 1.5	6.3 ± 0.9 ^c	9.8 ± 1.0	7.4 ± 0.8 ^c
Adiponectin (μg/ml) ^a	3.0 ± 0.3	3.2 ± 0.3	3.2 ± 0.3	3.8 ± 0.03	3.4 ± 0.3	3.7 ± 0.3

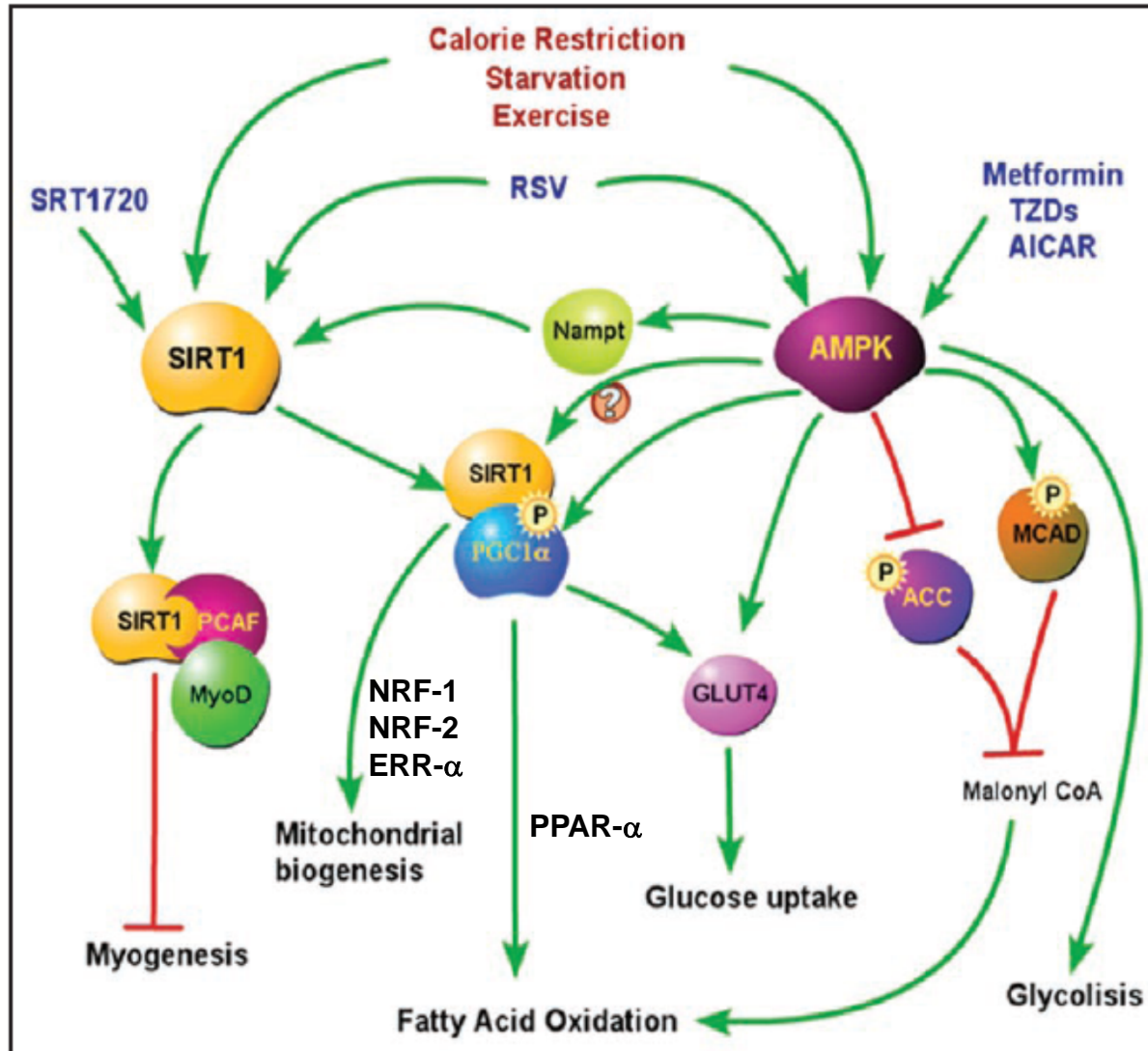
Changes in Skeletal Muscle Gene Expression for Key Mitochondrial Proteins



Effects of CR on Mitochondrial Energetics



Critical Effectors of Effects of Calorie Restriction and Exercise (and Resveratrol?)



Metabolic Pathways of Growth and Proliferation: Where Research on Aging and Cancer Converge

