Supplemental Materials to Change in the rate of biological aging in response to caloric restriction: CALERIE Biobank Analysis

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Supplementary materials are divided into two sections.

Section I reports further details on the CALERIE randomized trial.

Section II reports additional analyses supporting those reported in the main text of this article.

I. The CALERIE Study

Details on the CALERIE Trial have been published previously (1–3). Here we reproduce selected pieces of information published in those articles and also on the CALERIE Biobank website (https://calerie.duke.edu/).

The following text is quoted from the CALERIE Biobank website (https://calerie.duke.edu/about-study)

Chronic caloric restriction extends average life span in animals and delays age-related diseases. The CALERIE (**C**omprehensive **A**ssessment of **L**ong-term **E**ffects of **R**educing **I**ntake of **E**nergy) Study was designed to test the hypothesis that two years of caloric restriction (CR) in humans would yield the same results.

CALERIE Phase 2 was a two-year intervention of a 25% calorie restriction (CR) in young and middle-aged non-obese men and women to test important study outcomes: feasibility, safety, effects on quality of life, effects on disease risk factors, and effects on biological predictors of life span.

Potential participants were screened during a series of physical and psychological tests and interviews to identify healthy individuals who agreed to make the necessary commitments to participate in a two-year intensive CR-oriented lifestyle modification program. Two-hundred eighteen healthy volunteers across three sites (Tufts University, Pennington Biomedical Research Center, and Washington University School of Medicine) were recruited beginning in May 2007. The study Coordinating Center was at the Duke Clinical Research Institute. CALERIE participant's requirements were:

- Be relatively healthy
- Be ages 20-50 (inclusive) for men and ages 20-47 (inclusive) for women
- Have a body mass index (BMI) of 22-27.9 (lean to slightly overweight)
- Be free of diabetes, cancer, heart and liver disease, and AIDS
- No recent and substantial weight loss
- If women, using an acceptable form of contraception (barrier method, oral contraceptive, intrauterine device, or similar form), and to continue use while enrolled in the study.

The overall aim of CALERIE Phase 2 was to test the hypothesis that two years of sustained CR would result in the same adaptive changes occurring in rodents subjected to CR. Particular emphasis was on the adaptive responses thought to be involved in slowing the aging process and protecting against age-related disease processes. An important secondary aim was to identify potential adverse effects of CR in humans.

Study results were published in 2015: A 2-Year Randomized Controlled Trial of Human Caloric Restriction: Feasibility and Effects on Predictors of Health Span and Longevity.

Prior to the CALERIE study, the duration of the intervention and the randomized nature of the treatment assignment had never been attempted in a human study. These factors, combined with the extensive biorepository of samples from study participants and careful attention to detail in the collection and assessment of data, make the CALERIE biorepository an invaluable resource for investigators. We have a rich biological and clinical data repository for the investigation of innumerable hypotheses about the role of calorie restriction on the human aging biology. We aim to provide CALERIE Network Investigators with all the tools they need to further study the biological mechanisms related to aging and longevity.

A CONSORT diagram illustrating enrollment and retention in CALERIE was published in The Journal of Gerontology A: Biological Sciences in 2015 (3). That article is freely available through PubMed Central (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4841173/). The CONSORT diagram can be viewed at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4841173/figure/F1/.

II. Supplementary Analysis

Re-analysis of CALERIE data using eight-biomarker biological age algorithms

We repeated our analysis of the CALERIE database using Klemera-Doubal method (KDM) Biological Age and homeostatic dysregulation measures based on the subset of eight biomarkers used in previous studies and also available in CALERIE. The eight biomarkers were serum albumin, alkaline phosphatase, C-reactive protein, total cholesterol, creatinine, glycated hemoglobin (estimated from serum glucose), and systolic blood pressure. Results from the eight-biomarker versions of the biological age algorithms were essentially the same as results from the ten-biomarker versions of the algorithms. Results are reported in **Supplemental Table 6**.

Tests of biological age algorithms defined from 2007-8 NHANES in data from 2009-10 NHANES

To evaluate the performance of the biological age algorithms in an independent dataset, we re-computed all parameters using only data from the 2007-8 NHANES. We then implemented the resulting algorithms in the 2009-10 NHANES data. We examined data from all 2009-10 NHANES participants aged 30-75 years with complete biomarker data (N=3,969).

