Science Translational Medicine
AAAS

www.sciencetranslationalmedicine.org/cgi/content/full/9/377/eaai8700/DC1

Supplementary Materials for

Fasting-mimicking diet and markers/risk factors for aging, diabetes, cancer, and cardiovascular disease

Min Wei, Sebastian Brandhorst, Mahshid Shelehchi, Hamed Mirzaei, Chia Wei Cheng, Julia Budniak, Susan Groshen, Wendy J. Mack, Esra Guen, Stefano Di Biase, Pinchas Cohen, Todd E. Morgan, Tanya Dorff, Kurt Hong, Andreas Michalsen, Alessandro Laviano, Valter D. Longo*

*Corresponding author. Email: vlongo@usc.edu

Published 15 February 2017, *Sci. Transl. Med.* **9**, eaai8700 (2017)
DOI: 10.1126/scitranslmed.aai8700

This PDF file includes:

Materials and Methods
Table S1. Complete metabolic panel.
Table S2. Arm-specific markers of adherence and changes in risk factors, including arm 1 after crossover to FMD, and summary of FMD arms 1 and 2.
Table S3. Changes in risk factors and metabolic markers of adherence after the first FMD.
Table S4. Changes in risk factors and metabolic markers of adherence 3 months after intervention.
Fig. S1. Subject self-reported adverse effects based on CTCAE.
Fig. S2. Comparison of participants who completed the trial versus dropouts.
Fig. S3. Baseline to 3 months before/after comparison of individual subjects in the control cohort and all subjects who completed the FMD.
Fig. S4. Nutritional information of the FMD.
CONSORT checklist
Trial protocols

Materials and Methods

Common Terminology Criteria for Adverse Events

Study participants were asked about adverse events at each study visit and were graded according to the general CTCAE guidelines: 0= no symptoms; 1 “Mild”= asymptomatic or mild symptoms, clinical/diagnostic observations only with no intervention indicated; 2 “Moderate”= minimal, local or noninvasive intervention indicated; 3 “Severe”= medically significant but not immediately life-threatening, hospitalization or prolongation of hospitalization indicated, disabling; 4 “Life-threatening consequences”= urgent intervention indicated; 5 “Death”.

Blood tests and Serum Markers

Complete metabolic panels and lipid panel were ordered at the Clinical Laboratories at the Keck Medical Center of USC. Glucose (diabetes, hypoglycemia), calcium (muscle contraction, neuro-transmitter, hormone imbalance, problems with kidneys, bones, or pancreas), albumin and total protein (maintenance of muscles, bones, blood, and organ tissue), albumin (liver or kidney disease, or nutritional problems), sodium, potassium, carbon dioxide, and chloride (heart function, muscle contraction, brain function, fluid levels and acid-base balance), blood urea nitrogen and creatinine (kidney function), alkaline phosphatase, alanine amino transferase, aspartate amino transferase, and bilirubin (liver function) were evaluated for safety. Commercially available kits for CRP (R&D Systems DCRP00) and β-hydroxybutyrate (Cayman Chemicals 700190) were used following the manufacturers’ protocols. Plasma IGF-I and IGFBP-1 levels were analyzed by an in-house ELISA.

Table S1. **Complete Metabolic Panel**

Variable	N=	Baseline Mean ± SD	Baseline (95% CI)	After Completion of 1st FMD cycle Mean ± SD	After Completion of 1st FMD cycle (95% CI)	After Completion of 1st FMD cycle p-value§	After Completion of 1st FMD cycle Δ*	CTRL: 3 months after Baseline / FMD: 5 Days After 3rd FMD Cycle Mean ± SD	CTRL: 3 months after Baseline / FMD: 5 Days After 3rd FMD Cycle (95% CI)	CTRL: 3 months after Baseline / FMD: 5 Days After 3rd FMD Cycle p-value§	CTRL: 3 months after Baseline / FMD: 5 Days After 3rd FMD Cycle Δ*	Efficacy (comparing Δ) p-value#
Albumin *(g/dL)*												
Control Diet, Arm 1	*22*	4.7 ± 0.3	*(4.5 - 4.8)*					4.4 ± 0.4	*(4.3 - 4.6)*	0.0013	-0.3 ± 0.3	0.087
FMD, Arm 2	*31*	4.4 ± 0.3	*(4.3 - 4.5)*	4.7 ± 0.2	*(4.6 - 4.7)*	< 0.0001	0.3 ± 0.2	4.3 ± 0.3	*(4.2 - 4.4)*	0.033	-0.1 ± 0.2	
Alkaline phosphatase *(units/L)*												
Control Diet, Arm 1	*22*	59.3 ± 17.7	*(51.7 - 66.9)*					56.6 ± 13.7	*(50.9 - 62.3)*	0.37	-2.7 ± 13.3	0.819
FMD, Arm 2	*31*	58.5 ± 14.5	*(53.4 - 63.7)*	59.7 ± 14.2	*(54.5 - 64.8)*	0.13	1.2 ± 5.6	55.2 ± 13.4	*(50.4 - 60.0)*	0.0059	-3.3 ± 6.1	
Alanine transaminase *(units/L)*												
Control Diet, Arm 1	*22*	19.7 ± 8.2	*(16.1 - 23.3)*					19.2 ± 15.7	*(12.6 -25.7)*	0.95	-0.5 ± 10.2	0.19
FMD, Arm 2	*31*	25.7 ± 23.0	*(17.6 - 33.8)*	30.1 ± 29.5	*(19.5 - 40.6)*	0.12	4.4 ± 13.8	19.4 ± 11.8	*(15.2 - 23.5)*	0.078	-6.3 ± 19.4	
Aspartate transaminase *(units/L)*												
Control Diet, Arm 1	*22*	22.4 ± 6.4	*(19.7 - 25.2)*					24.2 ± 9.2	*(20.4 - 28.1)*	0.28	1.8 ± 8.5	0.27
FMD, Arm 2	*31*	27.9 ± 24.3	*(19.3 - 36.4)*	31.6 ± 17.4	*(25.4 - 37.8)*	0.34	3.7 ± 20.3	24.2 ± 8.8	*(21.1 - 27.3)*	0.37	-3.7 ± 22.6	
Bilirubin (Total) *(mg/dL)*												
Control Diet, Arm 1	*22*	0.6 ± 0.4	*(0.4 - 0.8)*					0.5 ± 0.3	*(0.4 - 0.6)*	0.14	-0.1 ± 0.2	0.50
FMD, Arm 2	*31*	0.6 ± 0.5	*(0.4 - 0.8)*	0.9 ± 0.9	*(0.6 - 1.3)*	0.00035	0.3 ± 0.4	0.5 ± 0.3	*(0.4 - 0.6)*	0.027	-0.1 ± 0.3	
Blood Urea Nitrogen *(mg/dL)*												
Control Diet, Arm 1	*22*	13.6 ± 5.0	*(11.4 - 15.7)*					13.3 ± 5.0	*(11.2 - 15.4)*	0.96	-0.3 ± 4.4	0.68
FMD, Arm 2	*31*	13.8 ± 4.9	*(12.0 -15.5)*	9.8 ± 3.3	*(8.6 - 11.0)*	< 0.0001	-4.0 ± 2.8	13.3 ± 4.6	*(11.6 - 14.9)*	0.41	-0.5 ± 3.3	
Calcium *(mg/dL)*												
Control Diet, Arm 1	*22*	9.5 ± 0.4	*(9.3 - 9.6)*					9.4 ± 0.3	*(9.5 - 9.5)*	0.38	-0.1 ± 0.4	0.66
FMD, Arm 2	*31*	9.3 ± 0.4	*(9.2 - 9.5)*	9.5 ± 0.4	*(9.4 - 9.6)*	0.0093	0.2 ± 0.3	9.3 ± 0.4	*(9.2 - 9.4)*	0.46	0.0 ± 0.2	
Chloride *(mmol/L)*												
Control Diet, Arm 1	*22*	101.5 ± 2.1	*(100.7 - 102.4)*					101.3 ± 1.7	*(100.6 - 102.0)*	0.64	-0.2 ± 2.1	0.23
FMD, Arm 2	*31*	101.5 ± 2.1	*(100.8 - 102.3)*	99.6 ± 2.7	*(98.5 - 102.7)*	0.00011	-1.9 ± 2.4	102.0 ± 2.3	*(101.2 - 102.8)*	0.15	0.5 ± 1.6	
CO_2 *(mmol/L)*												
Control Diet, Arm 1	*22*	27.3 ± 2.2	*(26.2 - 28.4)*					26.9 ± 1.5	*(26.2 - 27.7)*	0.57	-0.4 ± 2.6	0.47
FMD, Arm 2	*31*	26.9 ± 2.5	*(26.0 -27.8)*	25.8 ± 2.4	*(24.9 - 26.7)*	0.0029	-1.1 ± 1.9	27.1 ± 2.8	*(26.0 - 28.1)*	0.73	0.2 ± 2.1	
Creatinine *(mg/dL)*												
Control Diet, Arm 1	*22*	0.8 ± 0.2	*(0.7 - 0.9)*					0.8 ± 0.2	*(0.7 - 0.9)*	0.36	0.0 ± 0.1	0.082
FMD, Arm 2	*31*	0.8 ± 0.2	*(0.7 - 0.9)*	0.9 ± 0.2	*(0.8 - 0.9)*	0.0001	0.1 ± 0.2	0.8 ± 0.2	*(0.8 - 0.9)*	0.11	0.0 ± 0.1	
Potassium *(mmol/L)*												
Control Diet, Arm 1	*22*	4.3 ± 0.5	*(4.1 - 4.5)*					4.3 ± 0.4	*(4.2 - 4.5)*	0.47	0.0 ± 0.5	0.82
FMD, Arm 2	*31*	4.2 ± 0.3	*(4.1 - 4.3)*	4.3 ± 0.3	*(4.1 -4.4)*	0.39	0.1 ± 0.5	4.3 ± 0.5	*(4.1 -4.5)*	0.13	0.1 ± 0.4	
Sodium *(mmol/L)*												
Control Diet, Arm 1	*22*	138.7 ± 2.0	*(137.9 - 139.6)*					137.6 ± 1.6	*(136.9 - 138.2)*	0.047	-1.1 ± 2.5	0.024
FMD, Arm 2	*31*	138.5 ± 2.2	*(137.7 - 139.3)*	137.7 ± 3.0	*(136.6 - 138.8)*	0.13	-0.8 ± 2.3	138.8 ± 2.5	*137.9 - 139.7)*	0.43	0.3 ± 2.0	
Total protein *(g/dL)*												
Control Diet, Arm 1	*22*	7.5 ± 0.4	*(7.4 - 7.7)*					7.3 ± 0.5	*(7.1 - 7.5)*	0.042	-0.2 ± 0.5	0.56
FMD, Arm 2	*31*	7.2 ± 0.5	*(7.0 - 7.4)*	7.5 ± 0.4	*(7.3 -7.6)*	0.00059	0.3 ± 0.4	7.1 ± 0.7	*(6.9 - 7.2)*	0.030	-0.1 ± 0.4	

§ p-values comparing within-group changes were calculated using paired two-tailed Student's t-test compared to the baseline.

** Plus-minus values are mean± SD rounded to the nearest tenth.*

 # Between-arm comparison was calculated using two-tailed two-sample equal variance t-tests.

Table S2. **Arm-specific Markers of Adherence and Changes in Risk factors; including Arm 1 after Cross-over to FMD, and Summary of FMD Arm 1 and 2.**

