

Inflammation and Frailty in Older Adults (and Mice)

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Introduction

- Frailty: The Opposite of Longevity
- Inflammation in Geriatrics and Frailty
- Inflammatory cytokines as markers of vulnerability
- A mouse model of chronic inflammation

Frailty

- A geriatric syndrome of weakness, weight loss, and low activity associated with adverse health outcomes
- An age-related, biological vulnerability to adverse outcomes that stems from alterations in multiple physiological systems

A Clinical and Research Tool

■ In clinical practice

- To identify those at highest risk for adverse clinical outcomes
- develop interventions to decrease risk

■ As a research tool

- to explore the biological etiologies of late life decline, chronic disease, and aging biology
- to study age-related, multisystem decline

Why Does Frailty Matter?

- Slowest recovery rate
- Most iatrogenic complications
- Highest mortality rate
- Key to understanding late life decline and the connections between aging and chronic diseases

Key Observations by Clinicians

- Shrinking, weight loss, low activity correlated with vulnerability to getting sick and dying in older adults
- Frail, older adults often resemble other patients with malignancy or inflammatory diseases

Frailty Phenotype Development

- Weight loss
- Weakness
- Exhaustion
- Slowed walking speed
- Low activity

* Frail if 3 of 5 are present

Frailty Status Predicts Adverse Outcomes

	CHS	WHAS
Incident Fall	1.29 (1.00 – 1.68)	1.18 (0.63, 2.19) (NS)
Worsening Mobility	1.50 (1.23, 1.82)	10.44 (3.51, 31.00)
Worsening ADL Disability	1.98 (1.54 – 2.55)	15.79 (5.83, 42.78)
First Hospitalizations	1.29 (1.09, 1.54)	0.67 (0.33, 1.35) (NS)
Death	2.24 (1.51, 3.33)	6.03 (3.00, 12.08)

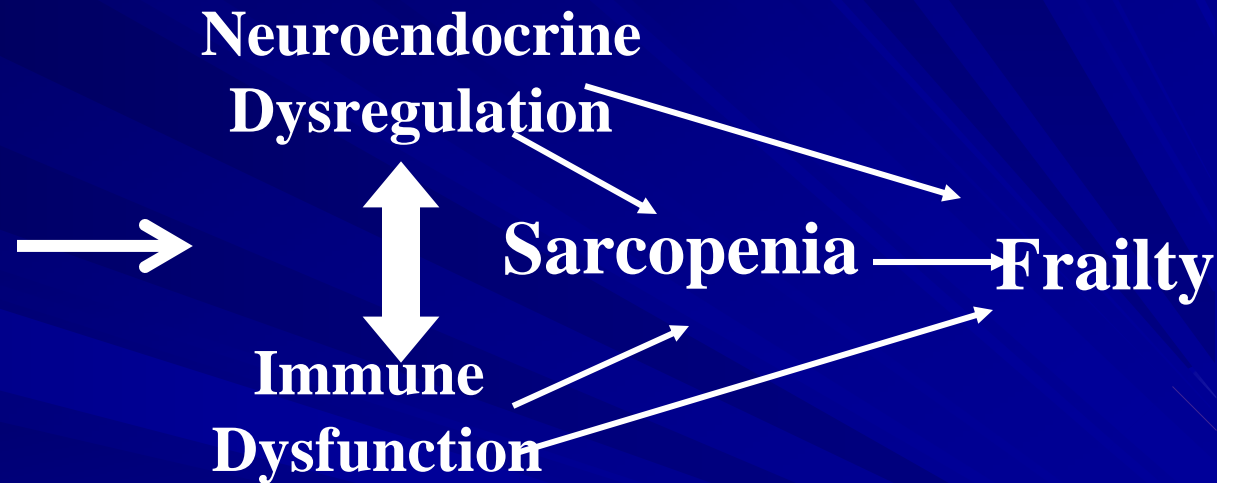
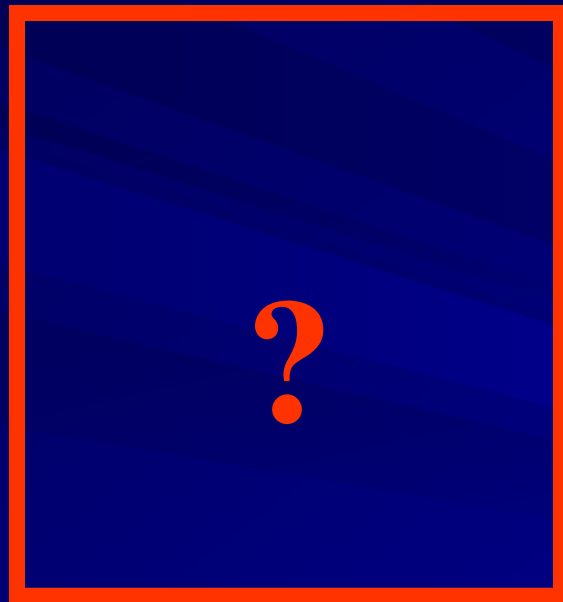
Hazard Ratios Estimated Over 3 Years, covariate adjusted, $p > 0.01$

Fried, L. P., et al, J Ger Med Sci, 2001

Bandein-Roche et al, J Ger Med Sci, 2006

Pathophysiologic Hypotheses

Physiology



Inflammatory Biomarkers of Frailty

Characteristic	Not Frail (n=2289)	Intermediate (n=2147)	Frail (n=299)	P Value
C-reactive protein (mg/L)	2.7 (4.0)	3.7 (6.5)	5.5 (9.8)	<.001
Fibrinogen (mg/dL)	313.3 (60.9)	324.1 (66.7)	340.7 (78.6)	<.001
Factor VII (mg/dL)	124.0 (28.3)	125.5 (29.4)	124.9 (33.1)	.42
Factor VIII (mg/dL)	118.6 (34.6)	123.3 (37.8)	137.9 (44.8)	<.001
* Including those with CVD & diabetes				

Metabolic Biomarkers of Frailty

Characteristic	Not Frail (n=2289)	Intermediate (n=2147)	Frail (n=299)	P Value
Fasting Glucose level (mg/L)	107.3 (28.8)	111.4 (34.8)	119.8 (57.2)	<.001
2-hour Glucose level (mg/dL)	141.4 (55.6)	151.4 (59.3)	160.6 (65.0)	<.001
Fasting Insulin level (IU/mL)	15.8 (23.8)	18.2 (26.5)	18.0 (29.9)	.001
2-hour Insulin level (IU/mL)	79.9 (61.7)	87.5 (64.8)	89.0 (63.6)	<.001

* Including those with CVD & diabetes

Walston, et al. 2002, Arch Intern Med

Cortisol and Frailty

- Significantly higher PM cortisol levels in frail compared to non frail
 - beta = 0.11, p = .04
- No differences early in AM
- Rate of decline significantly lower from morning to afternoon in frail
 - beta = 0.04, p = .02

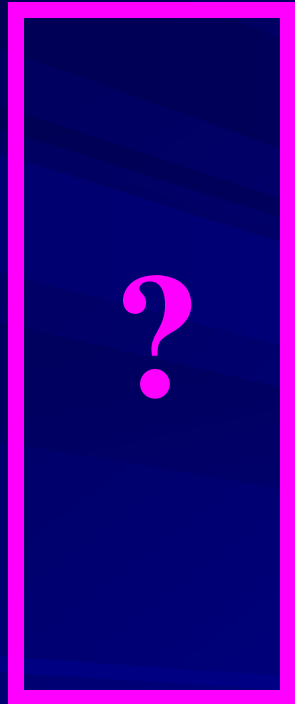
Chronic Disease Influence

- What diseases most associate with frailty?
 - Type 2 Diabetes Mellitus
 - Congestive Heart Failure
 - Hypertension
 - Peripheral vascular disease