Association with chronological age. KDM Biological Age and homeostatic dysregulation increased with chronological age among NHANES participants (r=0.92 for KDM Biological Age; r=0.41 for homeostatic dysregulation, **Supplemental Figure 3**).

Association with physical limitations. Next, we tested if biological aging measures predicted increased physical limitation as measured by the 16-item NHANES ADL scale (4). We counted the number of items on which the respondent indicated “much difficulty” or “unable to do.” Resulting scores ranged from 0-14, M=1, SD=2 in the sample aged 30-75 years. Chronologically older NHANES participants reported more ADLs (r=0.21, p<0.001). After accounting for chronological age differences, accelerated biological aging was associated with having more ADLs (for KDM Biological Age, r=0.34, p<0.001; for homeostatic dysregulation r=0.20, p<0.001).

Association with history of low educational attainment. Finally, we tested if biological aging measures were accelerated by an established risk factor for early disability and death, low educational attainment. Compared to individuals of the same age and sex who held a bachelor's or higher degree, KDM Biological Ages were increased by 1.17 [0.77, 1.56] "years" for those with only a high school diploma or GED and by 1.44 95 % CI [0.99, 1.89] "years" for those with no high school equivalency. Homeostatic dysregulation was increased in parallel (by 0.27 [0.21, 0.33] standard deviations for the high school diploma group, by 0.42 [0.36, 0.49] standard deviations for those with no high school equivalency).

Comparison with biological age algorithms including measured lung function. As a sensitivity analysis, we evaluated the potential impact of differences in biomarker sets between the version of KDM Biological Age analyzed in CALERIE and the version analyzed in previous studies (5,6). We compared the 10-biomarker KDM Biological Age analyzed in CALERIE to a 9-biomarker version more closely matching KDM Biological Ages used in previous studies. The 9-biomarker version excluded uric acid and white blood cell count, and include forced expiratory volume in 1 second (FEV1). Cytomegalovirus optical density, the tenth biomarker used in previous analyses of KDM Biological Age, was not available in the NHANES 2009-10 dataset. Correlations between the two versions of the KDM Biological Age were high (r=0.98 after controlling for chronological age).

Supplementary Table 1. Parameters used to calculate biological age measures in the CALERIE Dataset. Panel A shows parameters used to calculate the Klemera-Doubal Method Biological Age. **Panel B** shows parameters used to calculate homeostatic dysregulation.

Panel A.

		Klemera-Doubal Method Biological Age Algorithm Parameters									
	Sex	RMSE s	Age Coefficient k	Intercept q	Model R-squared r	r1	r2	n2	rchar	sr	sBA2
Biomarker Parameters											
Albumin	F	0.309	0.000	4.199	0.000	1.66E-05	1.18E-03	1.38E-06			
	M	0.287	-0.006	4.659	0.065	5.74E-03	2.25E-02	5.05E-04			
Alkaline Phosphatase*	F	0.303	0.006	3.863	0.060	5.14E-03	2.10E-02	4.42E-04			
	M	0.278	0.001	4.154	0.001	7.01E-05	2.44E-03	5.96E-06			
Blood Urea Nitrogen*	F	0.306	0.011	1.997	0.154	1.39E-02	3.56E-02	1.26E-03			
	M	0.293	0.006	2.331	0.064	5.60E-03	2.22E-02	4.92E-04			
Creatinine*	F	0.098	0.002	0.464	0.060	5.13E-03	2.10E-02	4.41E-04			
	M	0.105	0.001	0.607	0.025	2.17E-03	1.37E-02	1.87E-04			
C-reactive Protein*	F	0.314	0.001	0.239	0.002	1.74E-04	3.82E-03	1.46E-05			
	M	0.283	0.002	0.140	0.006	5.18E-04	6.64E-03	4.41E-05			
Glycated Hemoglobin*	F	0.102	0.003	1.751	0.092	8.05E-03	2.66E-02	7.05E-04			
	M	0.116	0.003	1.770	0.062	5.41E-03	2.18E-02	4.74E-04			
Systolic Blood Pressure	F	15.774	0.620	88.660	0.182	1.68E-02	3.93E-02	1.54E-03			
	M	15.080	0.307	108.511	0.054	4.75E-03	2.04E-02	4.15E-04			
Total Cholesterol	F	39.525	0.608	172.903	0.033	2.79E-03	1.54E-02	2.37E-04			
	M	41.164	-0.391	218.716	0.012	1.05E-03	9.49E-03	9.01E-05			
Uric Acid*	F	0.202	0.004	1.521	0.065	5.63E-03	2.20E-02	4.86E-04			
	M	0.178	0.000	1.941	0.000	1.24E-05	1.03E-03	1.05E-06			
White Blood Cell Count	F	2.087	-0.021	8.191	0.014	1.19E-03	1.00E-02	1.00E-04			
	M	2.016	-0.008	7.551	0.002	1.78E-04	3.89E-03	1.51E-05			
Summary Parameters											
	F					0.053	0.174		0.306	181.508	83.398
	M					0.025	0.123		0.207	417.099	140.612