Variable	N=	**Baseline** Mean ± SD *(95% CI)*		**CTRL:** 3 months after Baseline **FMD:** 5 Days After 3rd FMD Cycle Mean ± SD *(95% CI)*		p-value§	Δ*	**Efficacy** p-value# (**FMD** Arm 2 *vs.*) **CTRL** (Arm 1)	**FMD** (Arm 1)
Body Weight (kg)									
Control, Arm 1	*43*	77.2 ± 16.5	*(72.1 - 82.2)*	77.3 ± 17.0	*(72.0 - 82.5)*	0.72	0.1 ± 2.1	< 0.0001	0.12
FMD, Arm 2	*39*	74.1 ± 15.5	*(69.3 - 78.9)*	71.6 ± 14.6	*(67.0 - 76.1)*	< 0.0001	-2.6 ± 2.5		
FMD, Arm 1	*32*	79.0 ± 18.4	*(72.6 - 85.3)*	77.3 ± 17.5	*(71.2 - 83.3)*	< 0.0001	-1.7 ± 2.0		
FMD, Arm 1 and 2	*71*	76.3 ± 16.9	*(72.3 - 80.3)*	74.1 ± 16.1	*(70.3 - 77.9)*	< 0.0001	-2.2 ± 2.3		
Body-mass Index‡									
Control, Arm 1	*43*	27.4 ± 4.8	*(25.9 - 28.9)*	27.4 ± 5.0	*(25.9 - 28.9)*	0.82	0.0 ± 0.7	< 0.0001	0.071
FMD, Arm 2	*39*	26.2 ± 4.4	*(24.8 - 27.6)*	25.3 ± 4.3	*(24.0 - 26.5)*	< 0.0001	-0.9 ± 0.9		
FMD, Arm 1	*32*	27.4 ± 5.3	*(25.5 - 29.2)*	26.8 ± 5.1	*(25.0 - 28.6)*	< 0.0001	-0.6 ± 0.7		
FMD, Arm 1 and 2	*71*	26.7 ± 4.8	*(25.6 - 27.9)*	26.0 ± 4.5	*(24.9 - 27.0)*	< 0.0001	-0.7 ± 0.8		
Total Body Fat¥ (abs. Volume)									
Control, Arm 1	*43*	23651 ± 8155	*(21142 - 26161)*	23607 ± 8337	*(21041 - 26173)*	0.83	-44 ± 1365	0.0002	0.67
FMD, Arm 2	*38*	20643 ± 8459	*(17953 - 23332)*	19249 ± 7792	*(16772 - 21726)*	< 0.0001	-1393 ± 1786		
FMD, Arm 1	*32*	22841 ± 8733	*(19185 - 25866)*	21618 ± 8567	*(18650 - 24587)*	0.0001	-1222 ± 1484		
FMD, Arm 1 and 2	*70*	21648 ± 8594	*(19598 - 23697)*	20332 ± 8182	*(18381 - 22283)*	< 0.0001	-1315 ± 1646		
Total Body Fat¥ (rel. Volume %)									
Control, Arm 1	*43*	33.4 ± 8.6	*(30.7 - 36.0)*	33.3 ± 9.0	*(30.5 - 36.1)*	0.68	-0.1 ± 1.5	0.043	0.72
FMD, Arm 2	*38*	30.3 ± 10.0	*(27.2 - 33.5)*	29.4 ± 9.8	*(26.3 - 32.6)*	0.0095	-0.9 ± 2.0		
FMD, Arm 1	*32*	31.4 ± 8.9	*(28.3 - 34.5)*	30.3 ± 8.8	*(27.3 - 33.4)*	0.0031	-1.1 ± 1.8		
FMD, Arm 1 and 2	*70*	30.8 ± 9.5	*(28.6 - 33.1)*	29.8 ± 9.3	*(27.6 - 32.1)*	< 0.0001	-1.0 ± 1.9		
Trunk Fat¥ (abs. Volume)									
Control, Arm 1	*43*	8429 ± 4742	*(6969 - 9888)*	8395 ± 4776	*(6925 - 9865)*	0.83	-33 ± 1046	0.018	0.77
FMD, Arm 2	*38*	6573 ± 4877	*(5022 - 8124)*	5938 ± 4295	*(4572 - 7303)*	0.0023	-636 ± 1198		
FMD, Arm 1	*32*	7807 ± 4848	*(6127 - 9486)*	7092 ± 4578	*(5506 - 8678)*	0.0004	-715 ± 1015		
FMD, Arm 1 and 2	*70*	7137 ± 4869	*(5976 - 8298)*	6465 ± 4432	*(5408 - 7522)*	< 0.0001	-672 ± 1111		
Trunk Fat¥ (rel. Volume %)									
Control, Arm 1	*43*	11.7 ± 5.7	*(9.92 - 13.44)*	11.7 ± 6.0	*(9.81 - 13.52)*	0.96	0.0 ± 1.3	0.069	0.47
FMD, Arm 2	*38*	9.5 ± 6.1	*(7.52 - 11.40)*	8.9 ± 5.6	*(7.12 - 10.69)*	0.016	-0.6 ± 1.4		
FMD, Arm 1	*32*	10.5 ± 5.9	*(8.46 - 12.55)*	9.7 ± 5.4	*(7.84 - 11.57)*	0.0037	-0.8 ± 1.5		
FMD, Arm 1 and 2	*70*	9.9 ± 6.0	*(8.51 - 11.37)*	9.2 ± 5.5	*(7.96 - 10.58)*	0.0002	-0.7 ± 1.4		
Lean Body Mass¥ (abs. Volume)									
Control, Arm 1	*43*	45016 ± 11318	*(41533 - 48500)*	45188 ± 11885	*(41531 - 48846)*	0.44	172 ± 1441	0.0004	0.081
FMD, Arm 2	*38*	45321 ± 11794	*(41571 - 49071)*	44231 ± 11247	*(40655 - 47807)*	0.0002	-1089 ± 1613		
FMD, Arm 1	*32*	47451 ± 12323	*(43151 - 51691)*	47016 ± 11555	*(43006 - 51014)*	0.14	-411 ± 1575		
FMD, Arm 1 and 2	*70*	46281 ± 11997	*(43240 - 49142)*	45501 ± 11392	*(42785 - 48218)*	0.0001	-780 ± 1620		
Lean Body Mass¥ (rel. Volume %)									
Control, Arm 1	*43*	63.9 ± 8.2	*(61.4 - 66.4)*	64.0 ± 8.7	*(61.3 - 66.7)*	0.64	0.1 ± 1.5	0.070	0.67
FMD, Arm 2	*38*	66.8 ± 9.6	*(63.7 - 69.8)*	67.6 ± 9.4	*(64.6 - 70.6)*	0.016	0.8 ± 2.0		
FMD, Arm 1	*32*	65.8 ± 8.6	*(62.8 - 68.8)*	66.8 ± 8.4	*(63.9 - 69.7)*	0.0052	1.0 ± 1.8		
FMD, Arm 1 and 2	*70*	66.3 ± 9.1	*(64.2 - 68.5)*	67.2 ± 9.2	*(65.1 - 69. 3)*	0.0002	0.9 ± 1.9		
Waist Circumference (cm)									
Control, Arm 1	*28*	95.4 ± 14.2	*(89.9 - 100.9)*	94.6 ± 14.5	*(88.9 - 100.2)*	0.10	-0.8 ± 25	0.0035	0.073
FMD, Arm 2	*28*	92.1 ± 11.2	*(87.9 - 96.2)*	87.9 ± 120	*(83.5 - 92.4)*	0.0003	-4.1 ± 5.2		
FMD, Arm 1	*24*	97.6 ± 15.5	*(91.4 - 103.8)*	95.8 ± 13.7	*(90.3 - 101.3)*	0.022	-1.8 ± 3.6		
FMD, Arm 1 and 2	*52*	94.6 ± 13.5	*(90.9 - 98.4)*	91.6 ± 13.3	*(87.9 - 95.3)*	< 0.0001	-3.0 ± 4.6		
Fasting Glucose (mg/dL)									
Control, Arm 1	*41*	88.1 ± 8.9	*(85.3 - 90.9)*	90.3 ± 9.7	*(87.3 - 93.4)*	0.14	2.2 ± 9.5	0.27	0.35
FMD, Arm 2	*36*	89.7 ± 8.5	*(86.5 - 92.1)*	89.0 ± 8.0	*(86.4 - 91.6)*	0.87	-0.8 ± 9.9		
FMD, Arm 1	*30*	93.0 ± 9.1	*(89.8 - 96.3)*	90.2 ± 8.4	*(87.4 - 93.5)*	0.14	-2.8 ± 8.8		
FMD, Arm 1 and 2	*66*	91.0 ± 9.0	*(88.8 - 93.2)*	89.7 ± 8.2	*(87.7 - 91.7)*	0.28	-1.3 ± 9.8		
β-Hydroxybutyrate (mM)									
Control, Arm 1	*42*	0.5 ± 0.4	*(0.35 - 0.61)*	0.5 ± 0.6	*(0.28 - 0.68)*	0.99	0.0 ± 0.8	0.82	0.67
FMD, Arm 2	*38*	0.4 ± 0.3	*(0.34 - 0.55)*	0.4 ± 0.3	*(0.33 - 0.50)*	0.55	0.0 ± 0.3		
FMD, Arm 1	*31*	0.4 ± 0.3	*(0.32 - 0.55)*	0.4 ± 0.2	*(0.30 - 0.44)*	0.26	0.0 ± 0.3		
FMD, Arm 1 and 2	*69*	0.4 ± 0.3	*(0.36 - 0.52)*	0.4 ± 0.2	*(0.34 - 0.45)*	0.23	0.0 ± 0.3		
IGF-1 (ng/mL)									
Control, Arm 1	*41*	180.2 ± 84.5	*(153.5 - 206.9)*	188.9 ± 91.0	*(160.2 - 217.7)*	0.14	8.7 ± 36.9	0.0017	0.54
FMD, Arm 2	*38*	168.6 ± 69.1	*(146.6 - 190.5)*	146.9 ± 62.3	*(127.0 - 166.7)*	0.0063	-21.7 ± 46.2		
FMD, Arm 1	*31*	191.3 ± 75	*(165.9 - 217.7)*	162.9 ± 71.0	*(137.9 - 187.9)*	0.0011	-28.4 ± 43.6		
FMD, Arm 1 and 2	*69*	178.8 ± 72.2	*(161.5 - 196.1)*	154.1 ± 66.4	*(138.1 - 170.0)*	< 0.0001	-24.7 ± 44.9		
IGFBP-1 (ng/mL)									
Control, Arm 1	*41*	35.5 ± 43.6	*(21.8 - 49.3)*	36.5 ± 47.9	*(21.4 - 51.7)*	0.81	1.0 ± 26.9	0.53	0.84
FMD, Arm 2	*38*	24.2 ± 17.1	*(18.8 - 29.7)*	22.2 ± 16.4	*(17.0 - 27.4)*	0.35	-2.0 ± 13.0		
FMD, Arm 1	*31*	33.8 ± 51.2	*(15.8 - 51.8)*	33.9 ± 42.7	*(18.9 - 49.0)*	0.99	0.1 ± 62.2		
FMD, Arm 1 and 2	*69*	28.5 ± 36.6	*(19.7 - 37.3)*	27.5 ± 31.4	*(19.9 - 35.0)*	0.84	-1.0 ± 42.4		

Table S2. *Continued*

Variable	N=	Baseline Mean ± SD *(95% CI)*		**CTRL:** 3 months after Baseline **FMD:** 5 Days After 3rd FMD Cycle Mean ± SD *(95% CI)*		p-value[§]	Δ*	**Efficacy** p-value[#] (**FMD** Arm 2 *vs.*) **CTRL** (Arm 1)	**FMD** (Arm 1)
Systolic Blood Pressure (mmHg)									
Control, Arm 1	*43*	116.5 ± 12.3	*(112.7 - 120.3)*	115.8 ± 13.6	*(111.6 - 120.0)*	0.60	-0.7 ± 8.4	0.023	0.32
FMD, Arm 2	*38*	118.0 ± 13.4	*(113.7 - 122.2)*	113.5 ± 13.2	*(109.3 - 117.7)*	< 0.0001	-4.5 ± 6.0		
FMD, Arm 1	*32*	116.5 ± 12.6	*(112.6 - 120.9)*	113.6 ± 11.5	*(109.7 - 117.6)*	0.039	-2.9 ± 7.6		
FMD, Arm 1 and 2	*70*	117.4 ± 13.0	*(114.2 - 120.4)*	113.6 ± 12.4	*(110.6 - 116.5)*	< 0.0001	-3.8 ± 6.8		
Diastolic Blood Pressure (mmHg)									
Control, Arm 1	*43*	75.5 ± 9.6	*(72.5 - 78.5)*	74.8 ± 10.0	*(71.7 - 77.9)*	0.45	-0.7 ± 6.2	0.053	0.96
FMD, Arm 2	*38*	75.7 ± 8.0	*(73.2 - 78.3)*	72.6 ± 8.7	*(70.5 - 76.0)*	0.0089	-3.1± 4.7		
FMD, Arm 1	*32*	75.6 ± 9.8	*(72.2 - 79.1)*	73.1 ± 9.0	*(70.0 - 76.2)*	0.021	-2.6 ± 5.9		
FMD, Arm 1 and 2	*70*	75.7 ± 8.8	*(73.6 - 77.8)*	72.8 ± 8.8	*(70.7 - 74.9)*	0.0004	-2.9 ± 5.3		
Triglycerides (mg/dL)									
Control, Arm 1	*37*	100.5 ± 68.2	*(77.7 - 123.2)*	101.5 ± 57.1	*(82.5 - 120.6)*	0.85	1.0 ± 35.0	0.27	0.86
FMD, Arm 2	*30*	83.0 ± 39.5	*(69.1 - 96.9)*	74.9 ± 37.6	*(61.7 - 88.2)*	0.19	-8.1 ± 33.5		
FMD, Arm 1	*25*	114.6 ± 56.4	*(92.4 - 136.8)*	108.3 ± 51.8	*(88.0 - 128.6)*	0.48	-6.2 ± 43.4		
FMD, Arm 1 and 2	*55*	97.4 ± 50.4	*(83.74 - 111.0)*	90.2 ± 47.5	*(77.3 - 103.0)*	0.16	-7.2 ± 38.2		
Total Cholesterol (mg/dL)									
Control, Arm 1	*37*	195.9 ± 38.9	*(182.9 - 208.9)*	183.9 ± 35.2	*(172.1 - 195.6)*	0.0015	-12.0 ± 21.3	0.81	0.73
FMD, Arm 2	*30*	175.3 ± 25.3	*(166.4 - 184.2)*	164.4 ± 23.4	*(156.1 - 172.6)*	0.0012	-10.9 ± 17.0		
FMD, Arm 1	*25*	199.3 ± 35.2	*(185.3 - 213.1)*	190.2 ± 29.8	*(178.5 - 201.9)*	0.05	-9.1 ± 22.3		
FMD, Arm 1 and 2	*55*	186.1 ± 32.5	*(177.3 - 194.9)*	176.2 ± 29.4	*(168.2 - 184.1)*	0.0004	-9.9 ± 19.5		
LDL Cholesterol (mg/dL)									
Control, Arm 1	*37*	111.2 ± 35.6	*(99.4 - 123.1)*	104.0 ± 31.8	*(93.4 - 114.6)*	0.018	-7.2 ± 17.7	0.50	0.46
FMD, Arm 2	*30*	94.1 ± 23.0	*(86.0 - 102.2)*	89.7 ± 22.8	*(81.7 - 97.7)*	0.13	-4.4 ± 16.0		
FMD, Arm 1	*25*	117.8 ± 36.3	*(103.6 - 132.1)*	110.1 ± 29.0	*(98.7 - 121.5)*	0.039	-7.8 ± 17.8		
FMD, Arm 1 and 2	*55*	104.9 ± 32.0	*(96.2 - 113.5)*	99.2 ± 27.6	*(91.8 - 106.7)*	0.0011	-5.7 ± 16.8		
HDL Cholesterol (mg/dL)									
Control, Arm 1	*37*	64.3 ± 16.1	*(59.2 - 69.9)*	59.3 ± 14.9	*(54.3 - 64.3)*	0.0002	-5.3 ± 7.8	0.90	0.033
FMD, Arm 2	*30*	64.8 ± 17.2	*(58.6 - 70.6)*	59.6 ± 12.8	*(55.1 - 64.2)*	0.0097	-5.0 ± 10.0		
FMD, Arm 1	*25*	58.4 ± 16.2	*(52.1 - 64.8)*	58.4 ± 15.6	*(52.3 - 64.5)*	0.97	0.0 ± 5.8		
FMD, Arm 1 and 2	*55*	61.7 ± 16.9	*(57.1 - 66.3)*	58.9 ± 14.1	*(55.1 - 62.7)*	0.020	-2.8 ± 8.7		
C-reactive Protein (mg/L)									
Control, Arm 1	*42*	1.5 ± 1.9	*(0.92 - 2.11)*	1.9 ± 2.7	*(1.07 - 2.75)*	0.31	0.4 ± 2.5	0.27	0.096
FMD, Arm 2	*38*	1.1 ± 1.3	*(0.71 - 1.52)*	1.0 ± 1.2	*(0.61 - 1.37)*	0.61	-0.1 ± 1.5		
FMD, Arm 1	*31*	1.9 ± 3.0	*(0.89 - 2.98)*	0.9 ± 1.2	*(0.47 - 1.35)*	0.053	-1.0 ± 2.8		
FMD, Arm 1 and 2	*69*	1.5 ± 2.2	*(0.95 - 2.02)*	1.0 ± 1.2	*(0.66 - 1.25)*	0.052	-0.5 ± 2.2		

‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.

¥ Analysed by dual energy x-ray absorptiometry.

[§] p-values comparing within-group changes were calculated using paired two-tailed Student's t-test.

** Plus-minus values are mean± SD rounded to the nearest tenth.*

[#] Between-arm comparison was calculated using two-tailed two-sample equal variance t-tests.