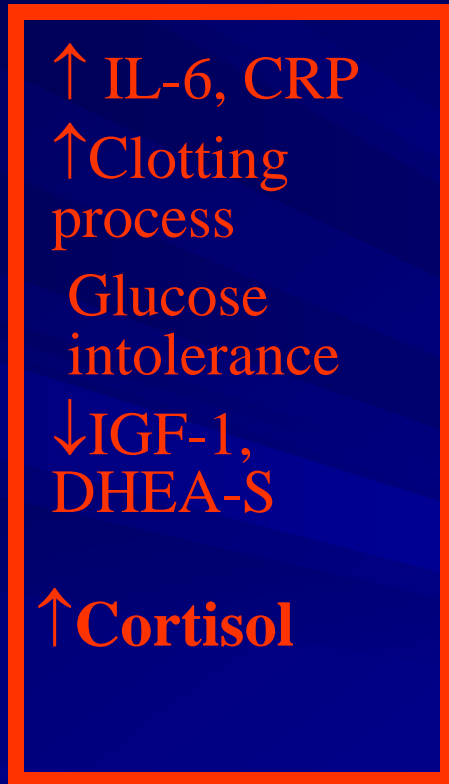
Blaum C et al, JAGS 2009; Newman A, et al, Journal of Gerontology 2001

