CALERIE Research Network Workshop

William E. Kraus, MD
for the CALERIE Research Network and CALERIE
CALERIE is supported by the following grants from the NIA U01AG022132, U01AG020478, U01AG020487, U01AG020480 and NIDDK. The CALERIE Research Network is supported by U24AG047121

Outline

- Bill Kraus
  - Background: CALERIE Phase 2 Design and Results Overview
  - CALERIE Research Network
  - Our Workhorse: The Website
- Dan Belsky: an example
- Carl Pieper & Manju Bhapkar: data repository and using the database: an example
- Chhanda Dutta (NIA): funding opportunities

Background: Design and Results Overview

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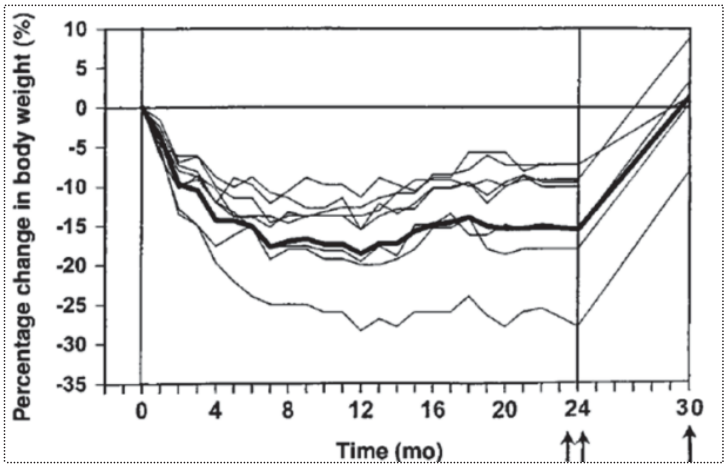
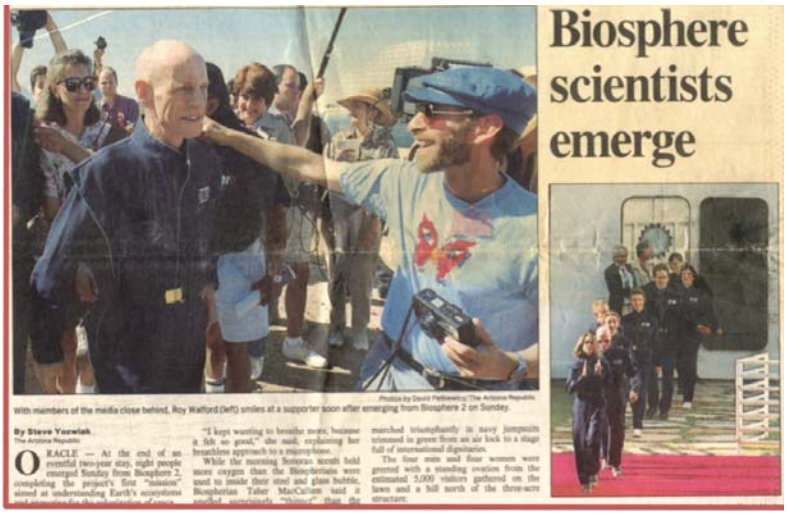
Results from Biosphere 2

- The approximate 750 calorie per day deficit resulted in an average weight loss of 15%.





Biosphere scientists emerge

With members of the media close behind, Roy Walford (left) smiles at a supporter soon after emerging from Biosphere 2 on Sunday.

By Steve Yozwiak
The Arizona Republic

ORACLE — At the end of an eventful two-year stay, eight people emerged Sunday from Biosphere 2, completing the project's first "mission" aimed at understanding Earth's ecosystems

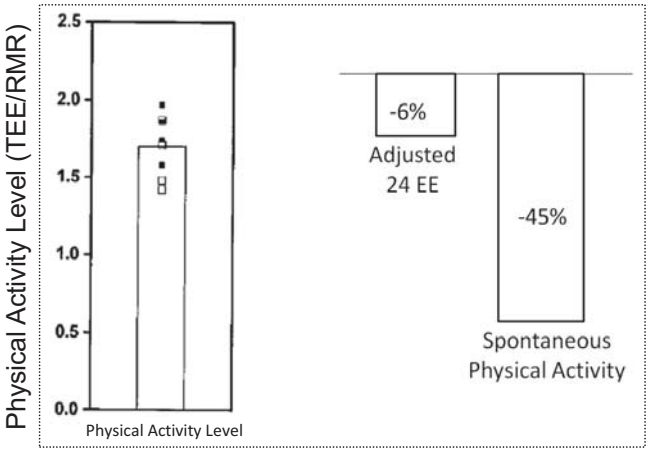
"I kept wanting to breathe more, because it felt so good," she said, explaining her breathless approach to a microphone.

While the morning Sonoran scouts held more oxygen than the Biospherians were used to inside their steel and glass bubble, Biospherian Taber MacCallum said it

marched triumphantly in navy jumpsuits trimmed in green from an air lock to a stage full of international dignitaries.

The four men and four women were greeted with a standing ovation from the estimated 5,000 visitors gathered on the lawn and a hill north of the three-acre structure.

- The weight loss was associated with many beneficial physiological, hematological, biochemical and metabolic alterations consistent with CR in rodents and primates.



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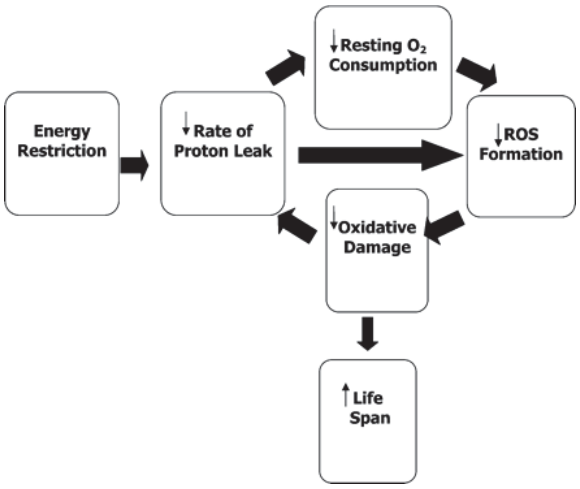
CALERIE
Metabolic adaptation and oxidative stress

Rate of Living and Oxidative Stress Theories of Aging

•Free radicals produced during aerobic respiration cause cumulative oxidative damage resulting in aging (Harmon, 1957)

– CR reduces ROS production



Potential Mechanism for anti-aging effects of CR

- CR induces a lowering of metabolic rate that is lower than expected for reduced body size and changed body composition
- CR reduces markers of oxidative stress (serum protein carbonyls, DNA damage, urinary isoprostanes)

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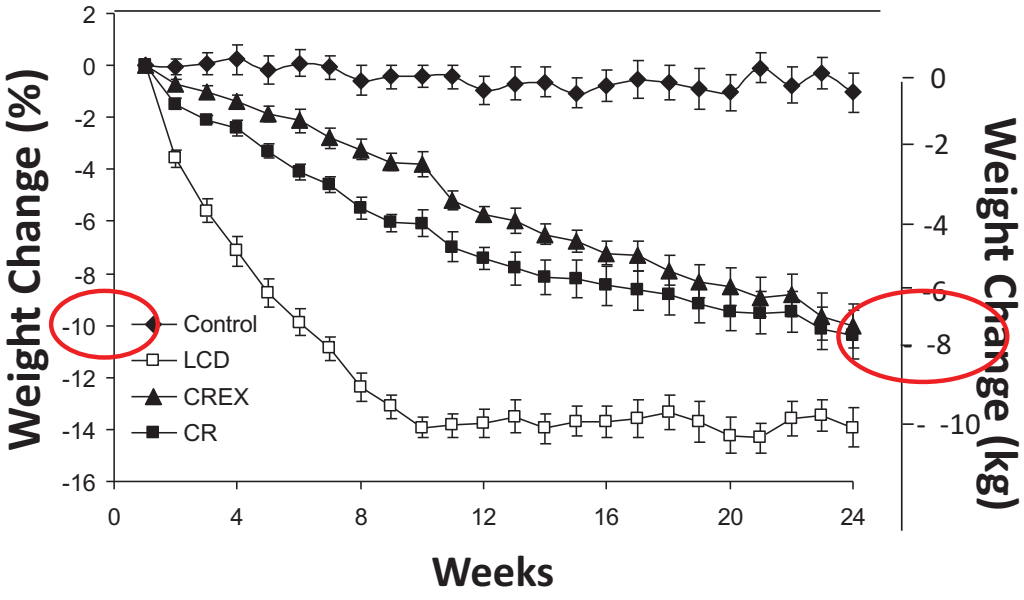
CALERIE I

calerie

Effect of 6-Month Calorie Restriction on Biomarkers of Longevity, Metabolic Adaptation, and Oxidative Stress in Overweight Individuals

A Randomized Controlled Trial

JAMA, April 5, 2006—Vol 295, No. 13



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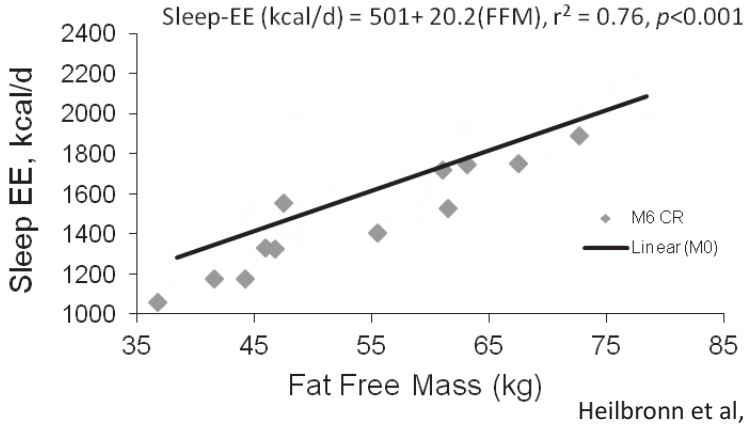
Evidence of metabolic adaptation

Table 2. Absolute Energy Expenditures (24-Hour Sedentary and Sleeping) Measured in a Metabolic Chamber At Baseline, Month 3, and Month 6*

Month	Mean (SEM), kcal: Actual 24-Hour Energy Expenditure	Mean (SEM), kcal: Predicted Energy Expenditure	*P* Value	Mean (SEM), kcal: Sleep Energy Expenditure	Mean (SEM), kcal: Predicted Sleep Energy Expenditure	*P* Value
Control						
Baseline	2129 (102)	2110 (80)		1654 (69)	1642 (60)	
Month 3	2119 (109)	2118 (84)	.89	1642 (92)	1698 (63)	.86
Month 6	2092 (97)	2110 (84)	.38	1513 (37)	1642 (63)	.26
Calorie restriction						
Baseline	2079 (102)	2100 (95)		1600 (88)	1635 (72)	
Month 3	1900 (101)	2048 (91)	.001	1472 (75)	1595 (69)	<.001
Month 6	1899 (101)	2034 (88)	.002	1473 (77)	1585 (66)	.001
Calorie restriction with exercise						
Baseline	2106 (102)	2085 (93)		1615 (78)	1623 (70)	
Month 3	1972 (101)	2057 (89)	.04	1524 (76)	1602 (67)	.02
Month 6	1917 (91)	2034 (86)	.008	1511 (62)	1585 (65)	.03
Very low-calorie diet						
Baseline	2085 (90)	2055 (92)		1658 (78)	1600 (69)	
Month 3	1842 (60)	1965 (82)	.007	1489 (54)	1533 (62)	.13
Month 6	1852 (71)	1977 (87)	.006	1479 (73)	1542 (66)	.19

*P values indicate differences between actual vs predicted values. Predicted energy expenditures were calculated as follows: 24-hour energy expenditure = 596 + 26.8 × fat-free mass (r^2 = 0.86, *P*<.001); sleep energy expenditure = 501 + 20.2 × fat-free mass (r^2 = 0.76, *P*<.001). The measured – predicted values for 24-hour energy expenditure and sleep energy expenditure are calculated as the difference between the measured and the predicted values.

