Inflammation and Nutrition: Rheumatoid Arthritis and anti-TNF Therapy as a Case Study

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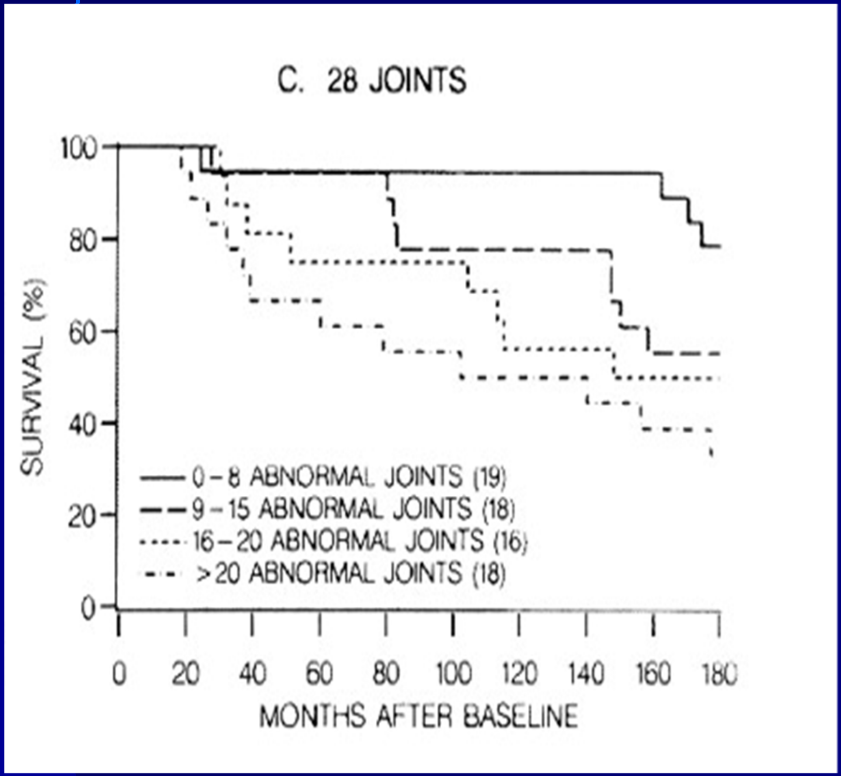
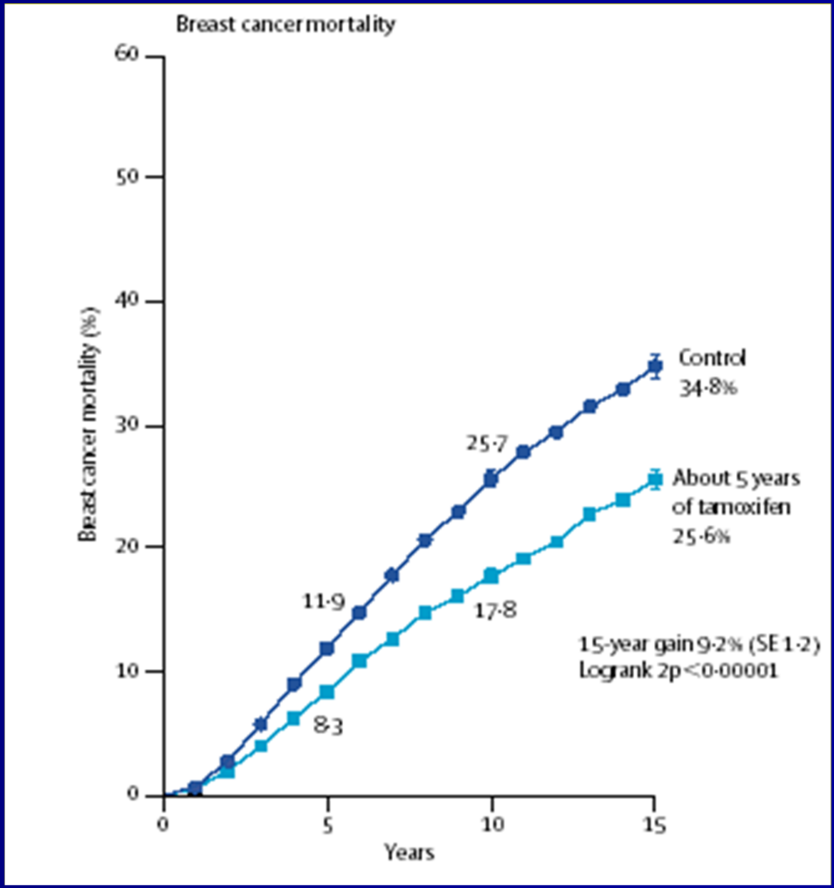
RA is the Most Common Inflammatory Arthritis

Images courtesy of J. Cush, 2002.

People Die Faster With (OF??) RA





Pincus, T. et. al. Ann Intern Med 1994;120:26-34;
EBCTCG, Lancet 2005; 365:1687-1717

Nutritional Complications of RA

- Macronutrients
  - Rheumatoid cachexia
  - Cachectic obesity
- Micronutrients
  - B6 deficiency
  - Folate deficiency
  - Oxidative stress

Rheumatoid Cachexia

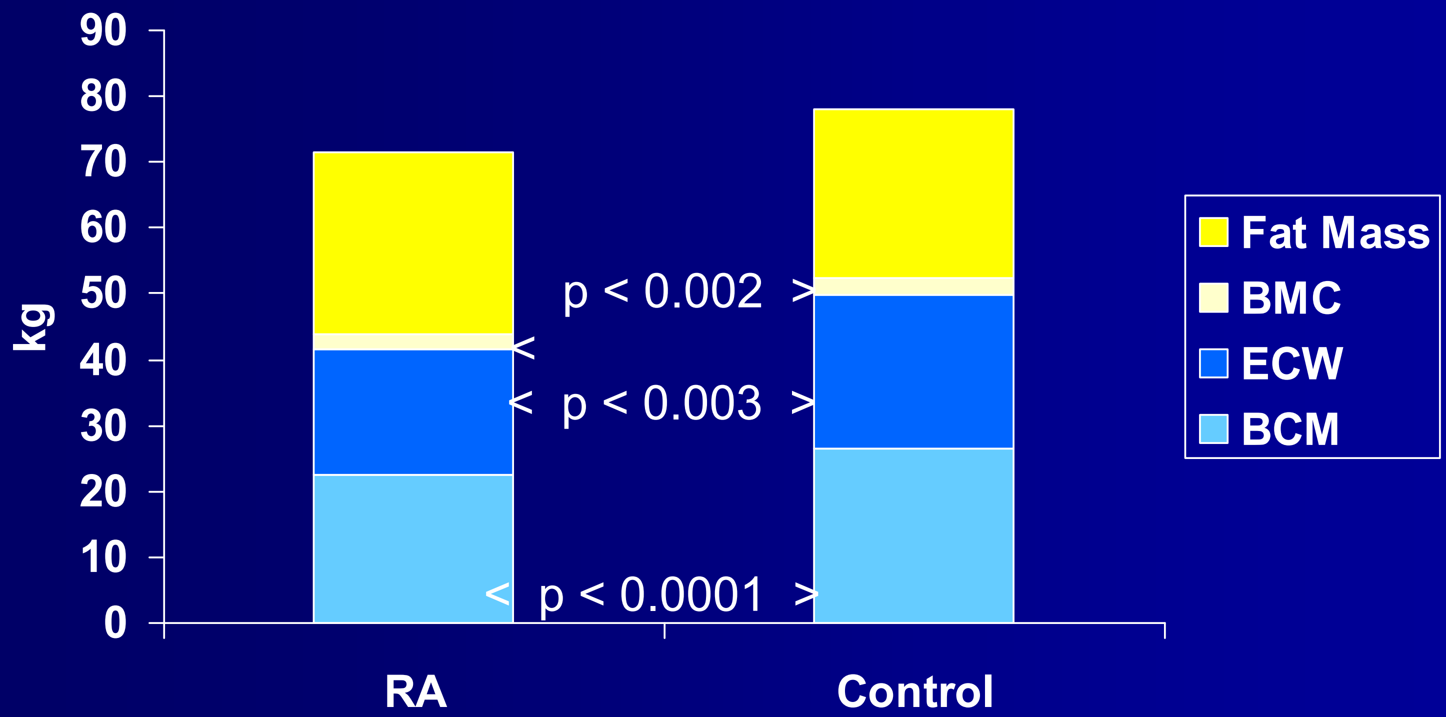
- Loss of body cell mass - predominantly in skeletal muscle - that occurs in RA.

- Compromises strength and functional capacity, and is accompanied by:

↑ TNF-α & IL-1β (systemic) ↑ REE ↑ Whole-body protein catabolism

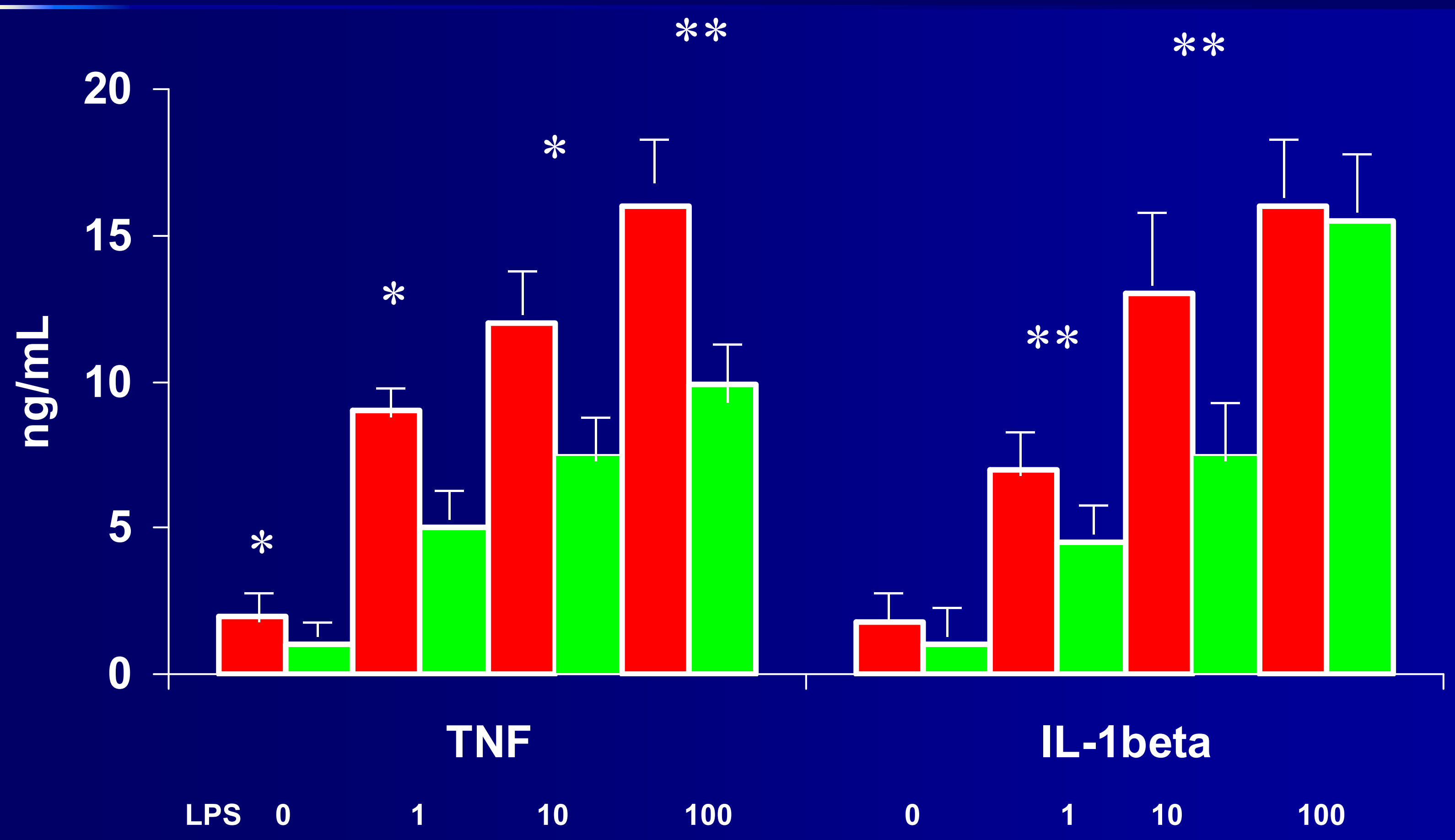
- Excess fat mass and low physical activity.

Body Composition in RA

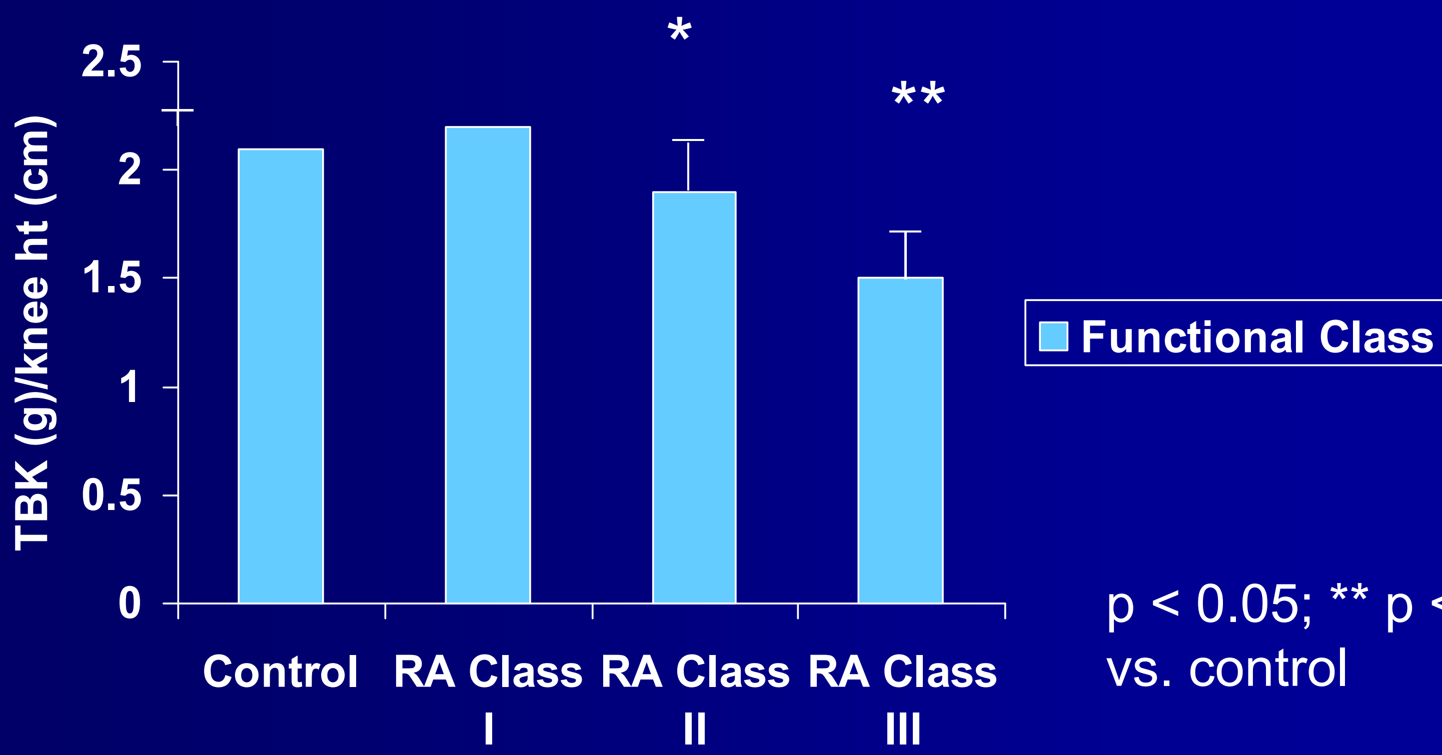


Roubenoff et al. *JCI* 1994; 93: 2379

PBMC Production of IL-1β and TNF-α in RA and Matched Controls



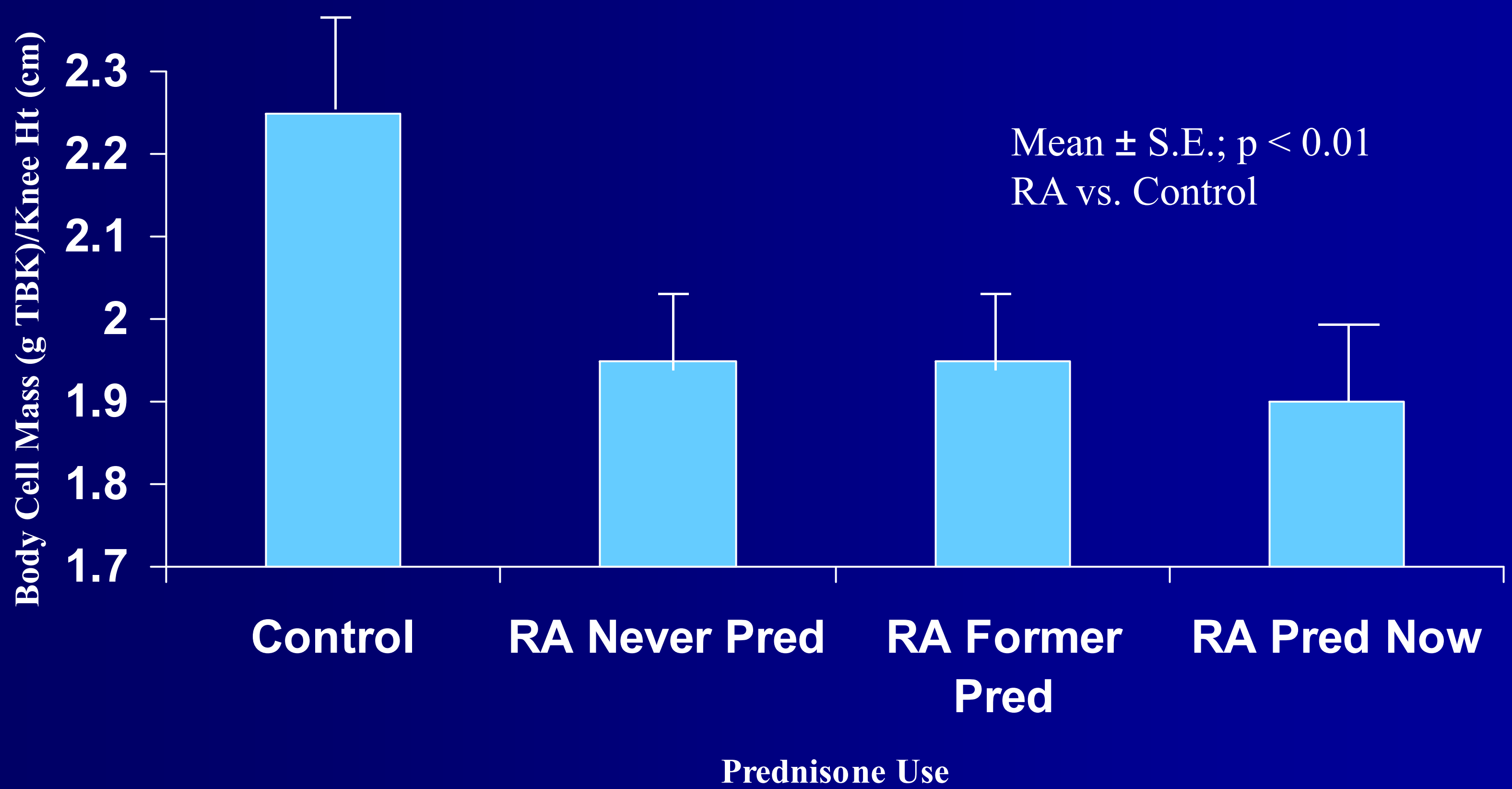
Rheumatoid Cachexia and Functional Class