Modal Pathway- 2010

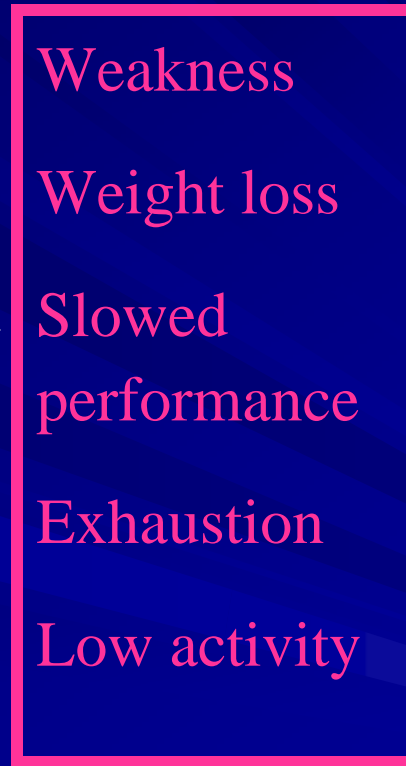
Molecular & Genetic



Physiology



Syndrome



Outcomes



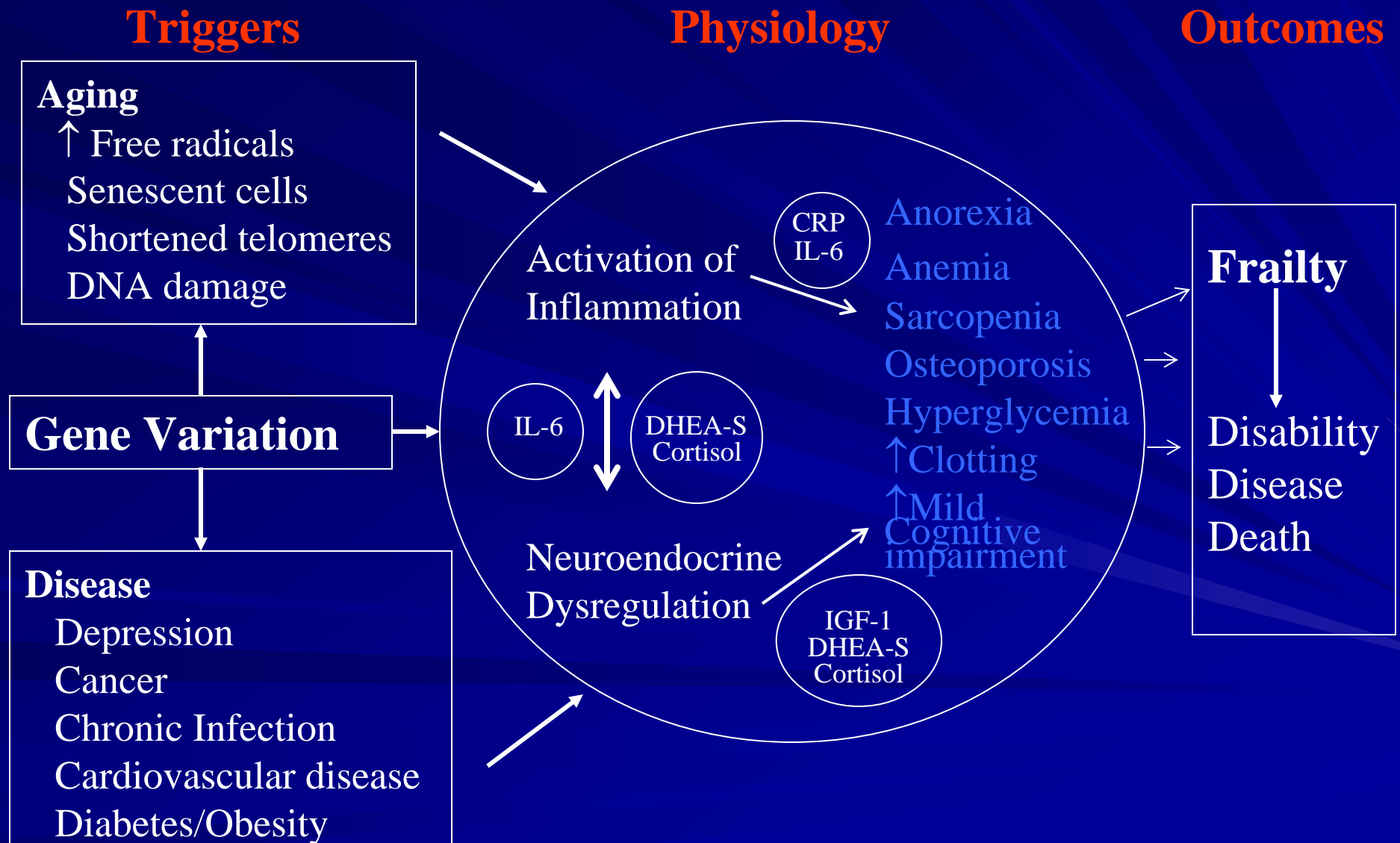
Fried, et al., Gerontology 2001

Walston, et al., Archives of IM 2002

Leng, et al., JAGS 2002

Leng, et al., Aging 2004

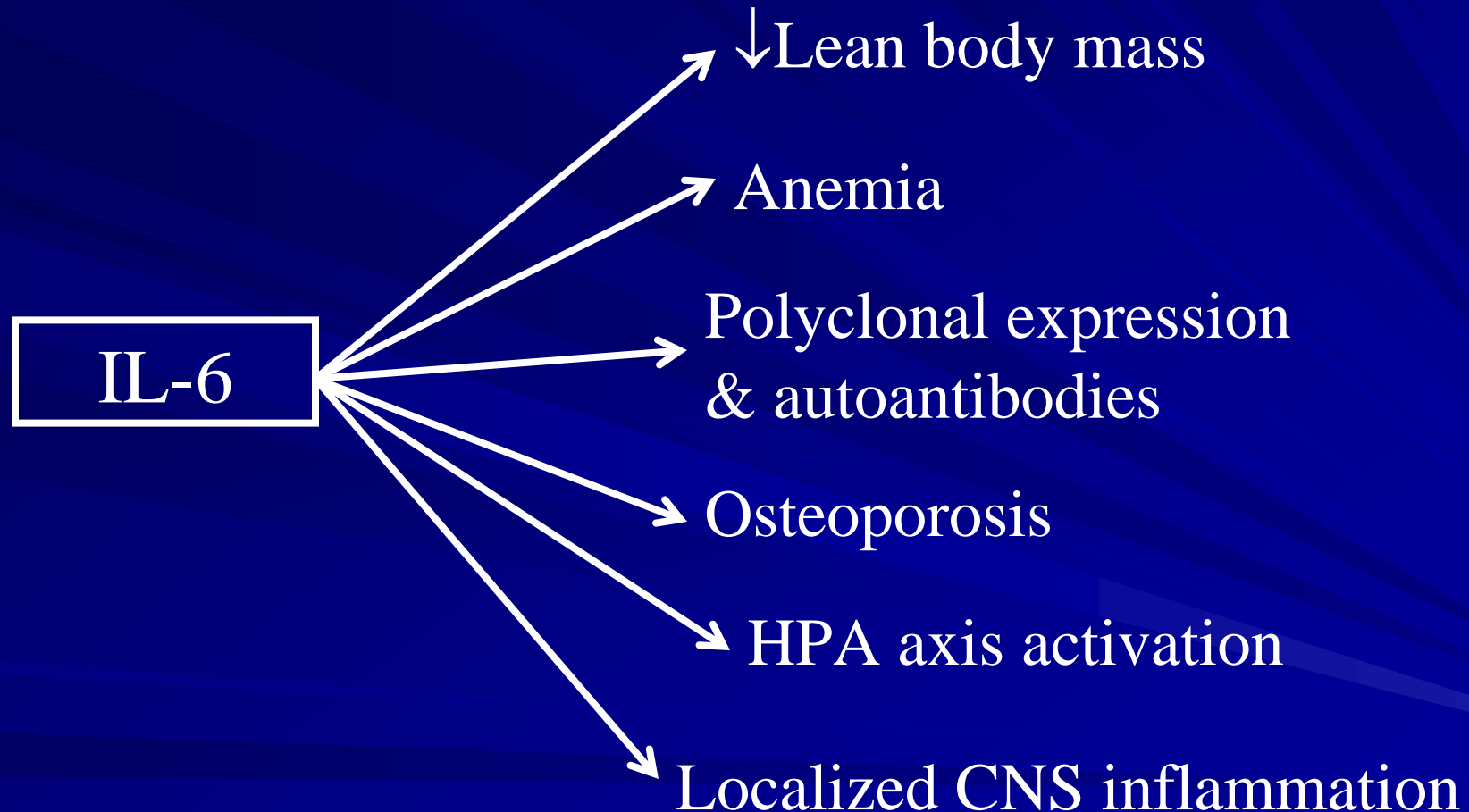
Pathophysiologic Model for Adverse Outcomes in Older Adults



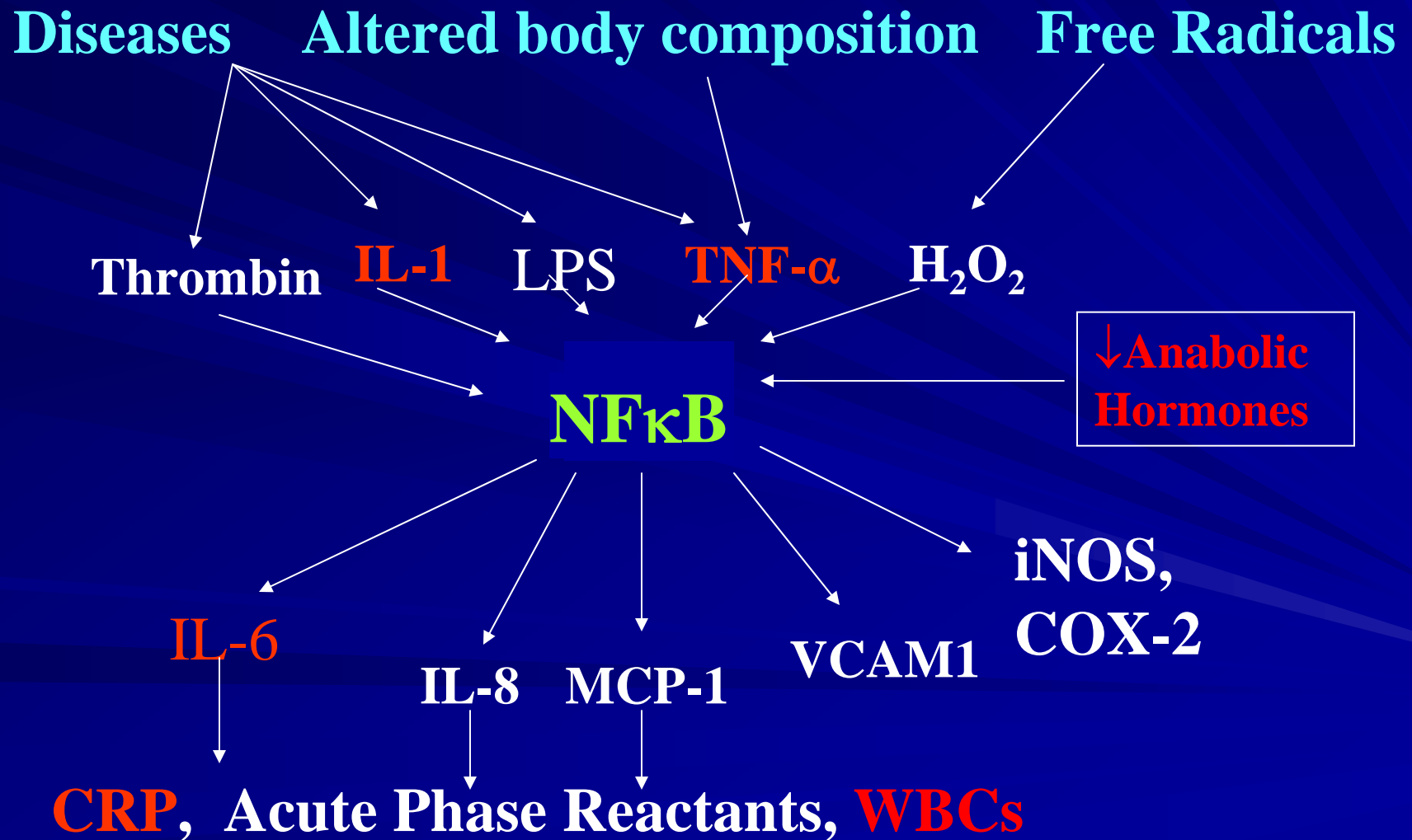
Inflammation and Poor Health

- Dozens of association studies
- Molecular studies demonstrating direct pathological impact of inflammatory mediators on multiple cell and tissue types
- Biological evidence that inflammation contributes to specific disease states

IL-6 and Multisystem Dysregulation



Inflammatory Mediators and Aging



Clinical Translation

- identify those at most risk for poor outcomes
- assess risk for peri- and post-operative complications in those undergoing surgery or anesthesia
- Monitor effectiveness of therapies

Developing Inflammatory Phenotype Markers

- Access to In CHIANTI and Cardiovascular Health Study samples and phenotypic data
- Baseline serum cytokines measured first in In CHIANTI and determined relationship between cytokines and mortality
- Developed aggregate phenotypes in CHS and validated in In CHIANTI

Best Serum Inflammatory Markers of Vulnerability?