~6%



Heilbronn et al, JAMA, April 2006

CALERIE Phase 2 - Aims

Primary Aim

25% CR results in sustained metabolic adaptation, i.e., a reduction in core body temperature and reduced RMR adjusted for changes in body composition

Secondary Aims

- 25% CR reduces serum triiodothyronine (T^3) – mediators of metabolic adaptation and reduces plasma TNF-α concentration.
- Investigate the safety implications of sustained CR in humans

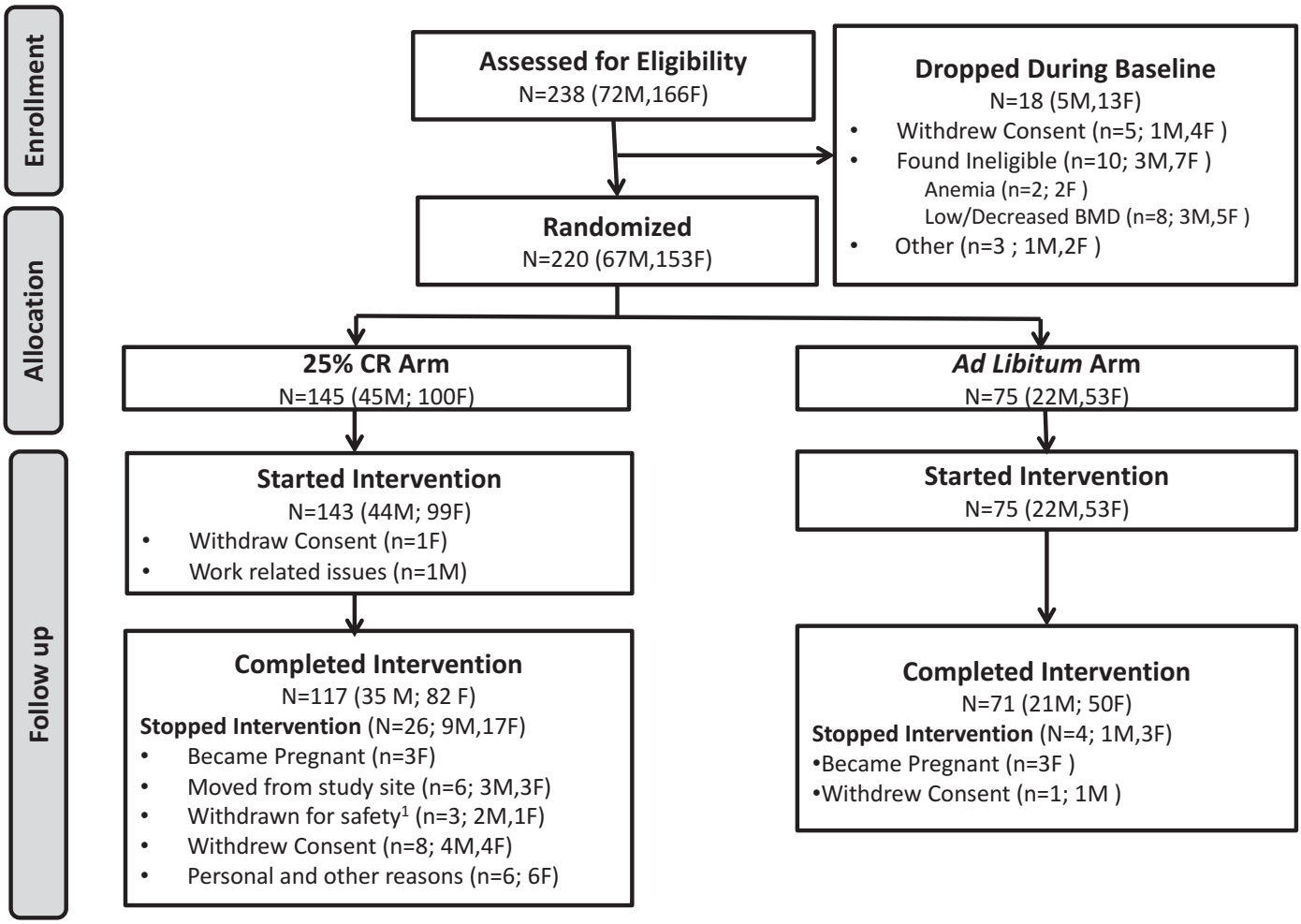
Exploratory Aims

- Body composition
- Serum hormones and inflammatory cytokines
- Plasma growth factor concentrations
- Risk factors for atherosclerosis and Type 2 diabetes
- Measures of oxidative stress
- Immune function measures
- Psychological, QoL and cognitive functioning
- VO_2max, muscular strength and endurance
- Cardiometabolic Risk Factors

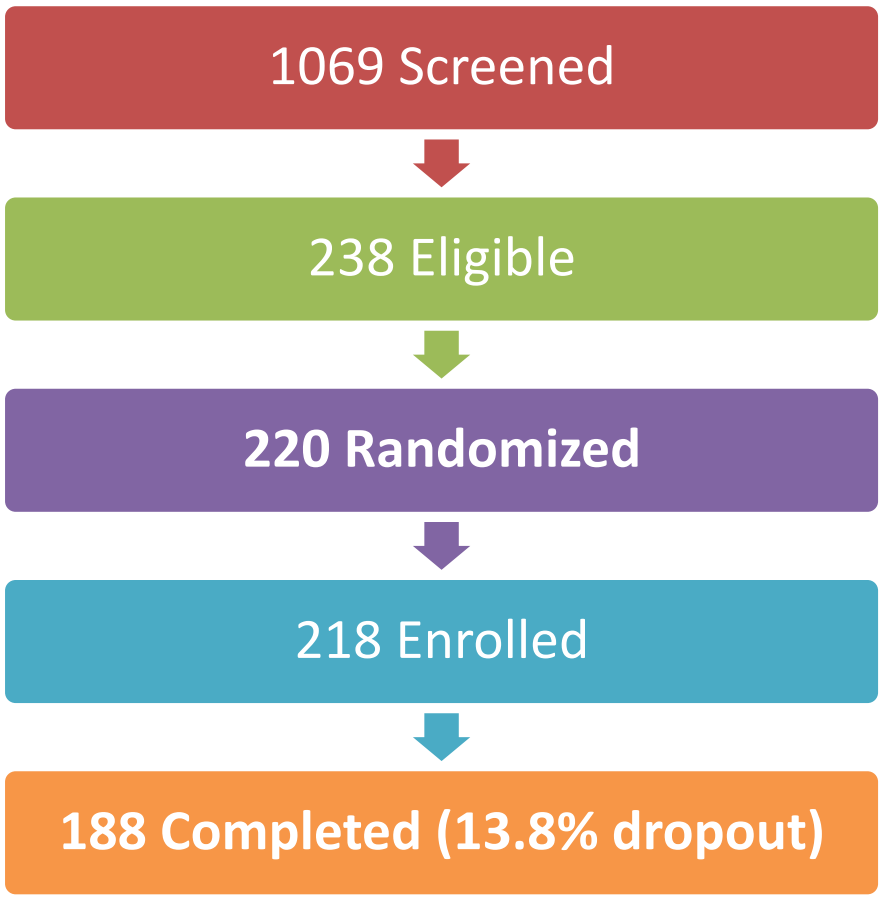
CALERIE Phase 2 - Design

- Multi-center, parallel-group, RCT
- Participants assigned at random to 25% CR or *ab libitum* control.
  BMI: 22.0 ≤ BMI < 28 kg/m^2
  Age: 21-50y for Men | 21-47y for women

- *N* = 218 participants started the intervention (188 completers)


Enrollment
Allocation
Follow up
Assessed for Eligibility
N=238 (72M,166F)
Dropped During Baseline
N=18 (5M,13F)
• Withdrew Consent (n=5; 1M,4F)
• Found Ineligible (n=10; 3M,7F)
Anemia (n=2; 2F)
Low/Decreased BMD (n=8; 3M,5F)
• Other (n=3 ; 1M,2F)
Randomized
N=220 (67M,153F)
25% CR Arm
N=145 (45M; 100F)
Ad Libitum Arm
N=75 (22M,53F)
Started Intervention
N=143 (44M; 99F)
• Withdraw Consent (n=1F)
• Work related issues (n=1M)
Started Intervention
N=75 (22M,53F)
Completed Intervention
N=117 (35 M; 82 F)
Stopped Intervention (N=26; 9M,17F)
• Became Pregnant (n=3F)
• Moved from study site (n=6; 3M,3F)
• Withdrawn for safety[1] (n=3; 2M,1F)
• Withdrew Consent (n=8; 4M,4F)
• Personal and other reasons (n=6; 6F)
Completed Intervention
N=71 (21M; 50F)
Stopped Intervention (N=4; 1M,3F)
•Became Pregnant (n=3F)
•Withdrew Consent (n=1; 1M)