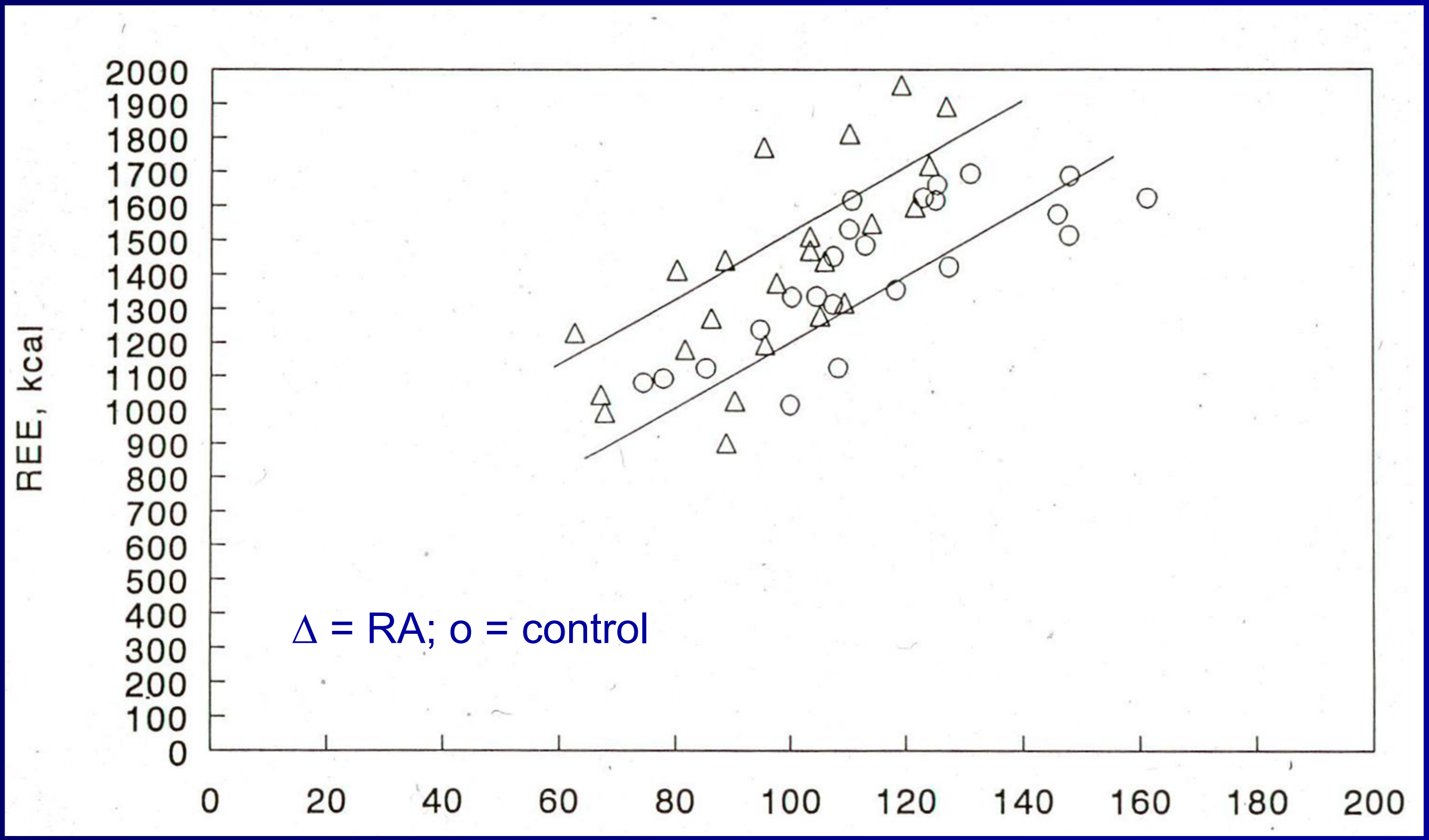
p < 0.05; ** p < 0.01 vs. control

Roubenoff et al., *J Clin Invest* 1994

Body Cell Mass and Prednisone History in RA

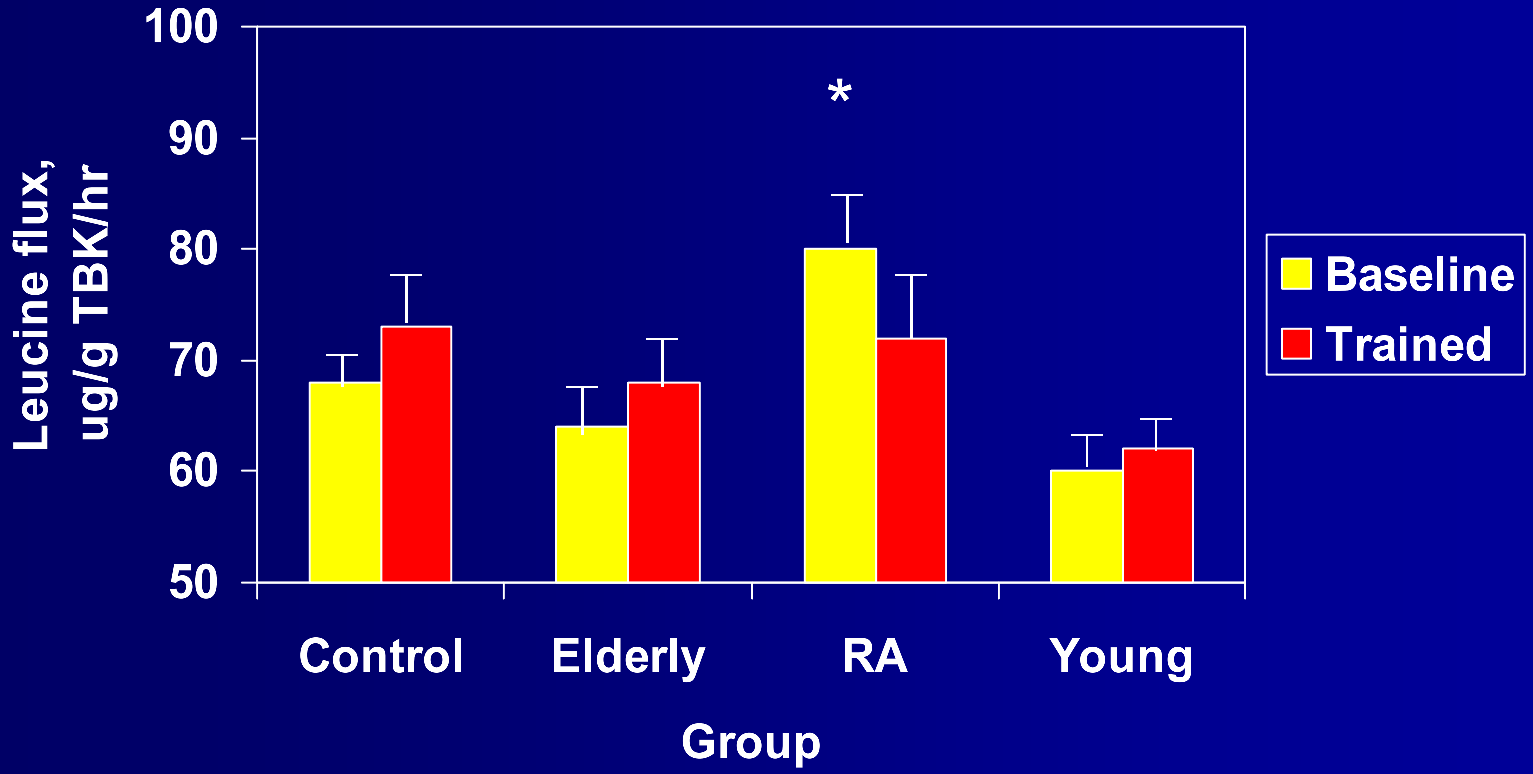


Hypermetabolism: REE per g TBK in RA vs. Matched Controls



Roubenoff et al., *J Clin Invest* 1994

Whole-Body Protein Metabolism in RA and Aging

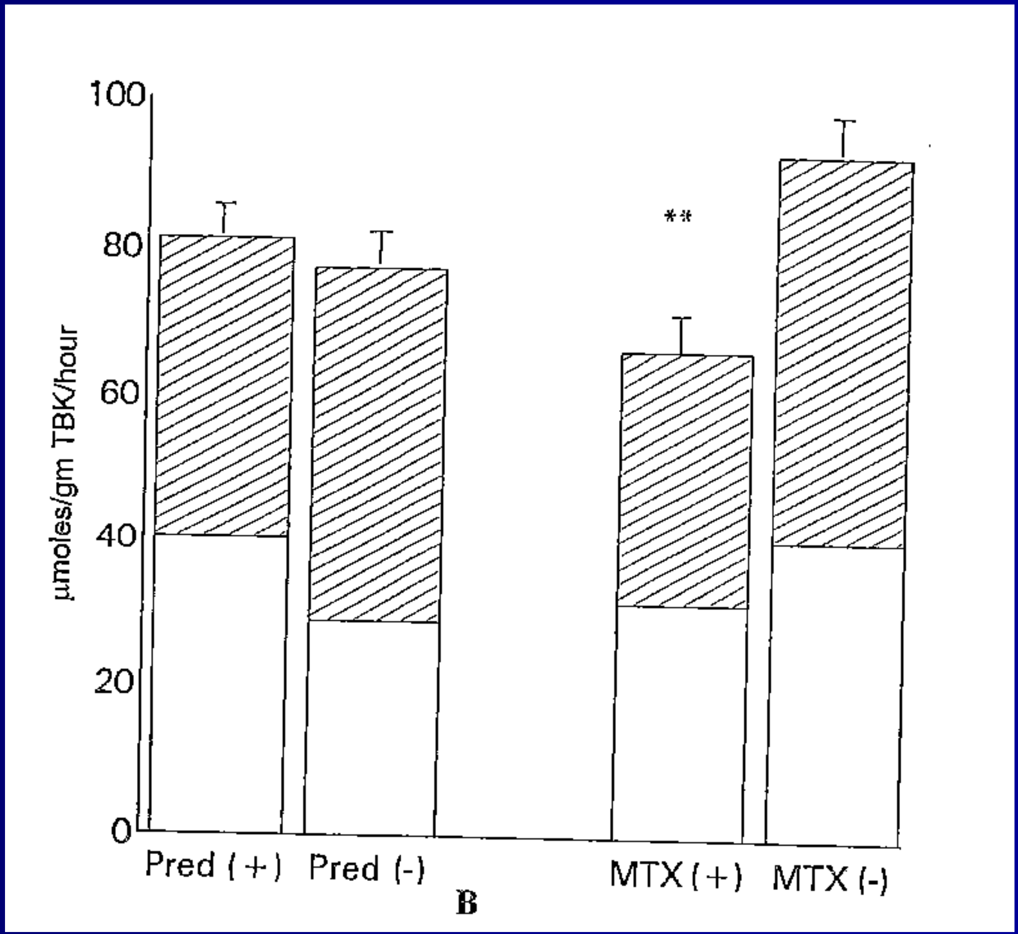


Rall et al., *Arthr Rheum* 1996

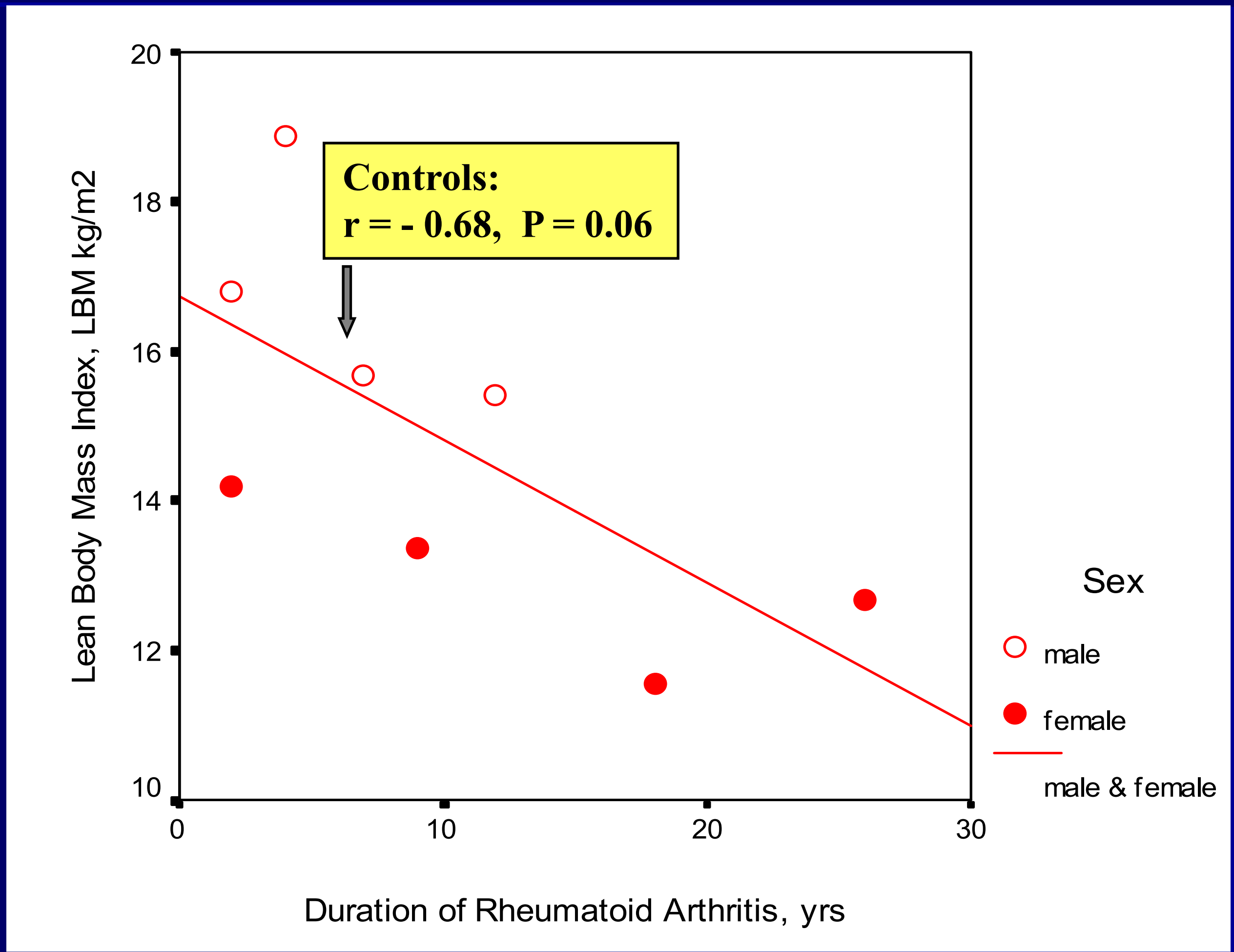
* $p < 0.05$

Methotrexate Normalizes Accelerated Protein Breakdown in Rheumatoid Cachexia

Rall et al. Arthr Rheum 1996; 39: 1115-1124



Lean Body Mass Declines with Duration of Rheumatoid Arthritis

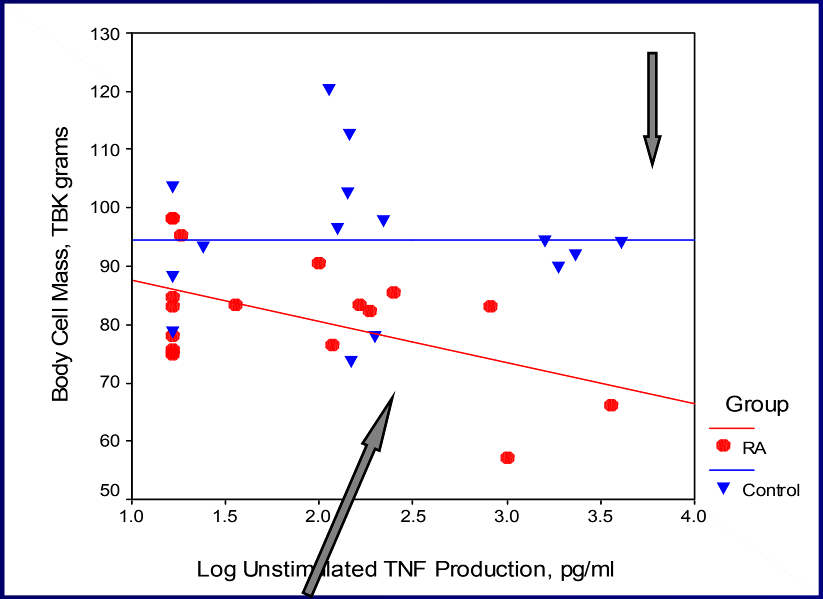


Walsmith et al., J Rheumatol 31:23-29, 2004.