* Analysis value is the natural log of the obsered biomarker value

Panel B.

	Variance - Covariance Matrix used to Calculate Homeostatic Dysregulation									
Values reflect NHANES 2007-8 & 2009-10 participants aged 20-30 years with complete biomarker data and body-mass index <30.**	Albumin	Alkalin Phosphatase	Blood Urea Nitrogen	C-reactive Protein	Creatinine	Glycated Hemoglobin	Systolic Blood Pressure	Total Cholesterol	Uric Acid	White Blood Cell Count
Albumin	0.97	-0.06	0.04	-0.30	0.00	-0.04	-0.01	0.04	0.04	0.01
Alkaline Phosphatase*	-0.06	1.01	-0.03	0.18	-0.10	0.16	-0.02	-0.04	0.08	0.17
Blood Urea Nitrogen*	0.04	-0.03	1.00	-0.01	0.14	0.01	0.04	0.12	0.00	-0.04
C-reactive Protein*	-0.30	0.18	-0.01	0.99	-0.06	0.01	0.01	0.02	0.13	0.24
Creatinine*	0.00	-0.10	0.14	-0.06	0.98	0.04	0.04	-0.06	0.26	-0.07
Glycated Hemoglobin*	-0.04	0.16	0.01	0.01	0.04	1.01	-0.02	0.03	-0.10	0.08
Systolic Blood Pressure	-0.01	-0.02	0.04	0.01	0.04	-0.02	0.98	0.01	0.06	0.06
Total Cholesterol	0.04	-0.04	0.12	0.02	-0.06	0.03	0.01	1.01	-0.01	-0.07
Uric Acid*	0.04	0.08	0.00	0.13	0.26	-0.10	0.06	-0.01	1.05	0.13
White Blood Cell Count	0.01	0.17	-0.04	0.24	-0.07	0.08	0.06	-0.07	0.13	1.01