CALERIE 2 - CONSORT



PARTICIPANTS

	Calorie Restriction (n=143)		Ad Libitum (n=75)	
Race				
White	111 (77.6%)		57 (76%)	
African American	15 (10.5%)		11 (14.7%)	
Other	17 (11.9%)		7 (9.3%)	
Sex (F/M)	99	44	53	22
Age, y	36.8 (7.2)	40.5 (7.2)	37.9 (6.9)	37.8 (7.1)
Weight, kg	67.7 (6.3)	81.6 (8.3)	68.0 (6.9)	79.8 (6.6)
BMI, kg/m^2	24.8 (1.7)	26.0 (1.6)	24.9 (1.6)	25.6 (1.7)
% Fat	36.0 (4.3)	26.1 (3.1)	36.8 (4.2)	25.7 (4.0)
FFM, kg	43.2 (4.1)	60.3 (6.0)	42.8 (3.6)	59.3 (5.3)
FM, kg	24.4 (4.3)	21.3 (3.7)	25.2 (4.8)	20.5 (3.9)
Blood pressure				
SBP, mmHg	110.3 (10.1)	116.2 (8.2)	108.4 (9.4)	117.9 (7.6)
DBP, mmHg	71.4 (7.5)	73.6 (7.5)	70.4 (6.8)	73.2 (7.6)
Laboratory Values				
Glucose, mg/dL	80.5 (5.5)	85.2 (5.1)	82.4 (5.7)	86.5 (6.2)
Insulin, uU/mL	5.2 (2.4)	5.7 (2.4)	5.9 (2.4)	5.6 (2.8)
HDL-C, mg/dL	53.7 (13.0)	38.8 (6.8)	51.9 (11.4)	42.7 (9.8)
LDL-C, mg/dL	92.6 (25.2)	110.1 (25.5)	100.7 (25.3)	117.4 (32.7)
TG, mg/dL	92 (48)	129 (48)	90 (34)	147 (84)

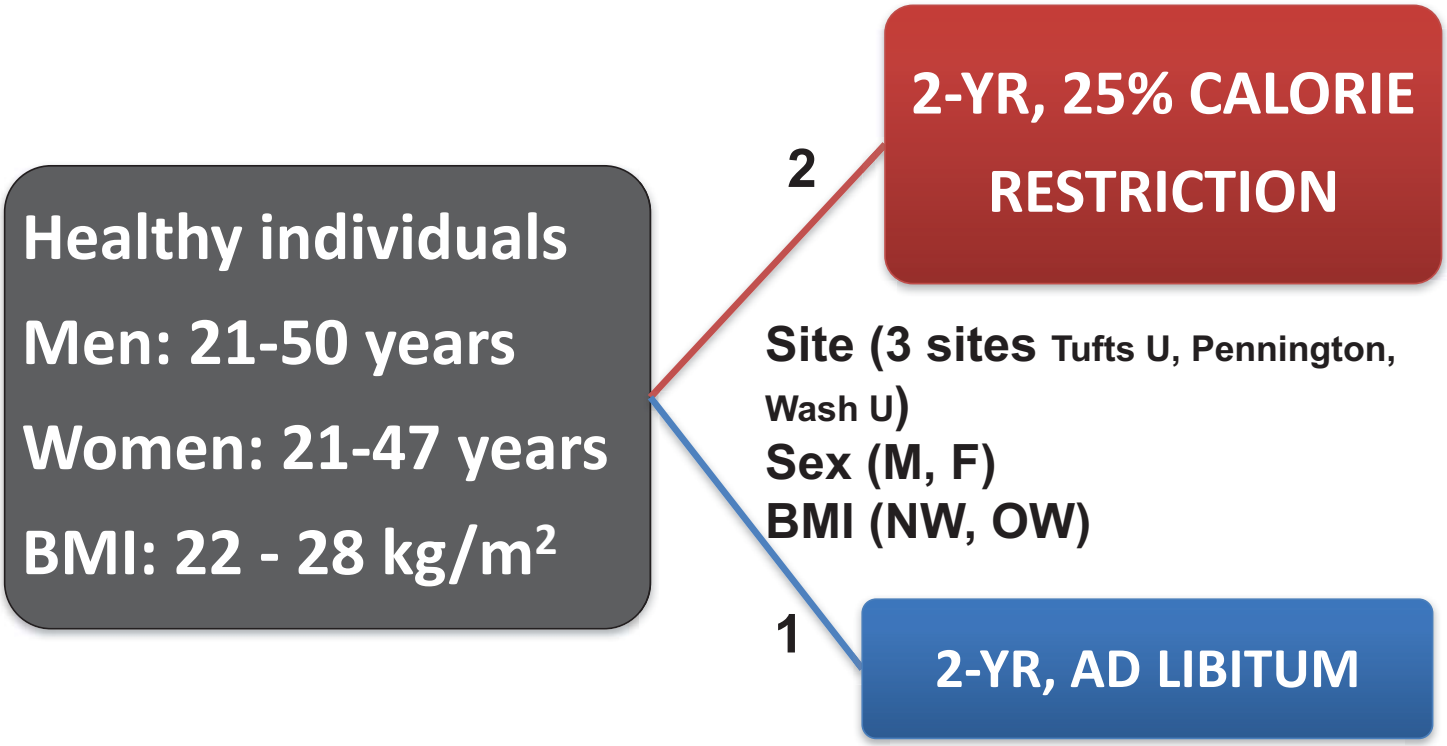
CALERIE 2 - Methods

- Clinical Endpoints (metabolic & behavioral outcomes)
  - CR: M6, M12, M18, M24
  - AL: M12, M24

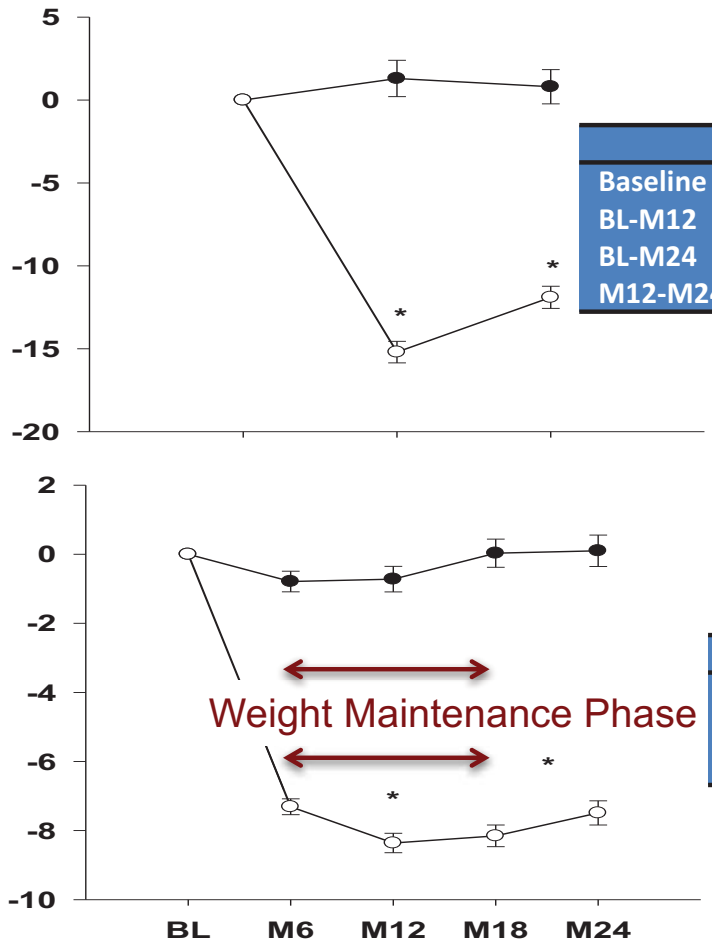
Testing Schema

Outpatient		Inpatient			
Day 1	**Day 2**	**Day 1**	**Day 2**	**Day 3**	
DLW VO_2max Strength	QOL PAR Food record	6pm Admit	24h Core temp	24h urine	12pm Discharge
			RMR DXA OGTT Questionnaires	Muscle bx Fat bx	

Experimental Design



RESULTS
%CR and Weight Change



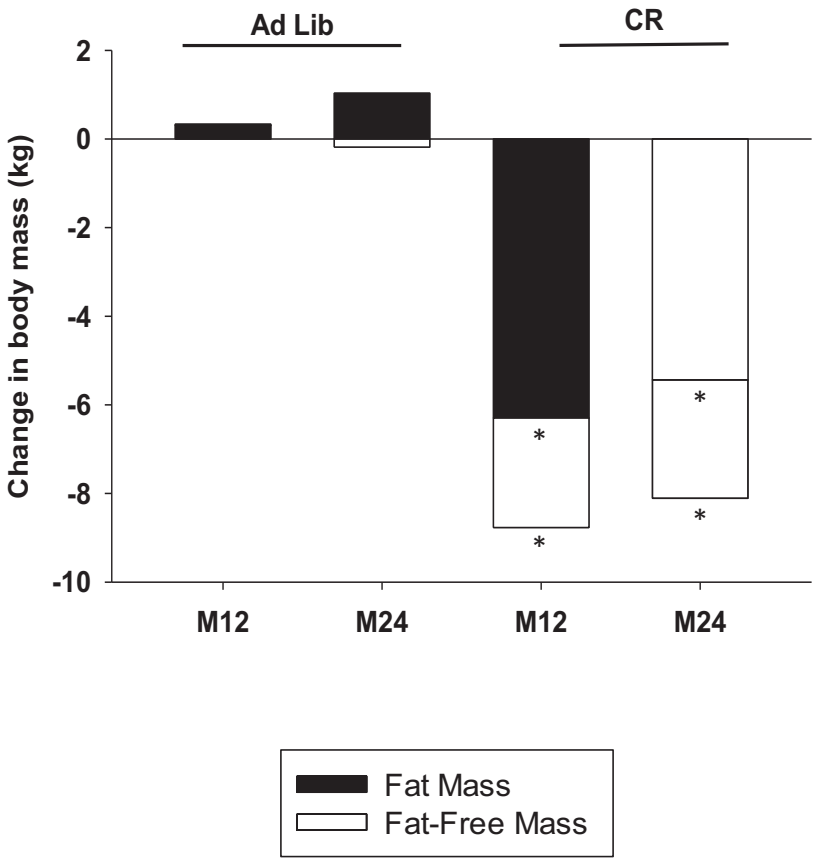
Energy Intake (kcal/d)

	AL (n=75)	CR (n=143)	p
Baseline	2422 (50)	2409 (38)	.88
BL-M12	-22 (25)	-341 (19)	<.0001
BL-M24	-14 (23)	-258 (18)	<.0001
M12-M24	-8 (11)	-83 (18)	<.0001