Body Cell Mass And Spontaneous PBMC TNF-α and IL-1β Production

TNF-α

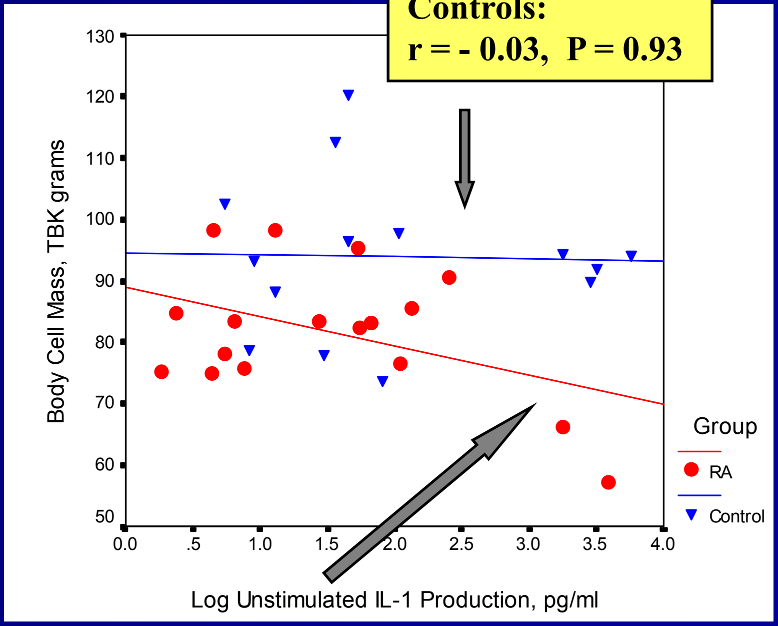
Controls:
r = - 0.002, P = 0.99



Patients:
r = - 0.51, *P = 0.03

IL-1β

Controls:
r = - 0.03, P = 0.93



Patients:
r = - 0.43, P = 0.08

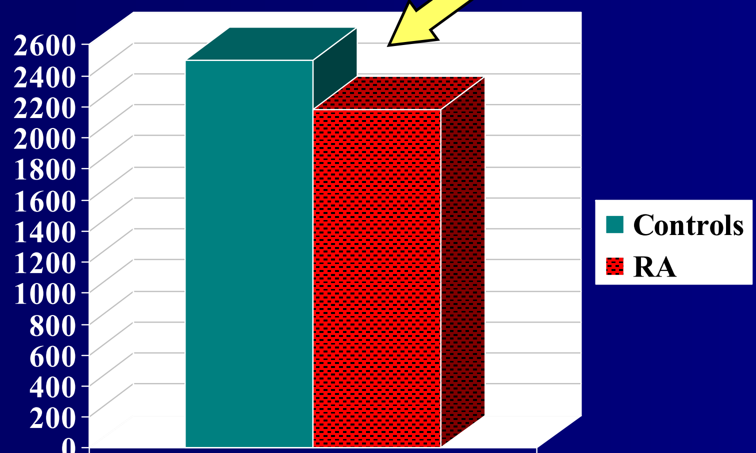
Walsmith et al., J Rheumatol 31:23-29, 2004.

Total Energy Expenditure is Reduced in RA due to Lower Physical Activity

kcal/d

TEE

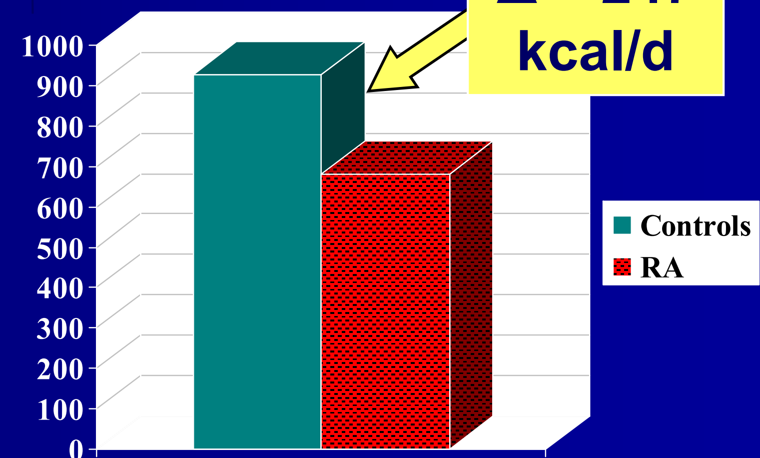
Δ = 321 kcal/d



p < 0.02

Physical Activity

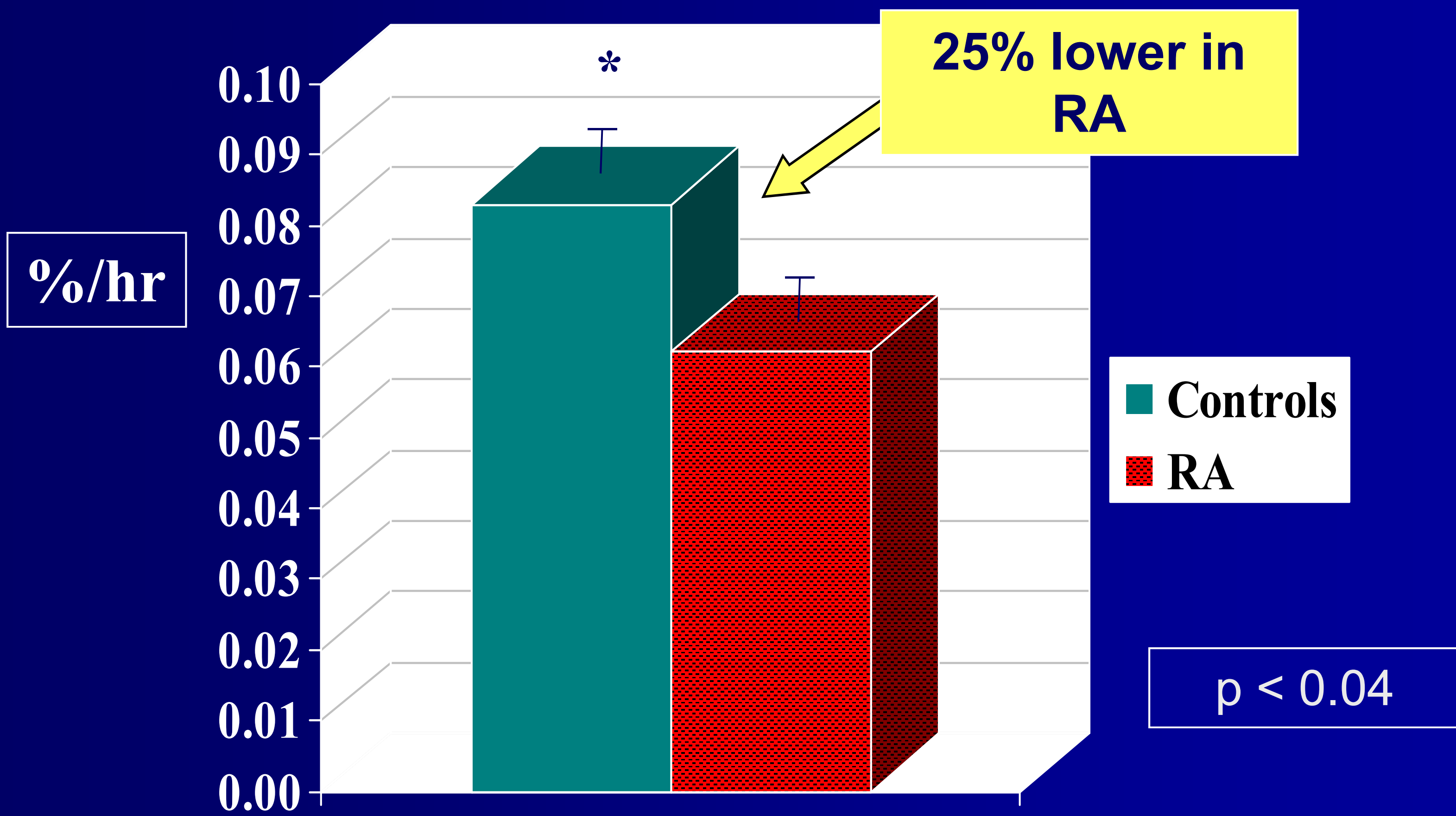
Δ = 247 kcal/d



p < 0.04

Walsmith et al., J Rheumatol 31:23-29, 2004.

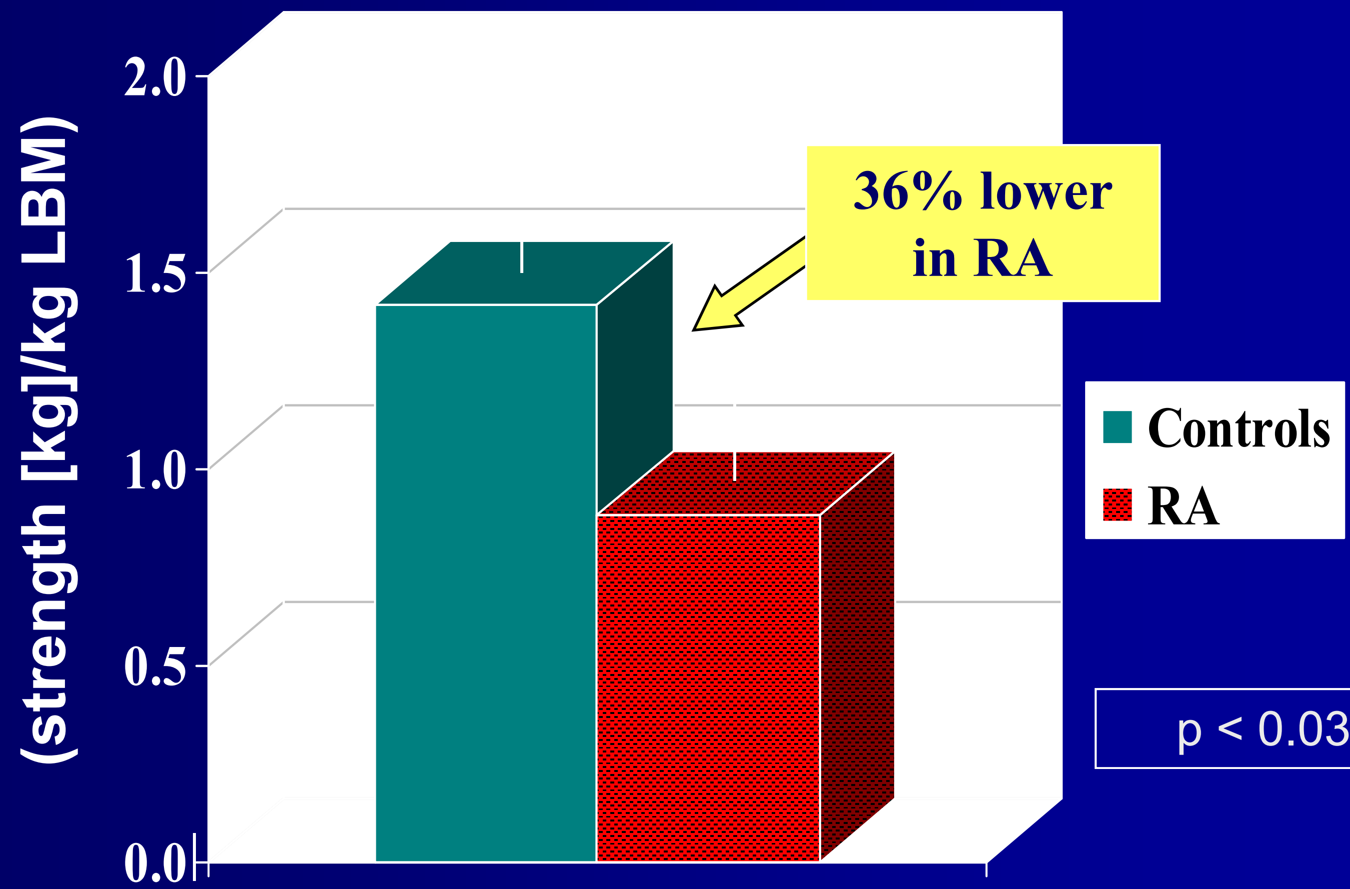
In Vivo Rate of Mixed Skeletal Muscle Protein Synthesis



mean±SEM

Walsmith et al., J Rheumatol 31:23-29, 2004.

Skeletal Muscle Quality in RA (strength per unit mass)

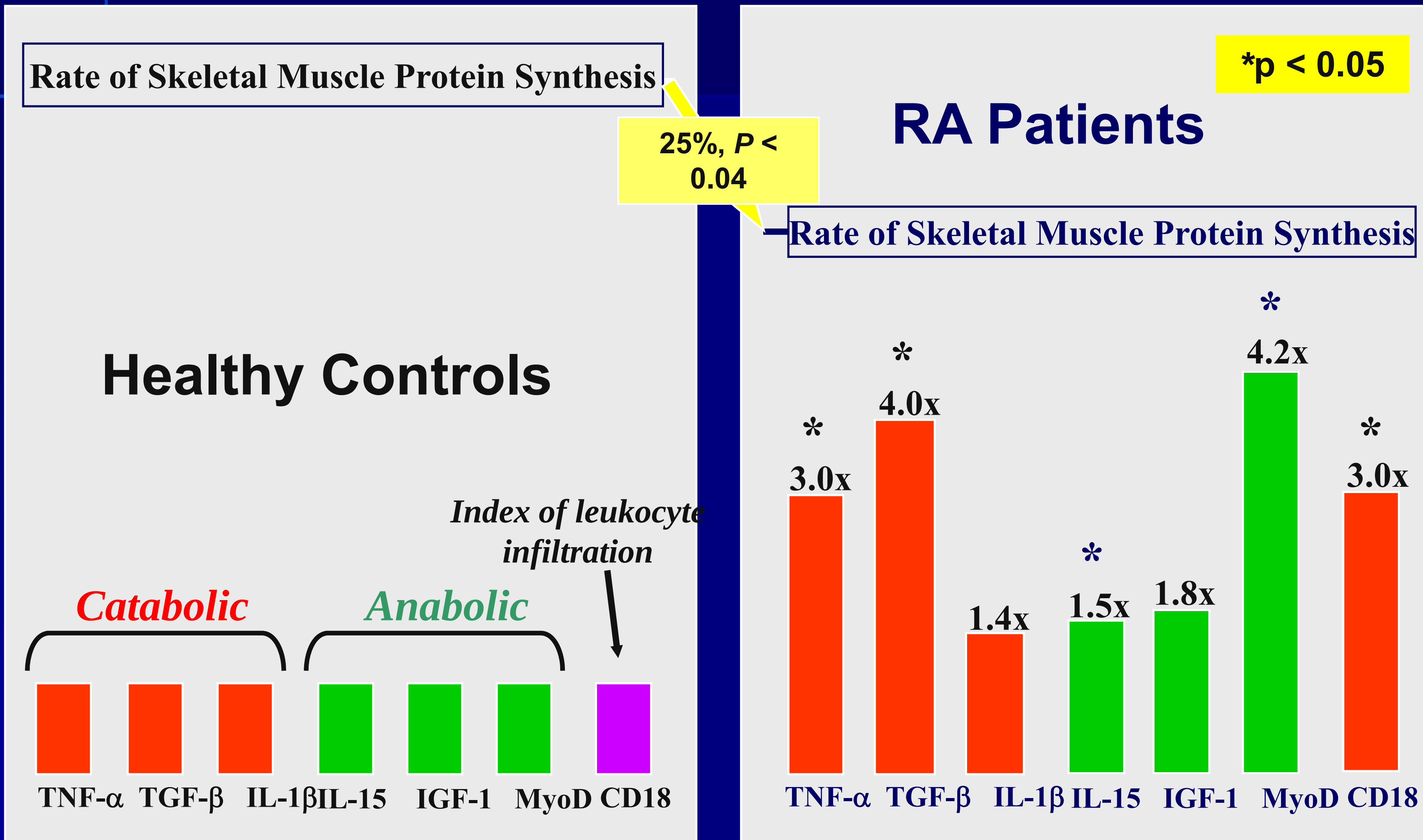


p < 0.03

mean±SEM

Walsmith et al., J Rheumatol 31:23-29, 2004.

Gene Expression in Skeletal Muscle of RA Patients vs. Controls



Walsmith et al., J Rheumatol 31:23-29, 2004.

Effect of TNF inhibition on cachexia: Disappointing!

