Supplementary Data

Supplementary Methods

Study eligibility criteria

Primary eligibility requirements included body mass index (BMI) between 20.0 and 30.0 kg/m^2, age between 19 and 30, stable weight (change less than $\pm 10\%$) for 3 months immediately prior to the study, and no history of metabolic disorders (*e.g.*, non-diabetic), cardiovascular disease (CVD), or thyroid dysfunction. Additional exclusion criteria were consistent with prior studies[1] and are listed below:

1. Pregnant (assessed by urine human chorionic gonadotropin [hCG] test for female participants), planning to become pregnant, breastfeeding.
2. Taking any medications to regulate blood sugar or lipids, or medications for metabolic conditions.
3. History of metabolic disorders or CVD, including, but not limited to, diabetes, peripheral artery disease, and thyroid dysfunction.
4. History of eating disorders, including, but not limited to, anorexia nervosa and bulimia nervosa.
5. History of an acute or chronic inflammatory disorder.
6. Heavy alcohol consumption (more than 15 drinks/week)
7. Tobacco use tobacco (assessed by urine cotinine test) or recreational drug use within past 3 months.
8. Unwillingness to abstain from tobacco, alcohol, or recreational drugs for duration of study.
9. Unwillingness to abstain from anti-oxidants for duration of study. These include vitamin supplements, daily vitamins, resveratrol, minerals, or other herbal supplements.
10. Unwillingness to eat only study-provided food during pre-conditioning and intervention periods.
11. Unstable weight (change greater than $\pm 10\%$) in 3 months immediately prior to the study.
12. Food allergies.
13. Dietary restrictions (*e.g.*, vegetarianism).
14. More than moderate physical activity (*i.e.*, < 3 hr/week of light exercise sessions for the past 3 months).

Eligibility for the study was assessed following a physical examination and patient history performed by a study physician.

Sample size

The sample size was determined by power analyses and available study budget. We estimated that at a two-sided p value of 0.05 and with 80% power, we would need 16 participants to complete the study to detect an effect size of 0.75 standard deviations. The predicted effect size was estimated based on prior studies.[2] To allow for participant dropout and still maintain power, we enrolled an additional eight participants (24 total).

Blinding

Study participants were randomly assigned by an independent statistician to complete either the intermittent fasting (IF) or the IF plus anti-oxidant supplementation (IFAO) study trials first. Study participants and study investigators were blinded to the trial order assignments (*i.e.*, double blinded). The blind was only broken for statistical analysis upon completion of the study and all downstream assays.

Additional study design details

For the duration of their trial period, participants were instructed to maintain their normal habits of sleep, exercise, and other habitual activities. They were instructed to avoid strenuous exercise and not to consume any alcohol, tobacco, recreational drugs, resveratrol, vitamins, minerals, or other non-study anti-oxidant supplements.

All meals were provided to the participants by the metabolic kitchen at the Clinical Research Center of the University of Florida with the exception of the 2-week washout period between trial periods. Participants were encouraged to eat all study-provided food, but instructed to leave food uneaten if it made them uncomfortable. During the study trial periods, participants were instructed to not eat additional food outside of the study-provided food. Participants were provided a daily food log to record supplemental food intake and estimates of the amount of study food not consumed. Participants were allowed to consume calorie-free beverages, tea, black coffee, sugar-free gum, and were encouraged to drink water as desired.

Vital signs (blood pressure, pulse, respiration rate [RR], weight, and temperature) were measured weekly along with administration of a safety survey to identify potentially hazardous side effects. Body weight was measured weekly to ensure consistent weight and to allow for adjustment of caloric intake to maintain weight.

Study data were managed using the REDCap electronic data capture platform hosted at the University of Florida.[3]

Peripheral blood mononuclear cell isolation

Ten milliliters of whole blood was collected in an EDTAVacutainer tube and incubated at room temperature in 40 mL of red blood cell (RBC) lysis buffer (89.9 grams NH_4Cl, 10.0 grams $KHCO_3$, 2.0 mL of 0.5 M EDTA in 1 L of ddH_2O at pH 7.3). Cells were pelleted at $600 \times g$ for 10 min at 4°C, and the pellet was resuspended in 1 mL of RBC lysis buffer. Cells were then pelleted at 3000 rpm, and washed twice in phosphate-buffered saline (PBS). Following removal of supernatant, cell pellets were stored at -80°C for storage.

qPCR primer sequences

qPCR primer sequences are provided below. All qPCR primers were validated by serial dilutions of input cDNA across a 1000-fold dilution range and all primers had $> 80\%$ efficiency.

Primer	*Sequence (5′ to 3′)*
18S-FWD	GTAACCCGTTGAACCCCATT
18S-REV	CCATCCAATCGGTAGTAGCG
SOD2-FWD	GCTCCGGTTTTGGGGTATCTG
SOD2-REV	GCGTTGATGTGAGGTTCCAG
SIRT1-FWD	AAGTTGACTGTGAAGCTGTACG
SIRT1-REV	GGACATCGAGGAACTACCTGAT
SIRT3-FWD	TGGAAAGCCTAGTGGAGCTTCTGGG
SIRT3-REV	TGGGGGCAGCCATCATCCTATTTGT
TFAM-FWD	ATGGCGTTTCTCCGAAGCAT
TFAM-REV	CAGATGAAAACCACCTCGGTAA

FWD, forward; REV, reverse/

Statistical methods

We compared the percent change in levels from the start of the IF to the end of the IF by computing percent change and then applying a one-sample, two-sided t-test. Note that this comparison is an observational one, in that the order is fixed (normal diet first).