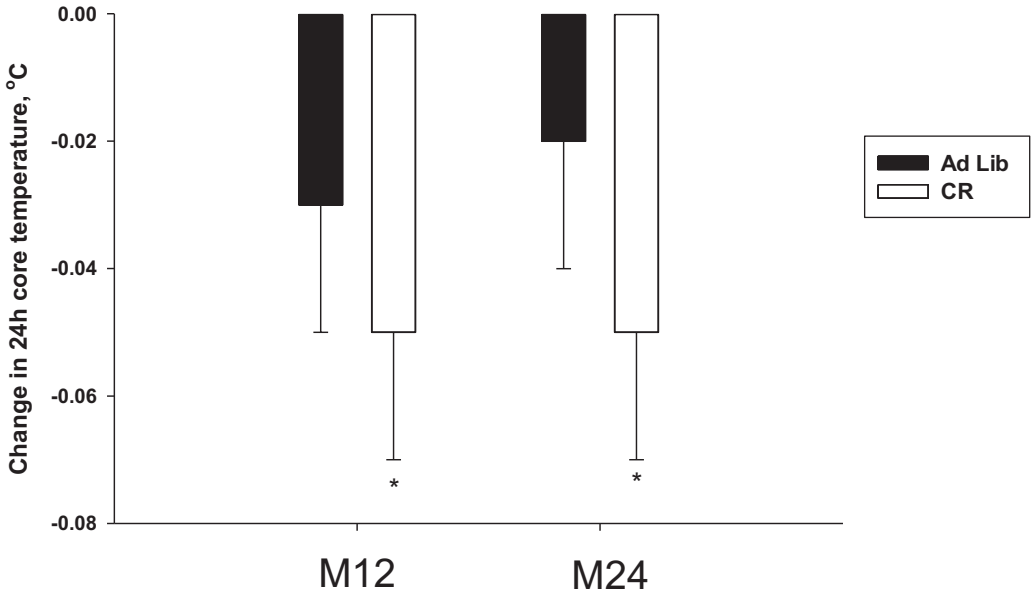
% Weight Loss: BL-M24

	AL	CR
≥5%	18	91
≥10%	3	56
≥15%	-	14

RESULTS
Change in body composition



RESULTS
Change in Core Temperature

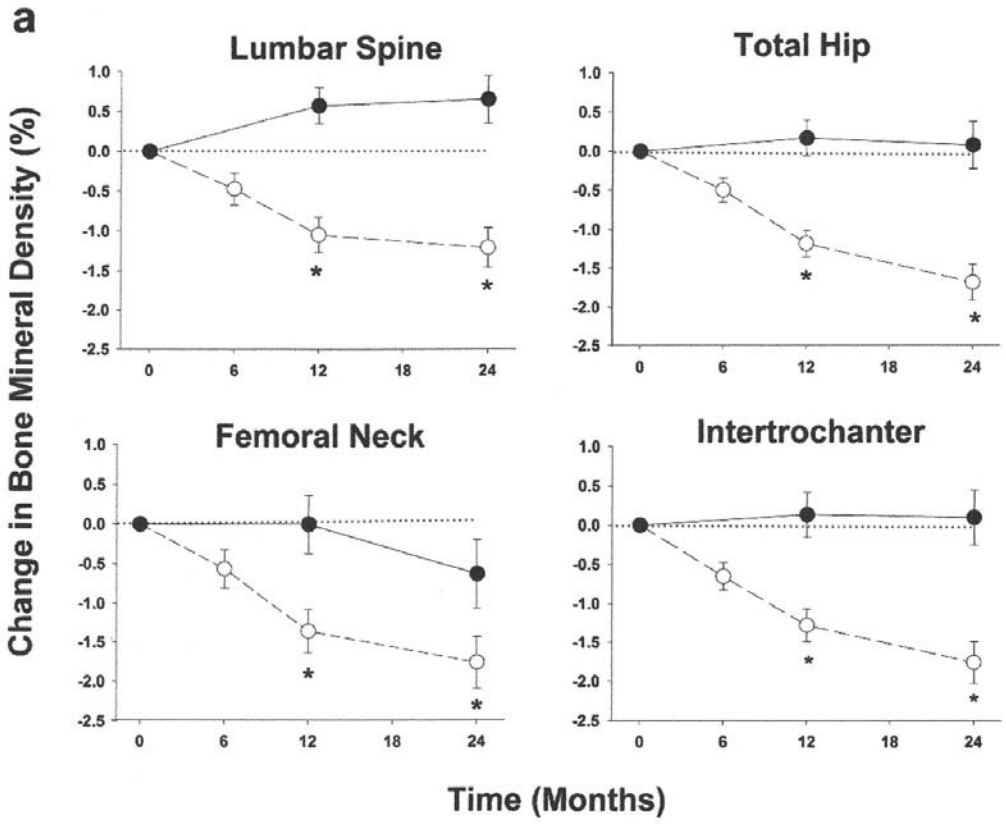


ITT

M12: AL v CR, p=0.35

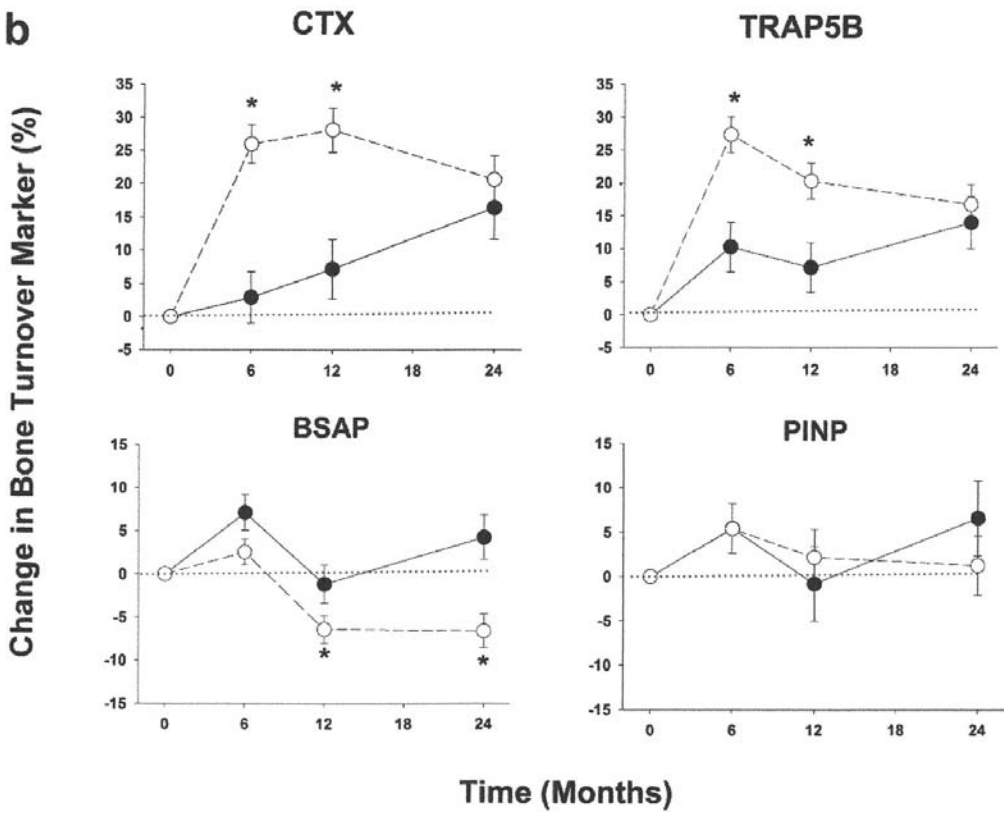
M24: AL v CR, p=0.42

RESULTS – Change in Bone Density



Villareal, J Bone Mineral Res, 2015

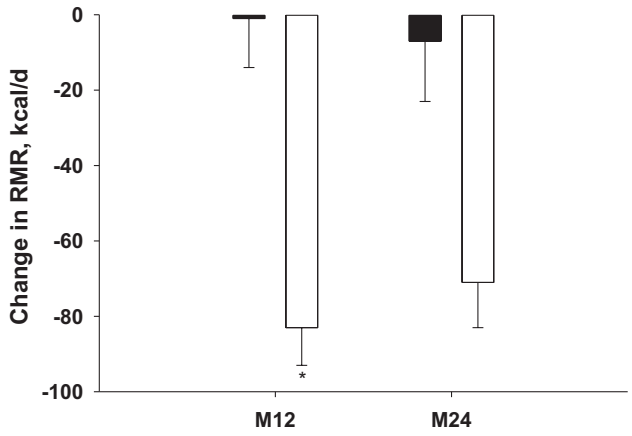
RESULTS – Change in Bone Markers



Villareal, J Bone Mineral Res, 2015

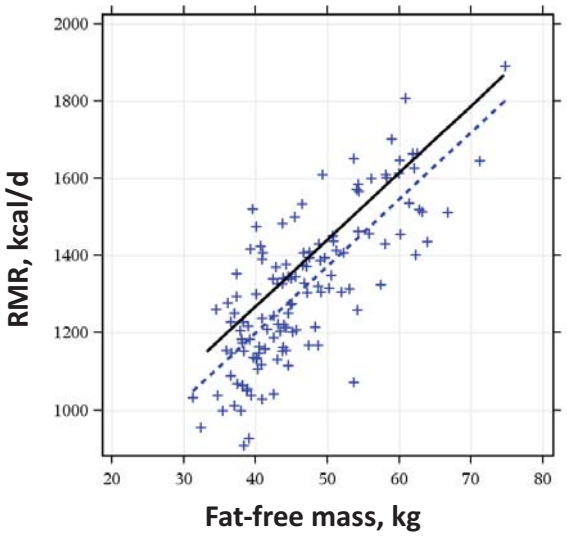
RESULTS - RMR

Metabolic adaptation at 12 months



ITT

M12: AL v CR, p=<.0001



RMR Residual

ITT

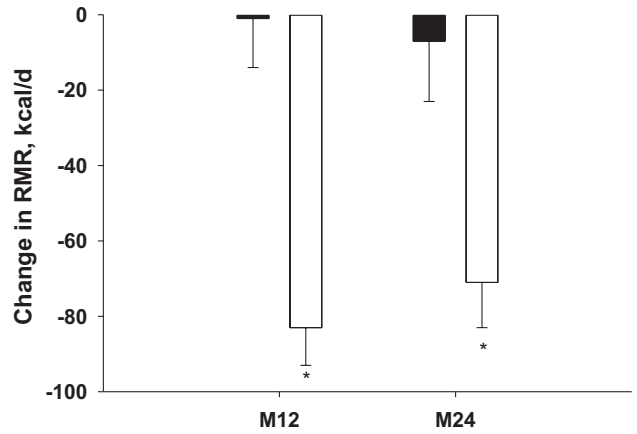
Ab Lib: -14±12 kcal/d

25% CR: -48±9 kcal/d*

AL vs CR, p=0.06

RESULTS - RMR

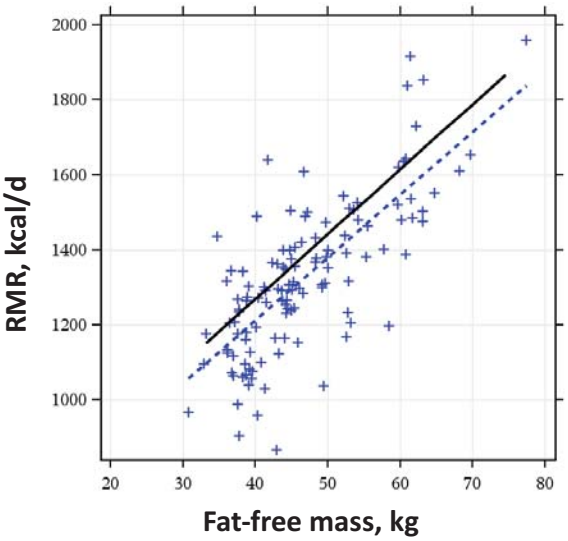
Metabolic adaptation at 24 months



ITT

M12: AL v CR, p=<.0001

M24: AL v CR, p=0.0012