TABLE 1
Effects of 24 wk of treatment with etanercept or methotrexate on body mass and composition in patients with early rheumatoid arthritis[1]

Outcome variable	Etanercept group ($n = 12$)	Methotrexate group ($n = 12$)	P Treatment × time[2]
Body mass (kg)			
Pretest	76.4 ± 14.4	73.4 ± 18.9	
Midtest	76.6 ± 14.6	73.7 ± 18.3	
Posttest	77.5 ± 16.1	74.5 ± 18.1	0.99
Arms lean mass (kg)[3]			
Pretest	3.50[a] ± 1.68	3.52[a] ± 1.29	
Midtest	3.73[b] ± 1.74	3.50[a] ± 1.27	
Posttest	3.84[b] ± 1.89	3.56[a] ± 1.29	0.05
Legs lean mass (kg)			
Pretest	12.3 ± 2.8	12.2 ± 3.0	
Midtest	12.5 ± 3.0	12.1 ± 2.9	
Posttest	12.4 ± 3.1	12.4 ± 3.0	0.08
Total lean mass (kg)			
Pretest	41.3 ± 9.7	41.2 ± 8.5	
Midtest	42.1 ± 9.6	40.9 ± 8.0	
Posttest	41.9 ± 10.5	41.3 ± 8.3	0.22

Marcora et al. Am J Clin Nutr 2006; 84:1463

TABLE 2. Mean ± S.D. and differences in the studied body composition and disease-related variables between the three different times of assessment

	Baseline	Time-1 (2 weeks)	Time-2 (12 weeks)	P
Body composition assessment				
Weight (kg)	79.4 ± 15.6	80.4 ± 16.2	78.8 ± 16.6	>0.05
BMI (kg/m^2)	28.3 ± 3.7	28.6 ± 3.8	28.1 ± 4.1	>0.05
Total body fat (%)	38.8 ± 7.5	36.5 ± 6.9	36.0 ± 7.4	>0.05
Truncal fat (%)	35.9 ± 6.7	37.4 ± 6.3*	36.7 ± 6.4	0.036
FFM (kg)	50.9 ± 12.7	50.5 ± 12.4	51.1 ± 12.5	>0.05
RA-related assessments				
CRP (mg/l)	33.7 ± 34.4	17.7 ± 11.9	15.3 ± 18.9	>0.05
ESR (mm/1st h)	41.7 ± 25.6	22.1 ± 16.9**	18.3 ± 15.4**	0.002
HAQ	1.83 ± 0.3	1.54 ± 0.3**	1.41 ± 0.4**	<0.001
DAS28	5.66 ± 0.7	4.64 ± 0.6**	3.59 ± 0.7**	<0.001
TNF-α (pg/ml)	38.1 ± 41.1	22.2 ± 26.8	8.9 ± 10.2*	0.024

P = level of significance between times of assessment using repeated-measures ANOVA. Difference from baseline assessment: **$P < 0.001$ and *$P < 0.05$.

Metsios et al. Rheumatology 2007; 46: 1824

Could TNF inhibition support LBM gain *in the setting of weight gain?*

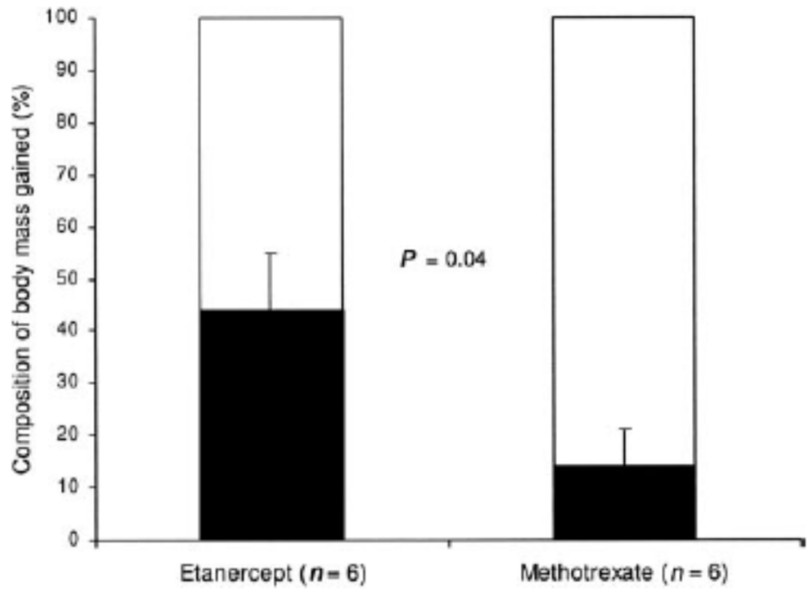
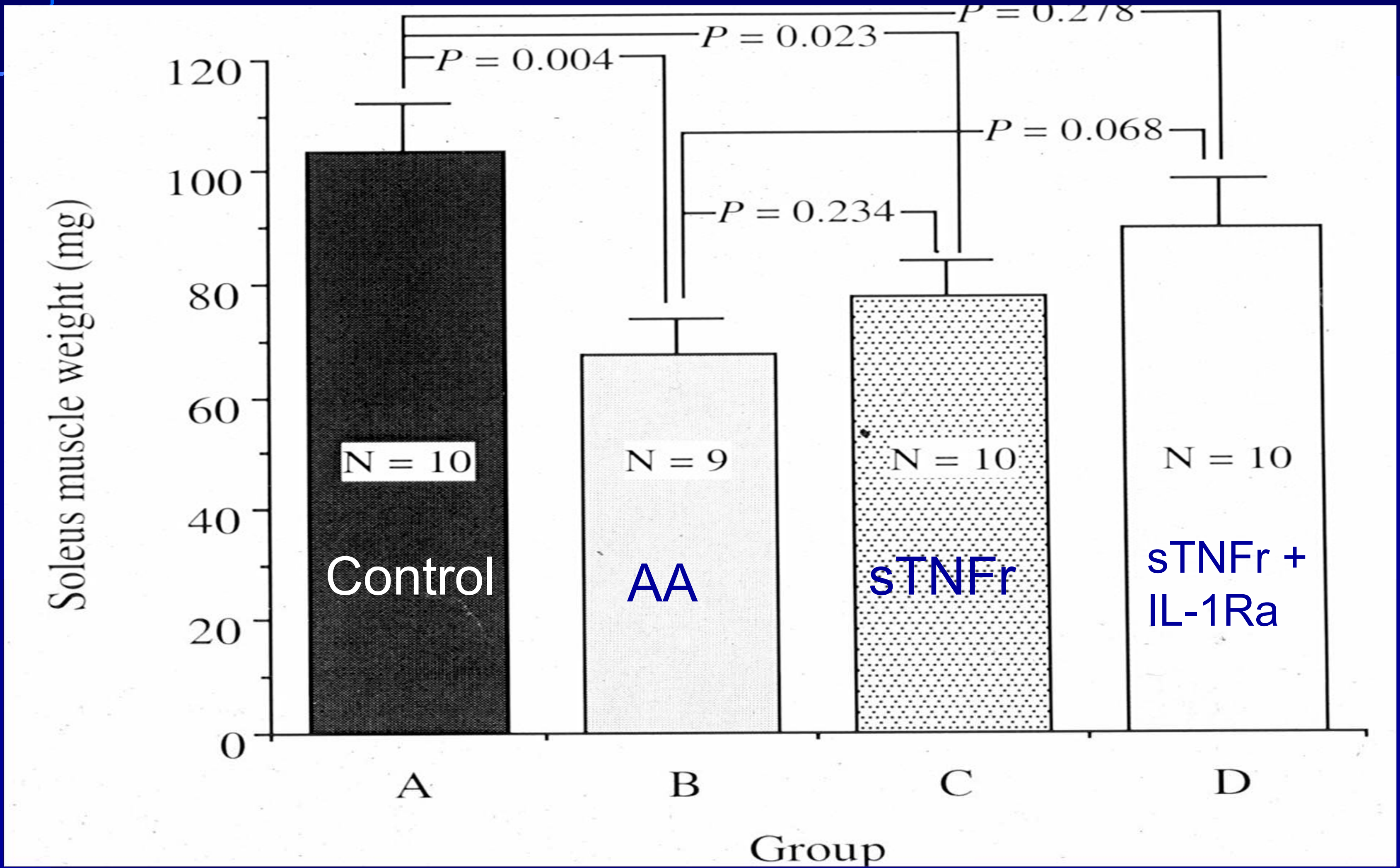


FIGURE 3. Mean (±SE) effect of treatment with etanercept or methotrexate on the composition of body mass gained [% of fat-free mass (FFM)] in the 12 patients with early rheumatoid arthritis who had a significant increase in body weight over the 6-mo follow-up period. □, Fat mass; ■, FFM. Data were analyzed by unpaired *t* test.

Marcora et al. Am J Clin Nutr 2006; 84:1463

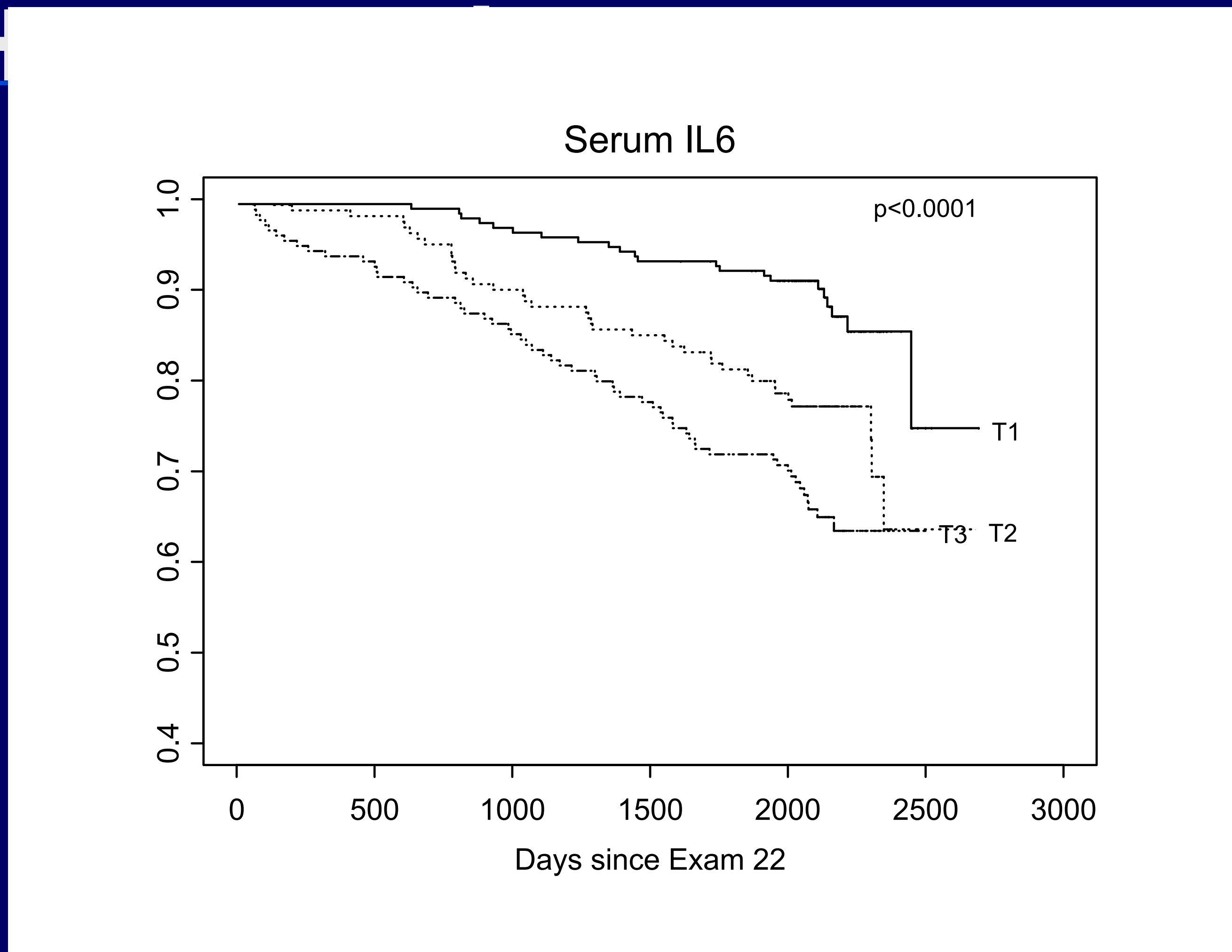
Both TNF & IL-1 Blockade are Needed to Inhibit Soleus Atrophy in Rat Adjuvant Arthritis



Hamada et al., FASEB J, 2000

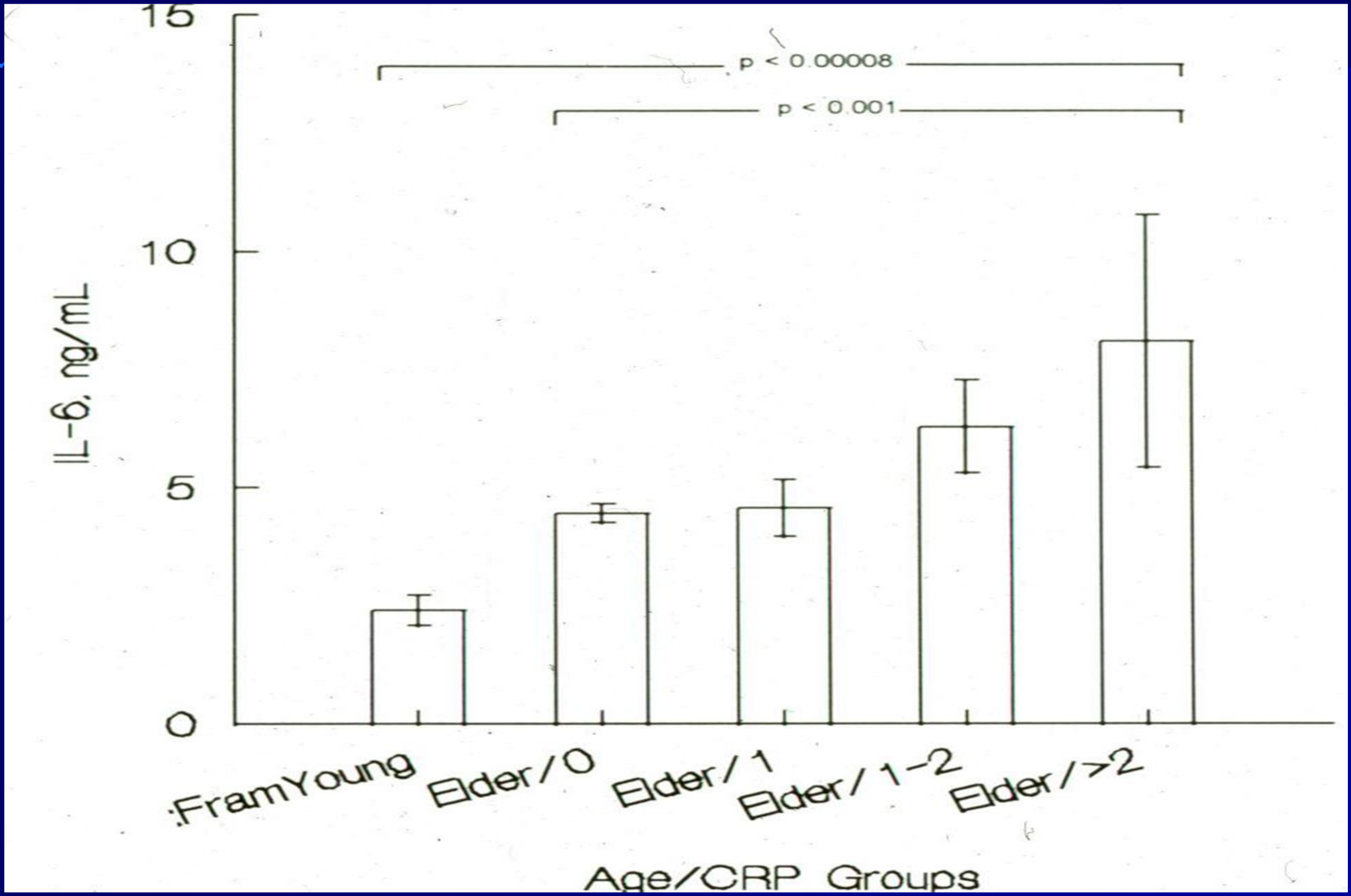
IL-6 and Aging…

IL-6 Predicts All-Cause Mortality: Framingham H



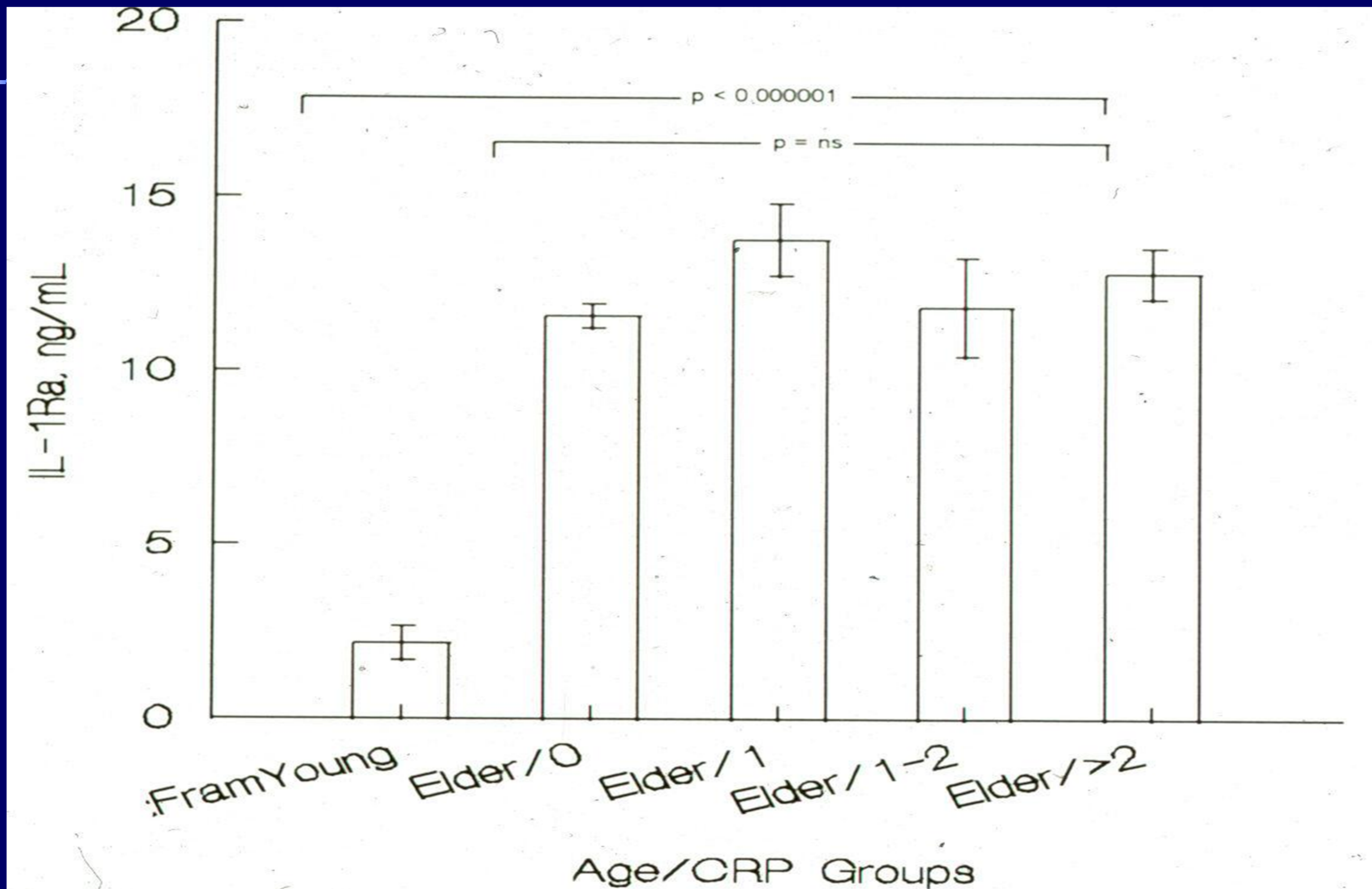
Roubenoff et al., Am J Med, 2003

IL-6 Production in the Elderly: The Framingham Heart Study



Roubenoff et al., *J Gerontol* 1998

IL-1Ra Production in the Elderly: The Framingham Heart Study



Roubenoff et al., *J Gerontol 1998*

Determinants of 2-yr changes in fat-free mass

Framingham Study n=539, 72-94 yrs

	β	SE	P
Age (yrs)	-.02	.02	.40
FFM (kg) at baseline	-.04	.01	.0001
Cell. Il-6 (ng/ml)	-.21	.10	.04