■ Candidate Inflammatory Biomarkers

- IL-1 RA
- IL-6, 15, 18, 1beta
- TNF-alpha and R1, R2
- CRP
- MCP-1
- WBC

Five Finalists Based on Mortality and Age Associations in IN CHIANTI

- IL-1 RA
- IL-6
- TNF-alpha R1
- IL-18
- CRP

Endophenotype Development

- Three methods utilized and evaluated for mortality risk analysis of inflammatory phenotypes in 10 years CHS survival data.
- Validated same method in an independent study - 10 years InCHIANTI data

Weighted Summary Score (WSS)

$$WSS = \sum_{k=1}^5 w_k (Y_{ik} - \bar{Y}_k) \quad w_k = \frac{1/\sigma_k}{\sum_{k=1}^5 (1/\sigma_k)}$$

- Where Y_{ik} is each log cytokine
 $k = \text{cytokines}, 1, 2 \dots 5; i = \text{obs}, 1, 2 \dots n, n = \text{total obs.}$
- mortality risk assessed using age adjusted Cox model
- Weights are:

logCRP

0.08

logIL6

0.24

LogTNFR1

0.37

logIL18

0.18

logIL1RA

0.13

Principle Component Score (PCS):

- Uses the first principle component of correlation matrix

$$PCS = \sum W_k * Y_{ik}$$

- Where W_k is the loading of the first principle component .
 $k = \text{cytokines, } 1, 2 \dots 5;$
- $\sum w_k^2 = 1$ by the property of principle component.
- Weights (loading) are:

logCRP	logIL6	logTNFRI	logIL18	logIL1RA
0.48	0.50	0.44	0.31	0.47

Mortality Risk Score (MRS):

- Model selection for 5 cytokines by BIC

where $k=0$ is for age, $k=1, 2, \dots, 5$ for cytokines

$$h_i(t) = h_0(t) \exp(\beta_0 Y_{i0} + \beta_1 Y_{i1} + \dots + \beta_k Y_{ik})$$

- IL6 and TNFaR1 were selected by strength of prediction, others dropped from model as didn't add additional information
- mortality risk score assessed using age adjusted Cox model

$$\text{MRS} = \beta_{\log\text{IL6}} * \log\text{IL6} + \beta_{\log\text{TNFa}} * \log\text{TNFa}$$

- Weights (coefficients) :

$\beta_{\log\text{IL6}}$
0.48

$\beta_{\log\text{TNFaR1}}$
0.94

Results in CHS

Parameter	Chi-Square	Pr > ChiSq	Hazard Ratio
logCRP	72	<.0001	1.22
logIL6	287	<.0001	1.44
logTNFRI	274	<.0001	1.48
logIL18	24	<.0001	1.12
logIL1RA	56	<.0001	1.19
age	772	<.0001	1.8
WSS	281	<.0001	1.47
PCS	237	<.0001	1.43
MRS	433	<.0001	1.64

- Each predictor is adjusted by age, except age by its own.
- To make HRs comparable between predictors, all predictors are standardized.
- From looking at the hazard ratios for age, the model indicates that increase 1 unit of standardized age, the rate of mortality increases by 80 %.

Validation in InCHIANTI

Parameter	Chi-Square	Pr > ChiSq	Hazard Ratio
logCRP	24	<.0001	1.3
logIL6	19	<.0001	1.26
logTNFAR1	25	<.0001	1.32
logIL18	16	<.0001	1.25
logIL1RA	18	<.0001	1.29
age	366	<.0001	2.78
WSS	33	<.0001	1.23
PCS	27	<.0001	1.24
MRS	33	<.0001	1.37

- Each predictor is adjusted by age, except age by its own.
- To make HRs comparable between predictors, all predictors are standardized .
- From looking at the hazard ratios for age, the model indicates that increasing 1 unit of standardized age, the rate of mortality increases by 178%.

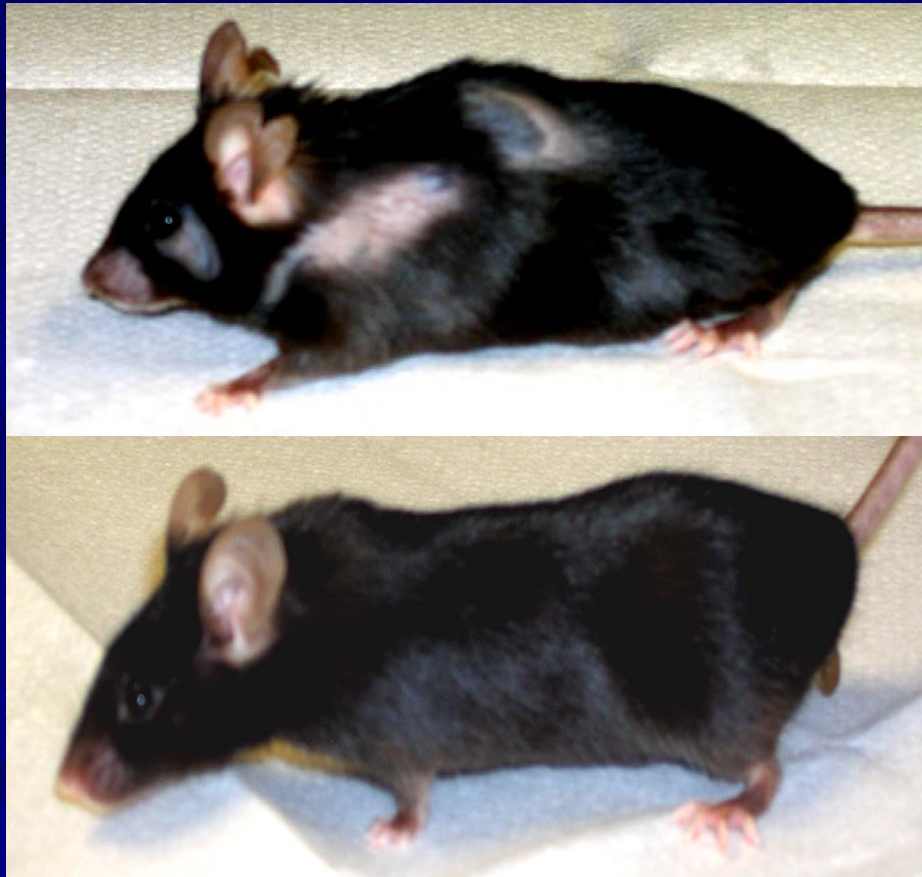
Next Steps:

Study predictive power of MRS for other adverse outcomes and frailty

Use in clinical translational studies to assess risk of adverse outcomes to surgery, anesthesia, other procedures

Use in genetic studies as aggregate inflammatory phenotype

A Mouse Model for Frailty And Chronic Inflammation:



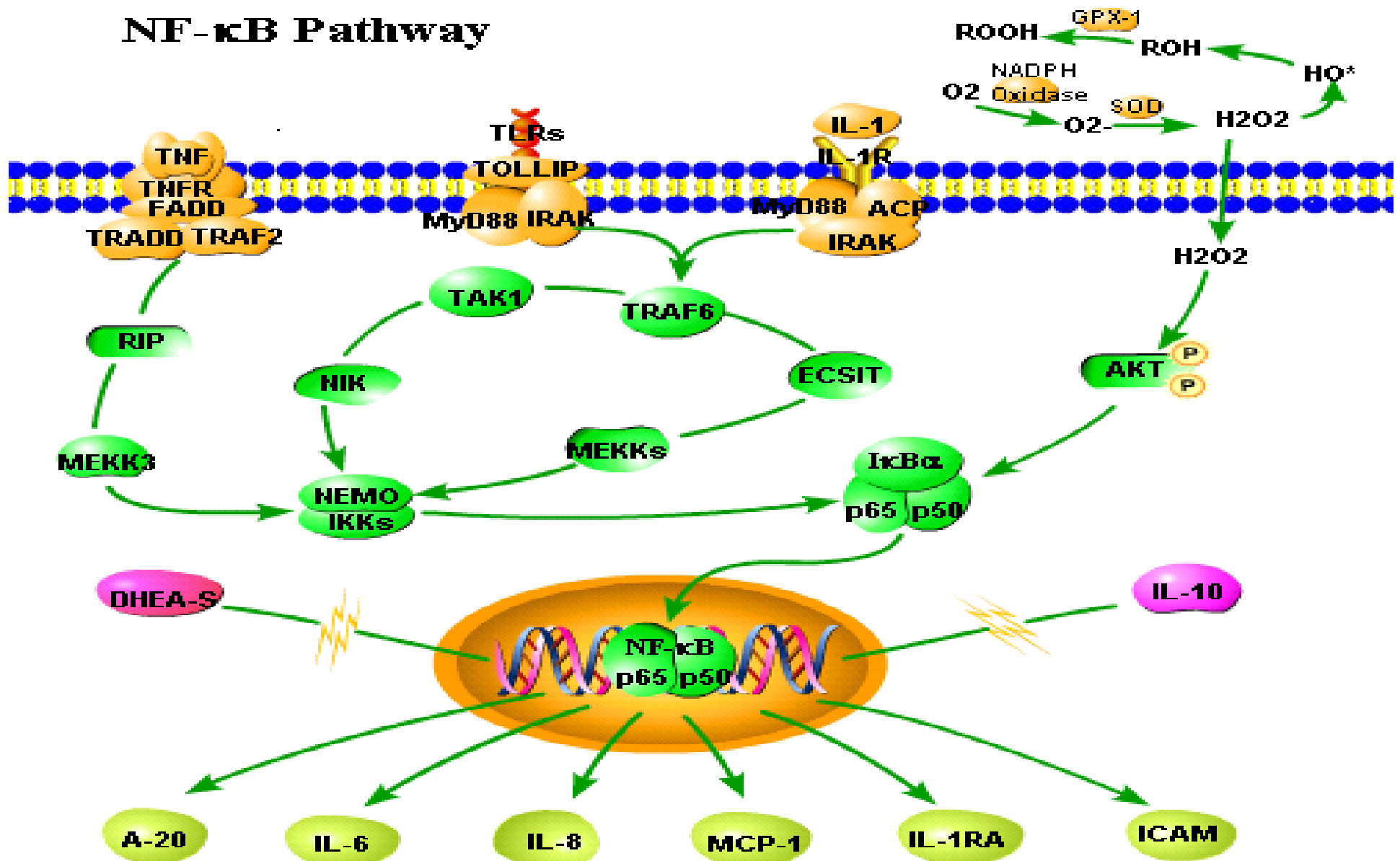
IL-10^{tm/tm}

C57B1/6J

Walston J et al, Gerontology Med Sciences, 2008

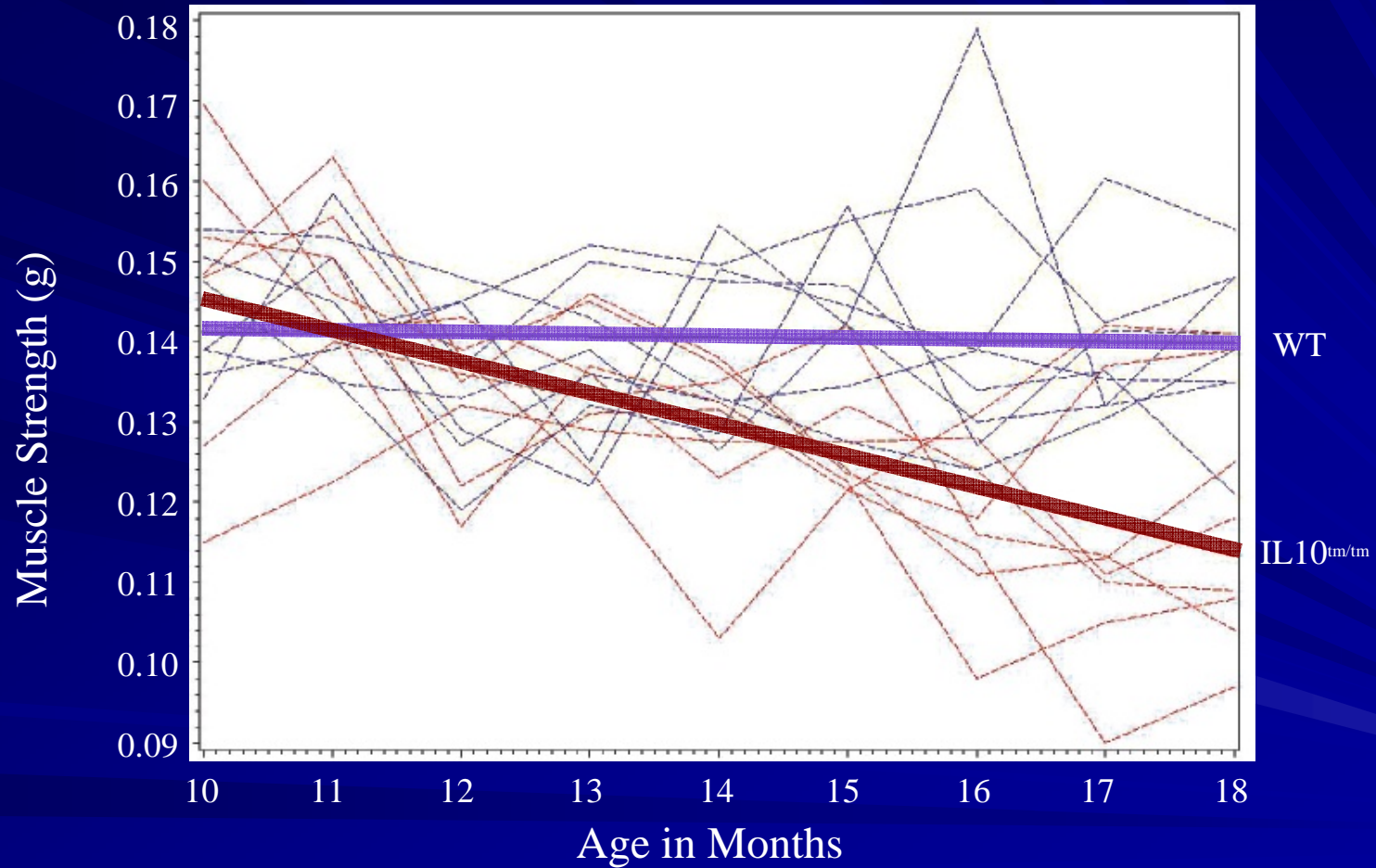
Molecular Gateway to Inflammation

NF- κ B Pathway



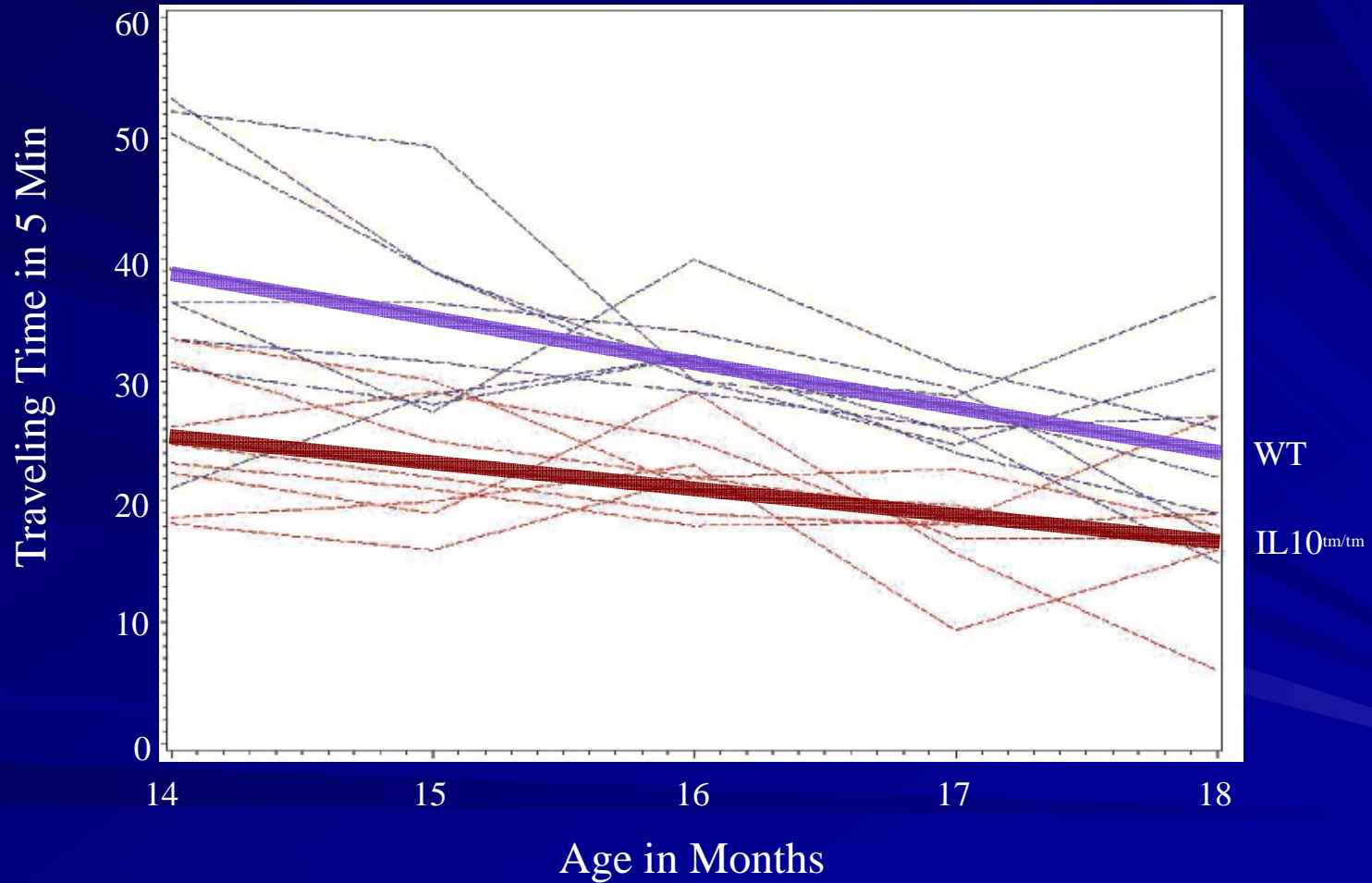