Table S3. **Changes in Risk factors and Metabolic markers of Adherence after the first FMD.**

Variable	N=	Baseline Mean ± SD *(95% CI)*		After Completion of 1st FMD cycle Mean ± SD *(95% CI)*		p-value§	Δ*
Body Weight							
kg	*71*	76.3 ± 16.9	*(72.30 - 80.32)*	73.8 ± 16.3	*(69.98 - 77.66)*	< 0.0001	-2.5 ± 1.1
Body-mass Index‡							
All Subjects	*71*	26.7 ± 4.8	*(25.58 - 27.86)*	25.9 ± 4.7	*(24.76 - 26.96)*	< 0.0001	-0.8 ± 0.3
< 25	*27*	22.4 ± 1.7	*(21.68 - 23.03)*	21.6 ± 1.6	*(20.98 - 22.25)*	< 0.0001	-0.7 ± 0.3
25- 30	*30*	27.1 ± 1.4	*(26.58 - 27.60)*	26.3 ± 1.5	*(25.71 - 26.78)*	< 0.0001	-0.8 ± 0.3
> 30	*14*	34.4 ± 3.5	*(32.37 - 36.36)*	33.2 ± 3.2	*(31.37 - 35.10)*	< 0.0001	-1.2 ± 0.4
Total Body Fat¥							
abs. Volume	*70*	21648 ± 8594	*(19598 - 23697)*	20320 ± 9326	*(17582 - 23058)*	0.075	-1328 ± 1714
rel. Volume %	*70*	30.8 ± 9.5	*(28.55 - 33.06)*	30.1 ± 10.2	*(27.05 - 33.06)*	0.047	-0.7 ± 1.6
Lean Body Mass¥							
abs. Volume	*70*	46281 ± 11997	*(43240 - 49142)*	44469 ± 11554	*(41077 - 47862)*	< 0.0001	-1812 ± 1997
rel. Volume %	*70*	66.3 ± 9.1	*(64.15 - 68.50)*	66.9 ± 9.9	*(63.99 - 69.79)*	0.020	0.9 ± 9.5
Waist Circumference							
cm	*52*	94.6 ± 13.5	*(90.86 - 98.38)*	91.8 ± 14.5	*(86.66 - 96.91)*	< 0.0001	-2.8 ± 13.1
Fasting Glucose							
mg/dL	*66*	91.0 ± 9.0	*(88.78 - 93.19)*	80.1 ± 9.2	*(77.78 - 82.31)*	< 0.0001	-10.9 ± 10.1
beta-Hydroxybutyrate							
mM	*69*	0.4 ± 0.3	*(0.36 - 0.52)*	1.3 ± 0.9	*(1.12 - 1.54)*	< 0.0001	0.9 ± 0.9
IGF-1							
ng/ml	*69*	178.8 ± 72.2	*(161.5 - 196.1)*	134.5 ± 67.6	*(118.2 - 150.7)*	< 0.0001	-44.3 ± 51.6
IGFBP-1							
ng/ml	*69*	28.5 ± 36.6	*(19.73 - 37.32)*	48.3 ± 43.6	*(37.79 - 58.73)*	< 0.0001	19.7 ± 32.7
Systolic Blood Pressure							
mmHg	*70*	117.4 ± 13.0	*(114.2 - 120.4)*	115.7 ± 13.8	*(112.4 - 118.9)*	0.076	-1.7 ± 7.8
Diastolic Blood Pressure							
mmHg	*70*	75.7 ± 8.8	*(73.59 - 77.78)*	73.3 ± 9.7	*(70.98 - 75.62)*	0.0003	-2.4 ± 5.3
Triglycerides							
mg/dL	*55*	97.4 ± 50.4	*(83.74 - 111.0)*	74.1 ± 28.2	*(66.47 - 81.71)*	< 0.0001	-23.3 ± 39.3
Cholesterol							
Total, mg/dL	*55*	186.1 ± 32.5	*(177.3 - 194.9)*	189.8 ± 38.1	*(179.5 - 200.1)*	0.12	3.7 ± 17.7
LDL, mg/dL	*55*	104.9 ± 32.0	*(96.23 - 113.5)*	112.4 ± 39.2	*(101.8 - 123.0)*	0.0026	7.5 ± 17.8
HDL, All subjects	*55*	61.7 ± 16.9	*(57.12 - 66.26)*	62.5 ± 16.5	*(58.06 - 67.00)*	0.29	0.8 ± 5.8
C-reactive Protein							
mg/L	*69*	1.5 ± 2.2	*(0.95 - 2.02)*	1.6 ± 1.9	*(1.10 - 2.03)*	0.75	0.1 ± 2.1

‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.

¥ Analysed by dual energy x-ray absorptiometry.

§ p-values comparing within-group changes were calculated using paired two-tailed Student's t-test.

** Plus-minus values are mean± SD rounded to the nearest tenth.*

Table S4. **Changes in Risk factors and Metabolic markers of Adherence 3 months after Intervention**

Variable	N=	Baseline Mean ± SD *(95% CI)*		3 Months After 3rd FMD Cycle Mean ± SD *(95% CI)*		p-value§	Δ*
Body Weight (kg)							
All subjects	*48*	77.0 ± 17.9	*(71.79 - 82.17)*	75.6 ± 17.3	*(70.54 - 80.60)*	0.0002	-1.4 ± 2.5
Body-mass Index‡							
All subjects	*48*	26.7 ± 4.8	*(25.33 - 28.10)*	26.2 ± 4.6	*(24.88 - 27.57)*	0.0002	-0.5 ± 0.9
< 25	*18*	22.5 ± 1.8	*(21.62 - 23.39)*	22.1 ± 1.9	*(21.14 - 23.05)*	0.041	-0.4 ± 0.8
25- 30	*20*	26.8 ± 1.3	*(26.22 - 27.41)*	26.5 ± 1.5	*(25.78 - 27.19)*	0.094	-0.3 ± 0.8
> 30	*10*	34.1 ± 3.7	*(31.46 - 36.72)*	33.1 ± 3.6	*(30.56 - 35.70)*	0.010	-1.0 ± 0.9
Total Body Fat¥							
abs. Volume	*45*	21267 ± 8582	*(18689 - 23845)*	20388 ± 8393	*(17866 - 22910)*	0.0065	-879 ± 2064
rel. Volume %	*45*	30.3 ± 9.5	*(27.41 - 33.10)*	29.6 ± 9.5	*(26.76 - 32.43)*	0.060	-0.7 ± 2.3
Lean Body Mass¥							
abs. Volume	*45*	47054 ± 12829	*(43200 - 50909)*	46460 ± 12377	*(42741 - 50178)*	0.0040	-595 ± 1315
rel. Volume %	*45*	66.9 ± 9.1	*(64.14 - 69.62)*	67.5 ± 9.1	*(64.73 - 70.18)*	0.089	0.6 ± 2.2
Waist Circumference (cm)							
All subjects	*36*	93.2 ± 13.6	*(88.63 - 97.81)*	91.4 ± 13.3	*(86.92 - 95.89)*	0.0048	-1.8 ± 3.6
Fasting Glucose (mg/dL)							
All subjects	*44*	91.2 ± 9.1	*(88.40 - 93.61)*	89.6 ± 7.8	*(87.23 - 91.95)*	0.31	-1.6 ± 10.0
> 99 mg/dL	*9*	105.1 ± 5.1	*(101.2 - 109.0)*	94.6 ± 5.4	*(90.40 - 98.71)*	0.0019	-10.6 ± 7.0
IGF-1 (ng/mL)							
All subjects	*41*	175.7 ± 69.3	*(153.8 - 197.6)*	161.1 ± 59.3	*(142.3 - 179.8)*	0.061	-14.7 ± 48.8
> 225 ng/mL	*9*	282.7 ± 47.0	*(246.6 - 318.8)*	212.0 ± 59.0	*(166.6 - 257.4)*	0.0007	-70.7 ± 39.9
Systolic Blood Pressure (mmHg)							
All subjects	*48*	118.2 ± 13.9	*(114.2 - 122.2)*	116.1 ± 12.3	*(112.6 - 119.7)*	0.046	-2.1 ± 7.0
> 120 mmHg	*17*	133.3 ± 9.8	*(128.2 - 138.2)*	128.0 ± 9.6	*(123.1 - 132.9)*	0.0064	-5.2 ± 6.9
Diastolic Blood Pressure (mmHg)							
All subjects	*48*	75.9 ± 9.4	*(73.13 - 78.56)*	73.1 ± 9.0	*(70.52 - 75.76)*	0.0005	-2.8 ± 5.0
> 80 mmHG	*11*	89.4 ± 7.3	*(84.44 - 94.29)*	83.9 ± 10.5	*(76.85 - 90.92)*	0.010	-5.5 ± 5.7
Triglycerides (mg/dL)							
All subjects	*37*	96.0 ± 46.3	*(80.58 - 111.5)*	88.6 ± 45.5	*(73.40 - 103.7)*	0.065	-7.5 ± 23.8
> 100 mg/dL	*14*	144.2 ± 35.9	*(123.5 - 164.9)*	132.6 ± 41.6	*(108.6 - 156.6)*	0.22	-11.6 ± 33.4
Cholesterol (mg/dL)							
Total, All subjects	*37*	181.8 ± 29.6	*(171.9 - 191.6)*	182.3 ± 26.2	*(173.5 - 191.0)*	0.86	0.5 ± 17.1
Total, > 199 mg/dL	*8*	222.1 ± 24.3	*(201.8 - 242.4)*	208.8 ± 28.8	*(184.7 - 232.8)*	0.11	-13.4 ± 21.0
LDL, All subjects	*37*	98.8 ± 28.8	*(89.17 - 108.4)*	98.4 ± 25.5	*(90.66 - 107.0)*	0.98	-0.4 ± 11.5
LDL, > 199 mg/dL Total Cholesterol	*8*	134.1 ± 27.5	*(111.1 - 157.1)*	124.9 ± 28.9	*(100.7 - 149.0)*	0.085	-9.3 ± 13.0
HDL, All subjects	*37*	63.8 ± 16.9	*(58.18 - 69.44)*	65.7 ± 15.7	*(60.46 - 70.95)*	0.24	1.9 ± 9.7
HDL, < 50 mg/dL	*10*	44.9 ± 4.1	*(41.93 - 47.87)*	48.5 ± 5.4	*(44.64 - 52.36)*	0.021	3.6 ± 4.1
C-reactive Protein (mg/L)							
All subjects	*47*	1.3 ± 2.1	*(0.63 - 1.88)*	1.0 ± 1.1	*(0.63 - 1.30)*	0.39	-0.3 ± 2.2
> 1 mg/L	*16*	3.0 ± 2.9	*(1.46 - 4.57)*	1.6 ± 1.2	*(0.99 - 2.32)*	0.13	-1.4 ± 3.4

‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.

¥ Analysed by dual energy x-ray absorptiometry.

§ p-values comparing within-group changes were calculated using paired two-tailed Student's t-test.

** Plus-minus values are mean± SD rounded to the nearest tenth.*

Supplementary Figures

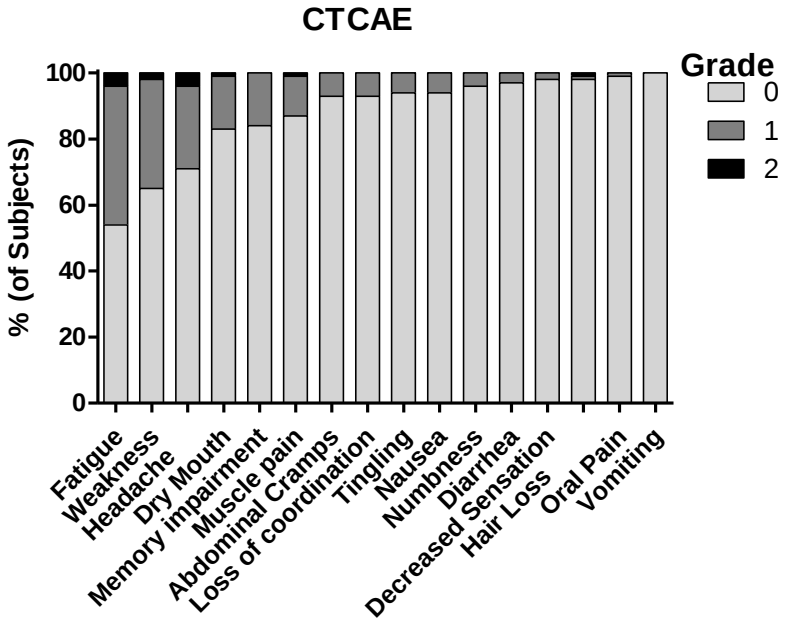


Fig. S1. Subject self-reported adverse effects based on CTCAE. (CTCAE; 1 = Mild, 2 = Moderate, 3 = Severe, 4 = Life-threatening, 5 = Death). Percentage of subjects reporting no symptoms (grade 0), grade 1 or grade 2 adverse effects; grades 3 to 5 were not reported.

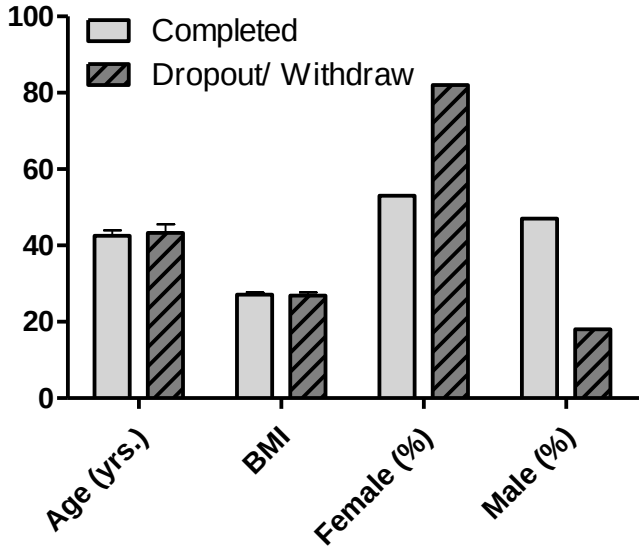
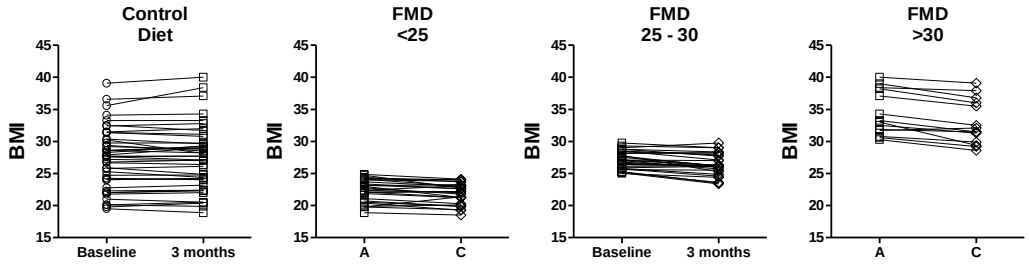
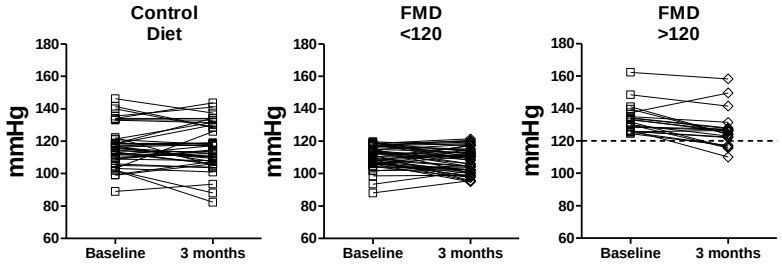


Fig. S2. Comparison of participants who completed the trial versus dropouts. Average age in years, BMI, and percentages of female and male subjects. Mean ± SEM.