* Analysis value is the natural log of the obsered biomarker value

** Biomarker values were transformed to have M=0, SD=1 separately for men and women

Supplemental Table 2. Description of CALERIE Biobank data used for analysis

Panel A. Summary statistics of biomarkers as reported in the CALERIE database

		Control			Caloric Restriction			Total		
		M	SD	N	M	SD	N	M	SD	N
Baseline				*75*			*145*			*220*
Albumin	g/dL	4.40	0.29	75	4.39	0.27	145	4.40	0.28	220
Alkaline Phosphatase	IU/L	61.61	16.34	75	59.32	18.17	145	60.10	17.56	220
Blood Urea Nitrogen	mg/dL	13.03	3.26	75	12.64	3.04	145	12.77	3.12	220
C-reactive Protein	ug/mL	1.09	1.34	74	1.46	3.71	145	1.34	3.12	219
Creatinine	mg/dL	0.84	0.16	75	0.87	0.15	145	0.86	0.15	220
Glycated Hemoglobin*	pct	4.63	0.21	75	4.63	0.23	145	4.63	0.22	220
Systolic Blood Pressure	mm/Hg	111.19	9.86	75	112.09	9.89	145	111.78	9.87	220
Total Cholesterol	mg/dL	176.15	34.79	74	168.01	29.65	145	170.76	31.64	219
Uric Acid	mg/dL	4.50	1.26	75	4.53	1.27	145	4.52	1.26	220
White Blood Cell Count	10^3/uL	5.88	1.29	69	5.96	1.57	139	5.93	1.48	208
12-month Follow-up				*70*			*130*			*200*
Albumin	g/dL	4.36	0.28	70	4.41	0.31	130	4.39	0.30	200
Alkaline Phosphatase	IU/L	60.71	16.47	70	55.02	16.38	129	57.03	16.59	199
Blood Urea Nitrogen	mg/dL	12.97	3.69	70	13.23	3.31	130	13.14	3.45	200
C-reactive Protein	ug/mL	1.73	3.57	71	1.10	2.62	129	1.32	3.00	200
Creatinine	mg/dL	0.82	0.15	70	0.86	0.16	130	0.85	0.16	200
Glycated Hemoglobin*	pct	4.57	0.26	70	4.52	0.23	130	4.54	0.24	200
Systolic Blood Pressure	mm/Hg	113.71	11.49	70	110.23	10.67	130	111.45	11.06	200
Total Cholesterol	mg/dL	174.37	32.43	71	157.76	26.00	129	163.66	29.46	200
Uric Acid	mg/dL	4.52	1.08	70	4.48	1.21	130	4.49	1.16	200
White Blood Cell Count	10^3/uL	5.99	1.48	69	5.64	1.63	122	5.77	1.58	191
24-month Follow-up				*71*			*120*			*191*
Albumin	g/dL	4.34	0.29	71	4.43	0.27	120	4.39	0.28	191
Alkaline Phosphatase	IU/L	62.63	15.90	71	54.49	14.55	120	57.52	15.53	191
Blood Urea Nitrogen	mg/dL	12.69	3.12	71	13.18	3.53	120	12.99	3.38	191
C-reactive Protein	ug/mL	1.45	2.54	71	0.88	1.36	120	1.09	1.90	191
Creatinine	mg/dL	0.76	0.14	71	0.83	0.15	120	0.81	0.15	191
Glycated Hemoglobin*	pct	4.56	0.24	71	4.51	0.24	120	4.53	0.24	191
Systolic Blood Pressure	mm/Hg	113.71	12.89	71	110.24	10.37	120	111.53	11.46	191
Total Cholesterol	mg/dL	178.25	34.53	71	160.46	29.12	120	167.07	32.32	191
Uric Acid	mg/dL	4.54	1.13	71	4.49	1.20	120	4.51	1.17	191
White Blood Cell Count	10^3/uL	5.88	1.52	70	5.40	1.50	119	5.58	1.52	189

*Estimated as (glucose mg/dL +46.7) /28.7

Panel B. Summary statistics of log-transformed values for biomarkers log-transformed for analysis

		Control		Caloric Restriction		Total	
		M	SD	M	SD	M	SD
Baseline							
Alkaline Phosphatase	IU/L	4.10	0.26	4.06	0.29	4.07	0.28
Blood Urea Nitrogen	mg/dL	2.61	0.24	2.59	0.22	2.60	0.23
C-reactive Protein	ug/mL	0.61	0.47	0.63	0.57	0.62	0.54
Creatinine	mg/dL	0.60	0.09	0.62	0.08	0.62	0.08
Glycated Hemoglobin*	pct	1.73	0.04	1.73	0.04	1.73	0.04
Uric Acid	mg/dL	1.68	0.22	1.68	0.22	1.68	0.22
12-month Follow-up							
Alkaline Phosphatase	IU/L	4.09	0.26	3.99	0.28	4.02	0.27
Blood Urea Nitrogen	mg/dL	2.60	0.27	2.63	0.24	2.62	0.25
C-reactive Protein	ug/mL	0.70	0.65	0.49	0.56	0.57	0.60
Creatinine	mg/dL	0.60	0.08	0.62	0.08	0.61	0.08
Glycated Hemoglobin*	pct	1.72	0.05	1.71	0.04	1.71	0.04
Uric Acid	mg/dL	1.69	0.19	1.68	0.22	1.68	0.21
24-month Follow-up							
Alkaline Phosphatase	IU/L	4.12	0.24	3.98	0.27	4.03	0.27
Blood Urea Nitrogen	mg/dL	2.59	0.23	2.62	0.24	2.61	0.24
C-reactive Protein	ug/mL	0.65	0.60	0.48	0.48	0.55	0.53
Creatinine	mg/dL	0.56	0.08	0.60	0.08	0.59	0.08
Glycated Hemoglobin*	pct	1.71	0.04	1.70	0.04	1.71	0.04
Uric Acid	mg/dL	1.69	0.21	1.68	0.22	1.68	0.21