Adj. R^2 = .04, p=.001

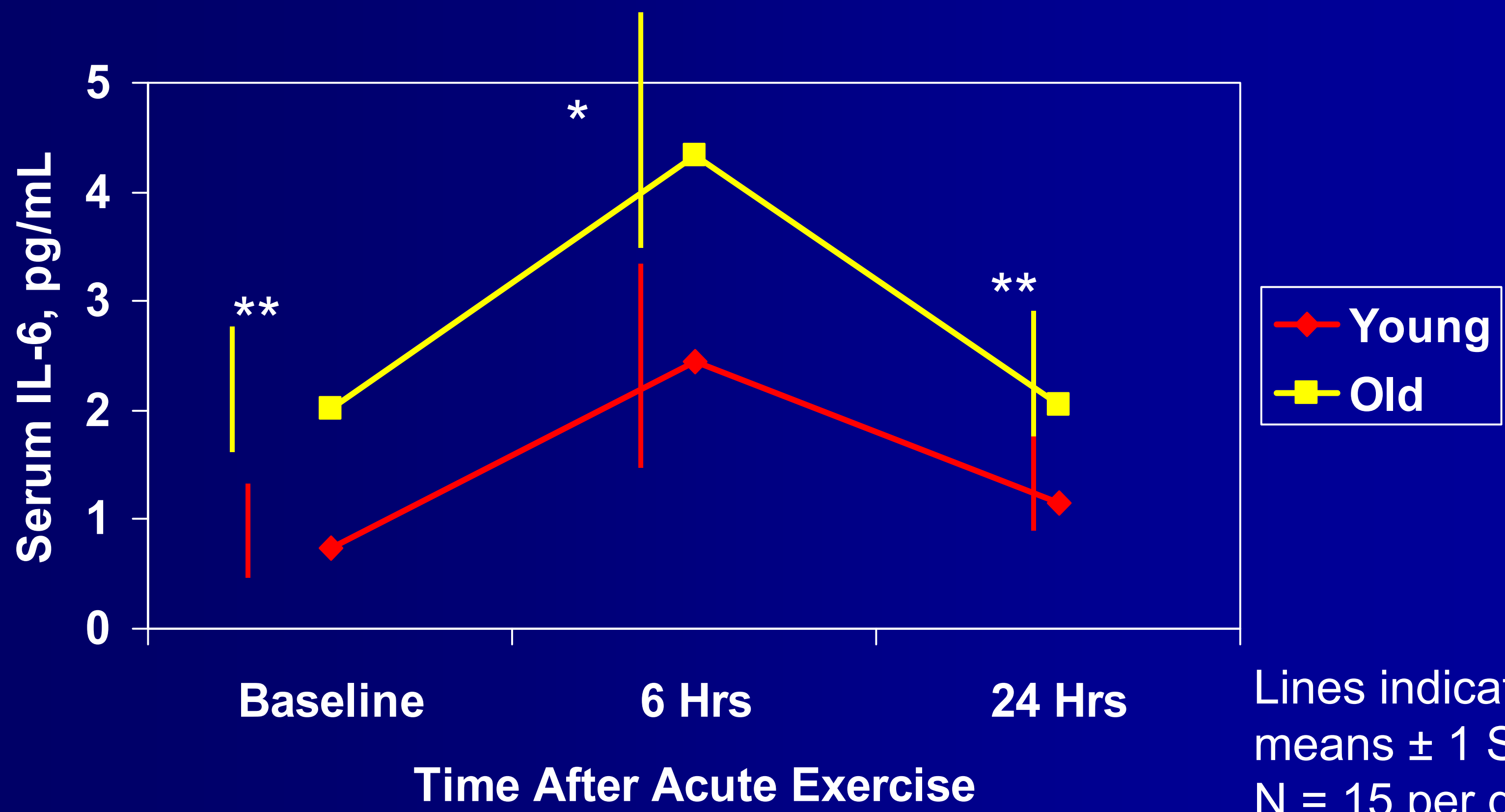
Payette, et al., **J Am Geriatr Soc** 51: 1237-1243, 2003.

Predictors of Death in the Elderly: Framingham Heart Study

- 4 –year all cause mortality, ages 72-92
  - High serum IL-6
  - High PBMC TNF-alpha
  - Low serum IGF-1
  - Greater loss of fat-free mass

(Adjusted for smoking, diabetes, CVD, arthritis, high CRP, being bedridden)

Effect of Acute Exercise on Serum IL-6 in Young and Old Men

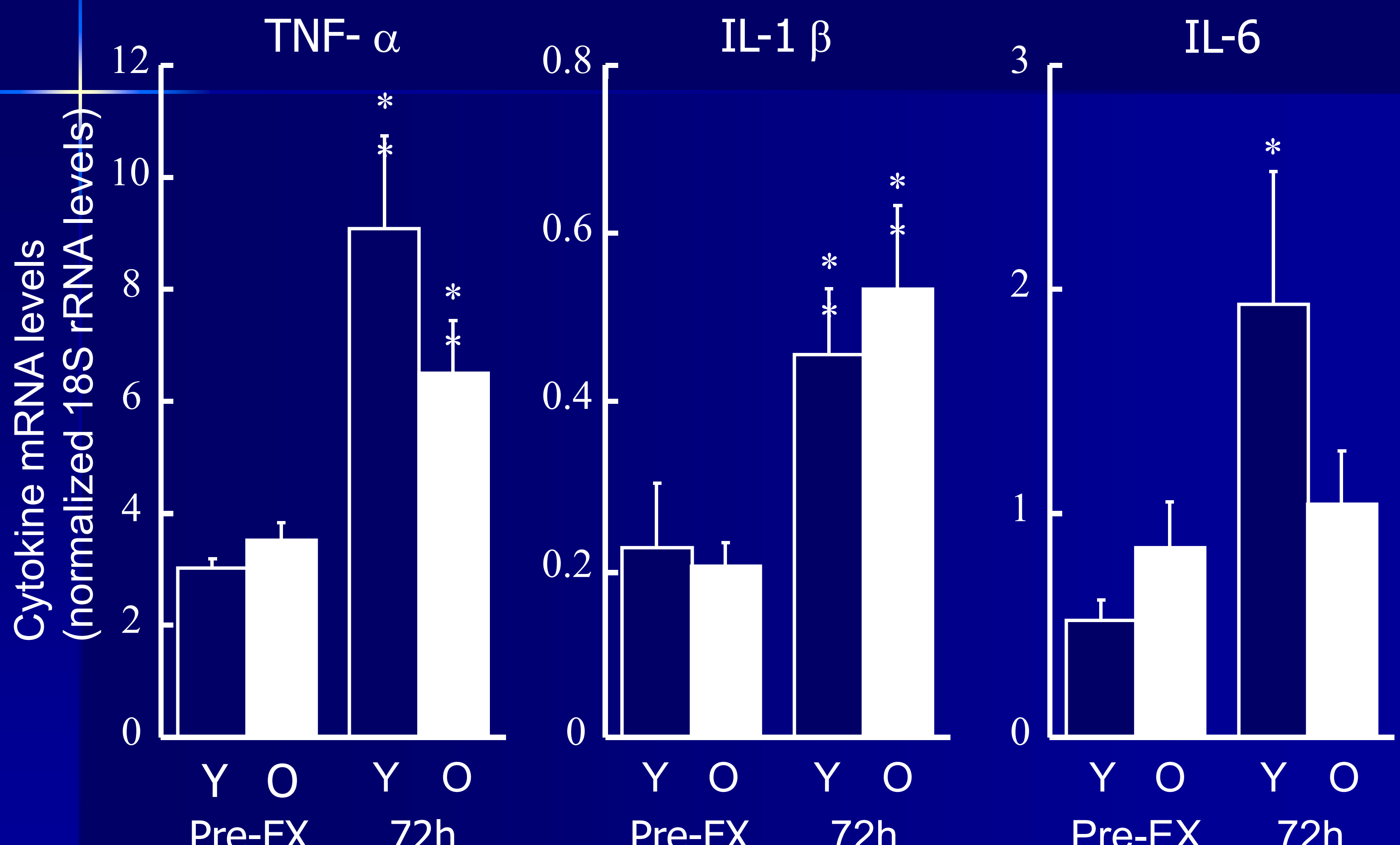


Lines indicate means ± 1 SD, N = 15 per group

•p < 0.02; ** p < 0.001

Sacheck et al.,**Am J Physiol**, 291: E340-349, 2006

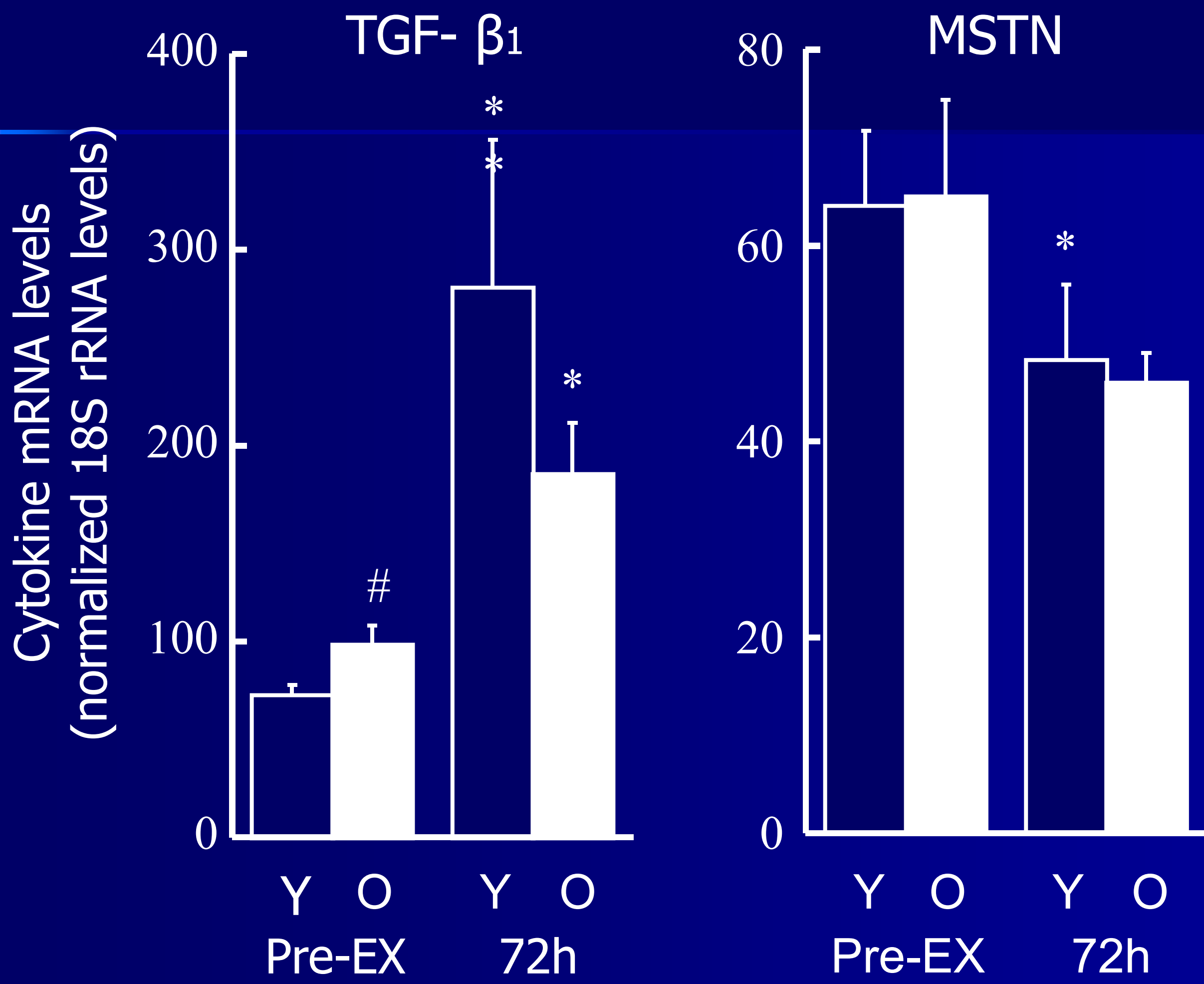
Effect of Acute Exercise on TNF-α, IL-1 β, and IL-6 mRNA Levels in Vastus Lateralis from Young and Old Men



* $P < 0.05$ *vs.* Pre-EX; ** $P < 0.01$ *vs.* Pre-EX.

Hamada et al., **FASEB J** 19: 264-266, 2005

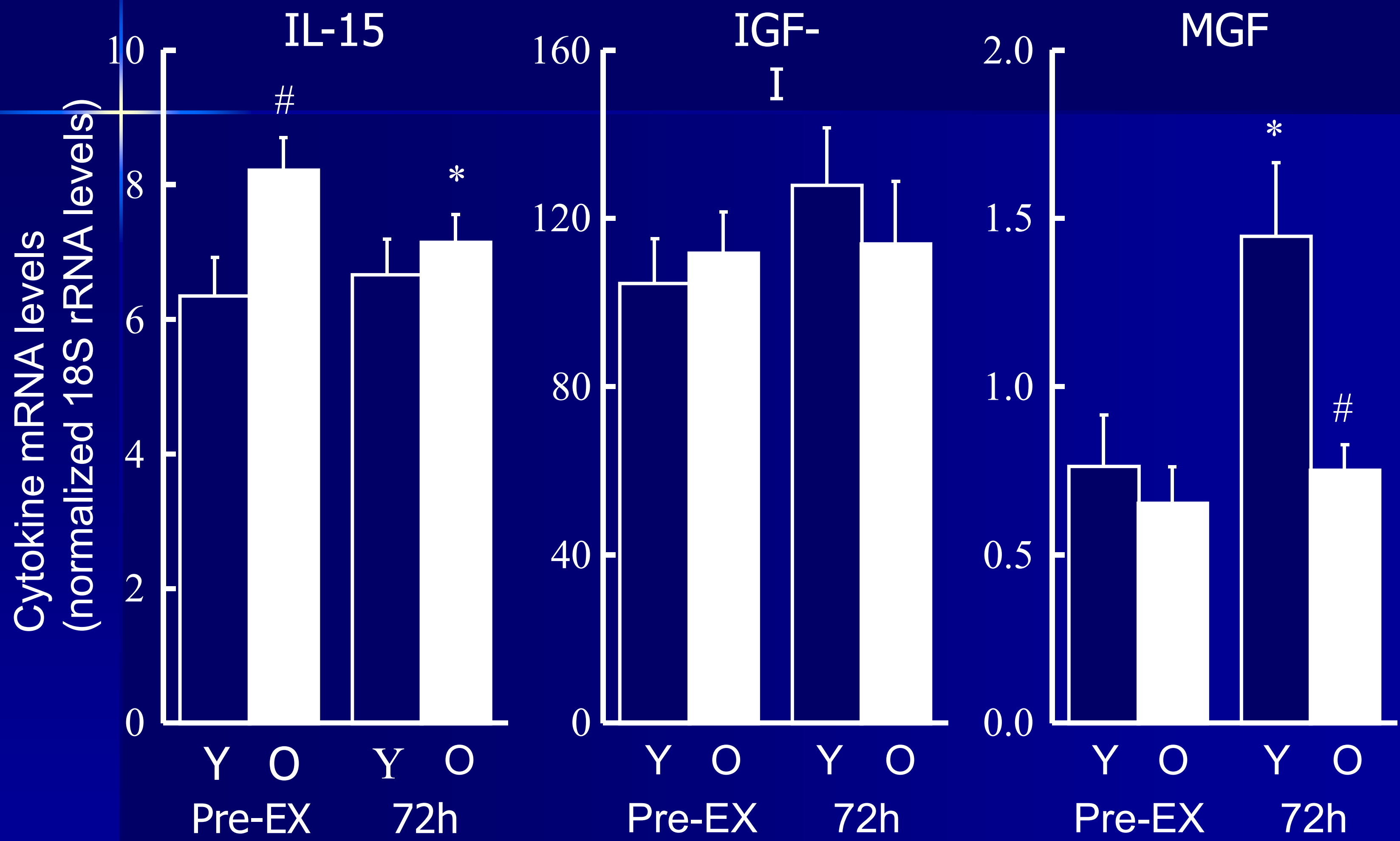
Effect of Acute Exercise on TGF-β_1 and MSTN mRNA Levels in Vastus Lateralis from Young and Old Men



*$P < 0.05$ *vs.* Pre-EX; ** $P < 0.01$ *vs.* Pre-EX; # $P < 0.05$ *vs.* Y.