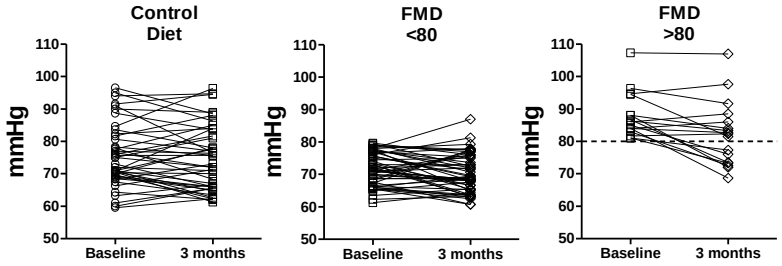
A) BMI



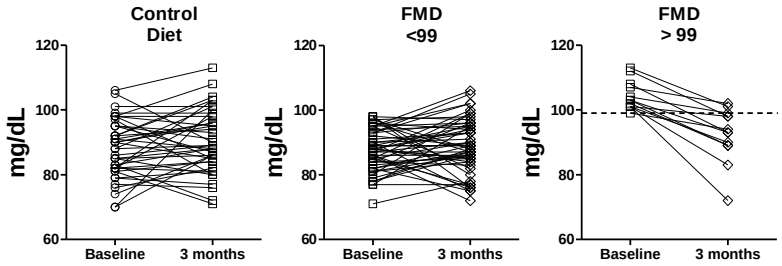
B) Systolic Blood Pressure



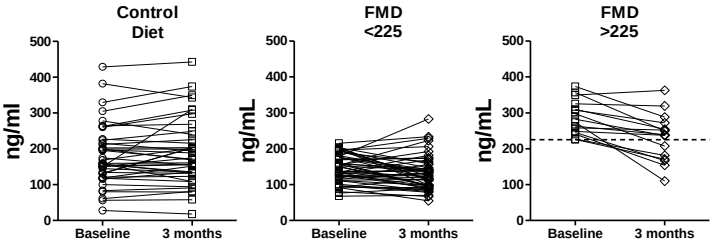
C) Diastolic Blood Pressure



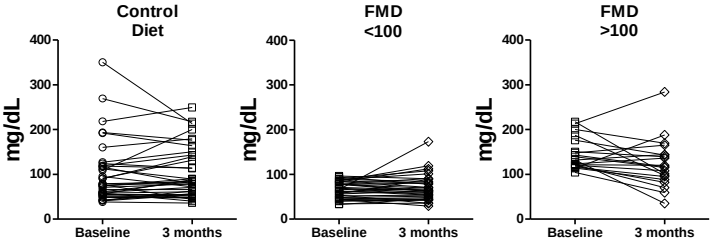
D) Fasting Glucose



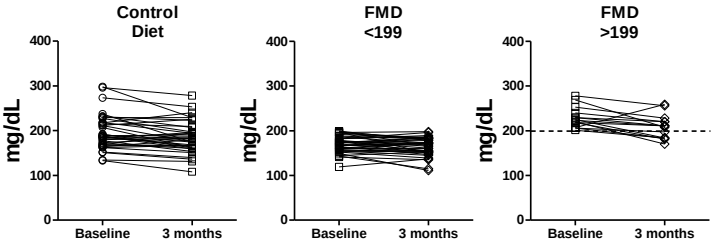
E) IGF-1



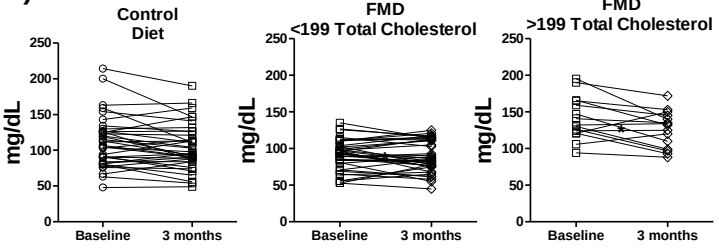
F) Triglycerides



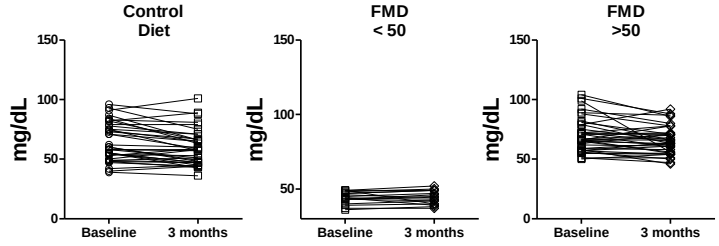
G) Total Cholesterol



H) LDL



I) HDL



J) CRP

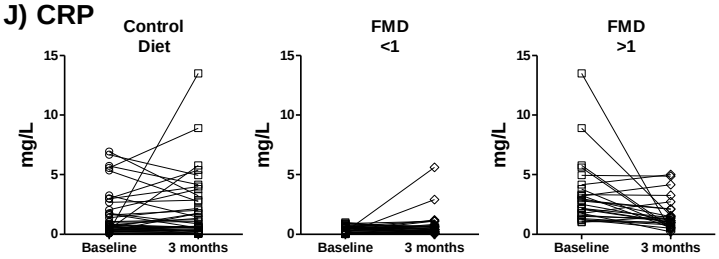


Fig. S3. Baseline to 3 months before/after comparison of individual subjects in the control cohort and all subjects who completed the FMD. Subjects in the FMD cohort were grouped based on their baseline status for the investigated risk factor in either the normal range or at-risk range. Cutoffs for risk range as indicated for the risk factor in graph. (**A**) Body mass index (BMI), control N= 43, <25 N= 27, 25-30 N=30, >30 N=14; (**B**) Systolic blood pressure, control N= 43, <120 N= 49, >120 N=21; (**C**) Diastolic blood pressure, control N= 43, <80 N= 53, >80 N=17 ; (**D**) Fasting glucose, control N= 41, <99 N= 53, >99 N=13 ; (**E**) Serum IGF-1, control N= 41, <210 N= 51, >210 N=18 ; **F**) Triglycerides, control N= 37, <100 N= 34, >100 N=21; (**G**) Total cholesterol, control N= 37, <199 N= 40, >199 N=15 ; (**H**) Low density lipoprotein in the control and FMD subjects with more than 199mg/dL total cholesterol, control N= 37, <199 N= 40, >199 N=15 ; **I**) High density lipoprotein, control N= 37, <50 N= 17, >50 N=38 ; (**J**) C-reactive protein, control N= 42, <1 N= 43, >1 N=26.

Fig. S4. Nutritional information of the FMD.

SOUPS

Vegetable Soup Mix

INGREDIENTS: Rice Flour, Dried Onion, Inulin (Chicory Fiber), Dried Tomato, Dried Carrot, Salt, Dried Red Pepper, Dried Leek, Potato Starch, Olive Oil, Freeze Dried Basil, Spinach Powder, Dried Parsley, Natural Flavor

Nutrition Facts

Serving Size 1.2 oz (33g); (10 fl oz Prepared)
Servings Per Package 1

Amount Per Serving		
Calories 100		
Calories from Fat 20		
		% Daily Value*
Total Fat	2 g	**3**%
Saturated Fat	1 g	**5**%
Trans Fat	0 g	
Cholesterol	0 mg	
Sodium	650 mg	**27**%
Total Carbohydrate	19 g	**6**%
Dietary Fiber	5 g	**20**%
Sugars	5 g	
Proteins	2 g	
Vitamin A (RAE)		45%
Vitamin C		45%
Calcium		4%
Iron		6%

* Percent Daily Values are based on a 2,000 calorie diet.

SOUPS

Mushroom Soup Mix

INGREDIENTS: Rice Flour, Carrot Powder, Dried Onion, Champignon Mushroom Powder, Inulin (Chicory Fiber), Dried Champignon Mushroom, Salt, Yeast Extract, Potato Starch, Olive Oil, Dried Parsley, Natural Flavor

Nutrition Facts

Serving Size 1.2 oz (33g); (10 fl oz Prepared)
Servings Per Package 1

Amount Per Serving		
Calories 100		
Calories from Fat 20		
		% Daily Value*
Total Fat	2 g	**3**%
Saturated Fat	1 g	**5**%
Trans Fat	0 g	
Cholesterol	0 mg	
Sodium	910 mg	**38**%
Total Carbohydrate	18 g	**6**%
Dietary Fiber	4 g	**16**%
Sugars	9 g	
Proteins	3 g	
Vitamin A (RAE)		90%
Vitamin C		4%
Calcium		2%
Iron		2%

* Percent Daily Values are based on a 2,000 calorie diet.

SOUPS

Tomato Soup Mix

INGREDIENTS: Rice Flour, Dried Tomato Powder, Dried Onion, Inulin (Chicory Fiber), Potato Starch, Dried Tomato Pieces, Olive Oil, Salt, Yeast Extract, Dried Basil, Dried Parsley, Natural Flavor

Nutrition Facts

Serving Size 1.2 oz (33g); (10 fl oz Prepared)
Servings Per Package 1

Amount Per Serving		
Calories 110		
Calories from Fat 10		
		% Daily Value*
Total Fat	1 g	**1**%
Saturated Fat	0 g	**0**%
Trans Fat	0 g	
Cholesterol	0 mg	
Sodium	800 mg	**33**%
Total Carbohydrate	25 g	8%
Dietary Fiber	6 g	**24**%
Sugars	6 g	
Proteins	3 g	
Vitamin A (RAE)		0%
Vitamin C		6%
Calcium		10%
Iron		10%

* Percent Daily Values are based on a 2,000 calorie diet.

Energy Drink Mix
INGREDIENTS: Purified Water, Natural Vegetable Glycerin, Polylysine (Natural Preservative).

Nutrition Facts
Serving Size 0.6 fl oz (17mL)
Servings Per Package 4

Amount Per Serving	
Calories 20	
	% Daily Values*
Total Fat 0g	0%
Sodium 0g	0%
Total Carbohydrate 5g	2%
Proteins 0g	

Not a significant source of fat, cholesterol, fiber, sugars, vitamin A, vitamin C, calcium and iron.
*Percent Daily Values (DV) are based on a 2,000 calorie diet.

Energy Bar

INGREDIENTS: Almond Meal, Macadamia Nut Butter, Honey, Pecan, Coconut, Flaxseed Meal, Coconut Oil, Vanilla, Sea Salt.

Nutrition Facts

Serving Size 1.6 oz (45g)

Serving Per Package 1

Amount Per Serving		
Calories 270		
Calories from Fat 200		
		% Daily Value*
Total Fat	23 g	**35**%
Saturated Fat	4.5 g	**24**%
Trans Fat	0 g	
Cholesterol	0 mg	
Sodium	350 mg	**15%**
Total Carbohydrate	13 g	**4**%
Dietary Fiber	3 g	**13**%
Sugar	8 g	
Proteins	5 g	
Calcium		4%
Iron		14%

Not a significant source of vitamin A and vitamin C.

* Percent Daily Values are based on a 2,000 calorie diet.

Chip Snack

INGREDIENTS: Kale, Red Bell Peppers, Cashews, Sunflower Seeds, Nutritional Yeast, Lemon Juice, Cayenne Pepper, Sea Salt

Nutrition Facts

Serving Size 1.05 oz (30g)

Serving Per Package 1

Amount Per Serving		
Calories 160		
Calories from Fat 110		
		% Daily Value*
Total Fat	11 g	**17**%
Saturated Fat	1 g	**5**%
Trans Fat	0 g	
Cholesterol	0 mg	
Sodium	190 mg	**8%**
Total Carbohydrate	13 g	**4**%
Dietary Fiber	4 g	**16**%
Sugar	1 g	
Proteins	7 g	
Vitamin A (RAE)		60%
Vitamin C		40%
Calcium		8%
Iron		15%

Not a significant source of vitamin A and vitamin C.

* Percent Daily Values are based on a 2,000 calorie diet.

Algal Oil
INGREDIENTS: Gelatin, Glycerin, Purified Water, Turmeric (Color), Annatto Extract (Color)

Nutrition Facts
Serving Size 1 Softgel

Amount Per Serving	
Calories 6	
Calories from Fat 5	
	% Daily Values*
Total Fat 0.5g	< 1%
Sodium 0g	0%
Total Carbohydrate 0g	0%
Proteins 0g	
DHA Omega-3 (from Algal Oil) 200 mg**	

*Percent Daily Values (DV) are based on a 2,000 calorie diet.
**Daily value not established.

NR-1
Vegetable Powder with Vitamins and Minerals Supplements

OTHER INGREDIENTS: Stearic Acid, Microcrystalline Cellulose, Dicalcium Phosphate, Croscarmellose Sodium, Magnesium Stearate, Silicone Dioxide, Food-grade Shellac

Nutrition Facts
Serving Size 1 Tablet
Servings Per Container: 2

Amount per Serving		**% Daily Value***
Vitamin A (as Beta Carotene)	1,250 IU	25%
Vitamin C (Ascorbic Acid)	15 mg	25%
Vitamin D (as Cholecalciferol)	100 IU	25%
Vitamin E (as DL-Alpha Tocopherol Acetate)	7.5 IU	25%
Vitamin K (as Phytonadione)	20 mcg	25%
Thiamine (as Thiamine Mononitrate)	0.37 mg	25%
Riboflavin	0.42 mg	25%
Niacin (as Niacinamide)	5 mg	25%
Vitamin B6 (as Pyridoxine HCl)	0.5 mg	25%
Folic Acid	100 mcg	25%
Vitamin B12 (as Cyanocobalamin)	1.5 mcg	25%
Biotin	15 mcg	5%
Pantothenic Acid (as Calcium-D-Pantothenate)	2.5 mg	25%
Calcium (as Calcium Carbonate and Tribasic Calcium Phosphate)	100 mg	10%
Iron (as Ferrous Fumarate)	4.5 mg	25%
Phosphorous (as Tribasic Calcium Phosphate)	10 mg	1%
Iodine (as Potassium Iodine)	37.5 mcg	25%
Magnesium (as Magnesium Oxide)	26 mg	7%
Zinc (Zinc Oxide)	3.75 mg	25%
Selenium (as Sodium Selenate)	7.5 mcg	11%
Copper (as Cupric Sulfate)	0.25 mg	13%
Manganese (as Manganese Sulfate)	0.5 mg	25%
Chromium (as Chromium Picolinate)	17.4 mcg	15%
Molybdenum (as Sodium Molybdate)	18.75 mcg	25%
L-Nutra Power Blend (Beet Root, Spinach Leaf, Tomato Fruit, Carrot Root, Collard Leaf, Kale Leaf)	600 mg	**

* Percent Daily Values are based on a 2,000 calorie diet.
** Daily Values not established.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3-4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	13
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	13
	4b	Settings and locations where the data were collected	13
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	14
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	14
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	Supplement 1
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	13-14
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	13-14
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	NA
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Supp 1

Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	13
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	15,16, Sup1-2
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	15-16, Sup1-2
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Fig 1, page 5
	13b	For each group, losses and exclusions after randomisation, together with reasons	Fig 1, page 5
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the trial ended or was stopped	Supp 1
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	All applicable tables
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	All applicable tables
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	All applicable tables
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	Fig S1, p 5
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	10-13
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	10-13
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	10-13
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	Attached, p1
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	2

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Study Protocol

PROTOCOL NUMBER:	
TITLE:	DIETARY INTERVENTION IN HUMANS: A PILOT STUDY ON FEASIBILITY, SAFETY, AND EFFECTS ON COGNITIVE AND IMMUNE FUNCTIONS
SHORT TITLE:	Multicycle Dietary Intervention
STUDY PHASE:	Phase I
STUDY ARMS:	1
IND OR IDE #:	
PRINCIPAL INVESTIGATOR(S):	Min Wei, PhD Davis School of Gerontology 3715McClintock Ave, LA, CA 90089 213.740.1755
CO-INVESTIGATOR(S):	Mara Mather, Ph.D., mara.mather@usc.edu Elizabeth Zelinski, Ph.D., zelinski@usc.edu
SPONSOR:	
PARTICIPANTS/LOCATONS:	Davis School of Gerontology Dornsife Neuroscience Imaging Center USC Norris Comprehensive Cancer Center Facilities
AMENDMENTS/REVISIONS:	

TABLE OF CONTENTS

1.0	BACKGROUND AND HYPOTHESES	page
2.0	OBJECTIVES AND PURPOSE	page
3.0	STUDY DESIGN	page
4.0	DRUG/DEVICE INFORMATION	page
5.0	SELECTION AND WITHDRAWAL OF SUBJECTS	page
6.0	DESCRIPTIVE FACTORS/STRATIFICATION/RANDOMIZATION SCHEME	page
7.0	STUDY AGENT ADMINISTRATION OR INTERVENTION AND TOXICITY MANAGEMENT PLAN	page
8.0	ASSESSMENT OF EFFICACY AND SAFETY	page
9.0	CLINICAL AND LABORATORY EVALUATIONS	page
10.0	CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS	page
11.0	SPECIAL INSTRUCTIONS	page
12.0	DATA COLLECTION AND MONITORING	page
13.0	STATISTICAL CONSIDERATIONS	page
14.0	REGISTRATION GUIDELINES	page
15.0	BIOHAZARD CONTAINMENT	page
16.0	ETHICAL AND REGULATORY CONSIDERATIONS	page
17.0	REFERENCES	page

APPENDICES

1.0 Background and Hypotheses

Modern chemotherapy can improve the quality of life of cancer patients via palliation of cancer-related symptoms, and in many malignancies can significantly extend survival as well. However, the toxicity to normal cells poses a major clinical challenge, particularly when malignant cells have acquired resistance to chemotherapy. Evidence from bio-gerontology research from our laboratory and others have showed that short-term fasting/starvation (STS) can improve the efficacy of chemotherapy by protecting normal cells and tissues and potentially sensitizing malignant cells to chemo drugs. Cancer patients, however, may not be able or willing to incorporate fasting into existing chemotherapy.

The objective of the study is to obtain preliminary estimates of the feasibility, safety and impact of a calorie restricted special diet on adult subjects and to examine the neural correlates of these interventions. We hypothesize that the specially designed dietary regimen can induce similar changes, including the levels of glucose, IGF-1, IGFBPs and other bio-markers, as with short-term fasting.

This study is designed as a randomized cross-over trial, includes two arms: Control and multi-cycle special 5-day dietary regimen (Diet, 3 cycles). After 3 cycles, the two arms are crossed over such that the control group will under-go dieting and the Diet group will return to normal diet.

Statistical methods: Paired samples t-test and Mann-Whitney test will be used to compare between Control and Diet groups as well as pre- and end-fasting/diet values.

1.1 Dietary Restriction, Short-term Fasting, and Chemotoxicity

Calorie restriction has been associated with longevity in animals (1), and with lower rates of spontaneous cancer formation (2, 3). Cell culture experiments document a reduction in chemotherapy toxicity when healthy cells are incubated in low-glucose medium, but neoplastic cells do not experience the same protection, rather they may actually be sensitized to the chemotherapy cytotoxicity in the low-glucose environment (4-8). In mice with xenografts, short-term starvation for 48-60 hours with ad lib water consumption significantly reduced the toxicity and nearly eliminated mortality from high-dose chemotherapy, compared to mice fed standard diets prior to receiving chemotherapy (4). Furthermore, these fasting periods resulted in a more rapid return to normal weight in the post chemotherapy period. These intriguing findings led to the development of a clinical trial (0S-08-9) incorporating fasting (aka short-term starvation or STS) prior to chemotherapy administration in humans, which is currently accruing.

Metabolic alterations induced by STS in normal and cancer cells are pleotropic. Studies in healthy volunteers revealed that with 22 and 48 hours of fasting, blood glucose and insulin levels decreased significantly, and blood ketones increased (9, 10). Short-term Fasting in mice and humans (48-120 hours) has been shown to cause a 75% reduction in circulating insulin-like growth factor 1 (IGF1) as well as major changes in IGF1 binding protein (IGFBPs) levels (11, 12). There are 7 different IGF binding proteins, which differentially modulate IGF-1 stability, bioavailability and activity; and each of which has a unique pattern of modulation. For example, IGFBP-2 increases and IGFBP-3 decreases during prolonged fasting, whereas IGFBP-1 increases rapidly even with overnight fasting and is quickly suppressed by caloric intake (13). Decreased IGF-1 level and IGF-1 signaling (attenuated activities in TOR, S6K, AKT, and RAS) have been shown to protect simple organisms and cells against a variety of toxins and stress stimuli (14-16).

Recent animal studies suggest that low IGF-1 signaling may explain, in part, the beneficial effects of fasting. Mice with a liver IGF-1 deficiency experienced significantly less toxicity from multiple chemotherapy drugs including doxorubicin and showed prolonged tumor-bearing survival (**Figure 1**). In fact, the majority of IGF-1 deficient mice bearing melanomas and receiving chemotherapy achieved long-term survival whereas none of the ad lib fed mice was able to avoid either chemotherapy- or tumor-dependent death. Additional experiments showed that the protection against toxicity conferred by fasting was abrogated when IGF was administered (6).

Our ongoing phase I clinical trial of fasting in cancer patients receiving platinum chemotherapy has documented that fasting for 24 hours is feasible (escalation ongoing). However, enrollment has been slow in part because patients are already under a very stressful condition and can view fasting as an additional stress. If the differential stress resistance induced by fasting could be achieved with a restricted diet instead, it would be more acceptable to a broader range of patients and oncologists, since it would encourage compliance and minimize the risk of complete nutrients deprivation.

In this study, we will test a restricted diet in healthy adult subjects, to evaluate whether this dietary regimen will lead to similar physiological changes elicited by short-term fasting.

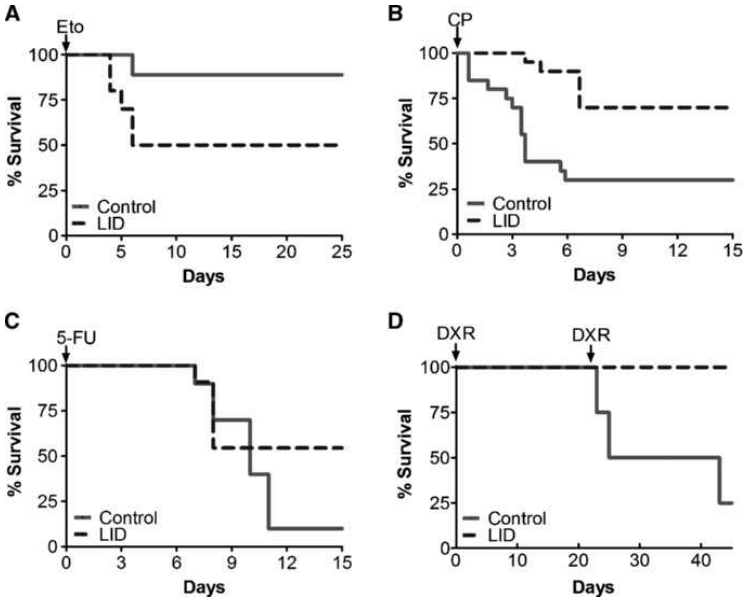


Figure 1. Mice with deficiency in liver IGF-1 (LID) has 70-80% lower circulating IGF-1 levels than the wild-type littler mates. LID mice experienced less chemo-associated toxicity: (A) etoposide (100 mg/kg, n=9, p=0.064), (B) cyclophosphamide (CP, 500 mg/kg, n=20, p=0.001), (C) 5-Fluorouracil (5-FU, 400 mg/kg, n=11, P=0.148), and (D) Doxorubicin (DXR, 2 injections at 20 mg/kg and 28 mg/kg, n=5, p=0.022).

Stress resistance testing in LID mice with various high-dose chemotherapeutic drugs. LID and control mice received (A) a single injection of 100 mg/kg etoposide (n = 10/LID, n = 9/control, P = 0.064), (B) a single injection of 500 mg/kg cyclophosphamide (CP; n = 20/group, P = 0.001), (C) a single injection of 400 mg/kg 5-fluorouracil (n = 11/LID, n = 10/control, P = 0.148), or (D) two injections of DXR. The first injection of 20 mg/kg was given on day 0, and the second injection of 28 mg/kg was given on day 22 (n = 5/LID, n = 4/control, P = 0.022). Toxicity evaluated by percent survival is shown. P values by Peto's log-rank test.

1.2 L-NUTRA CHEMOLIEVE™ AND PROLION™: NOVEL CALORIE RESTRICTED DIET REGIMENS

In rodent tumor models, it has been demonstrated that low glucose and low IGF-1 mediate much of the differential protective effects of fasting (6). Longo and colleagues also showed that 2-3 days of very low calorie substitution diet was noted to reduce IGF levels, similarly to fasting and conferred protection of mice against doxorubicin toxicity (data not shown; confidential until publication). A separate group of

mice treated with 3-5 days of no/low essential amino acids also showed similar benefit. Repeated cycles of the low calorie diet were also noted to retard tumor progression while allowing the mice to maintain their weight (manuscript in preparation). Based on these experiments and clinical trials in humans showing the effect of protein restriction on IGF-1, we have postulated that the beneficial effects of fasting are largely mediated by a low amount of proteins/essential amino acids and low glucose consumption. CHEMOLIEVE™ AND PROLION™ was developed as a calorie restricted, low protein diet regimen. The diet regimen consists of a high fat content diet on day 1 in order to minimize the calorie restriction burden on the patient so that the desired duration of very low calorie intake can be tolerated without causing adverse effects, while maintaining the induction of differential stress resistance to mimic the effects of fasting on reducing chemotherapy toxicity and enhancing chemotherapy efficacy.

The CHEMOLIEVE™/PROLION™ diet regimen consists of 100% ingredients which are generally regarded as safe (GRAS). It is comprised of bars, soups, and beverages aiming to achieve a consistent and effective short-term calorie restriction, while provide adequate micronutrients for 3-6 days.

2.0 OBJECTIVES AND PURPOSE

The objective of the study is to obtain preliminary estimates of the feasibility, safety and impact of a calorie restricted special diet on adult subjects and to examine the neural correlates of these interventions. We hypothesize that the specially designed dietary regimen can induce similar changes, including the levels of glucose, IGF-1, IGFBPs and other bio-markers, as with short-term fasting. We also hypothesize that the specially designed dietary regimen will influence hippocampal memory encoding function.

We are particularly interested in how the specific diet (Diet) will influence hippocampal function, as the hippocampus has a high density of insulin and ghrelin receptors and is a region of the brain that can show increases or decreases in structural volume across just days (17, 18). To investigate the effects of dieting on hippocampal function and brain function more broadly, we will be conducting optional functional neuroimaging scans before and after the intervention.

3.0 STUDY DESIGN

This study is designed as a randomized cross-over trial, includes two arms: Control and multi-cycle special 5-day dietary regimen (Diet, 3 cycles). After 3 cycles, the two arms are crossed over such that the control group will under-go dieting and the Diet group will return to normal diet.

68 subjects:

	Control	Diet
Sample size	20	20
Dropout/Incompliance	10 (50%)	10 (50%)
Technical problem	4 (20%)	4 (20%)
Subtotal	34	34

With an anticipated dropout rate of 50% (due to long study period of 6 months and participants' adherence to the regimen), and a 20% potential technical problem (including declining certain tests by the participants), a total of 64 subjects will be recruited for the each group.

For the Diet group, participants will be provided with a 5-day supply of special diet. The diet consists of ingredients (see Appendix L) which are Generally Regarded As Safe (GRAS) and includes:

The total length of subject participation is 6 months. For the control group, participants will be asked to come in for a base-line examination and tests, and a follow-up examination and tests at the end of 3rd month. For the Diet group, the diet regimen (one-month per cycle) is composed of 5 days special diet followed by normal diet. Participants will be asked to come in for a base-line examination and follow-up examinations at the end of the 1st and 3rd diet cycle. At the end of the 3rd month, the Control group will be switched to become the Diet group, and Diet group becomes Control (crossover).

Examination and tests will include:

- Physical examination (body composition): height, weight (dress weight), waist and hip circumferences, and upper arm length, the triceps skin fold (TSF), and the (mid-)upper arm circumference ((M)UAC), as well as 4-site skin fold (the abdominal, triceps, thigh and suprailiac skinfold sites).
- Body composition (optional): Whole body fat, soft lean tissue, and bone mineral content will be measured by dual energy x-ray absorptiometry (DEXA).
- Vital signs: (body temperature, blood pressure, pulse, respiratory rate).
- Blood draw through venipuncture (25 mL: 7.5 mL for serum, 7.5 mL for plasma, 10 mL for white blood cells) before (baseline) and after the dietary intervention. Note, the typical draw volume for blood donation in the US is 1 pint (~450 mL). 3 cycles of blood draw (~25 mL each and > 4 days apart) should be safe for the participants.
- Magnetic resonance imaging (MRI) (optional) (Dr. Mara Mather):
    - A maximum time of 100 minutes MRI scan time (Siemens full-body scanner).
    - Memory encoding session: After some initial scans, subject will be asked to view and try to remember a series of pictures and their locations on the screen. Some of these pictures may be emotionally intense and/or graphic. For instance, there may scenes involving bloody or disgusting images.
- Cognitive test (optional) (Dr. Elizabeth Zelinski): The protocol uses standardized neuropsychological tests developed and widely used to assess adult cognitive functioning. The total testing session will be no more than 1.5 hours, including breaks between tests. Participants will be tested in private sessions. The test forms will constitute the data. De-identified paper copies of the tests with coded subject numbers only will be used and researchers will be blind to the experimental condition of the participants.