*Estimated as (glucose mg/dL +46.7) /28.7

Supplemental Table 3. Regression model results for tests of caloric-restriction treatment effects on change over time in Klemera-Doubal Biological Age. Coefficients for age are reported for a 10y difference in age. Coefficients for weight are reported for a 5kg difference in weight.

	Base Model of Change (control arm only) n=75 (216)	Test of CR Treatment Effect n=220 (611) b [95% CI]	Control for Weight n=220 (611)
Follow-up (12-month increments)	0.71 [0.41 , 1.01]	0.72 [0.41 , 1.02]	0.72 [0.40 , 1.04]
Follow-up-by-Treatment Interaction (difference in effect of follow-up time in the caloric-restriction		-0.60 [-0.99 , -0.21]	-0.57 [-0.98 , -0.16]
Sex (male=1)	0.10 [-0.36 , 0.56]	0.09 [-0.20 , 0.37]	0.09 [-0.21 , 0.39]
Baseline Age (centered at 38y)	0.01 [-0.30 , 0.33]	-0.01 [-0.19 , 0.17]	-0.01 [-0.20 , 0.18]
Weight			0.06 [-0.03 , 0.14]
Treatment Group (CR=1)		-0.04 [-0.19 , 0.10]	-0.03 [-0.18 , 0.12]

Supplemental Table 4. Regression model results for tests of caloric-restriction treatment effects on change over time in homeostatic dysregulation. Coefficients for age are reported for a 10y difference in age. Coefficients for weight are reported for a 5kg difference in weight.

	Base Model of Change (control arm only) n=73 (211)	Test of CR Treatment Effect n=218 (601) b [95% CI]	Control for Weight n=218 (601)
Follow-up (12-month increments)	0.01 [-0.03 , 0.06]	0.01 [-0.03 , 0.06]	0.02 [-0.03 , 0.06]
Follow-up-by-Treatment Interaction (difference in effect of follow-up time in the caloric-restriction		-0.07 [-0.12 , -0.01]	-0.07 [-0.13 , -0.02]
Sex (male=1)	-0.01 [-0.04 , 0.03]	-0.03 [-0.06 , 0.00]	-0.03 [-0.06 , 0.00]
Baseline Age (centered at 38y)	-0.02 [-0.06 , 0.02]	-0.01 [-0.04 , 0.01]	-0.01 [-0.04 , 0.01]
Weight			0.00 [-0.01 , 0.01]
Treatment Group (CR=1)		0.00 [-0.02 , 0.01]	0.00 [-0.02 , 0.01]

Supplemental Table 5. Analysis of caloric-restriction dose-of-treatment effects on change over time in Klemera-Doubal Method Biological Age and homeostatic dysregulation. Panel A shows stratified estimates for caloric restriction-arm participants who achieved <10% caloric restriction on average across the 12- and 14-month follow-ups and caloric restriction-arm participants who achieved 10% or more caloric restriction. Mixed-effects growth models were estimated by including dummy variables for each group of caloric restriction-arm participants and computing interactions between each dummy variable and follow-up time (gray shaded coefficients). Panel B shows results from models testing the dose-response effect. Mixed-effects growth models were estimated by including dummy variables for randomization condition and for membership in the 10% or greater caloric-restriction group of caloric restriction-arm participants and computing interactions between randomization condition and follow-up time and randomization, follow-up time, and caloric restriction group (gray shaded coefficients). Coefficients for age are reported for a 10y difference in age. Coefficients for weight are reported for a 5kg difference in weight.