Muscle weakness

Individual Muscle Strength Change over Time



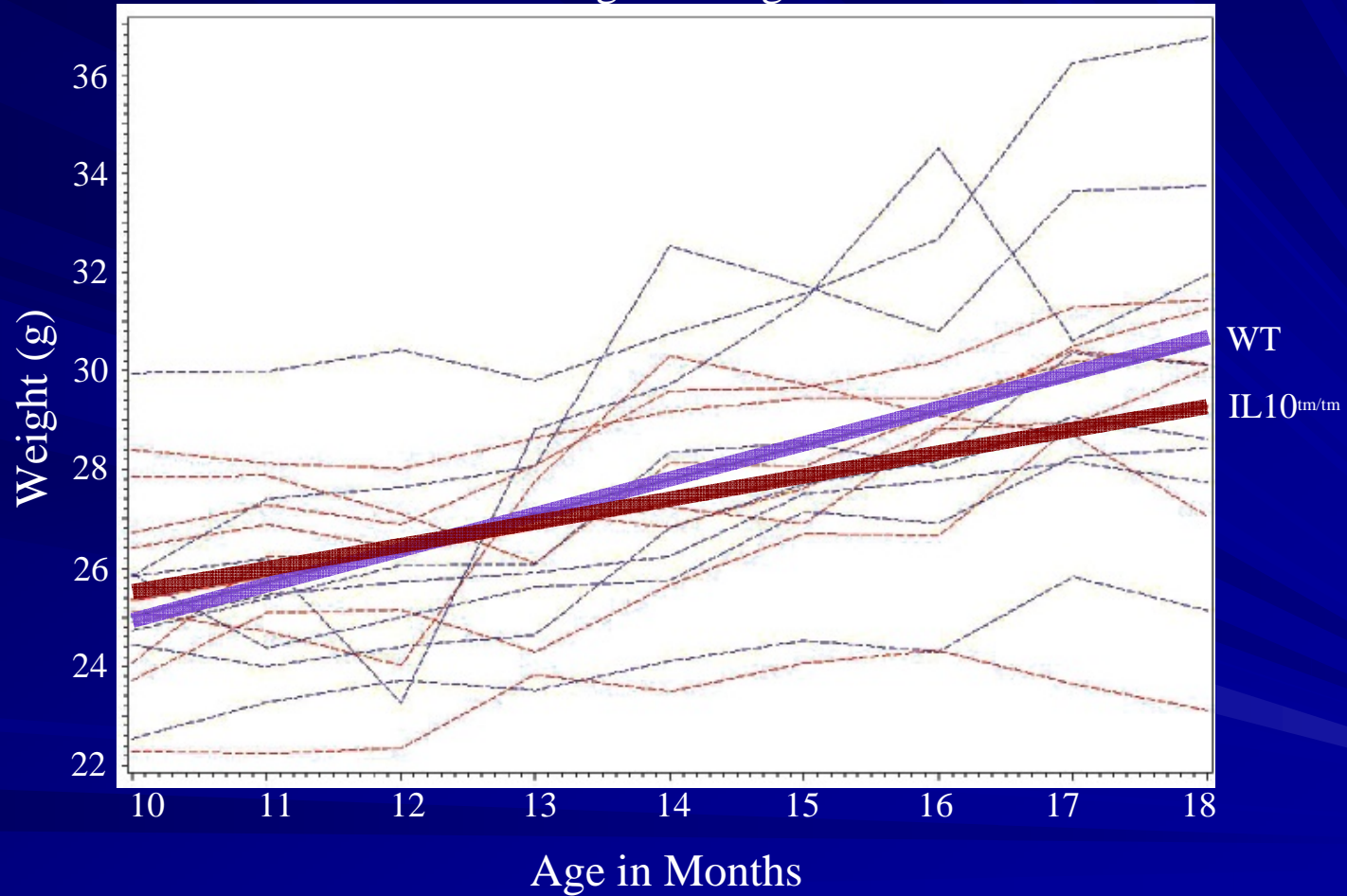
Decreased activity

Individual Change of Traveling in 5 Min over Time

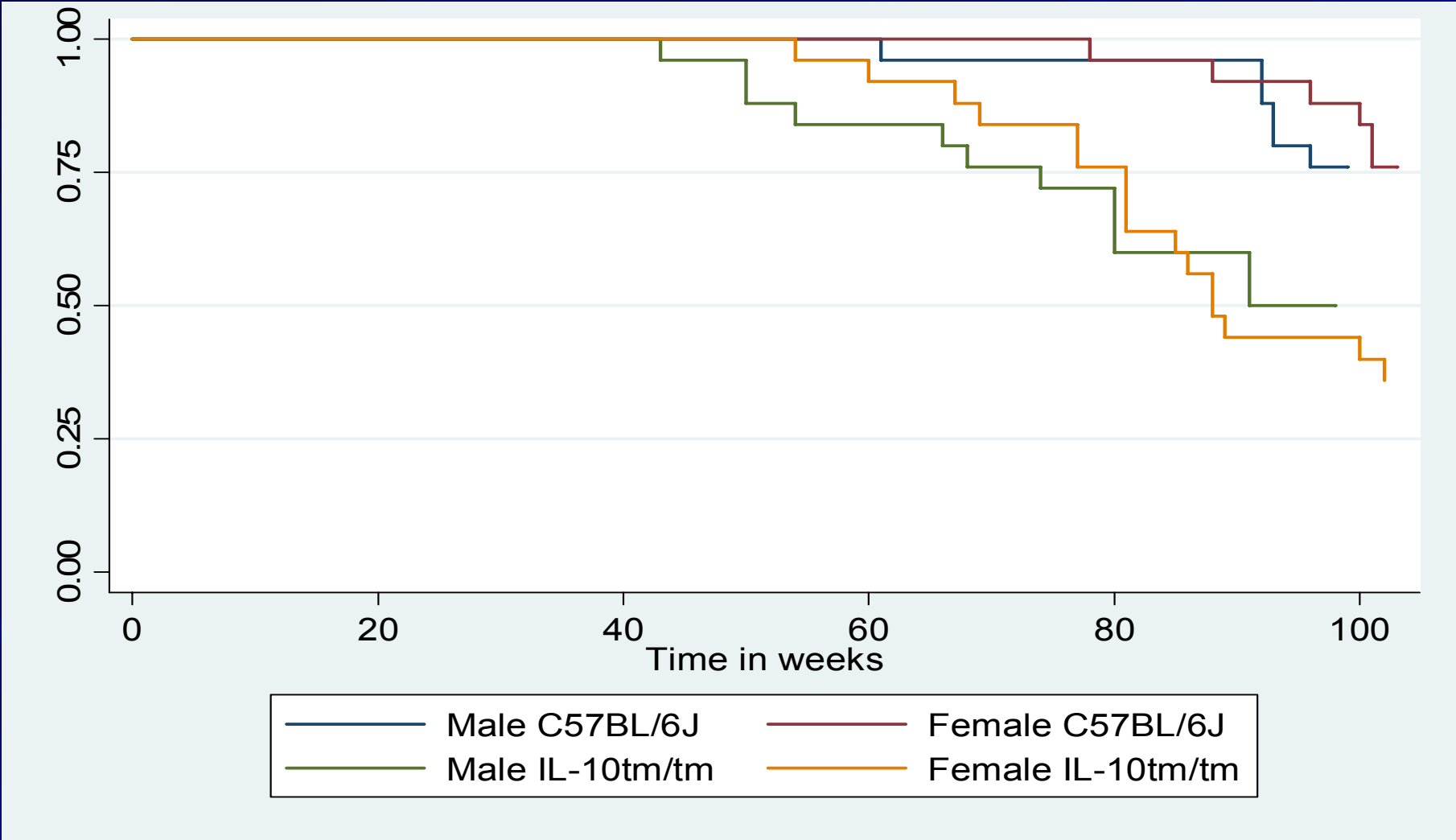


Weight loss