Surveys

- Health Habits Questionnaire (Appendix E)
- Mood & Anxiety Questionnaire - STAI PANAS (Appendix G)
- Self-evaluating survey based on Common Terminology Criteria for Adverse Events (CTCAE, v4.0) (Appendix H)
- Diet Diary (Appendix I)

4.0 DRUG/DEVICE INFORMATION

N/A

5.0 SELECTION AND WITHDRAWAL OF SUBJECTS

5.1 Inclusion Criteria:

- Generally healthy adult volunteers.
- Subjects of 18-70 years of age.

- Body mass index: 18.5-30 kg/m^2 (normal weight to overweight, but not obese).
- Ability and willingness to provide written informed consent (Appendix A and C).
- Ability and willingness to undergo multiple cycles of a 5-day dietary regimen.
- Ability and willingness to provide blood samples via venipuncture.

5.2 Exclusion Criteria

Age-based exclusion criteria: we will not include pediatric subjects because this is a pilot study on adult subjects. We are excluding subjects who are 65 years or older for safety and feasibility reasons and to minimize the complicating factors such as frailty and un-/under-diagnosed medical conditions.

Competing risk:

- Any major medical condition and chronic diseases
- Mental illness, including severe depression, dementia.
- Drug dependency.
- Hormone replacement therapy (DHEA, estrogen, thyroid, testosterone).

Safety reasons:

- Severe hypertension (systolic BP > 200 mm Hg and/or diastolic BP > 105 mm Hg).
- Underweight (BMI < 18.5 kg/m2).
- Females who are pregnant or nursing. Although there is no evidence that magnetic and electric fields associated with MRI interfere with human development, in vitro studies and theoretical predictions raise the question of whether exposure might pose risks to the developing embryo and fetus. We will not include females that are pregnant or nursing to minimize exposure of the mother and embryo/fetus to DEXA and MRI scans, short-term reduction in calorie intake and micronutrient deficiency during the dietary intervention.

  If you are pregnant or become pregnant, you will not be allowed to participate in the study. If you are a woman who could become pregnant, you must have a pregnancy test to make sure you are not pregnant.

  A pregnancy test will be performed before each DEXA and MRI scans. If you are pregnant, you will not have the DEXA and MRI scans performed.

Adherence or retention reasons:

- Unable or unwilling to participate in baseline or follow-up examinations.
- Unable or unwilling to complete the dietary intervention. Biochemical readouts will be used to determine compliance: e.g. plasma IGF-1 level, fasting blood glucose level, and blood β-Hydroxybutyrate level, etc.

Technical reasons:

- Don't have normal or corrected vision (for cognitive study).

Dietary reasons:

- Special dietary requirements incompatible with the study interventions.
- Significant food allergies which would make the subject unable to consume the food provided.
- Alcohol dependency (alcohol intake greater than two drinks per day for women and three drinks per day for men).

MRI exclusion criteria (see MRI screening questionnaire, Appendix D):

- Persons with certain conditions (including, but not limited to, post-traumatic stress disorders, panic disorder, heart disease, peripheral vascular disease, diabetes, Reynaoud's phenomenon, cryoglobulinemia, vasculities, lupus, or tingling or numbness in hands and/or feet) could be at risk for adverse consequences upon exposure to the stress-induced stimuli. Persons have any diseases or disorders that cause either clinical sensitivity or insensitivity to pain will be excluded from this study.
- Persons at secondary risk for exposure to the magnet include women who are pregnant.

5.3 Withdrawal Criteria

- Participants can withdraw from the study at any time.
- Participants of non-compliance will be removed from the study.

6.0 DESCRIPTIVE FACTORS/STRATIFICATION/RANDOMIZATION SCHEME

6.1 Stratification factors: N/A.

6.2 Descriptive factors: Gender, Body mass index (BMI).

6.3 Participants will be randomly enrolled into either the Control or the Diet group.

7.0 STUDY AGENT ADMINISTRATION OR INTERVENTION AND TOXICITY MANAGEMENT PLAN

For the Diet group, participants will be provided with a 5-day supply of special diet (per monthly cycle). The diet consists of ingredients (see Appendix L) which are Generally Regarded As Safe (GRAS).

8.0 ASSESSMENT OF EFFICACY AND SAFETY

8.1 Side effects/Toxicities to be monitored.

There are risks and discomforts associated with calorie restricted diet, such as hunger, anxiety, drowsiness, dizziness, headache, muscle aches, fatigue, low blood pressure and, in rare cases, fainting. These diet interventions may also cause abnormal heart rhythms, short-term nutrient deficiency, and a weakened immune response. Extended period of low calorie dieting can be especially dangerous in people who are already malnourished, such as those with some forms of advanced cancer.

Participants may stop dieting and resume normal diet at any time during the study. Participants should seek immediate medical care if they experience significant discomforts.

During the dieting period, participants should drink adequate water to prevent from dehydration and avoid strenuous tasks/exercises. Participants should avoid operating motor vehicle and heavy machinery. Participants should avoid exposure to high temperature environment, such as hot shower or bath and avoid alcohol consumption.

Participants should contact the investigator, consult personal physician, or seek immediate medical care, if they have any question regarding or experience discomfort.

At the end of the dieting, participants should avoid binge eating and resume the normal diet gradually, starting with liquid foods, such as soups and fruit juices, flowed by light meals.

Participants may feel light-headed from the blood draw. In rare events, participants may experience bruising, excessive bleeding, infection (a slight risk any time the skin is broken), dizziness, and fainting. Participants can stop the blood draw procedure at any time. Participants should contact personal physician or seek immediate medical care, if they experience blood draw-related bleeding and infection.

Each DEXA procedure will expose you to a very small amount of radiation, much less than most routine diagnostic x-ray procedures. Exposure to radiation can increase ones risk of developing cancer. The amount of radiation to which you will be exposed during a DEXA scan is known to be low. Participant can stop the DEXA scan session at any time by talking to the technician directly.

Viewing emotional images during memory test could cause some psychological stress, but participants may stop the task at any time.

There is a risk of a heating or burning sensation from the MRI scan. Participants should report any heating/burning sensation immediately. Participants may have the scan stopped by using the call button at any time if this occurs. Participants may experience a localized twitching sensation due to the magnetic field changes during the scan. This should not be painful. Dizziness and nausea may occur momentarily when participant's head is moved in or out of the tunnel of the magnet. The sensation should disappear quickly.

Other risks/discomforts associated with MRI include:

1) Lying and maintaining an unmoving posture while concentrating on visual stimuli can be tiring;
2) Participants may be uncomfortable for the first few minutes in the scanner. Participants may become anxious from lying in an enclosed space.
3) If participants have metal surgical implants or implanted electric/magnetic devices (pacemaker, orthodontic braces, aneurysm clips), they are at an increased risk. The study staff will ask participants questions and review participants' records to make it is safe for participants to have this scan.
4) Persons at secondary risk for exposure to the magnet include women who are pregnant.

The risks will be minimized in the following ways:

1) Effort will be made to position the participant in the most comfortable position possible. In addition, participant will be give short breaks between each scan to allow the participant to close eyes and relax.
2) The participant will be able to stop the session at any time by squeezing a bulb held in one hand. The bulb activates a buzzer in the control room. There is also an intercom voice link with the control room that allows verbal communications between the participant and staff at all time.
3) Individuals at primary risk will be excluded from the study based on the MRI screening form.
4) Although there is no evidence that magnetic and electric fields associated with MRI interfere with human development, in vitro studies and theoretical predictions raise the question of whether

exposure might pose risks to the developing embryo and fetus. Individuals at secondary risk (pregnant women or women who may be pregnant) will be excluded from the study.

The main risks of the cognitive tests are of fatigue during testing, which is accommodated by having breaks during the sessions. The tests given are standard psychological assessments of cognition, and many participants find them interesting.

8.2 Dosage change based on toxicity.

Participants may stop dieting and resume normal diet at any time during the study. Participants should seek immediate medical care if they experience significant discomforts.

8.3 Adverse Event Reporting: Procedures for reporting unexpected and fatal toxicity should be explained.

Following serious adverse event (SAE) or serious suspected adverse reaction will be reported to IRB within 10 working days after investigators becoming aware of the event:

- Death;
- Inpatient hospitalization;
- Persistent or significant incapacity;
- Substantial disruption of the ability to conduct normal life functions;
- May jeopardize the subject; or
- May require medical or surgical intervention to prevent one of the outcomes listed above.

9.0 CLINICAL AND LABORATORY EVALUATIONS

Participants will be evaluated before the dietary intervention, at the end of the dietary intervention, and at least one week after resuming normal diet.

Physical evaluation of body composition:

1) Body mass index (BMI)
2) The derived measures of anthropometry of the upper arm include the (mid-)upper arm muscle area ((M)UAMA), the (mid-)upper arm fat area ((M)UAFA), and the arm fat index. These measures will be used as rough indicators of body fat.
3) Body density will be determined using 4-site skinfold measurements (based on the abdominal, triceps, thigh and suprailiac skinfold sites) with the Slim Guide Skinfold Caliper (Creative Health Products) (19, 20)(Schmidt and Carter, 1990, Hum Biol). The level of percent body fat (%BF) will then be calculated using the Siri Equation (21, 22).
4) Optional body composition test: whole body fat, soft lean tissue, and bone mineral content will be measured by dual energy x-ray absorptiometry (DEXA) using a Hologic QDR 5400 densitometer (Hologic, Inc., Bedford, MA).

Clinical and biochemical evaluations:

1) Plasma lipids profile
2) Plasma mineral profile
3) Serum/plasma metabolites profile
4) Serum/plasma growth factors: IGF-1, IGFBP1/3, insulin, VEGF, CSF, G-CSF
5) Serum/plasma growth factor functional assay using cell-based in vitro analyses
6) Serum/plasma inflammatory markers: adiponectin, CRP, cytokine

7) Serum/plasma enzyme assays for liver and kidney functions: Alanine Transaminase (ALT), BCG Albumin, and Bilirubin assays, Creatinine and Urea assays
8) Blood tests. Hematologic evaluation – including hemoglobin, hematocrit, complete blood cell counts (CBC), morphological exam, and cellular macromolecular modifications assays (lipid/protein/DNA oxidation, DNA breakage, and hematopoietic stem cell markers analysis, etc.)

Magnetic resonance imaging (MRI)

1) Structural scan
2) Resting state scans (functional and ASL)
3) Memory task during functional magnetic resonance imaging (fMRI)

Cognitive tests

1) Verbal memory and learning will be assessed with the Rey Auditory-Verbal Learning Test, which includes 2 lists of15 words that are recalled over 5 trials each.
2) Speed of processing/attention is assessed in the Ruff 2's and 7's test, and the WAIS III Digit Symbol tests.
3) Working memory is assessed in the WAIS III Digits Backward test.
4) Executive function: Color Trails, FAS (letter fluency), and category fluency.
5) Visuospatial construction and memory (Rey Osterreith Complex Figure copy and delayed recall).
6) Frequency of Forgetting-10 Scale.

Each test takes 3-5 minutes to administer, except for the RAVLT, which takes about 25 min, with breaks given as necessary.

10.0 CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

Biochemical readouts will be used to determine compliance, e.g.

- Plasma IGF-1 level (<80% of baseline at the end of dietary intervention);
- Blood glucose level (<80% of baseline at the end of dietary intervention);
- Blood ketone bodies (β-Hydroxybutyrate level (>2-3 mM at the end of dietary intervention).

Note: all measurements are taken after an overnight fast of at least eight hours.

11.0 SPECIAL INSTRUCTIONS

Blood draw through venipuncture (25 mL: 7.5 mL for serum, 7.5 mL for plasma, 10 mL for white blood cells). Note, blood draw volume for blood donation in the US is 1 pint (~450 mL). Three blood draws (~25 mL each) should be safe for the participants.

Blood samples will be stored at -80°C until assayed.

12.0 DATA COLLECTION AND MONITORING

- Research procedures will be conducted in person in a private setting.
- Data will be captured and reviewed in a private setting.
- Only authorized research study personnel will be present during research related activities.
- The collection of information about participants is limited to the amount necessary to achieve aims of the research.

- Participants will not be approached in a setting or location that may constitute an invasion of privacy or could potentially stigmatize them.
- Data and/or specimens will be labeled with a code that the research team can link to personal identifying information. (Coded)
- At the conclusion of the study, data will be retained for study record keeping purposes per institutional policy.

13.0 STATISTICAL CONSIDERATIONS

A total of 40 participants will enter this randomized cross-over study. Using serum IGF-1 level as a primary outcome, the effects will be determined by comparing the Control and Dieting as well as before and after the dietary interventions. The probability is 70 percent that the study will detect a treatment difference at a 0.05 significance level, if the true difference between Dieting and Control is more than 25% (Note). All participants who are will be included in the analyses of the results. Participants who are removed from study for non-compliance will still have their data included in the final analysis, unless they withdraw consent to have their data included, and will not be replaced.

Note, Sample size analysis is based on the adult serum IGF-1 reference values (Leite, 2011, PMID: 12876414):

IGF-1 (ng/mL)	-1SD	Mean	+1SD
Male 26-30	140.47	205.41	300.37
Male 31-40	137.41	178.22	231.13
Female 26-30	143.31	203.36	288.59
Female 31-40	126.6	190.76	287.44

A sample size of 20 is needed for an α error of 5%, β error of 30%, to observe a 25% difference in IGF-1 level change assuming the control Mean of 194 ng/mL (mean of male and female age between 26-40 years) and a SD value of 97 ng/mL (maximum SD of the age group).

Statistical power analysis regarding the Dexa scan.

Based on prior published results, an N of 10-20 is sufficient for a medium-term calorie restriction study (Fontana, 2007, PMID: 17389710; Racette, 2006, PMID: 16960025). The lead author of the mentioned papers, Dr. Luigi Fontana, is one of our advisors for this trial. In the above mentioned study, a medium-term calorie restriction regimen led to significant reductions in BMI, body fat and body weight in a study group of 19 volunteers (Table is reproduced from Racette, 2006, PMID: 16960025).

	Weight, kg				% Fat mass			
	Mean	SE	SD*	%Baseline		SE	SD*	%Baseline
Baseline	78.5	2.3	10.0	100	33.1	1.1	4.8	100
3 mo CR	75.4	2.4	10.5	96.1	29.6	1.1	4.8	89.4
6 mo CR	73.3	2.4	10.5	93.4	27.9	1.0	4.4	84.3
12 mo CR	71.0	2.4	10.5	90.4	26.7	1.0	4.4	80.7

* SD is calculated form published SE and n=19 (Racette, 2006, PMID: 16960025).

Assuming a sigma of 0.2 (0.13 for weight and 0.15 for % fat mass in above cited study), a sample size of 20 has the power of 0.99 to detect a difference of 20% with an α of 0.05 (paired t test, two-tailed); and, with n=10, the power is 0.8.

As in any clinical trial, the participant can always withdraw from the study even with prior consent. To ensure that the trial will collect sufficient data for analysis, we have planned for an enrollment of 34 participants per group (total 68) anticipating dropout, incompliance, and potential technical problem (see 3.0 Study Design in the Protocol). This enrollment plan, we believe, will ensure that sufficient data will be collected for Dexa analysis. To further ensure that sufficient data is collected, we plan to keep the enrollment open till a minimum of 15 participants are tested with Dexa.