RMR Residual

ITT

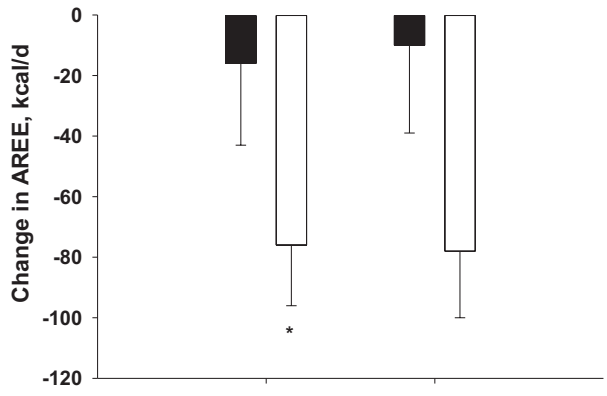
Ad Lib -23±15 kcal/d

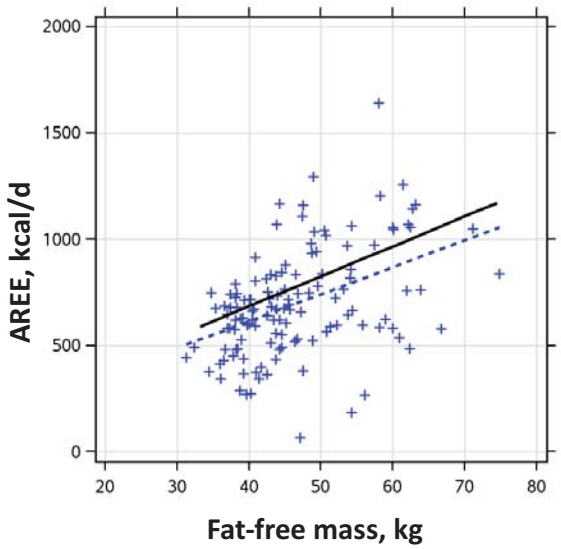
25% CR -38±11 kcal/d

AL vs CR, NS

RESULTS – Activity Energy Expenditure

12 months





Ad Lib
CR

ITT

M12: AL v CR, p=0.07

AREE Residual

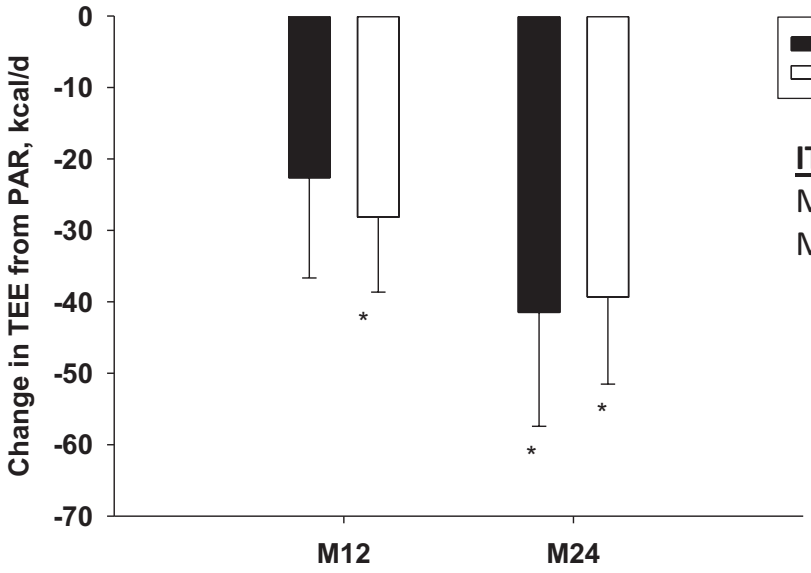
ITT

Ad Lib -40±26 kcal/d

25% CR -118±19 kcal/d*

AL vs CR, p=0.014

RESULTS – Change in Physical Activity

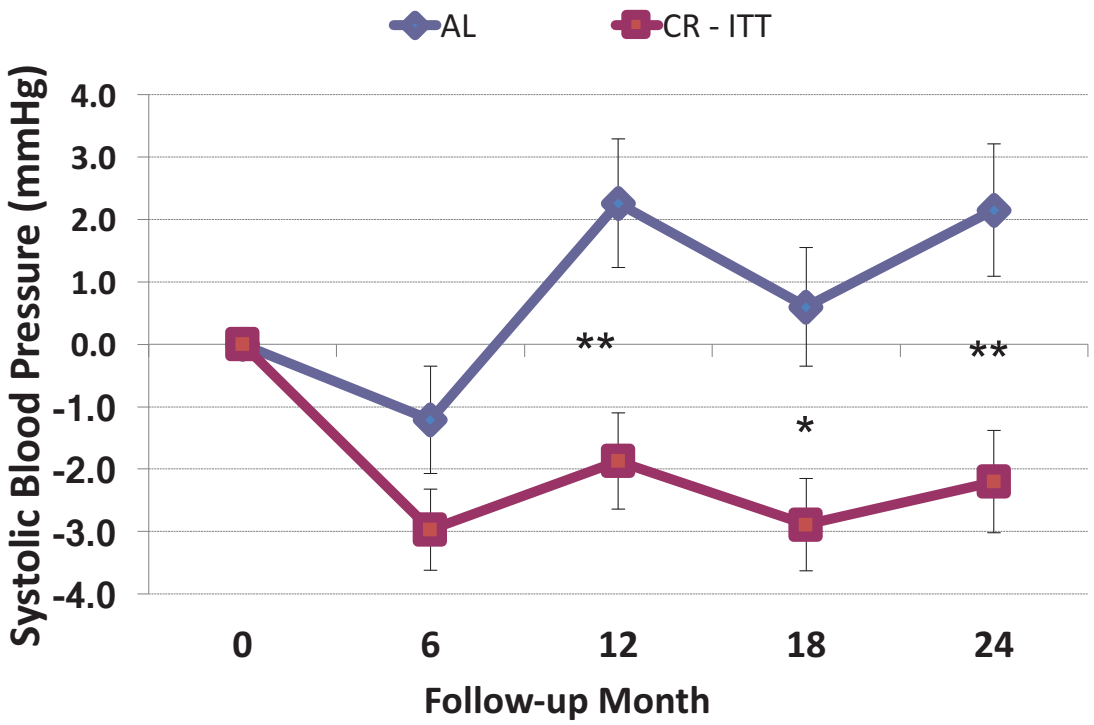


ITT

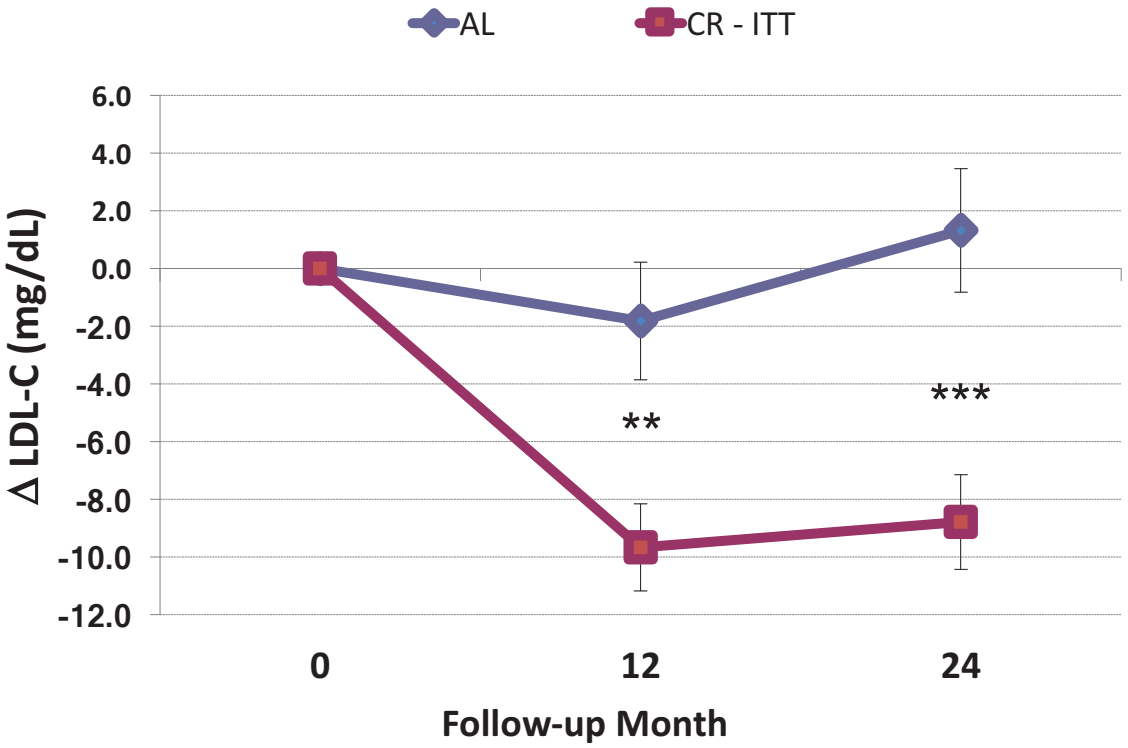
M12: AL v CR, p=0.75

M24: AL v CR, p=0.91

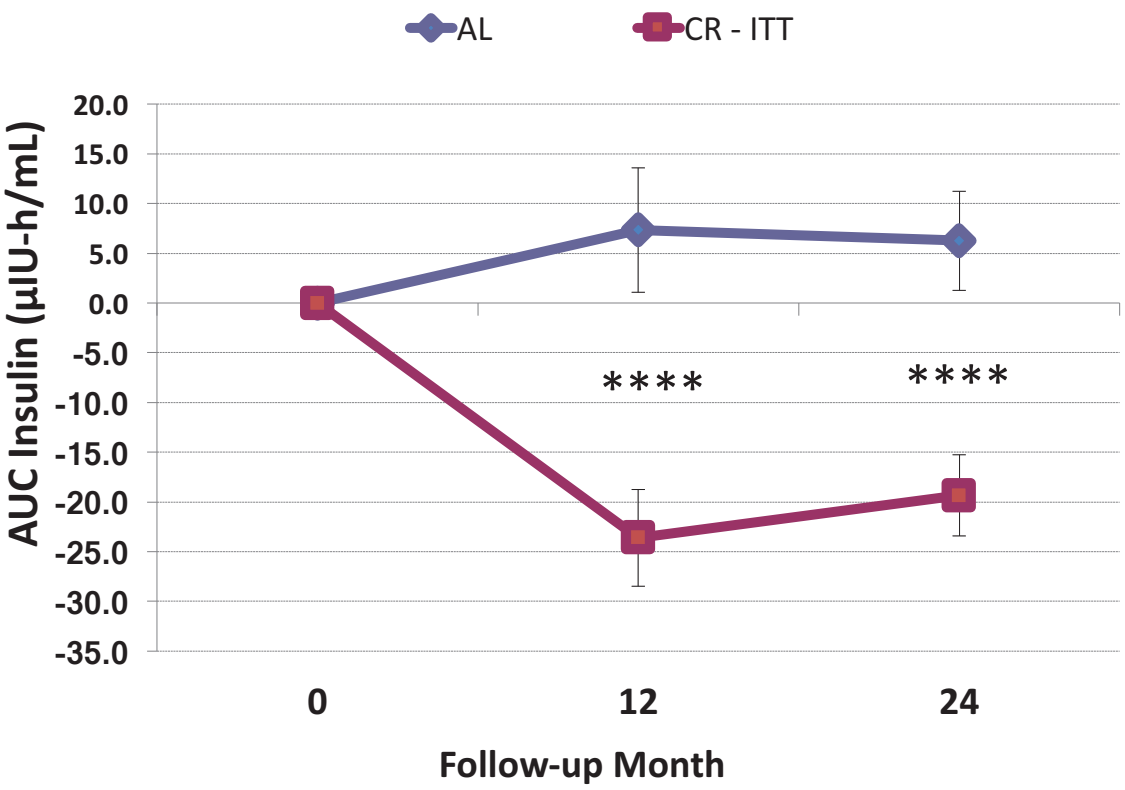
RESULTS –
Change in Systolic Blood Pressure



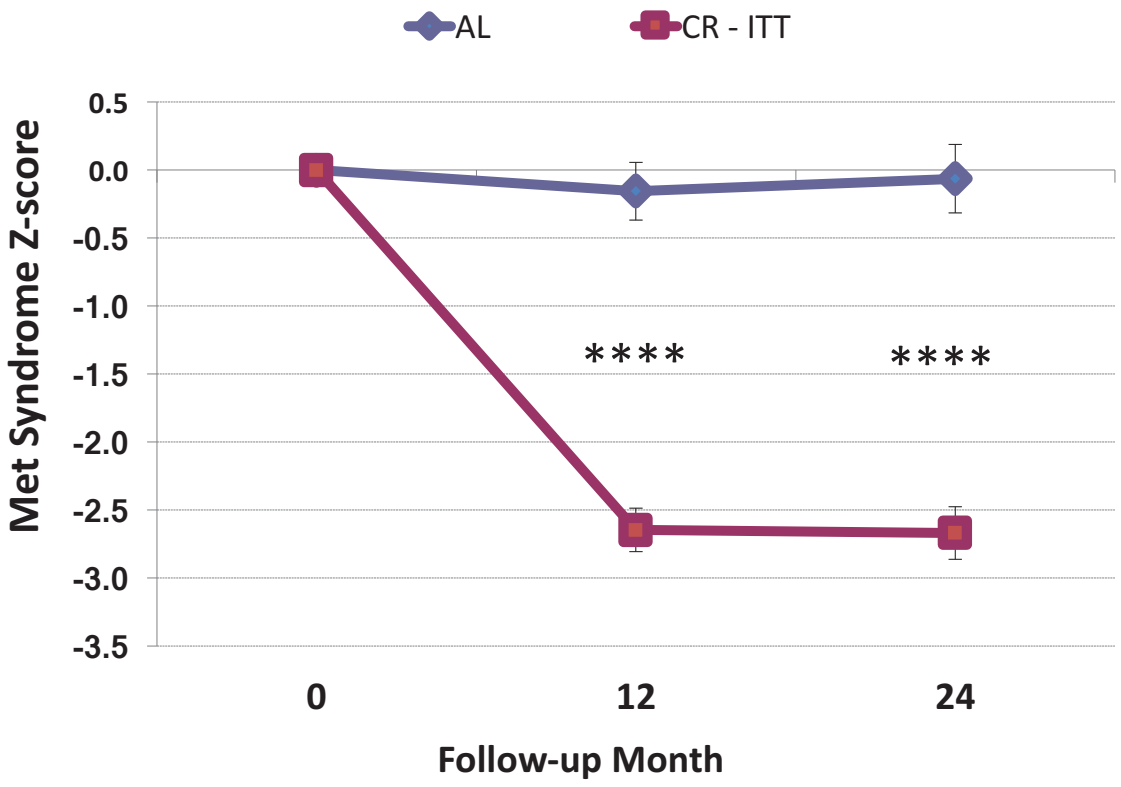
RESULTS – Change in LDL Cholesterol



RESULTS – Change in AUC Insulin (OGTT)



RESULTS –
Change in Metabolic Syndrome Score



Secondary Metabolic outcomes and changes in exploratory physiologic markers and disease risk factors