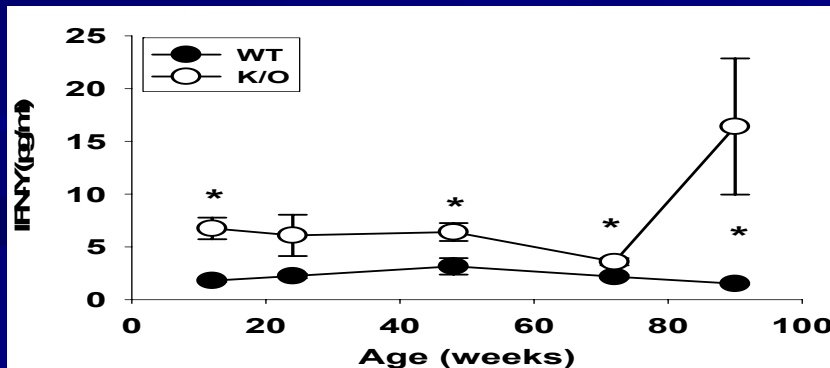
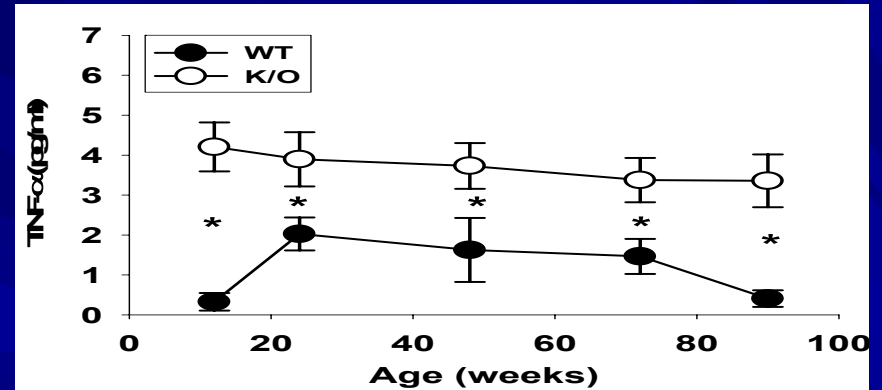
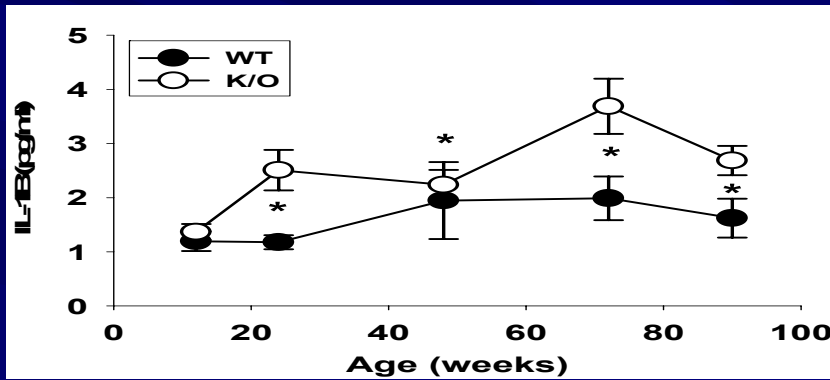
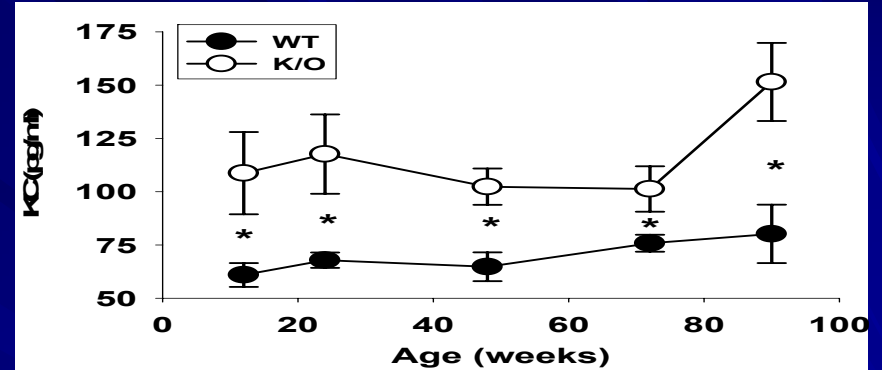
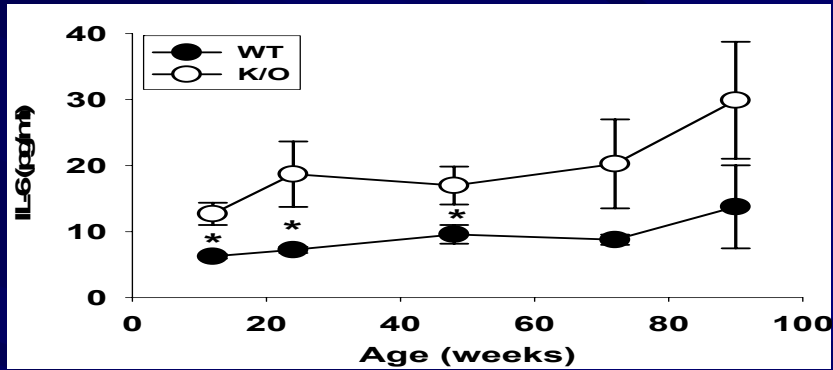
Individual Weight Change over Time