14.0 REGISTRATION GUIDELINES

14.1 Specify phone number to register the patients. Also identify whether the patients will be randomized or stratified.

Participants may contact Min Wei, PhD, at 213-740-1755, Mara Mather, PhD, at 213-821-1868, or Valter D Longo, PhD, at 213-740-6212 with any questions, concerns, or complaints about the research or their participation in this study. If the participant has questions, concerns, or complaints about the research and are unable to contact the research team, contact the Institutional Review Board (IRB) Office at 323-223-2340 between the hours of 8:00 AM and 4:00 PM, Monday to Friday. (Fax: 323-224-8389 or email at irb@usc.edu).

If participants have any questions about participants' rights as a research participant, or want to talk to someone independent of the research team, participants may contact the Institutional Review Board Office at the numbers above or write to the Health Sciences Institutional Review Board at LAC+USC Medical Center, General Hospital Suite 4700, 1200 North State Street, Los Angeles, CA 90033.

Participants will be randomly assigned to either the Control or the Diet group:

1) Control Group: participants will be their normal diet
2) Diet Group: participants will be provided with a 6-day supply of special low calorie diet. The special diet consists of ingredients which are Generally Regarded As Safe (GRAS), including energy bars, soups, and drink packets.
3) At the end of 3 month, the Control group will start dieting, and the Diet group will resume their normal diets (crossover).

14.2 Specify the forms and records needed for registration: Informed Consent, Registration/Eligibility Worksheet, Flow Sheet, etc.

- Appendix A: Informed Consent
- Appendix C: Informed Consent-Incidental Findings
- Appendix D: Dornsife MRI Screen Questionnaire

Note: At the time of registration, two copies of a signed and dated patient Informed Consent form with Bill of Rights must be available (an original for patient's medical chart; one copy for the patient; and the other for the PI's file).

15.0 BIOHAZARD CONTAINMENT

N/A

16.0 ETHICAL AND REGULATORY CONSIDERATIONS

All institutional and Federal regulations concerning the Informed Consent form will be fulfilled. The study will be conducted in adherence to ICH Good Clinical Practice.

Valter Longo, Ph.D. is a professor at the Davis School of Gerontology, USC. Dr. Longo is the founder of and has ownership stake in L-Nutra, Inc. This study will use food products developed by this company. The nature of this conflict and the management of the conflict of interest have been reviewed by the USC Conflict of Interest Review Committee (CIRC). The University of Southern California and L-Nutra may develop products that can be sold. If they make money from these products, participants will not receive any money.

17.0 REFERENCES

1. Everitt AV, Le Couteur DG. Life extension by calorie restriction in humans. Ann N Y Acad Sci. 2007;1114:428-33. PMID: 17717102.
2. Dunn SE, Kari FW, French J, Leininger JR, Travlos G, Wilson R, Barrett JC. Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p53-deficient mice. Cancer Res. 1997;57(21):4667-72. PMID: 9354418.
3. Kritchevsky D. Diet and cancer: what's next? J Nutr. 2003;133(11 Suppl 1):3827S-9S. PMID: 14608121.
4. Raffaghello L, Lee C, Safdie FM, Wei M, Madia F, Bianchi G, Longo VD. Starvation-dependent differential stress resistance protects normal but not cancer cells against high-dose chemotherapy. Proc Natl Acad Sci U S A. 2008;105(24):8215-20. PMID: 18378900. PMCID: 2448817.
5. Lee C, Raffaghello L, Brandhorst S, Safdie FM, Bianchi G, Martin-Montalvo A, Pistoia V, Wei M, Hwang S, Merlino A, Emionite L, de Cabo R, Longo VD. Fasting cycles retard growth of tumors and sensitize a range of cancer cell types to chemotherapy. Sci Transl Med. 2012;4(124):124ra27. PMID: 22323820.
6. Lee C, Safdie FM, Raffaghello L, Wei M, Madia F, Parrella E, Hwang D, Cohen P, Bianchi G, Longo VD. Reduced levels of IGF-I mediate differential protection of normal and cancer cells in response to fasting and improve chemotherapeutic index. Cancer Res. 2010;70(4):1564-72. PMID: 20145127. PMCID: 2836202.
7. Safdie FM, Dorff T, Quinn D, Fontana L, Wei M, Lee C, Cohen P, Longo VD. Fasting and cancer treatment in humans: A case series report. Aging (Albany NY). 2009;1(12):988-1007. PMID: 20157582. PMCID: 2815756.
8. Safdie F, Brandhorst S, Wei M, Wang W, Lee C, Hwang S, Conti PS, Chen TC, Longo VD. Fasting enhances the response of glioma to chemo- and radiotherapy. PLoS One. 2012;7(9):e44603. PMID: 22984531. PMCID: 3439413.
9. Cahill GF, Jr. Fuel metabolism in starvation. Annu Rev Nutr. 2006;26:1-22. PMID: 16848698.
10. Mansell PI, Macdonald IA. The effect of starvation on insulin-induced glucose disposal and thermogenesis in humans. Metabolism: clinical and experimental. 1990;39(5):502-10. PMID: 2186256.
11. O'Sullivan U, Gluckman PD, Breier BH, Woodall S, Siddiqui RA, McCutcheon SN. Insulin-like growth factor-1 (IGF-1) in mice reduces weight loss during starvation. Endocrinology. 1989;125(5):2793-4. PMID: 2792010.
12. Ngo TH, Barnard RJ, Tymchuk CN, Cohen P, Aronson WJ. Effect of diet and exercise on serum insulin, IGF-I, and IGFBP-1 levels and growth of LNCaP cells in vitro (United States). Cancer Causes Control. 2002;13(10):929-35. PMID: 12588089.

13. Smith WJ, Underwood LE, Clemmons DR. Effects of caloric or protein restriction on insulin-like growth factor-I (IGF-I) and IGF-binding proteins in children and adults. J Clin Endocrinol Metab. 1995;80(2):443-9. PMID: 7531712.
14. Longo VD, Fontana L. Calorie restriction and cancer prevention: metabolic and molecular mechanisms. Trends Pharmacol Sci. 2010;31(2):89-98. PMID: 20097433. PMCID: 2829867.
15. Fontana L, Vinciguerra M, Longo VD. Growth factors, nutrient signaling, and cardiovascular aging. Circ Res. 2012;110(8):1139-50. PMID: 22499903.
16. Lee C, Longo VD. Fasting vs dietary restriction in cellular protection and cancer treatment: from model organisms to patients. Oncogene. 2011;30(30):3305-16. PMID: 21516129.
17. Protopopescu X, Butler T, Pan H, Root J, Altemus M, Polanecsky M, McEwen B, Silbersweig D, Stern E. Hippocampal structural changes across the menstrual cycle. Hippocampus. 2008;18(10):985-8. PMID: 18767068.
18. Protopopescu X, Tuescher O, Pan H, Epstein J, Root J, Chang L, Altemus M, Polanecsky M, McEwen B, Stern E, Silbersweig D. Toward a functional neuroanatomy of premenstrual dysphoric disorder. Journal of affective disorders. 2008;108(1-2):87-94. PMID: 18031826.
19. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. The British journal of nutrition. 1978;40(3):497-504. PMID: 718832.
20. Jackson AS, Pollock ML, Ward A. Generalized equations for predicting body density of women. Medicine and science in sports and exercise. 1980;12(3):175-81. PMID: 7402053.
21. Siri WE. Body composition from fluid spaces and density: analysis of methods. 1961. Nutrition. 1993;9(5):480-91; discussion , 92. PMID: 8286893.
22. Brozek J, Grande F, Anderson JT, Keys A. Densitometric Analysis of Body Composition: Revision of Some Quantitative Assumptions. Ann N Y Acad Sci. 1963;110:113-40. PMID: 14062375.

APPENDICES

Appendix A: Informed Consent

Appendix C: Informed Consent-Incidental Findings

Appendix D: Dornsife MRI Screen Questionnaire

Appendix E: Health Habits Questionnaire

Appendix G: Mood & Anxiety Questionnaire STAI PANAS

Appendix H: Self-evaluation

Appendix I: Diet Diary

Appendix J: IAPS (International Affective Picture System) Thumbnails

Appendix K: Study Flyer

Appendix L: Diet Regime

Amendments

HS-12-00391-AM001

Multi-cycle diet regimen

HS-12-00391-AM002 **Multicycle**

Dietary Intervention co-PI

We propose to expand inclusion criteria:

- Change from Participants of 18-65 years of age to Participants of 18-70 years of age.
- Change from BMI limit of 19-30 to BMI>19 (remove upper limit).

We will maintain the exclusion criteria to ensure only generally healthy adults are enrolled. The exclusion criteria relevant to the proposed changes include:

- Any major medical condition and chronic diseases
- Drug dependency
- Severe hypertension (systolic BP > 200 mm Hg and/or diastolic BP > 105 mm Hg)

Rationale:

1. People are living longer. The percentage of persons 65 years and over has reached 13.7% in USC (US Census, 2012, http://quickfacts.census.gov/qfd/states/06/06037.html).
2. People are getting heavier. The latest Centers for Disease Control data indicate that more than 31% of the USC adult (20-74 years of age) are considered obese (See Table, CDC).
3. Our study have attracted a lot of interest from the general public, with more than the half respondents were either over the age of 65 and/or with a BMI exceeds 30, majority of which are generally healthy.
4. Based on the preliminary observation and conversations with current participants (with an age range between 28-64 years), the dietary intervention is well received. So far we have not observed any feasibility or safety issues.
5. To broaden the study population will better serve the objective of the study, which is to obtain preliminary estimates of the feasibility, safety and impact of a calorie restricted special diet on adult subjects and to examine the neural correlates of these interventions.

Table: Age-adjusted prevalence of overweight and obesity among U.S. adults, age 20-74 years.

	NHANES III 1988-94 n=16,679	**NHANES 1999-2000 n=4,117**	**NHANES 2001-02 n=4,413**	**NHANES 2003-04 n=4,431**
Overweight or obese (BMI≥ 25.0)	47.0%	55.9%	64.5%	65.7%
Obese (BMI ≥ 30.0)	15.0%	23.2%	30.9%	31.3%

CDC, http://www.cdc.gov/nchs/data/hestat/overweight/overweight_adult_03.htm

Statistical power analysis regarding the Dexa scan

Based on prior published results, an N of 10-20 is sufficient for a medium-term calorie restriction study (Fontana, 2007, PMID: 17389710; Racette, 2006, PMID: 16960025). The lead author of the mentioned papers, Dr. Luigi Fontana, is one of our advisors for this trial. In the above mentioned study, a medium-term calorie restriction regimen led to significant reductions in BMI, body fat and body weight in a study group of 19 volunteers (Table is reproduced from Racette, 2006, PMID: 16960025).

	Weight, kg				**% Fat mass**			
	Mean	**SE**	**SD***	**σ**	**Mean**	**SE**	**SD***	**σ**
Baseline	78.5	2.3	10.0	**0.127**	33.1	1.1	4.8	**0.145**
3 mo CR	75.4	2.4	10.5	0.139	29.6	1.1	4.8	0.162
6 mo CR	73.3	2.4	10.5	0.143	27.9	1.0	4.4	0.158
12 mo CR	71.0	2.4	10.5	0.148	26.7	1.0	4.4	0.165

* SD is calculated form published SE and n=19 (Racette, 2006, PMID: 16960025).

Assuming a sigma of 0.15 (0.14 for weight and 0.15 for % fat mass in above cited study, see Table), a sample size of 20 has the power of 0.99 to detect a difference of 20% with an α of 0.05 (paired t test, two-tailed); and, with n=10, the power is 0.8.

HS-12-00391-AM003

Multicycle Dietary Intervention - OGTT and MRI

2014-01-12 Prolon Trial Amendment

Add procedures:

(1) Oral Glucose Tolerance Test (OGTT). OGTT measures how well the body is able to break down glucose, or sugar. When the participant arrives at the lab, a member of the research team will take a sample of blood by pricking your fingertip or inserting a needle into a vein in your arm. This blood sample will be used to measure the fasting blood glucose level. The participant will drink about 8 ounces (237 milliliters) of a syrupy glucose solution containing 2.6 ounces (75 grams) of sugar. Two hours later, the blood glucose level will be measured again.

(2) MRI scan for liver and visceral fat. IDEAL fat-water separated imaging for liver fat is arguably the best MRI pulse sequence currently available for quantitative fat water separated imaging. It was originally developed by investigators at Stanford and GE Healthcare, and has been validated by investigators at USC on the equipment located at USC Keck Medical Center Healthcare Consultation Center II (HCC-II). It produces 3D maps of fat-signal-fraction, T2*, off-resonance, fat, water, etc. USC investigators have used IDEAL in hundreds of subjects to date, for example, to quantify liver fat content, pancreatic fat content, and/or bone marrow water content. The scan duration will be around 10 minutes. Some participants can experience a claustrophobic sensation during the procedure. The MRI staff will be nearby during MRI scan. The participant can readily communicate with the MRI staff if the participant cannot tolerate the scan.

Rationale:

- The trial has attracted a lot of public interest with interested participants that are overweight (BMI: 25-30) and obese (BMI >30), which is agree with the fact that people are getting heavier. The latest Centers for Disease Control data indicate that more than 31% of the USC adult (20-74 years of age) are considered obese (See Table, CDC).
- Obesity increases the risk of a number of health conditions including cardiovascular diseases (CVD), type 2 diabetes, hypertension, nonalcoholic fatty liver disease, osteoarthritis, and certain types of cancers.
- Oral glucose Tolerance Test measures how well your body is able to break down glucose, or sugar. We are interested to learn whether the diet regimen has an effect on glucose metabolism in normal, overweight and obese participants.
- OGTT is routinely used to screen for type 2 diabetes and is safe with no added risk considering the test (blood draw) included in the trial.
- IDEAL fat-water separated imaging for liver fat by MRI is arguably the best MRI pulse sequence currently available for quantitative fat water separated imaging. It was originally developed by investigators at Stanford and GE Healthcare, and has been validated by investigators at USC on the equipment located at USC Keck Medical Center Healthcare Consultation Center II (HCC-II). It produces 3D maps of fat-signal-fraction, T2, off-resonance, fat, water, etc. USC investigators have used IDEAL in hundreds of subjects to date, for example, to quantify liver fat content, pancreatic fat content, and/or bone marrow water content.
- Optional MRI for fatty liver and visceral fat has no added risk to participants as we already have brain MRI procedure included in the trial.

- These additional tests will not only better serve the objective of the study, which is to obtain preliminary estimates of the feasibility, safety and impact of a calorie restricted special diet, but also to provide preliminary data for a planned Phase II trial that focus on the dietary effect on cardiovascular and fatty liver disease risk in overweight and obese subject.

2014-01-15 IRB

1. AM5.1: Explain why current subjects should not be informed of the changes. Will the new study procedures only apply to newly enrolled subjects?