% Change from Baseline in CR Group		
	M12	M24
+T3		<0.001
TSH	<0.05	
+Tumor Necrosis Factor-alpha		
C-Reactive Protein, Leptin		
Interleukin-6		
Insulin Resistance (homeostatic model assessment, HOMA-IR), BP, TG, TChol		
Fasting Glucose & AUC; Norepinephrine		
LDL cholesterol		
HDL cholesterol		

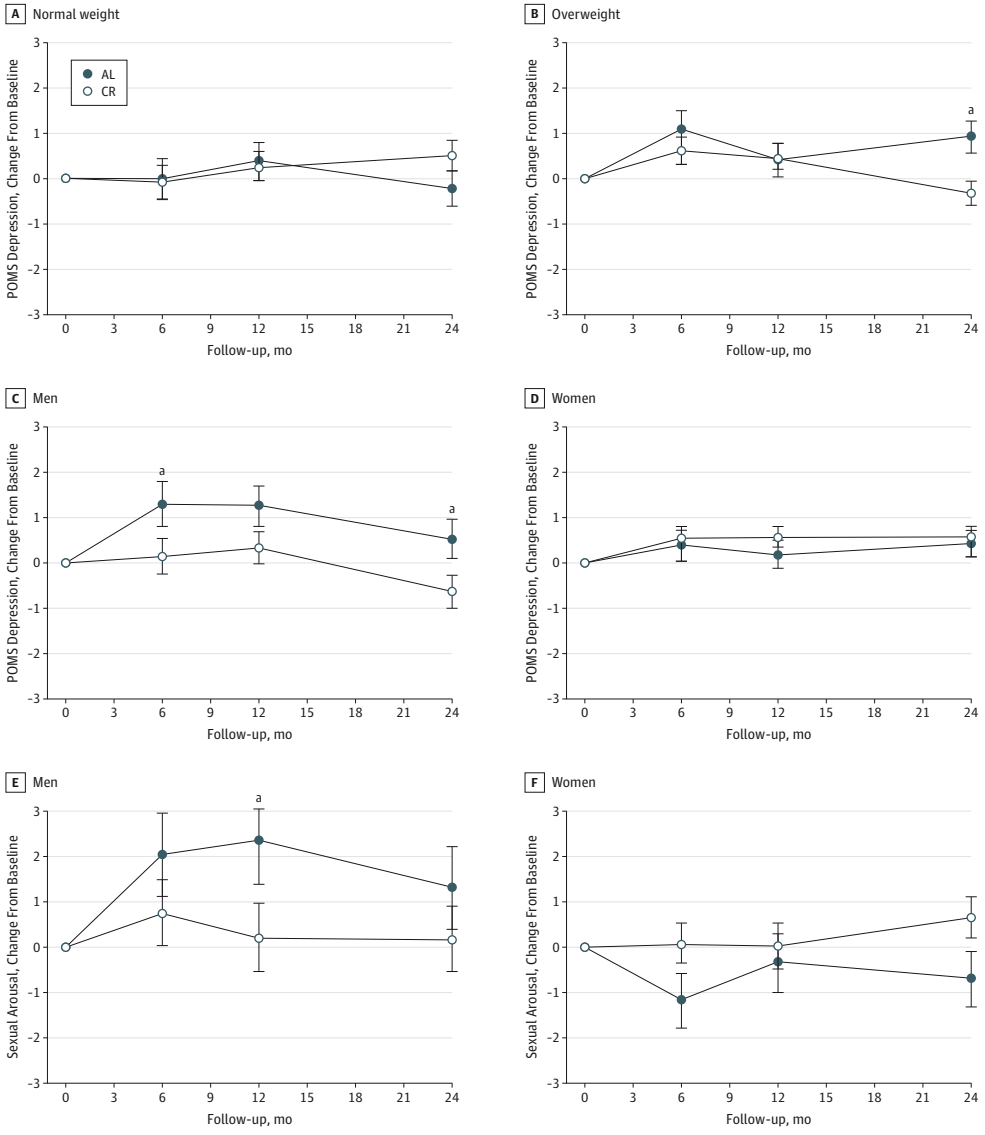
Ravussin E et al. 2015 J Gerontol Biol Sci Med

Behavioral Variables

- No eating disorders, depression or other psychological or psychiatric disorders were observed

- CR had no negative effect on quality of life (QOL) and cognitive function

- CR favorably affected some psychologic and QOL outcomes *with significant (Martin C et al. JAMA Internal Med 2016)*
    - Improvements in general health
    - Improvements in mood
    - Reduced tension
    - Improved sleep duration
    - Improved sexual drive and relationship