	Change in KDM Biological Age n=203 (591)		**Change in Homeostatic Dysregulation** n=201 (583)	
Panel A. Stratified Estimates				
Follow-up (12-month increments)	0.72	[0.42 , 1.02]	0.01	[-0.03 , 0.06]
Follow-up-by-Treatment				
<10% CR	-0.49	[-0.95 , -0.04]	-0.08	[-0.14 , -0.01]
10%+ CR	-0.72	[-1.19 , -0.25]	-0.06	[-0.12 , 0.00]
Sex (male=1)	0.05	[-0.24 , 0.35]	-0.03	[-0.07 , 0.00]
Baseline Age (centered at 38y)	0.03	[-0.16 , 0.21]	-0.02	[-0.04 , 0.01]
Treatment Group (<10% CR)	-0.05	[-0.23 , 0.13]	-0.01	[-0.03 , 0.02]
Treatment Group (10%+ CR)	-0.05	[-0.28 , 0.18]	0.00	[-0.03 , 0.03]
Panel B. Test of Dose-Response Effect				
Follow-up (12-month increments)	0.72	[0.41 , 1.04]	0.02	[-0.03 , 0.06]
Follow-up-by-Treatment Interaction	-0.50	[-0.96 , -0.04]	-0.08	[-0.15 , -0.02]
Follow-up-by-Treatment-by-Treatment Dose Interaction	-0.23	[-0.71 , 0.26]	0.02	[-0.04 , 0.08]
Sex (male=1)	0.08	[-0.22 , 0.39]	-0.03	[-0.07 , 0.00]
Baseline Age (centered at 38y)	0.00	[-0.18 , 0.18]	-0.01	[-0.04 , 0.01]
Treatment Group (CR=1)	-0.03	[-0.18 , 0.13]	0.00	[-0.02 , 0.02]
Treatment Group w/ 10%+ CR	-0.03	[-0.28 , 0.22]	0.00	[-0.03 , 0.04]

Supplemental Table 6. Analysis of caloric-restriction treatment effects on change over time in Klemera-Doubal Method Biological Age and homeostatic dysregulation based on the subset of 8 biomarkers matching those used in Levine's original paper. The Klemera-Doubal Method (KDM) Biological Age and homeostatic dysregulation algorithms were based on analysis of 10 biomarkers, 8 of which overlapped with the original set published by Levine (7). We repeated analysis using only those 8 biomarkers to form the KDM Biological Age and homeostatic dysregulation algorithms. Results were similar to those obtained for algorithms defined using the full set of 10 biomarkers. Coefficients for age are reported for a 10y difference in age. Coefficients for weight are reported for a 5kg difference in weight.

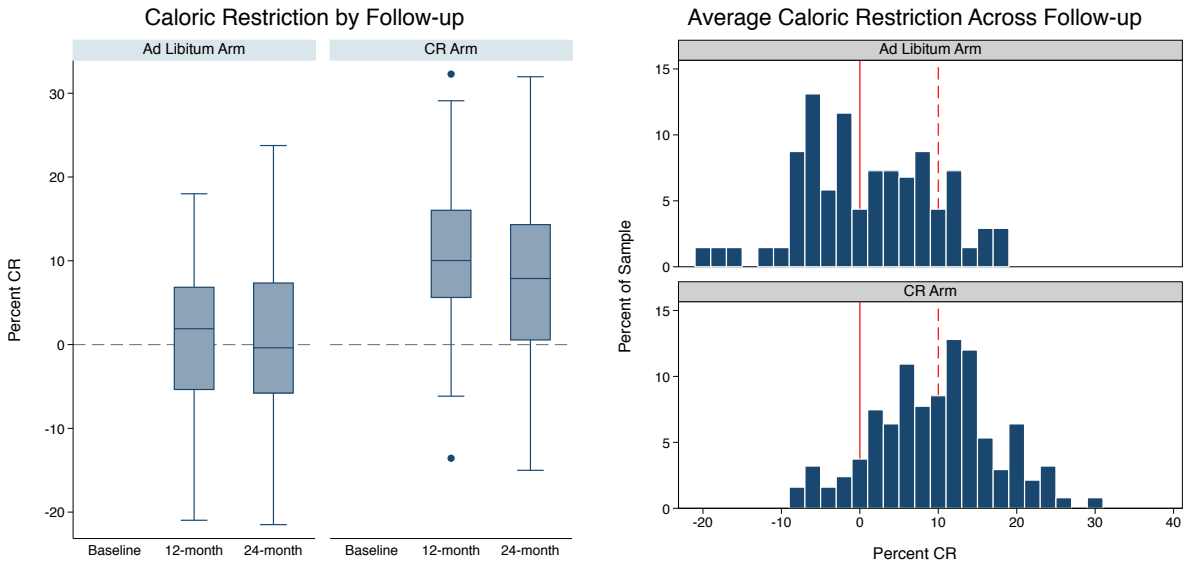
Eight-Biomarker Klemera-Doubal Method Biological Age