Hamada et al., **FASEB J** 19: 264-266, 2005

Effect of Acute Exercise on IL-15, IGF-I and MGF mRNA Levels in Vastus Lateralis from Young and Old Men



* *P* < 0.05 *vs.* Pre-EX; # *P* < 0.05 *vs.* Y.

Hamada et al., **FASEB J** 19: 264-266, 2005

Table 3. Correlations between changes in cytokine and CD18 mRNA levels within age groups

		CD18	TNF-α	IL-1β	IL-6	TGF-β1	MSTN	IL-15	IGF-I	MGF
CD18	*R*		0.84	0.66	0.73	0.93	-0.04	0.15	-0.23	0.23
	P value		<.001	0.01	<.01	<.001	0.89	0.61	0.41	0.41
TNF-α	*R*	0.62		0.60	0.80	0.84	-0.03	0.05	-0.09	0.29
	P value	0.01		0.02	<.001	<.001	0.92	0.86	0.75	0.30
IL-1β	*R*	-0.13	-0.22		0.75	0.77	0.01	-0.07	-0.09	0.45
	P value	0.65	0.45		<.001	<.001	0.98	0.82	0.74	0.09
IL-6	*R*	0.45	-0.31	-0.07		0.89	-0.09	-0.12	-0.19	0.47
	P value	0.09	0.27	0.81		<.001	0.76	0.68	0.50	0.08
TGF-β1	*R*	0.60	0.66	-0.27	-0.48		-0.12	0.03	-0.21	0.39
	P value	0.02	<.01	0.33	0.07		0.68	0.92	0.46	0.16
MSTN	*R*	0.07	0.02	0.39	0.13	0.03		0.58	-0.69	-0.51
	P value	0.80	0.94	0.15	0.64	0.90		0.02	<.01	0.05
IL-15	*R*	0.18	0.26	-0.05	-0.04	0.24	-0.01		-0.55	-0.38
	P value	0.54	0.36	0.88	0.88	0.41	0.97		0.03	0.16
IGF-I	*R*	-0.13	-0.08	-0.22	0.11	-0.07	0.20	-0.16		0.59
	P value	0.66	0.79	0.44	0.70	0.81	0.48	0.58		0.02
MGF	*R*	-0.27	0.00	-0.25	-0.12	0.23	0.10	-0.09	0.69	
	P value	0.34	1.00	0.38	0.68	0.42	0.74	0.76	<.01	

Y

O

P < 0.001, Y vs. O

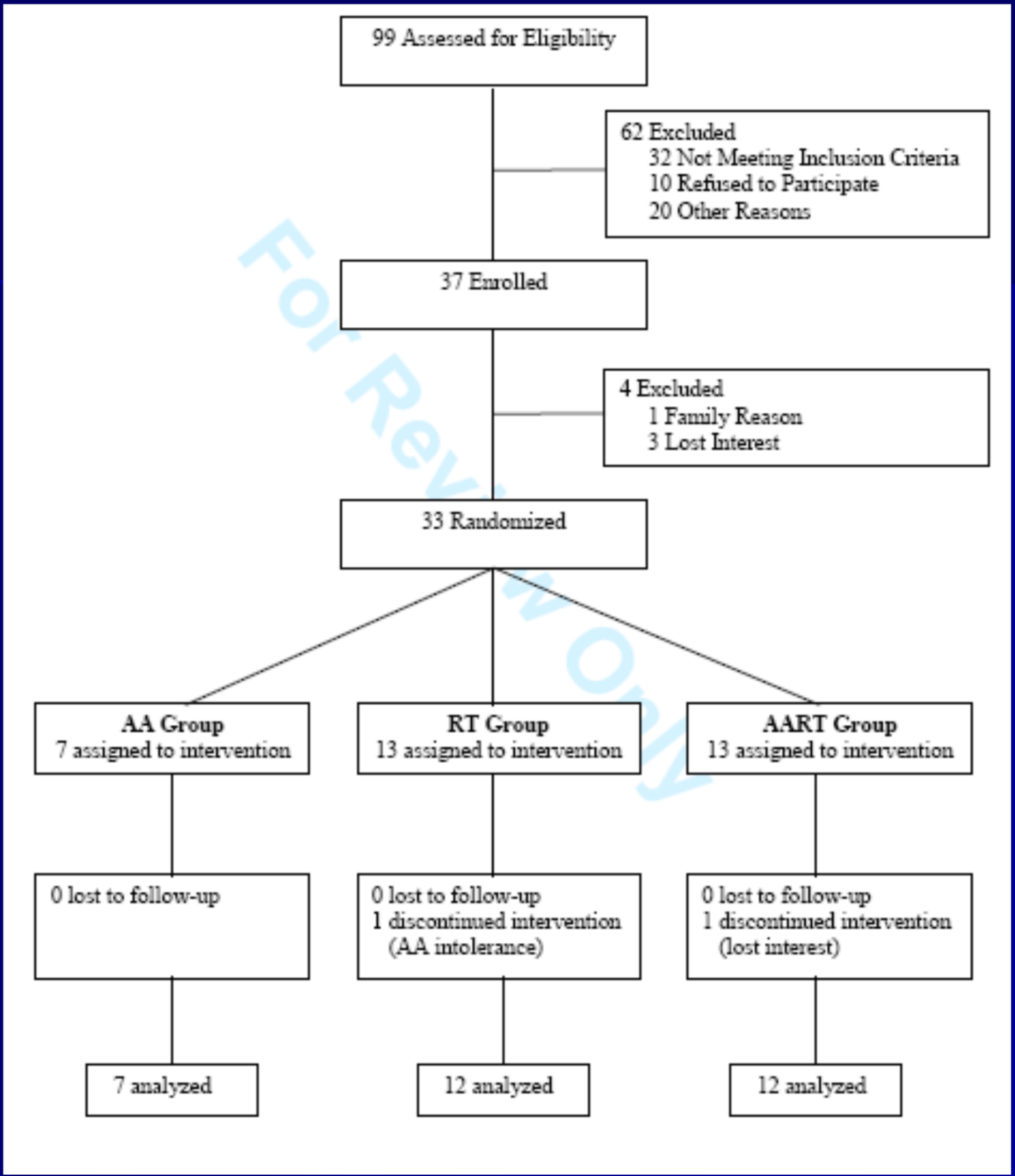
The gray blocks indicate significant correlations.

P < 0.06, Y vs. O

Muscle Anabolics in Aging

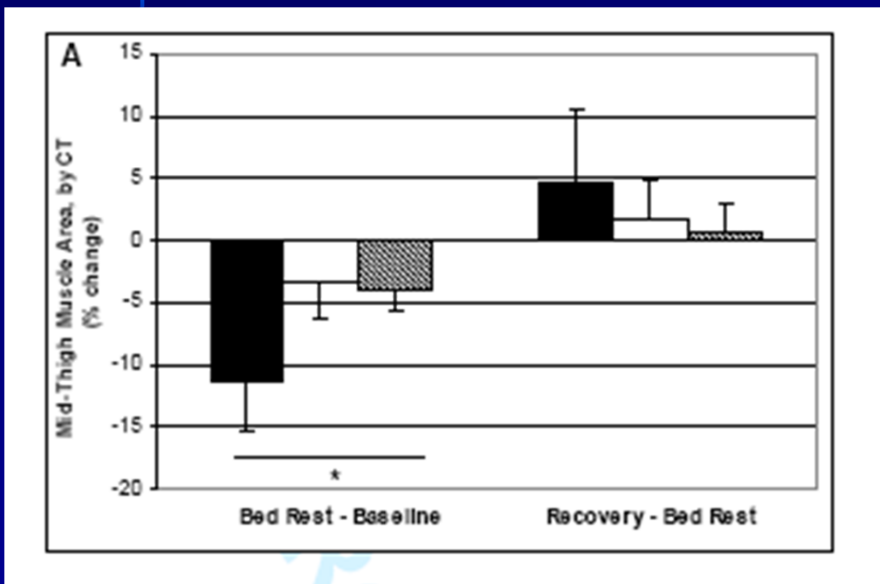
Assessing the Role of Nutrition and Exercise in Preventing Muscle Wasting

N. Brooks et al. 2008

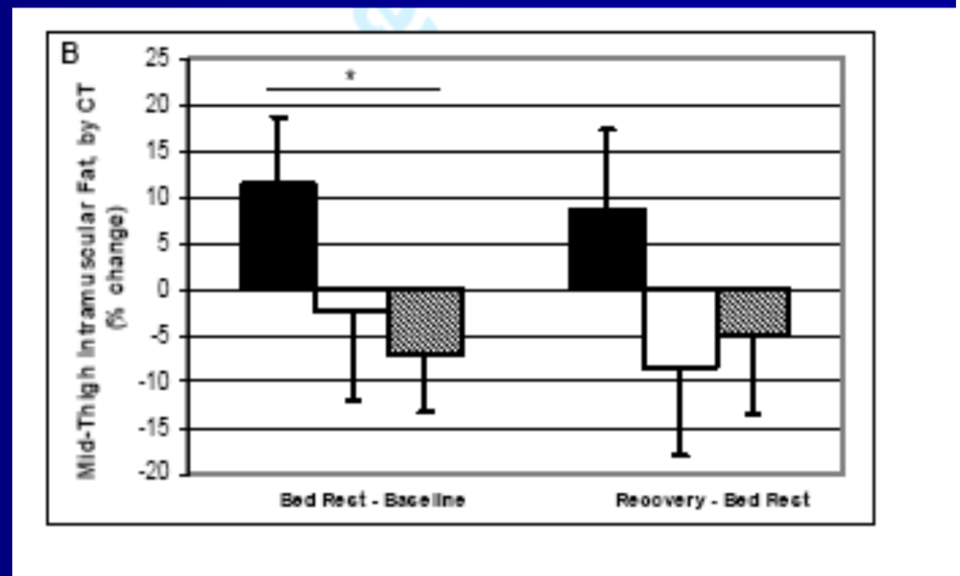


Bed Rest Mitigation: Thigh Muscle and Fat Areas

Mid-Thigh Muscle Area (CT)



Mid-Thigh Fat Area (CT)