Kaplan Meier Survival Curve for IL-10 -/- and Controls



Pro-inflammatory State



* $p < 0.05$. WT (n = 14-25), K/O (n = 9-20)

KC (IL-8) by Age and Strain

mouse type	age (week)	KC mean(SE)	p'
WT	12	60.94(22.44)	
KO		108.72(84.05)	9.8E-03
WT	24	67.87(15.32)	
KO		118.94(82.45)	5.6E-02
WT	48	65.83(25.90)	
KO		94.22(32.14)	6.0E-03
WT	72	75.82(15.79)	
KO		100.8(46.51)	9.4E-02
WT	90	80.19(53.07)	
KO		149.27(58.27)	3.2E-03
overall P''		2.39E-08	

P' = P value by strains in each age group, rank sum test

P'' = P value by strain adjusted by age, linear regression based on log transformed cytokines.

IL-1beta by Age and Strain

mouse type	age (week)	IL1B mean(SE)	p'
WT	12	1.20(0.73)	
KO		1.37(0.64)	4.0E-01
WT	24	1.18(0.55)	
KO		2.32(1.32)	1.2E-02
WT	48	1.24(1.44)	
KO		2.35(1.20)	1.8E-03
WT	72	1.99(1.61)	
KO		3.73(2.22)	9.8E-04
WT	90	1.62(1.39)	
KO		2.63(0.85)	2.7E-03
overall P''			4.51E-07

IL-6 by Age and Strain

mouse type	age (week)	IL6 mean(SE)	p'
WT	12	6.25(1.36)	
KO		12.7(7.34)	5.1E-04
WT	24	7.25(2.00)	
KO		19.32(21.98)	2.9E-01
WT	48	8.48(6.12)	
KO		15.68(11.84)	3.9E-03
WT	72	8.78(3.09)	
KO		20.63(29.43)	1.7E-01
WT	90	13.77(24.36)	
KO		30.36(28.34)	7.7E-04
overall P''			1.86E-08

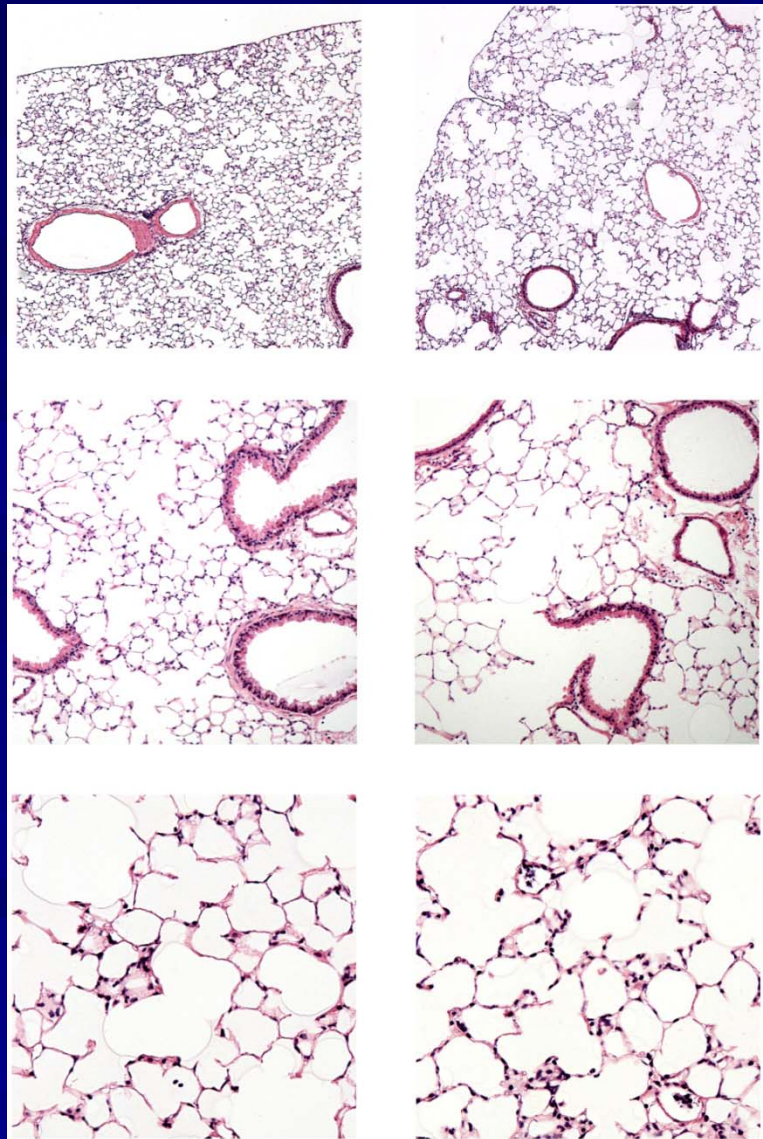
TNF-alpha by Age and Strain

mouse type	age (week)	TNFa mean(SE)	p'
WT	12	0.33(0.88)	
KO		4.21(2.67)	2.8E-04
WT	24	2.03(1.76)	
KO		4.10(2.92)	1.2E-02
WT	48	0.41(0.76)	
KO		3.82(2.27)	1.2E-05
WT	72	1.46(1.76)	
KO		3.22(2.32)	1.8E-02
WT	90	0.41(0.80)	
KO		3.65(1.90)	3.2E-04
overall P''			1.71E-09

Airspace Enlargement in IL10-deficient Lung

WT

IL10^{-/-}



Altered Gene Expression in Skeletal Muscle

- Upregulated
 - Apoptotic programming genes
 - DNA methylation related genes
- Downregulated
 - DNA repair
 - Biosynthesis
 - Transcription regulation

Altered Gene Expression in Aging Lung Parenchyma

- Upregulated
 - Apoptotic programming genes
 - Chemokines (CXCL1, 5, and 13)
 - DNA methylation related genes
- Downregulated
 - DNA repair
 - Biosynthesis
 - Transcription regulation

Other Phenotypes

- Bone
- Cognition
- Cardiac
- Vascular
- Muscle

Acknowledgments

Luigi Ferrucci - NIA

Linda Fried – Columbia Univ.

Richard Semba – Johns Hopkins

Alex Reiner – Univ. Washington

Russ Tracy – Univ. Vermont

Anne Newman – Univ. Pittsburgh

Acknowledgments

- National Institute on Health (NIA, NHLBI)
 - Johns Hopkins Older American Independence Center (OAIC)
 - R-21 Development of a Frail Mouse Model
 - R-01 Genetic Influences in NFkB Pathways on Adverse Health Outcomes
 - Women's Health and Aging Studies (WHAS)
 - In CHIANTI and Cardiovascular Health Studies

Biology of Frailty Program

Neal Fedarko
Brock Beamer
Fred Ko
Sean Leng
Cindy Roy

George Wang
Huanle Yang
Peter Abadir
Amy Matteni
Amy Unterman