	Base Model of Change (control arm only) n=75 (216)	Test of CR Treatment Effect n=220 (611) b [95% CI]	Control for Weight n=220 (611)
Follow-up (12-month increments)	0.73 [0.47 , 0.99]	0.72 [0.47 , 0.98]	0.72 [0.46 , 0.99]
Follow-up-by-Treatment Interaction (difference in effect of follow-up time in the caloric-restriction		-0.56 [-0.89 , -0.24]	-0.54 [-0.88 , -0.20]
Sex (male=1)	0.10 [-0.23 , 0.44]	0.12 [-0.09 , 0.34]	0.13 [-0.09 , 0.36]
Baseline Age (centered at 38y)	-0.05 [-0.30 , 0.20]	-0.01 [-0.16 , 0.13]	-0.01 [-0.17 , 0.14]
Weight			0.03 [-0.04 , 0.10]
Treatment Group (CR=1)		-0.05 [-0.17 , 0.07]	-0.05 [-0.17 , 0.08]

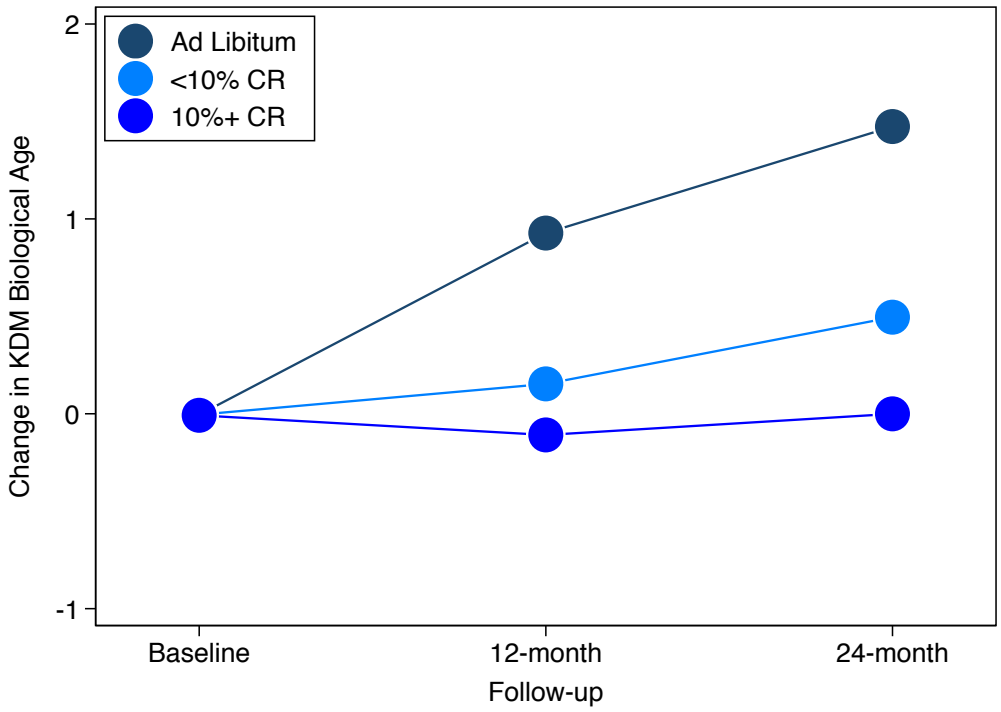
Eight-Biomarker Homeostatic Dysregulation