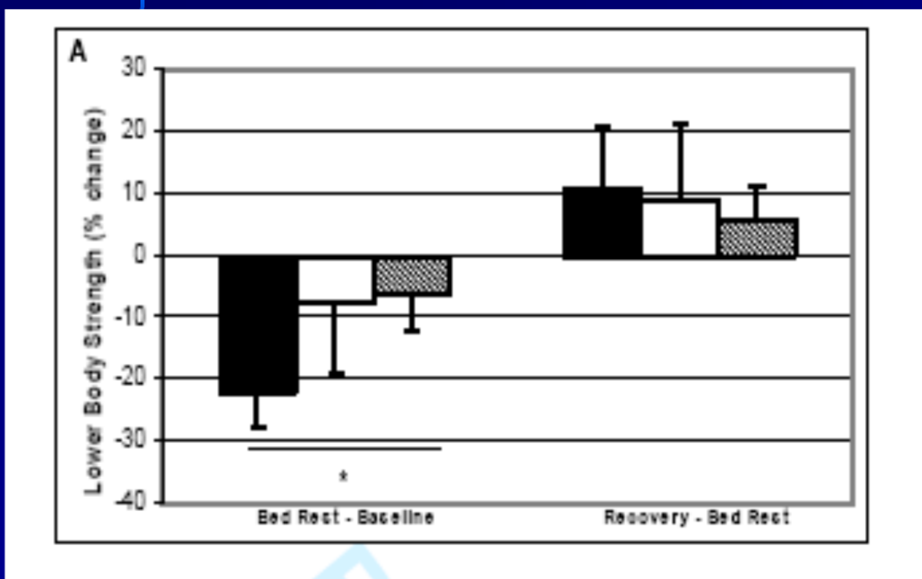


AA only RT only AA + RT

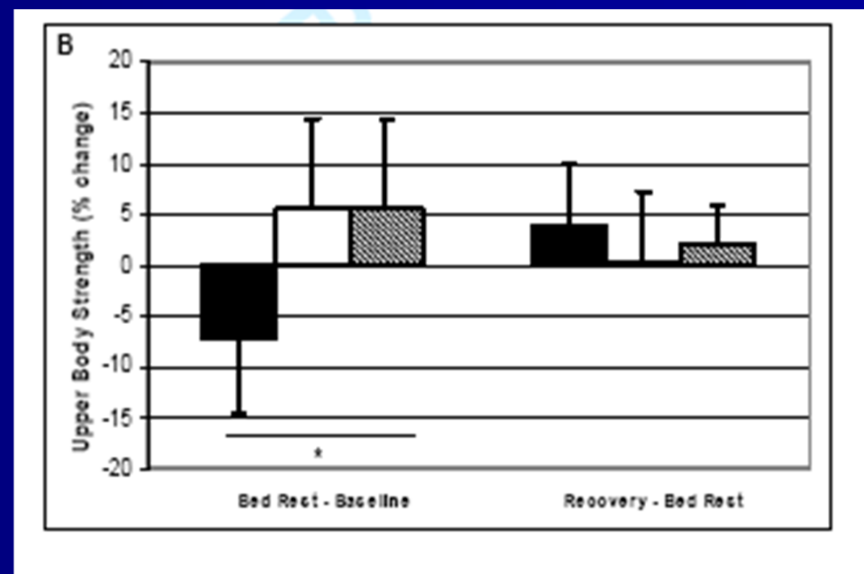
N. Brooks et al. 2008

Mitigation of Bedrest Weakness

Lower Body Strength



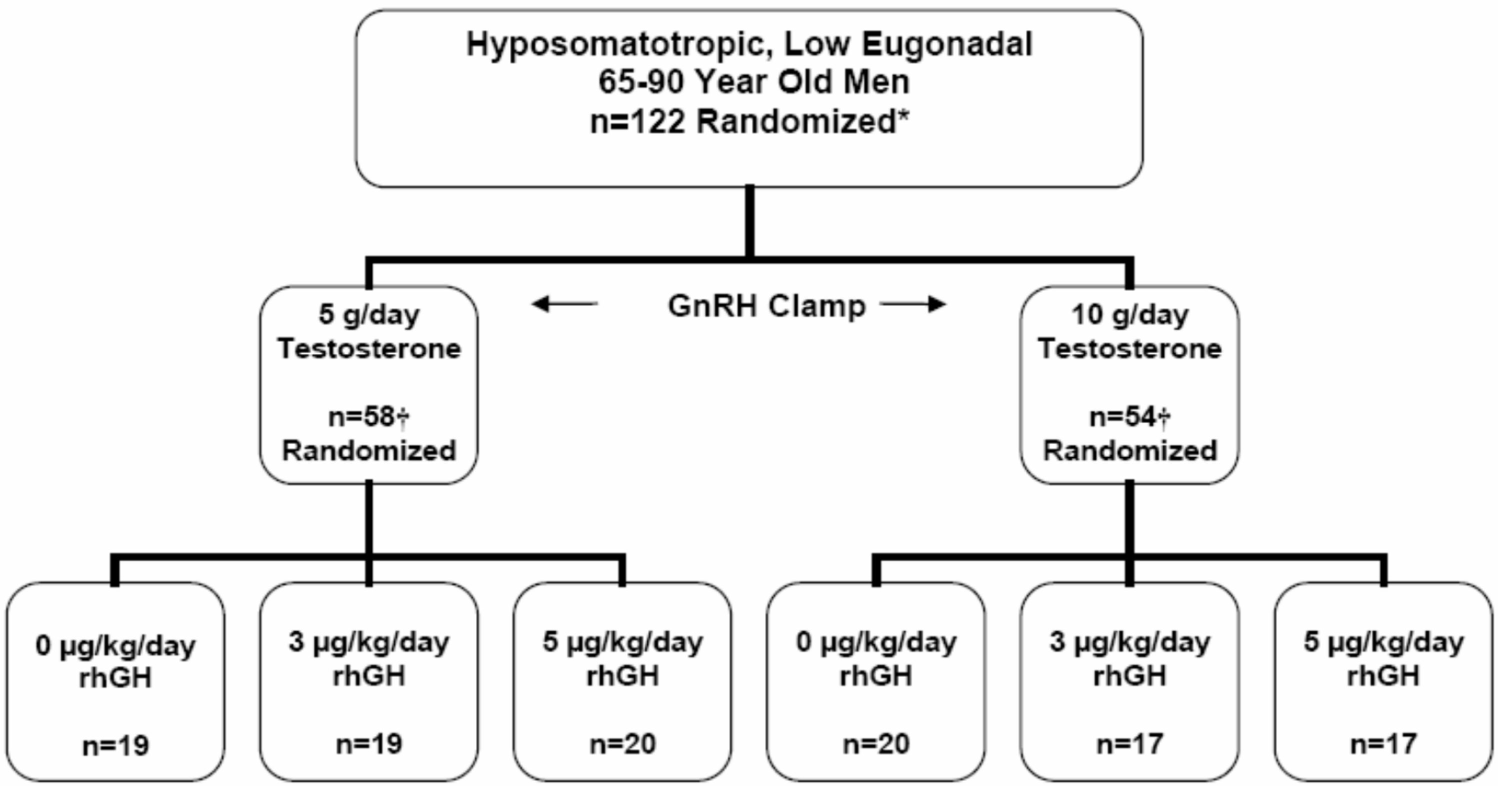
Upper Body Strength



■ AA only
□ RT only
▨ AA + RT

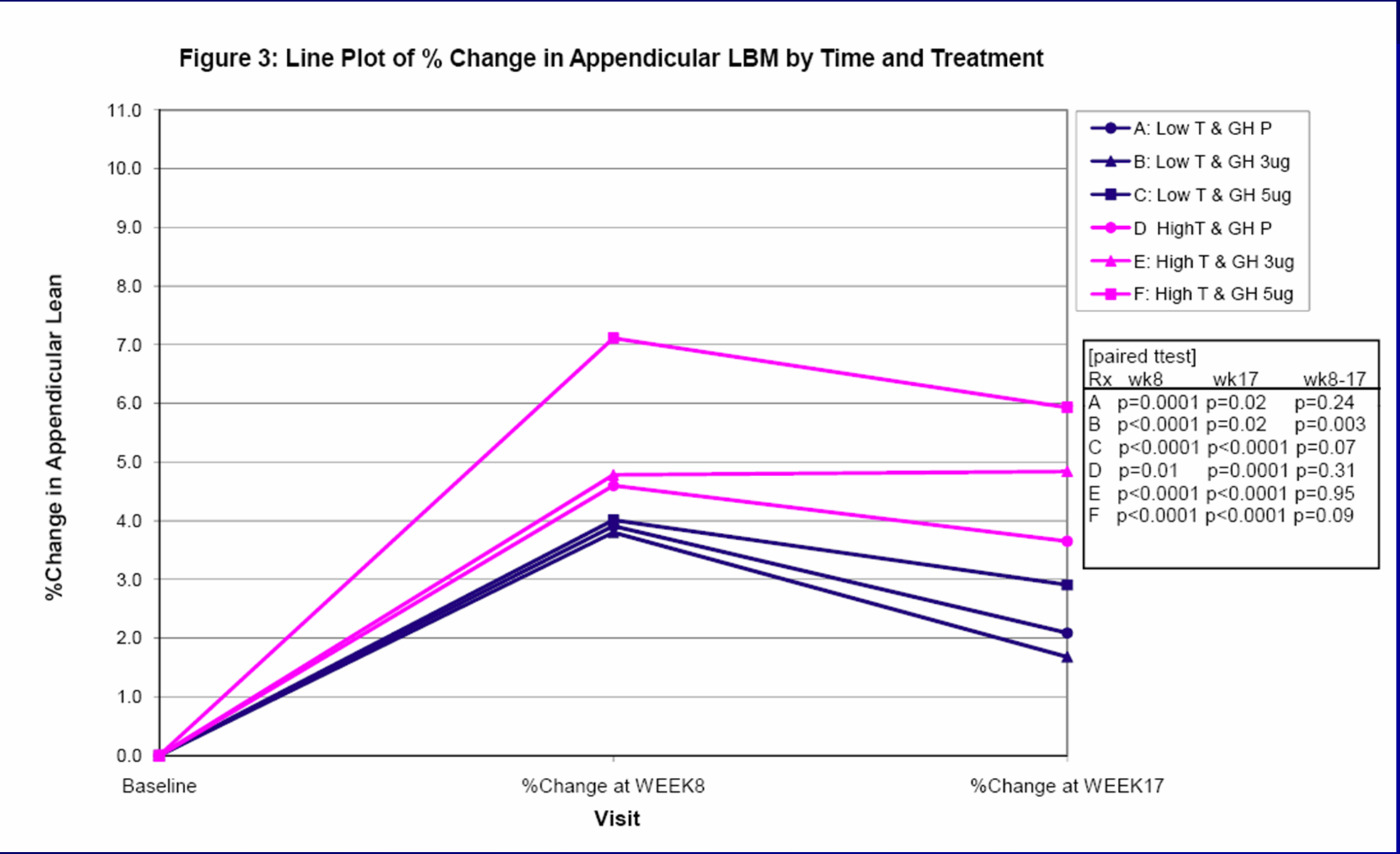
N. Brooks et al. 2008

Roles of GH and T in Sarcopenia



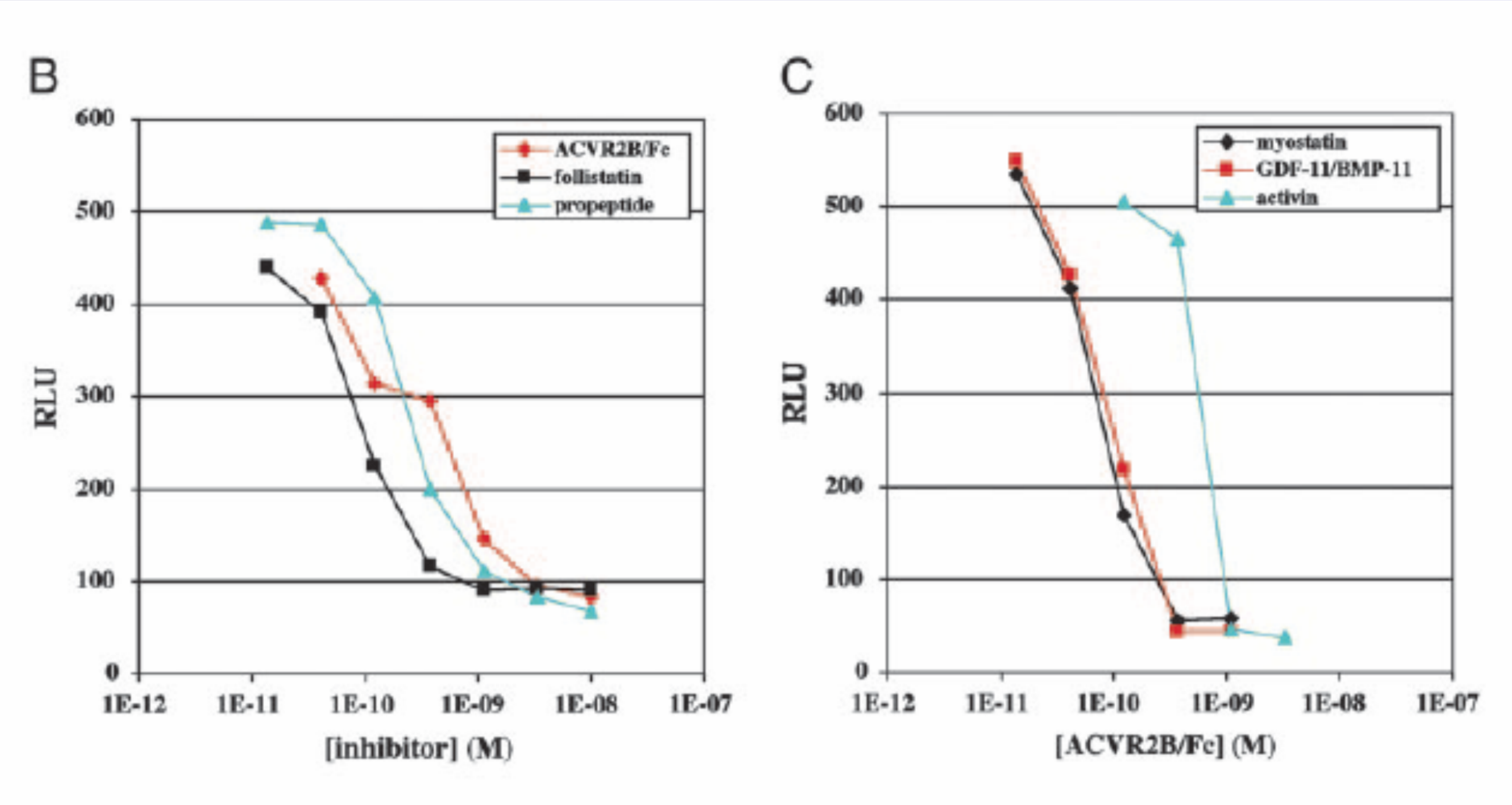
HORMA Investigators: JCEM, 2009

T > GH Effect



HORMA Investigators: JCEM 2009

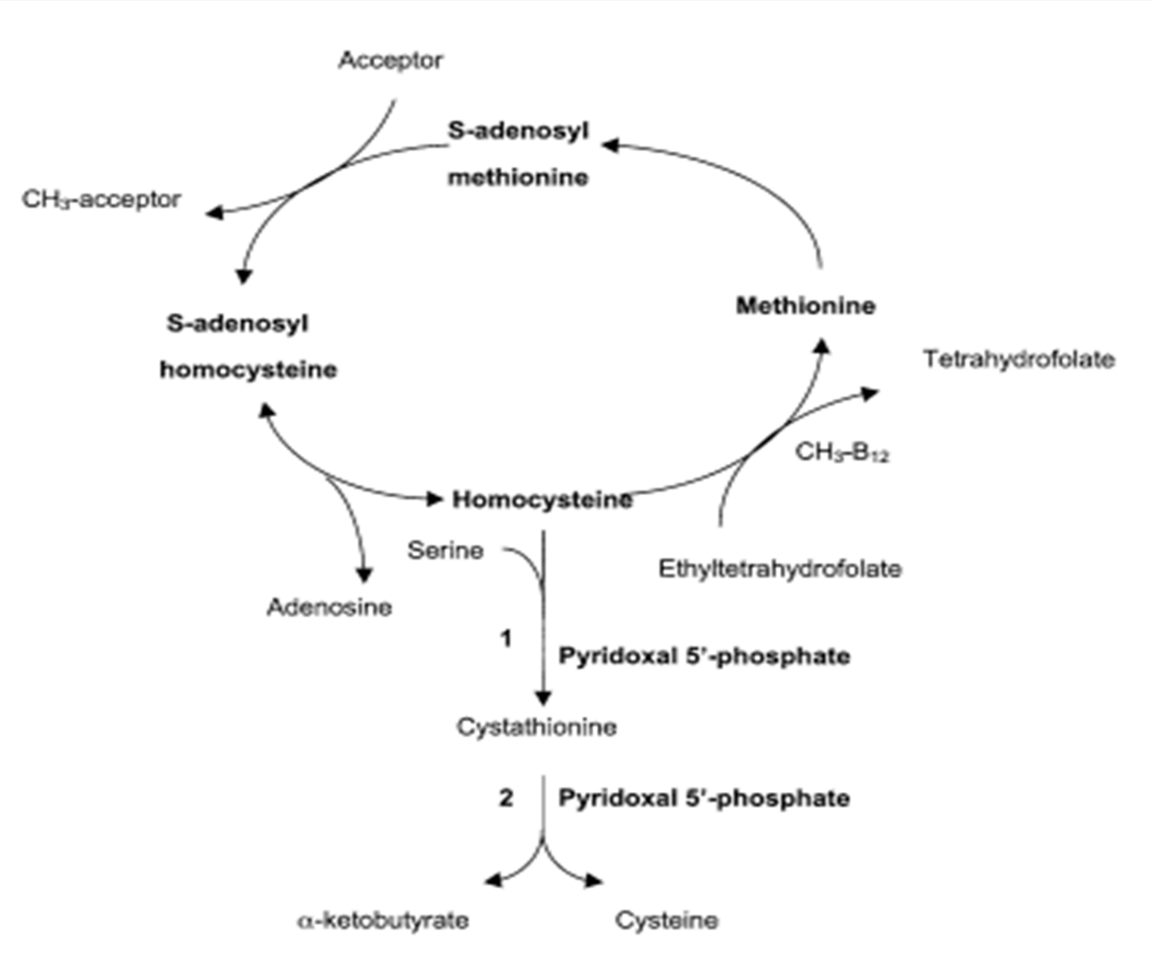
Activin Inhibition of Myostatin-Induced Muscle Wasting



Lee et al., PNAS 102:18117-18122, 2005

Micronutrients in RA: Vitamin B6 as a Case Study

B6, B12, and Folate Metabolism



Chaing, et al. Am J Med. 2003;114:283-287.

Homocysteine Elevation and PLP Depression are Common in RA

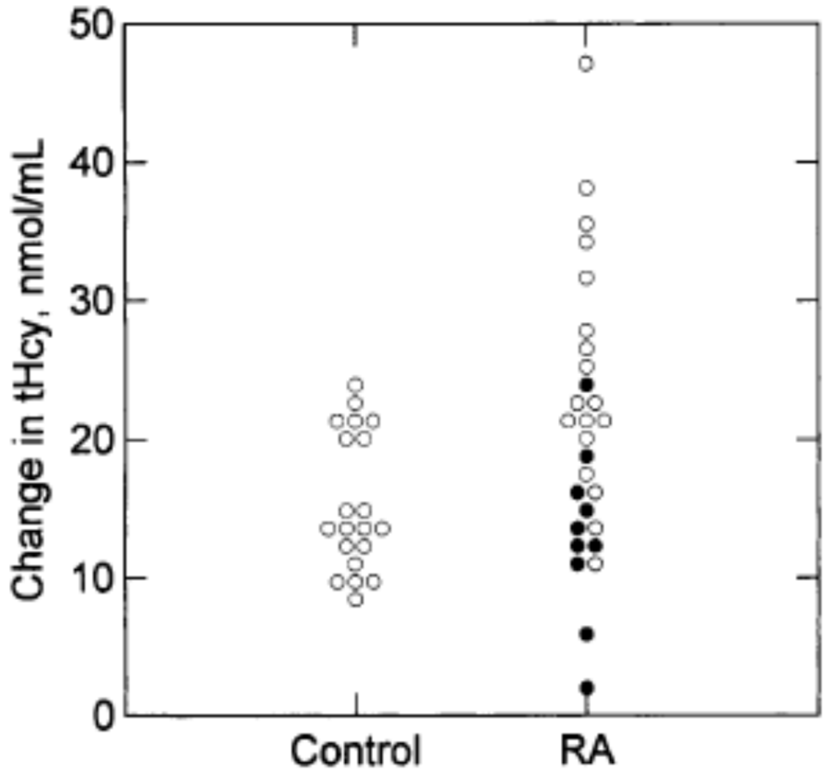


Figure 2. Distribution of change in total homocysteine (tHcy) levels in control subjects and patients with rheumatoid arthritis (RA). Within the RA group, patients taking methotrexate are indicated by closed circles and patients not taking methotrexate are indicated by open circles.

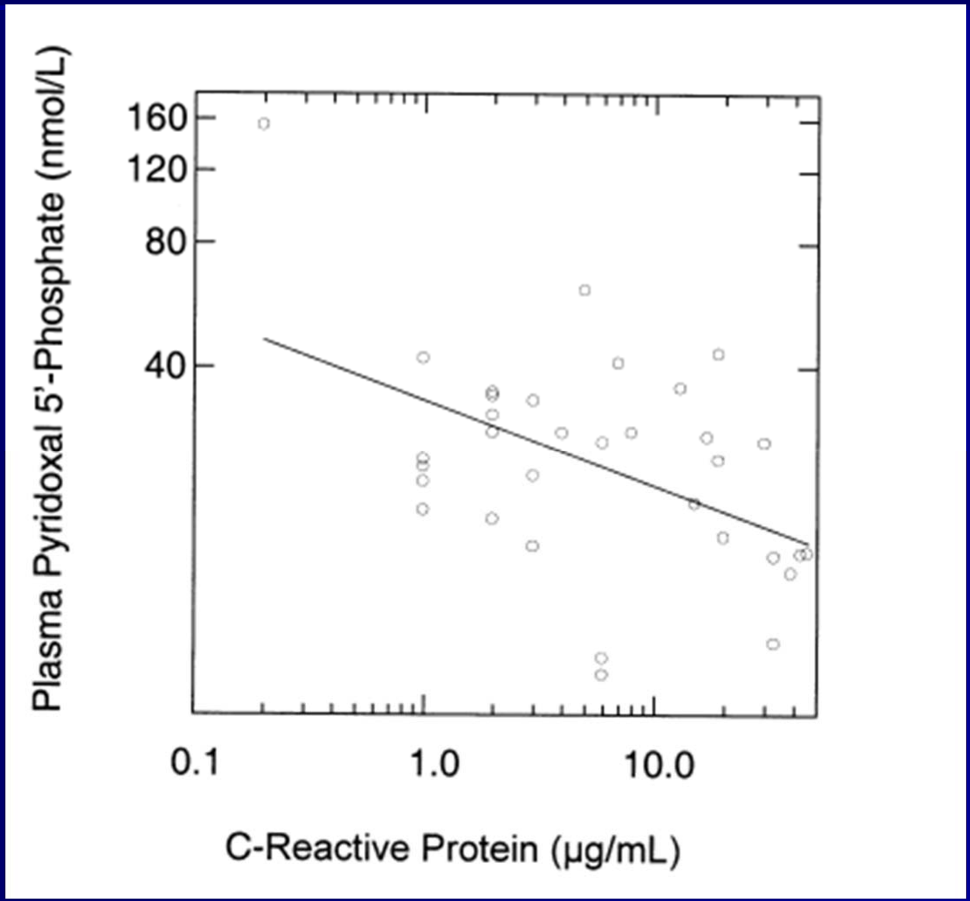
Table 2. Vitamin and total homocysteine (tHcy) levels in patients and controls*

Variable	RA group MTX+	RA group MTX−	Controls	P
Serum B_{12}, pg/ml	486.5 ± 127.6	351.0 ± 127.6	365.3 ± 134.1	0.029†
Plasma folate, ng/ml	4.83 ± 1.24	5.24 ± 1.17	6.00 ± 1.16	0.684
Plasma PLP, pmoles/ml	58.3 ± 1.1	59.3 ± 1.1	103.9 ± 1.1	0.0001‡
Serum creatinine, mg/dl	0.83 ± 0.06	0.90 ± 0.04	0.93 ± 0.05	0.228
Fasting tHcy, nmoles/ml	10.12 ± 1.12	12.60 ± 1.09	8.80 ± 1.08	0.015‡
4-hour tHcy, nmoles/ml	21.96 ± 1.12	36.89 ± 1.08	23.86 ± 1.08	0.0001§
ΔtHcy, nmoles/ml	12.90 ± 2.24	25.33 ± 1.67	15.45 ± 1.58	0.0001§

* Except where otherwise indicated, values are the mean ± SD. PLP = pyridoxal 5′-phosphate; see Table 1 for other definitions.
† Significant difference, MTX+ RA group compared with other 2 groups.
‡ Significant difference, controls compared with other 2 groups.
§ Significant difference, MTX− RA group compared with other 2 groups.