Cognition, Mood and QOL

From Martin CK et al. 2016 JAMA IM

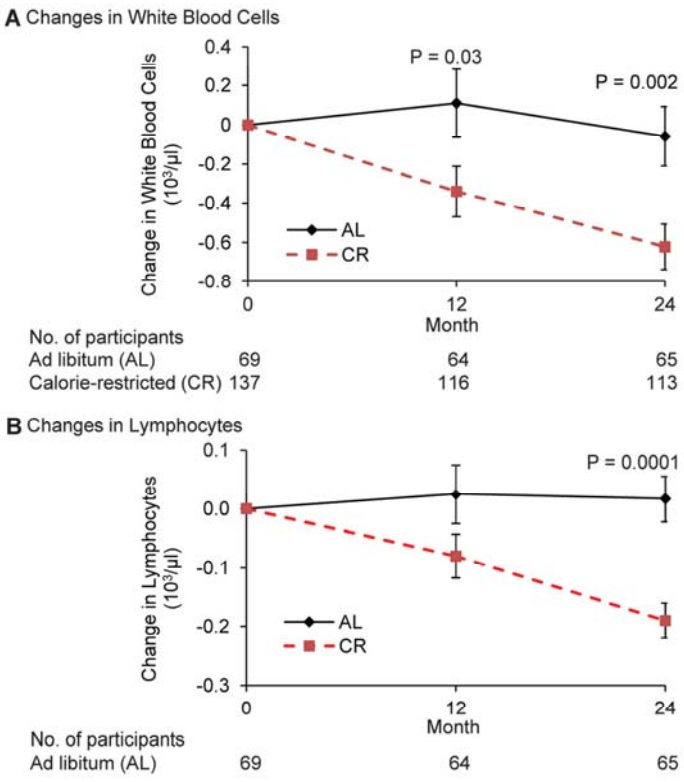
Table 4 Change from baseline in plasma concentrations of growth factors and cortisol at 12 and 24 months in AL and calorie restriction (CR) groups

	AL		CR		
Outcome	Mean (SE)†	Within-group *P*-value‡	Mean (SE)†	Within-group *P*-value‡	Between-group *P*-value‡
IGF-1 (ng mL^{-1})					
Baseline	183.1 (5.7)		175.5 (3.6)		0.589
Δ Month 12	−19.6 (4.9)	< 0.001	−7.1 (3.7)	0.108	0.072
Δ Month 24	−18.7 (4.1)	< 0.001	−15.1 (3.2)	< 0.001	0.919
IGFBP-1 (pg mL^{-1})					
Baseline	4477 (585)		5459 (523)		0.088
Δ Month 12	409 (636)	1.0	1839 (474)	< 0.001	0.065
Δ Month 24	−616 (573)	0.568	1391 (443)	0.004	0.005
IGFBP-3 (ng mL^{-1})					
Baseline	2528 (58.4)		2459 (33)		0.338
Δ Month 12	1 (43)	1.0	124 (32)	< 0.001	0.018
Δ Month 24	56 (49)	0.510	123 (38)	0.003	0.273
IGF-1/IGFBP-3 ratio					
Baseline	0.10 (0.00)		0.10 (0.00)		0.942
Δ Month 12	−0.008 (0.002)	< 0.001	−0.006 (0.002)	0.001	0.880
Δ Month 24	−0.008 (0.002)	< 0.001	−0.009 (0.002)	< 0.001	1.0
IGF-1/IGFBP-1 ratio					
Baseline	0.102 (0.012)		0.078 (0.008)		0.064
Δ Month 12	−0.007 (0.018)	1.0	−0.046 (0.014)	0.002	0.088
Δ Month 24	−0.020 (0.008)	0.018	−0.045 (0.006)	< 0.001	0.008
Cortisol (μg dL^{-1})					
Baseline	11.3 (0.69)		11.2 (0.41)		0.667
Δ Month 12	−0.91 (0.46)	0.102	0.78 (0.35)	0.055	0.003
Δ Month 24	−1.78 (0.51)	0.001	−0.44 (0.39)	0.530	0.312
PDGF-AB (pg mL^{-1})					
Baseline	20 000 (699)		18 131 (583)		0.018
Month 12	−398 (628)	1.0	−26 (469)	1.0	1.0
Month 24	−681 (515)	0.375	−1465 (398)	< 0.001	0.426
TGF-β1 (pg mL^{-1})					
Baseline	30 604 (932)		28 871 (813)		0.065
Δ Month 12	−3169 (932)	0.002	−3521 (697)	< 0.001	1.0
Δ Month 24	−5455 (707)	< 0.001	−6616 (549)	< 0.001	0.356

Growth Hormones and Cytokines

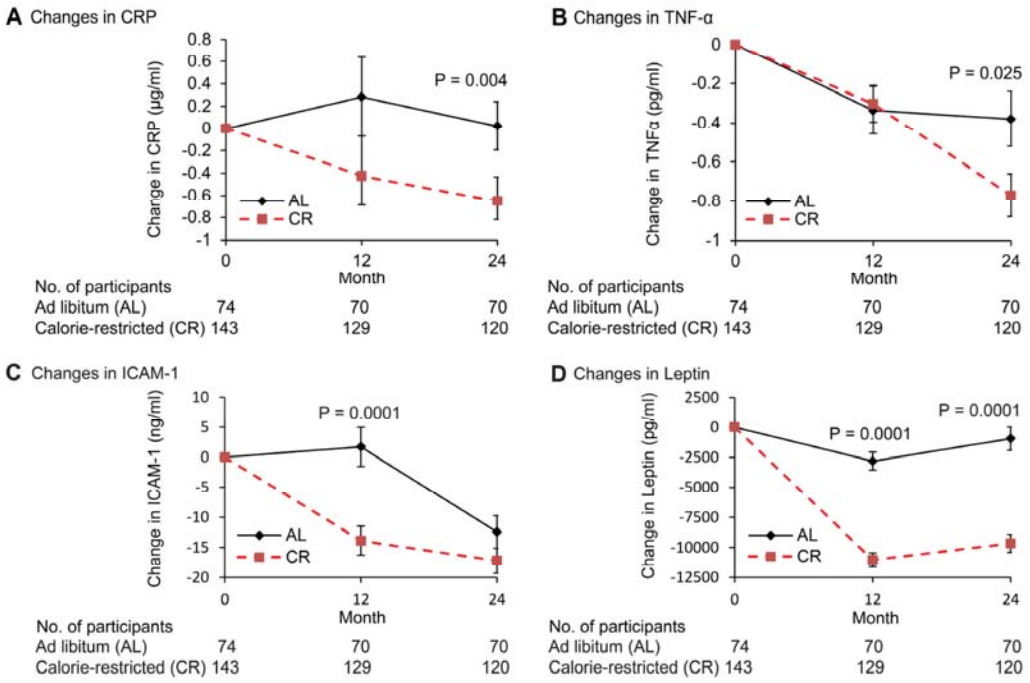
From Fontana L et al. 2016 Aging Cell

RESULTS – Change in Inflammatory Cells



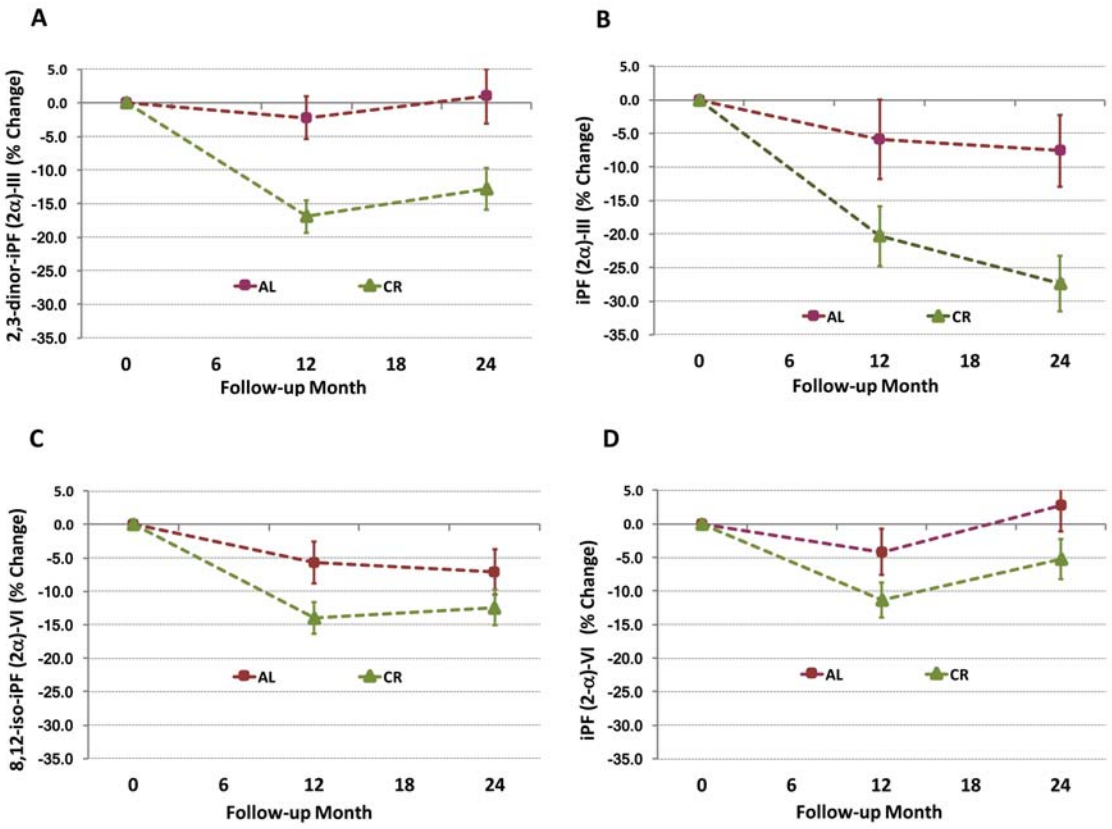
Meydani et al. 2016 Aging

RESULTS – Change in Inflammatory Cytokines

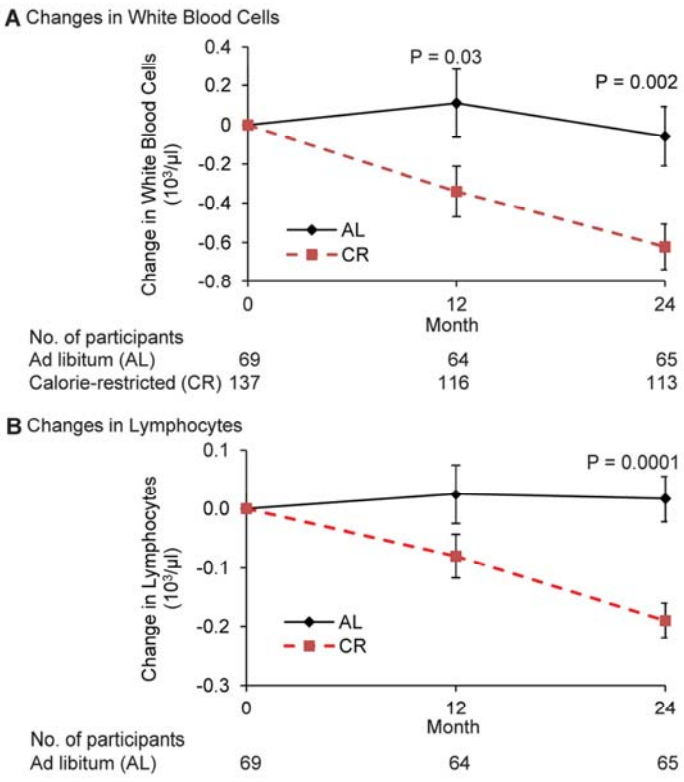


Meydani et al. 2016, Aging

RESULTS – Change in Isoprostanes



RESULTS – Change in Inflammatory Cytokines



Meydani et al. 2016 Aging

Summary

- Is 25% CR for 2 years is feasible in humans? Maybe but only ~15% or 400 kcal/d despite a lot of professional supervision
- Evidence for CR to induce a metabolic adaptation (change in metabolic rate larger than expected on the basis of weight loss) – BUT not significant from Ad Lib (controls) at 24 months
- Potential mechanisms – thyroid function, independent of SNS activity. Leptin????
- Evidence for a behavioral adaptation – not supported by self-reported physical activity