	Base Model of Change (control arm only) n=74 (213)	Test of CR Treatment Effect n=219 (606) b [95% CI]	Control for Weight n=219 (606)
Follow-up (12-month increments)	0.01 [-0.03 , 0.06]	0.02 [-0.03 , 0.06]	0.02 [-0.03 , 0.07]
Follow-up-by-Treatment Interaction (difference in effect of follow-up time in the caloric-restriction		-0.07 [-0.13 , -0.02]	-0.08 [-0.14 , -0.02]
Sex (male=1)	0.00 [-0.04 , 0.03]	-0.03 [-0.06 , 0.01]	-0.03 [-0.06 , 0.00]
Baseline Age (centered at 38y)	-0.02 [-0.06 , 0.02]	-0.01 [-0.04 , 0.01]	-0.01 [-0.04 , 0.01]
Weight			0.00 [-0.01 , 0.01]
Treatment Group (CR=1)		0.00 [-0.02 , 0.01]	0.00 [-0.02 , 0.01]

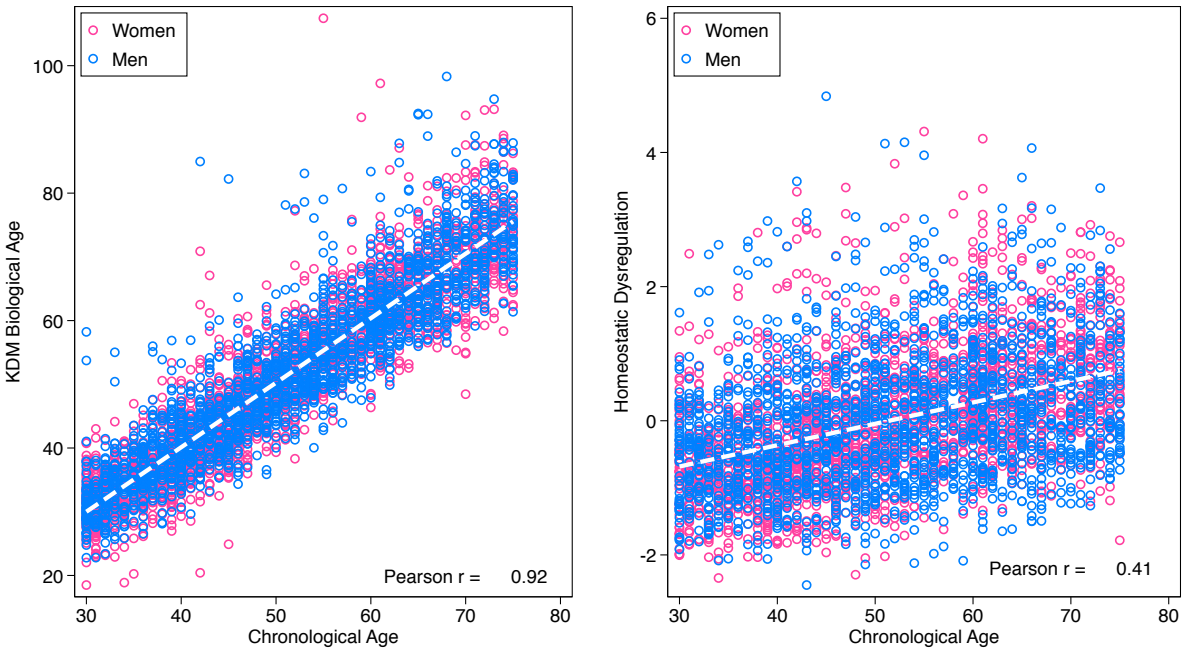
Supplemental Figure 1. Caloric restriction achieved by participants in the CALERIE Trial. The left-side panel shows box and whisker plots of the distribution of caloric restriction (CR) achieved at each follow-up for ad libitum- and caloric-restriction-arm (CR arm) participants. CR at baseline was 0 by definition. The right-side panel shows the distribution of average CR across 12- and 24-month follow-ups in ad libitum- and CR-arm participants. The vertical solid red line shows 0% CR. The vertical dashed red line shows 10% CR.



Supplemental Figure 2. Change in Klemera-Doubal method (KDM) Biological Age for participants in the ad libitum-arm and for caloric restriction-arm participants who achieved an average of <10% and 10% or more caloric restriction across 12- and 24-month follow-ups.



Supplementary Figure 3. Klemera-Doubal Method Biological Age (left) and homeostatic dysregulation (right) plotted against chronological age for participants in the 2009-10 NHANES. Plots show data for participants aged 30-75 with complete biomarker data.



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