Roubenoff et al. Arthr Rheum 1997; 40: 718-722

Vitamin B6 is an Acute Phase Reactant in RA



r = -0.52, p < 0.002

Chiang, et al. Am J Med. 2003;114:283-287.

NHANES: Relationship between Plasma PLP and Serum CRP concentrations

Population	N	Plasma Pyridoxal-5-Phosphate (nmol/L)[1] by CRP Category. CRP 1 (≤0.1mg/dL)	CRP 2 (0.11-0.3mg/dL)	CRP 3 (0.31-1.0mg/dL)	CRP 4 (≥1.0mg/dL)	P-Trend[2]
All	6013	**54.74** [a] (52.12, 57.49)	**45.42**[b] (43.21, 47.74)	**38.26**[c] (36.25, 40.38)	**28.40**[d] (26.20, 30.77)	**<0.001**
Males	2982	**63.30**[a] (59.66, 67.16)	**52.74**[b] (49.53, 56.15)	**48.46**[b] (45.03, 52.16)	**34.29**[c] (30.23, 38.89)	**<0.001**
Females	3031	**47.45**[a] (43.83, 51.38)	**39.13**[b] (36.21, 42.28)	**30.58**[c] (28.30, 42.28)	**23.54**[d] (21.08, 26.09)	**<0.001**

Not shown: No effect of supplement use.

Geometric Means (95% confidence interval) adjusted (or stratified) by sex, age, race, BMI smoking status, alcohol use, vitamin B6 intake, protein intake, and plasma/serum levels of folate, vitamin B12, homocysteine, albumin, creatinine and hormone use in women.

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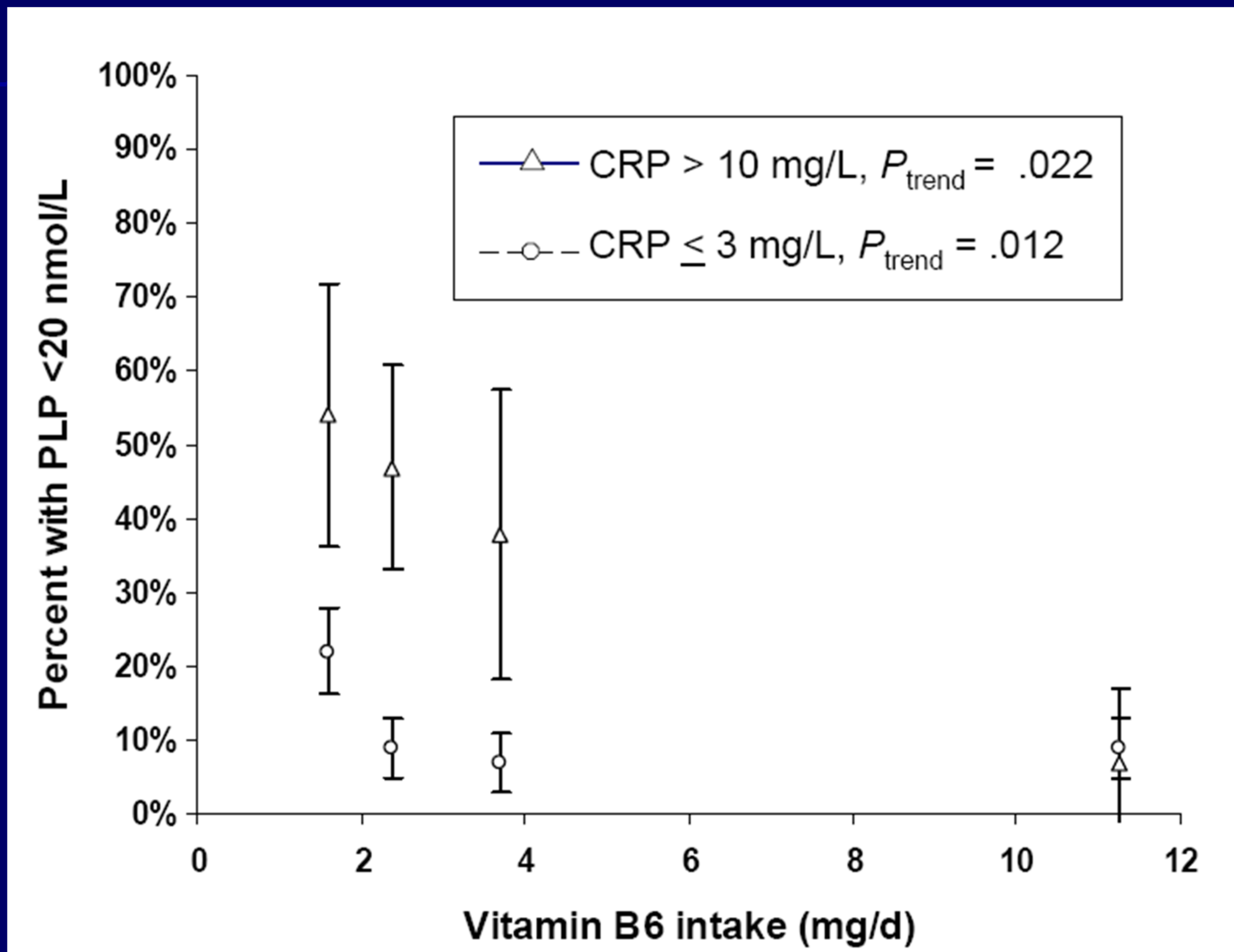
Prevalence of Low PLP by CRP status is Independent of Vitamin B6 Intake



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Relationship between plasma PLP and Inflammatory Biomarkers in the Framingham Offspring Study

Plasma Pyridoxal-5-Phosphate (nmol/L) by Quintile Category of Inflammatory Marker[1]						
Inflammatory Biomarker Quintiles	**1**	**2**	**3**	**4**	**5**	**p-trend[2]**
CRP	77.4[a] (72.4, 82.6)	72.8[a] (68.4, 77.6)	70.3[a] (66.1, 74.9)	62.0[b] (58.3, 65.9)	57.6[b] (54.0, 61.3)	<0.0001
CD40 Ligand	65.1 (61.2, 69.2)	68.1 (64.0, 72.5)	66.7 (62.7, 71.1)	67.3 (63.1, 71.9)	67.4 (63.2, 71.9)	0.6762
Fibrinogen	70.0[ab] (65.6, 74.7)	74.3[a] (69.7, 79.2)	65.4[bc] (61.4, 69.6)	65.2[bc] (61.1, 69.4)	61.9[bc] (58.2, 65.9)	<0.0001
Interlukin-6 (IL-6)	**77.9[a] (72.8, 83.2)**	**69.5[b] (65.2, 74.1)**	**70.0[b] (65.7, 74.5)**	**62.6[c] (58.9, 66.7)**	**59.6[c] (55.9, 63.4)**	**<0.0001**
Intracellular adhesion molecule-1 (ICAM-1)	**72.8[a] (68.0, 77.9)**	**70.0[a] (65.5, 74.8)**	**69.9[a] (65.6 (74.4)**	**68.2[a] (64.0, 72.7)**	**60.8[b] (57.3, 64.5)**	**<0.0001**
Lipoprotein Phospholipase-A2 Activity	**66.9[ab] (62.6, 74.6)**	**72.6[a] (68.1, 77.4)**	**70.3[a] (66.0, 75.0)**	**65.7[ab] (61.7, 70.0)**	**61.1[b] (57.4, 65.1)**	**0.0017**
MCP-1	66.7 (62.5, 71.2)	66.1 (62.0, 70.6)	68.4 (64.2, 72.8)	65.4 (61.4, 69.6)	67.3 (63.3, 71.6)	0.8661
Myeloperoxidase	66.5 (62.4, 70.8)	69.9 (65.6, 74.5)	68.9 (64.6, 73.4)	65.5 (61.6, 69.7)	64.1 (60.2, 68.2)	0.046
Osteoprotegerin	70.8[a] (66.2, 75.7)	69.9[ab] (65.6, 74.6)	67.4[ab] (63.2, 71.9)	65.3[ab] (61.4, 69.5)	63.1[b](59.1, 67.2)	0.0013
P-selectin	70.5[a] (66.0, 75.3)	67.9[ab](63.7, 72.4)	67.4[ab] (63.3, 74.8)	66.8[ab](62.7, 71.1)	63.6[b] (59.8, 67.6)	0.0057
Tumor Necrosis Factor Receptor 2	**76.5[a] (71.5, 81.8)**	**73.8[ab] (69.3, 78.5)**	**68.8[bc] (64.6, 73.2)**	**64.2[c] (60.3, 68.3)**	**56.5[d] (52.9, 60.3)**	**<0.0001**
Tumor Necrosis Factor-α	**72.9[a] (67.6, 78.6)**	**70.1[ab] (65.1, 75.5)**	**70.0[ab] (65.1, 75.3)**	**63.9[bc] (75.4, 68.7)**	**61.5[c](57.1, 66.3)**	**<0.0001**
Resistin	68.1[a] (63.5, 73.2)	71.9[a] (67.1, 77.1)	69.5[a] (64.8, 77.1)	67.4[ab] (62.8, 72.3)	61.3[b](57.2, 65.7)	0.0003
Adiponectin	63.2 (58.9, 67.9)	67.1 (62.5, 72.0)	67.2 (62.6, 72.2)	66.6 (62.1, 71.5)	70.2 (65.2, 75.6)	0.0556

[1]Geometric means (95% confidence intervals) adjusted for sex, age, BMI, plasma homocysteine, folate, vitamin B12, creatinine, total cholesterol, vitamin B6 intakes, protein intakes, calories, NSAID use, Cigarette use, and multivitamin use.

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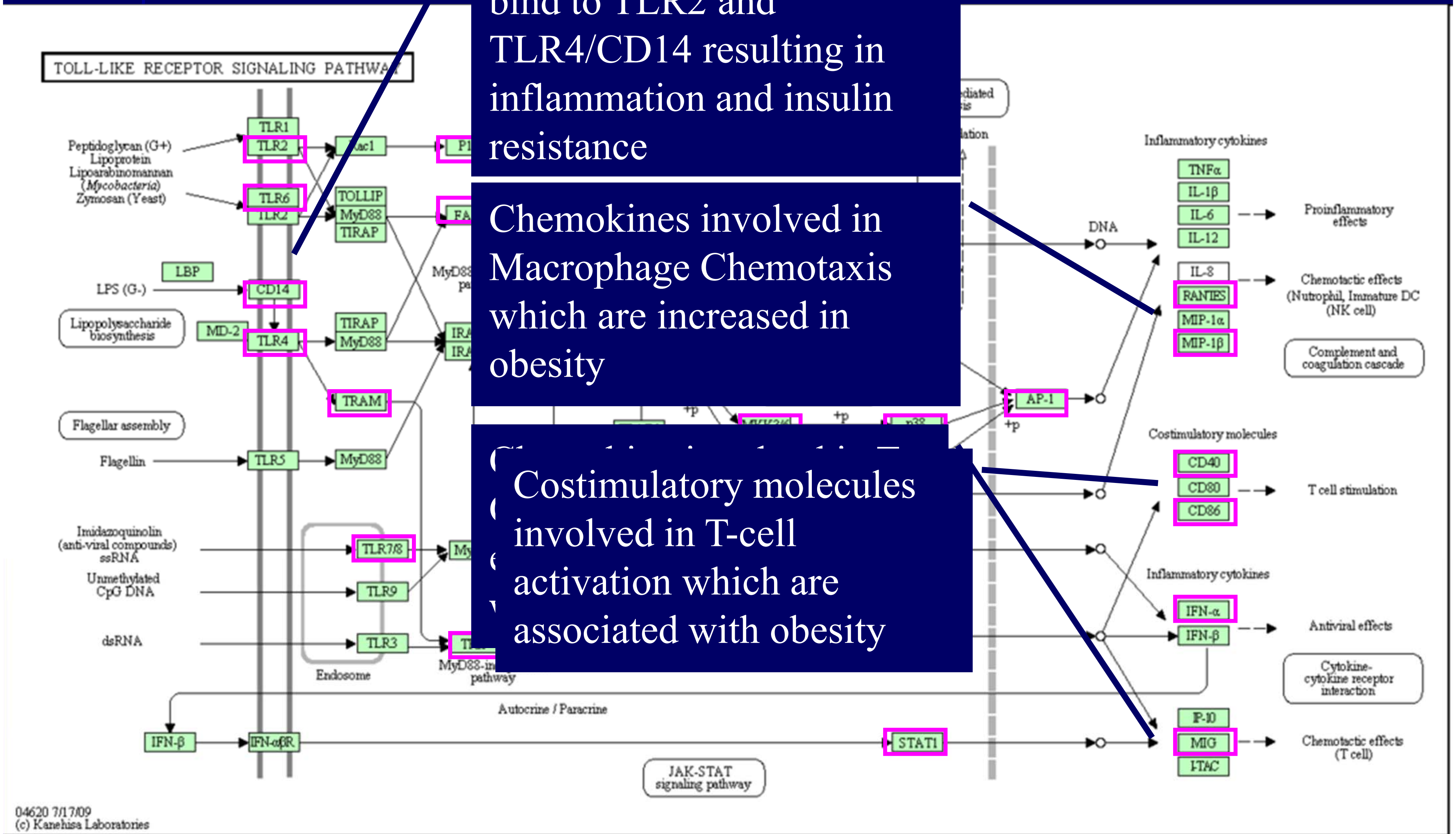
Effect of Vitamin B6 Restriction on Tissue PLP in a Mouse Obesity Inflammation Model

	Diet Group		
Characteristic	Low Fat	High Fat	High Fat, 50% B6
Plasma PLP (nmol/L)	223.3 (77.8)[a]	215.5 (49.1)[a]	73.3 (24.0)[b]
Liver PLP (pmol/g)	23852.3 (5670.4)	24387.1 (5490.6)	19459.1 (4331.3)
Adipose PLP (pmol/g)	420.8 (20.1)[a]	380.0 (102.9)[a]	163.7 (16.1)[b]

No effect of Low Vitamin B6 on the recruitment of macrophages, T-Cells, and NK cells to Adipose Tissue

Sakakeeny et al. 2009 in press

Table values are presented as unadjusted means (standard deviation). Different superscripts by column indicate a p-value < 0.05.



Free-Fatty Acids and LPS bind to TLR2 and TLR4/CD14 resulting in inflammation and insulin resistance

Chemokines involved in Macrophage Chemotaxis which are increased in obesity

Costimulatory molecules involved in T-cell activation which are associated with obesity

Nutritional Impact of Inflammation in RA: Signposts for Aging

- Cachexia is common and persists even when inflammation is controlled
- Increased fat mass and reduced lean mass (sarcopenic obesity) is common
- Overlapping cytokine effects are probably driving rheumatoid cachexia
- Plasma PLP is reduced in RA and age-related inflammation, independently of Vitamin B6 intake
- The effect of PLP reduction on inflammation is unclear

Back-Up Slides

PLP and Inflammation in NHANES III: Population Characteristics by CRP Category

	CRP 1 (≤0.1mg/dL)	CRP 2 (0.11-0.3mg/dL)	CRP 3 (0.31-1.0mg/dL)	CRP 4 (≥1.0mg/dL)
Gender (%)[1]				
Males (48.7)	50.9	27.9	16.8	4.3
Females (51.2)	43.6	23.4	22.7	10.4
Ages (%)[1]				
1-19 (27.4)	77.3	12.8	7.3	2.7
20-44 (36.4)	40.7	28.3	22.3	8.8
>45 (36.2)	29.0	33.4	27.6	9.9
Race (%)[1]				
Non-Hispanic White (69.7)	46.1	26.8	20.1	7.0
Non-Hispanic Black (12.6)	47.6	22.2	19.1	11.1
Mexican American (9.2)	47.6	24.9	20.6	6.9
Other Race- Including Multi-Racial (4.6)	58.3	21.6	16.0	4.1
Other-Hispanic (4.1)	51.5	19.6	21.8	7.1
BMI[2]	22.8 (22.6-23.0)	27.2 (27.0-27.5)	30.1 (29.8-30.4)	32.6 (32.1-33.1)
Protein Intake (g/d)[2]	78.6 (77.5-79.7)	82.3 (80.9-83.8)	81.1 (79.5-82.8)	74.9 (72.3-77.6)
Vitamin B6 Intake (mg/d)[2]	1.88 (1.85-1.91)	1.87 (1.83-1.91)	1.80 (1.75-1.85)	1.64 (1.56-1.71)
Plasma/Serum (95%CI)[3]				
PLP (nmol/L)	49.8 (48.2-51.4)	46.0 (44.2-47.8)	35.7 (34.2-37.3)	22.4 (20.9-24.1)
CRP (mg/dL)	0.034 (.033-.035)	0.181 (.177-.185)	0.514 (.501-.528)	1.792 (1.718-1.870)
Homocysteine (µmol/L)	7.0 (6.9-7.1)	8.3 (8.2-8.4)	8.2 (8.1-8.3)	8.1 (7.8-8.3)
B12 (pmol/L)	391.9 (385.9-398.0)	351.6 (345.1-358.2)	342.8 (335.6-350.2)	337.2 (325.7-349.1)
Folate (nmol/L)	28.4 (27.9-28.9)	27.7 (27.2-28.3)	27.1 (26.4-27.7)	24.3 (23.3-25.2)
Albumin (g/L)	44.3 (44.2-44.4)	43.1 (43.0-43.2)	41.6 (41.4-41.7)	39.5 (39.2-39.7)
Creatinine (mg/dL)	0.838 (0.829-0.846)	0.876 (0.866-0.885)	0.849 (0.839-0.860)	0.827 (0.810-0.843)

Sakakeeny et al. 2009 in press