ACKNOWLEDGMENTS
On behalf of the CALERIE Study Group

DCRI
William Kraus, PI
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CALERIE Research Network

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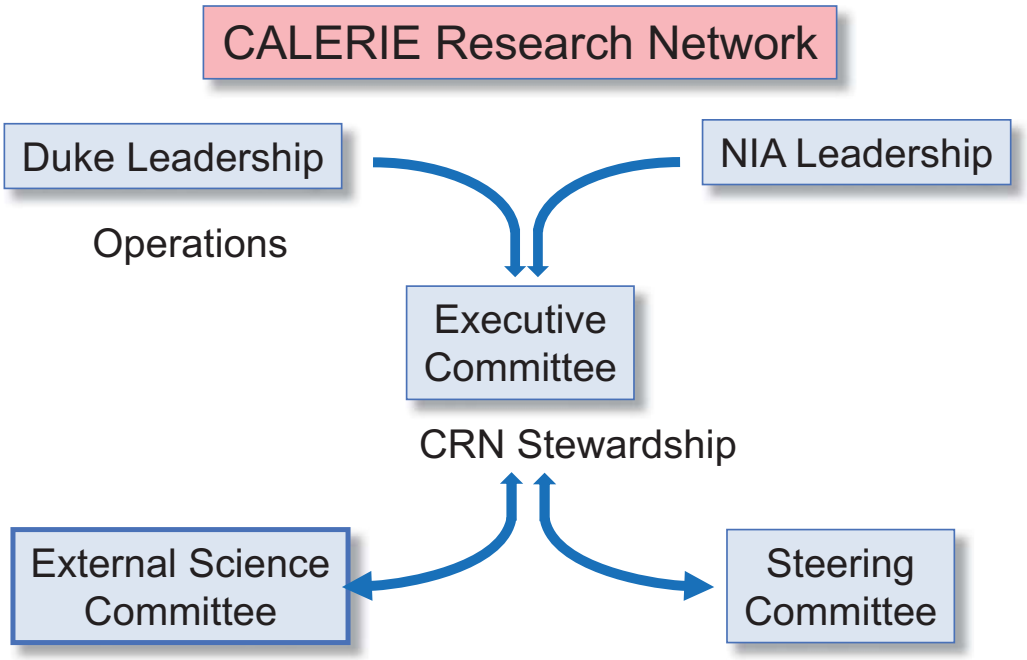
CALERIE is supported by the following grants from the NIA U01AG022132, U01AG020478, U01AG020487, U01AG020480 and NIDDK. The CALERIE Research Network is supported by U24AG047121

CALERIE Research Network

- Purpose of the CALERIE Research Network is to enhance CALERIE's scientific contributions by promoting studies by a broader range of researchers.
- **Web site: calerie.duke.edu**
- **CALERIE as a Platform for Further Aging Research**
  - Aging-related mechanistic factors in humans, e.g., progenitor cell populations in muscle and fat, cell senescence, telomere length, circulating factors, blood gene expression, DNA methylation.
  - Strategies to identify targets for new interventions influencing aging mechanisms.
  - Clarify mechanisms that mediate favorable effects in humans.

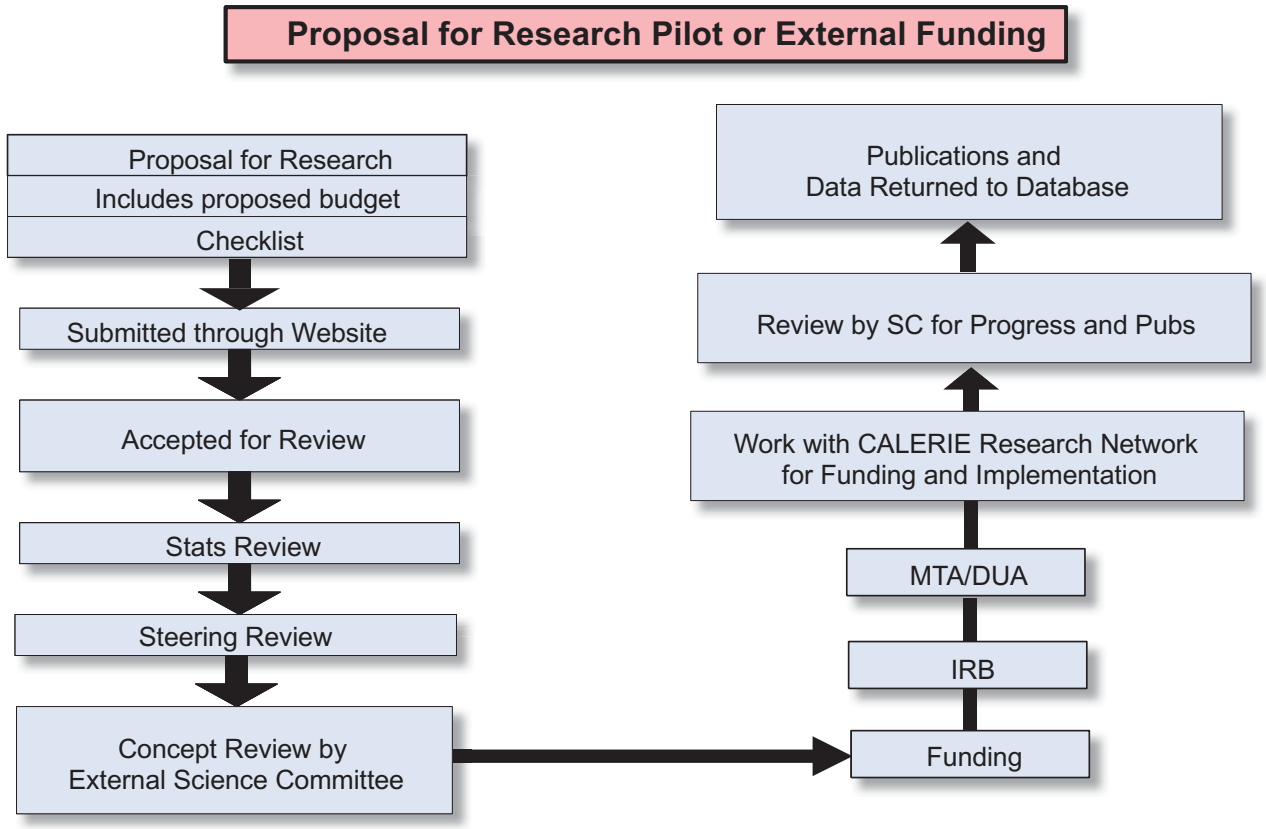
Who Are We?



Scientific Stewardship and Advice

Review and Approval of Ancillary Studies
Sample and Data Stewardship
Functions as Analysis and Publications Committee
Mentorship of New CALERIE Investigators
Consult on Direction of CALERIE Follow-up Studies

What Happens to Your Proposal?



Our Workhorse: The Website

William E. Kraus, MD
for the CALERIE Research Network and CALERIE
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calerie.duke.edu

Web site designed to:

- **Orient to CALERIE study**
- **Provide access to CALERIE resources:**
  - **Manuscripts**
  - **Databases**
  - **Samples**
  - **Statistical resources**

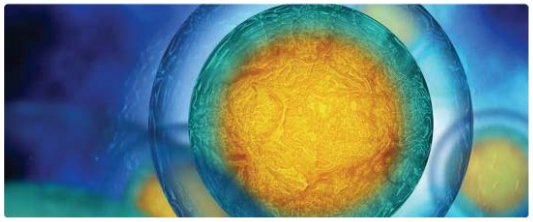
QUICK NAVIGATION

- Network Resources
- About the Study
- Database Documentation
- Biorepository
- Apply for Samples & Data Analysis
- Protocols & Procedures
- Ongoing Projects
- Publications
- Events

FUNDING OPPORTUNITIES

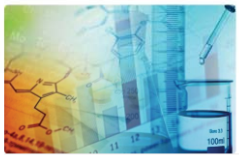
- CALERIE Research Network Pilot Projects
- NIA R01 Research Project Grant
- NIA R21 Exploratory/Developmental Grant

CALERIE CENTERS



CALERIE RESEARCH NETWORK

CALERIE (Comprehensive Assessment of Long term Effects of Reducing Intake of Energy) was a study designed to determine the biological effects of two years of prolonged caloric restriction in humans.



ABOUT THE STUDY

Rationale behind the study, the goals of the Phase 1 and Phase 2 trials, the design of each phase, and a brief summary of the findings. **Learn more.**

APPLY FOR SAMPLES AND DATA ANALYSIS

This section includes all the information needed to access and use the CALERIE Research Network resources, including the public use database and the application for access to biological samples. **Apply now.**

NEW PUBLICATIONS

A 2-Year Randomized Controlled Trial of Human Caloric Restriction: Feasibility and Effects on Predictors of Health Span and Longevity.

September 01, 2015

Effect of Two-Year Caloric Restriction on Bone Metabolism and Bone Mineral Density in Non-Obese Younger Adults: A Randomized Clinical Trial.

January 31, 2016

Effect of Calorie Restriction on Mood, Quality of Life, Sleep, and Sexual Function in Healthy Nonobese Adults: The CALERIE 2 Randomized Clinical Trial.

June 01, 2016

Effects of 2-year calorie restriction on circulating levels of IGF-1, IGF-binding proteins and cortisol in nonobese men and women: a randomized clinical trial.

April 24, 2016

Body-composition changes in the Comprehensive Assessment of Long-term Effects of Reducing Intake of Energy (CALERIE)-2 study: a 2-y randomized controlled trial

Come visit and explore

calerie.duke.edu

Help available at the site

calerie.duke.edu