The new study procedures only apply to newly enrolled subjects.

Why current subjects should not be informed of the changes?

1. The current subjects were recruited with BMIs between 18.5 and 25. New enrollment are open to participants that are overweight (BMI 25-30) and obese (BMI>30).
2. Obesity increases the risk of a number of health conditions including cardiovascular diseases (CVD), type 2 diabetes, hypertension, nonalcoholic fatty liver disease, osteoarthritis, and certain types of cancers.
3. Oral glucose Tolerance Test (OGTT) measures how well your body is able to break down glucose or sugar, and IDEAL fat-water separated imaging for liver fat by MRI is arguably the best MRI pulse sequence currently available for quantitative fat water separated imaging. We are interested to learn whether the diet regimen has an effect on glucose metabolism in normal, overweight and obese participants.
4. The new procedures will be administered matching dieting and lab visit schedule. The results will be compared between before and after diet as well. Thus, the new procedures are not applicable to current participants.

Why current subjects should not be informed of the changes?

2. iStar 24.7: You have not made the informed consent changes to the latest version of the IRB-approved document, dated 06/18/13. This means that all of the previous changes to the consent have not been carried through to the copy you attached at 24.7. You have also not updated the version date of the document to reflect the new changes. Please make the amendment changes to the 06/18/13 version of the consent and revise the version date accordingly. See attached for the 06/18/13 version. Remember to use the track-changes feature in Microsoft Word.

HS-12-00391-AM004

Multicycle Dietary Intervention - Cognitive Arm

We propose

1. to add a new placebo group for cognitive tests;
2. to increase enrollment (AM7);
3. to add nutrition education material in the study package (40.1);
    a. Nutrition Info 1:
4. to update the food brochure to reflect an supplemental pill in the dietary intervention (AM6, 40.1);
5. to add new research personnel (40.1);

1. Add a new placebo group for cognitive tests:

Rationale: The mid-term review of the preliminary results, we identified some interesting Diet-specific effect on cognitive functions. Although it is not known that the cognitive test can be learned or influenced by dietary intervention alone, we propose to add a placebo group (N=20, with a margin of 50%, 30 total), to control for our Diet group. The participant will

- be asked to maintain their own diet;
- be provided with some nutrition information (see attached documents adopted from USDA 10 Tips Nutrition Education Series);
- be asked to taste-test the foods that are included in the Diet regimen;
- receive dietary supplements;
- be asked to participate in the cognitive tests that matches the schedule of the diet group;

2. Increase enrollment

Rationale: Based on our analysis, our original estimation of the margin is appropriate. However, due to the possible seasonal variations in factors such as diet and physical activities, etc. that may influence the cognitive test outcomes, we propose to increase the enrollment of Diet arm of the study to match the new placebo group (N=20). Considering the dropout/non-compliance/technical difficulty (we expect the margin to be 50%), we will increase the enrollment by 64 (20*(1.5+0.1)+20*1.5).

3. Nutrition Education -USDA 10 Tips Nutrition Education Series and information sources on nutrition

Rationale: The participant will also be provided basic nutrition information that is adopted from USDA website, such that the placebo group serves a better control group for the Diet group in cognitive tests.

- Choose MyPlate - 10 tips to a great plate (PDF), http://www.choosemyplate.gov/food-groups/downloads/TenTips/DGTipsheet1ChooseMyPlate.pdf
- Build a healthy meal (PDF), http://www.choosemyplate.gov/food-groups/downloads/TenTips/DGTipsheet7BuildAHealthyMeal.pdf
- USDA, http://www.choosemyplate.gov/
- Nutrition for Everyone- Nutrition Basics, http://www.cdc.gov/nutrition/everyone/basics/index.html
- General Nutrition and Health Information, http://fnic.nal.usda.gov/dietary-guidance/general-nutrition-and-health-information

4. Add NR-1 supplement tablets

Rationale: We propose to add the supplement tablet, which is a blend of vegetable powder, vitamins and minerals, all of which are generally regarded as safe (GRAS). The supplement tablet will provide a~25% Daily Value of vitamins and minerals. Two supplement tables will be given on day 1 followed by 1 tablet each for the days 2 to 5. This addition will ensure adequate micronutrients during the dieting period. Moreover, the new placebo group, being provided with this supplement, may serve as a better placebo control arm in the cognitive test as compared to the Diet group.

An updated food insert is included in the study package. **40.1**

5. New personnel:

- Mahshid Shelechi
- Angel Maree

Follow-up survey

AM7. Number of Subjects

Add a new placebo group for cognitive tests N=20
Add to the diet group N=20

With a margin of 50% (or dropout, non-compliance and technical difficulty)
Total additional enrollment = 64

Rationale: The mid-term review of the preliminary results, we identified some interesting Diet-specific effect on cognitive functions. Although it is not known that the cognitive test can be learned or influenced by dietary intervention alone, we propose to add a placebo group (N=20, with a margin of 30%, 26 total), to control for our Diet group.
Based on our analysis, our original estimation of the margin is appropriate. However, due to the possible seasonal variations in factors such as diet and physical activities, etc. that may influence the cognitive test outcomes, we propose to increase the enrollment of Diet arm of the study to match the new placebo group (N=20).

AM15. Other

Added Nutrition Info documents (item 1 and 2), which are adopted from the USDA 10 Tips Nutrition Education Series, to be used for the new Placebo group and matching Diet group:

1. "Nutrition Info 1" (see attached PDF file in 40.1): Choose MyPlate - 10 tips to a great plate
   Source: http://www.choosemyplate.gov/food-groups/downloads/TenTips/DGTipsheet1ChooseMyPlate.pdf

2. Nutrition Info 2 (see attached PDF file in 40.1): Build a healthy meal
   Source: http://www.choosemyplate.gov/food-groups/downloads/TenTips/DGTipsheet7BuildAHealthyMeal.pdf

3. Updated the Appendix L_DietRegime.docx

The update reflects the addition to the regimen of NR-1 supplement tablets.
Rationale: We propose to add supplement tablets, which is a blend of vegetable powder, vitamins and minerals, all of which are generally regarded as safe (GRAS). The supplement tablet will provide a~25% Daily Value of vitamins and minerals. Two supplement tables will be given on day 1 followed by 1 tablet each for the days 2 to 5. This addition will ensure adequate micronutrients during the dieting period. Moreover, the new placebo group, being provided with this supplement, may serve as a better placebo control arm in the cognitive test as compared to the Diet group.

AM1.3 Will the changes you are making affect the accuracy of the study abstract? Yes
Revise the abstract

The diet part of the study is designed as a randomized cross-over trial, includes two arms: a Control arm and a multi-cycle special 5-day dietary regimen (Diet, 3 cycles) arm. After 3 cycles, the Control and Diet groups are crossed over such that the Control group will under-go dieting and the Diet group will return to normal diet.

There is one additional control group (Placebo group). The participants will be given nutrition information, food taste tests and cognitive function tests.

Statistical methods: Paired samples t-test and Mann-Whitney test will be used to compare between Control and Diet groups as well as pre- and post-diet values.

10.1
Maximum number of subjects: 68+64=132
132 subjects total.
The study will include Control, diet intervention (Diet) and the Placebo groups.
With an anticipated dropout rate of 50% (due to long study period of 6 months and participants' adherence to the regimen), and a 20% potential technical problem (including declining certain tests by the participants), a total of 132 subjects will be recruited for the each group.

	Control	Diet(ori)	Placebo
Sample size	20	20+20*	20
Dropout/	10 (50%)	20 (50%)	10 (50%)
Technical problem	4 (20%)	8 (20%)	0**
Subtotal	34	68	30

* Control vs. Diet comparison (20 participants each group); Placebo vs. Diet comparison (20 participants each group).
** We don’t expect there will be any dropout due to technical problem for the Placebo group.

12.1
For the Placebo group
The participants will be informed about the diet and the clinical procedures involved in the diet group. However the participant will only be given food samples for tasting test without altering their normal

diet. The participants will be given nutrition information and be asked to participant in the cognitive tests.

12.2
A total of 132 participants will be enrolled in this study (see 10.1 for detail).

Using serum IGF-1 level as a primary outcome, the effects will be determined by comparing the Control and Dieting as well as before and after the dietary interventions. The probability is 70 percent that the study will detect a treatment difference at a 0.05 significance level, if the true difference between Dieting and Control is more than 25% (see Note).
Note, Sample size analysis is based on the adult serum IGF-1 reference values (Leite, 2011, PMID: 12876414): a Mean of 194 ng/mL (male and female age between 26-40 years) and a SD value of 97 ng/mL (maximum SD of the age group).

2014-04-12 IRB request

Some places of the amendment do not mention the requirement of the new "placebo" group to take dietary supplements and other places indicate that the "placebo" group will be taking dietary supplements. Please clarify if the new "placebo" group will have to take dietary supplements. If so, the **abstract, protocol, and informed consent** must be revised so that they all reflect the same information.

- ✓ Updated 01 Abstract
- ✓ **Updated 12 Methods and Procedures - Prospective Studies;** "A Placebo group will be recruited to control for possible bias in the cognitive test. The participants will be given nutrition information, participating in food taste tests, taking dietary supplements and performing cognitive function tests."
- ✓ **Update 22a; Blood draw through venipuncture volume**
- ✓ Are the participants in the new "placebo" group healthy volunteers? Updated in AM5.1
- ✓ Revise 27.1 to indicate venipuncture risks.

Venipuncture risks and OGTT risk, http://www.nlm.nih.gov/medlineplus/ency/article/003466.htm

Some people feel nauseated, sweaty, light-headed, or may even feel short of breath or faint after drinking the glucose. Tell the staff if you have a history of these symptoms related to eating sugar. Serious side effects of this test are very uncommon.

When the needle is inserted to draw blood, some people feel moderate pain. Others feel only a prick or stinging. Afterward, there may be some throbbing.

Veins and arteries vary in size from one patient to another and from one side of the body to the other. Obtaining a blood sample from some people may be more difficult than from others.

Other risks associated with having blood drawn are slight but may include:

- Excessive bleeding
- Fainting or feeling light-headed
- Hematoma (blood accumulating under the skin)
- Infection (a slight risk any time the skin is broken)

- ✓ Also check 27.2 to indicate that a qualified person will be drawing the blood.

- ✓ Upaded 23.3; Venipuncture for the OGTT will be performed by individuals certified and privileged to perform the procedure.
- ✓ Upadated 10.3.1, with revised upper age.

HS-12-00391 Renewal

2014-05-18

2.2

The study has added a new arm (IRB approval: 04/20/14) that serves a Placebo group to the Diet group.

2.3

Considering the fact that this study involves a 3-month control (or observation pahse) and a 3-month dieting phase (6 month in total for an individual) and that this study does not compensate the participants financially, we have received very good response from the community. Despite the limits on the number of participants per lab visit (2-3 participants per visit in the morning only due to fasting blood draw requirement) and limited MRI capacity on campus, we ave enrolled, we have enrolled 65 participants in the study since the beginning of the study (IRB approval: 03/28/13). We do not experience low enrollment and are on track to complete the study.

3. Subject Withdrawals and Complaints

There are 6 participants withdrew from the study.
The reasons are as follows:
1 participant withdrew due to family objection.
1 participant withdrew after a traffic incidence (unrelated to study)
1 participant withdrew due to family reason (new born baby) and scheduling difficulty
1 participant (female) withdrew due to scheduling difficulty and potential effects of low calorie diet on her menstrual cycle.
2 participants thought the dieting is too hard.

5. Summary of Research Progress

5.1 brief summary of research progress

We have officially consented 59 participants with 6 more being scheduled for lab visits (total 65).
This is a randomized crossover dietary intervention study that involves 2-3 month of observation period for the Control group and 3 cycles of dieting (5 days dieting per month each cycle) for the Diet group.
We have 48, 36 and 30 participants successfully completed 1, 2 and 3 cycles of dieting, respectively.
We have experienced very few withdraws, only 2 of which is directly directly related (being too hard for the participants) and very few disqualifications(6 total, due to noncompliance, missed appointments, and technical difficulty - could not draw blood).
The survey results showed that the diet regimen is safe and feasible, and the diet is well-accepted by the participants.

We are on track to perform biochemical, cognitive and MRI analyses proposed in the initial application. Our preliminary data analyses on the biochemistry, cognitive tests and MRI, have already revealing interesting results.

10. Conflict Of Interest Information

10.6 Management of Potential Conflict of Interests: What will be done to manage the potential conflict(s) of interest disclosed above? (check all that apply)

	Name
☑	All relevant publications, proposals and presentations will contain a statement disclosing support received from, or financial interests in, any source outside of USC.
☑	All informed consent documents in the context of human subjects research vvill disclose support received from, or financial interests in, any source outside of USC.
☑	All person(s) with a potential conflict will not be involved in the informed consent process.
☐	All person(s) with a potential conflict will not participate in any intellectual property negotiations, or other contractual negotiations, between USC and the entity that the person(s) have an financial or management interest in.
☐	A qualified and non-conflicted party will oversee all data collection, analyses, and result interpretation.
☐	The person(s) with a potential conflict will divest from all financial interests.
☐	The person(s) with a potential conflict will give up all forms of management roles or sever outside relationships that create the potential conflict.
☐	All person(s) with a potential conflict must obtain departmental approval for all proposed consulting that compensation to be paid represents fair market value as defined by the Relationships with Industry policy.
☐	An independent IRB will be consulted to approve the management plan proposed by the CIRC.
☐	All person(s) with a potential conflict will report annually to their dean and chair, with a written copy provided to the CIRC, on the status of the studies and your relationship with the entity with which the conflict exists.
☐	Other (explain below)

Abstract

Modern chemotherapy can improve the quality of life of cancer patients via palliation of cancer-related symptoms, and in many malignancies can significantly extend survival as well. However, the toxicity to normal cells poses a major clinical challenge, particularly when malignant cells have acquired resistance to chemotherapy. Evidence from bio-gerontology research from our laboratory and others have showed that short-term fasting/starvation (STS) can improve the efficacy of chemotherapy by protecting normal cells and tissues and potentially sensitizing malignant cells to chemo drugs. Cancer patients, however, may not be able or willing to incorporate fasting into existing chemotherapy. The objective of the study is to obtain preliminary estimates of the feasibility, safety and impact of a calorie restricted special diet on adult subjects and to examine the neural correlates of these interventions. We hypothesize that the specially designed dietary regimen can induce similar changes, including the levels of glucose, IGF-1, IGFBPs and other bio-markers, as with short-term fasting. The diet part of the study is designed as a randomized cross-over trial, includes two arms: a Control arm and a multi-cycle special 5-day dietary regimen (Diet, 3 cycles) arm. After 3 cycles, the Control and Diet groups are crossed over such that the Control group will under-go dieting and the Diet group will return to normal diet. There is one additional control group (Placebo group). The participants will be given nutrition information, participating in food taste tests, taking dietary supplements and performing cognitive function tests. Statistical methods: Paired samples t-test and Mann-Whitney test will be used to compare between Control and Diet groups as well as pre- and post-diet values.