For gene expression analyses, we compared differences between treatment periods using the following approach. Let $D = 0.5*[100*(Y_2 - X_2)/X_2] - 0.5*[100*(Y_1 - X_1)/X_1]$, where X_1 and X_2 are the gene expression levels at the start of the first and second periods of IF, and Y_1 and Y_2 are the gene expression levels at the end of the first and second periods of IF, respectively. The two orderings were compared by a two-sample Student *t*-test. This is the two-sample method for crossover studies. The observed difference in the average D values for the two groups is an unbiased estimate of the true average effect size in periods 1 and 2, irrespective of carryover effects or period effects, and this un-biasedness is retained even when an unequal number are assigned to each group. The analysis of gene expression was performed separately for each gene.

For nucleotide oxidation analyses, we compared differences between the log-transformed oxidation ratios before and after each treatment (one-sample *t*-test) and this difference between treatments (two-sample *t*-test). The analysis of nucleotide oxidation was performed separately for each marker (8-oxo-7,8-dihydroguanosine [8-oxo-G] vs. 8-oxo-7,8-dihydro-2′-deoxyguanosine [8-oxo-dG]).

Survey Documents

Survey A

In the following survey, ‘‘feasting days’’ indicates the days during which you were asked to eat three larger meals with snacks. The ‘‘fasting days’’ were days where you were asked to eat only one meal during the afternoon. The ‘‘intermittent fasting diet’’ includes both the fasting and feasting days.

Please rate the degree to which you agree with each of the following statements (1 = Strongly disagree, 2 = Disagree, 3 = Neither agree nor disagree, 4 = Agree, 5 = Strongly agree). You can also indicate ‘‘6 = N/A’’ if the question does not apply to you. You can indicate ‘‘7 = No Response’’ if you choose not to answer a question or do not feel comfortable answering a question. Leaving a question blank will also indicate your desire not to answer a question. Please note that your responses will only be seen by study investigators and that your responses will not affect your compensation for this survey.

How strongly do you agree with the following statements?

1. Feasting days were more difficult than fasting days.
2. It was difficult to eat all the required food on most feasting days.
3. It was difficult to not eat much food on most fasting days.
4. On feasting days, I often could not finish all the food provided.
   a. I would eat less study food on feasting days in the second half of the study than the first half of the study.
5. On fasting days, I often would eat other food.
   a. I would eat more (non-study) food on fasting days in the second half of the study than the first half of the study.
6. The feasting days became more difficult as the study went on.
7. The fasting days became more difficult as the study went on.
8. On most fasting days, I consumed all my food in one short sitting.
9. On most fasting days, I consumed my food throughout the 2–6 pm time period.
10. While, on the intermittent fasting diet, I had less energy than usual.
11. I had less energy than usual on the feasting days.
12. I had less energy than usual on the fasting days.
13. ‘‘Feasting’’ made tasks at my work, school or at home more difficult.
14. ‘‘Fasting’’ made tasks at my work, school or at home more difficult.
15. I had difficulty falling asleep, staying asleep, or waking up on nights following a feasting day.
16. I had difficulty falling asleep, staying asleep, or waking up on nights following a fasting day.
17. I wish there were more meal options.
18. I wish meals had more flavor.
19. It was inconvenient coming in to the CRC for food two to three days of the week.
20. I enjoyed having meals prepared.
21. I would rather have to feast for several days and then fast for several days instead of alternating every day.
22. The foods provided in the study were very different from foods I ate before the study began.
23. I felt different during the pre-conditioning periods (week before intermittent fasting diet) compared to before beginning the study.
24. I exercised more in the 2 months before the study than during the study. I changed my exercise habits because of the diet.
25. I exercised more during the first half of the study than the second half of the study.
26. I took vitamins or supplements during the study other than those provided.
27. This diet was more difficult than other diets I have previously tried.

28. My overall quality of life was improved during the intermittent fasting diet.
29. If recommended by their doctor, I think many people would follow the intermittent fasting diet.
30. I would recommend this study to a friend.
31. I would participate in this study again.

For the following questions, please provide your thoughts. You may leave any question blank that you do not feel comfortable answering. You may write as much or as little as you wish. If you need additional space, feel free to write on the back or on additional sheets of paper [for paper version only].

1. What was most helpful in getting you through the fasting days?
2. What was most helpful in getting you through the feasting days?
3. If you felt different during the pre-conditioning period (as indicated on question number 23), could you describe how you felt different?
4. If your exercise habits were different between the first and second half of the study (as indicated on question number 25), please describe what may have caused this change.
5. Could you think of any changes to the intermittent fasting diet such that it might be more feasible for people to practice?
6. Any other comments?

Survey B

In the following survey, ''feasting days'' indicates the days during which you were asked to eat three larger meals with snacks. The ''fasting days'' were days where you were asked to eat only one meal during the afternoon. The ''intermittent fasting diet'' includes both the fasting and feasting days.

Please rate the importance of each of the following factors in your decision to withdraw from the study (1 = Not important, 2 = Somewhat important, 3 = Neither important nor unimportant, 4 = Somewhat important, 5 = Very important). You can also indicate ''6 = N/A'' if the question does not apply to you. You can indicate ''7 = No Response'' if you choose not to answer a question or do not feel comfortable answering a question. Leaving a question blank will also indicate your desire not to answer a question. Please note that your responses will only be seen by study investigators and that your responses will not affect your compensation for this survey.

Please rank the importance of the following factors in your decision to withdraw:

1. Difficulty of fasting days
2. Difficulty of feasting days
3. Meals not varied enough
4. Meals not flavorful
5. Meals different than what I typically eat
6. Restriction of exercise schedule
7. Restriction of vitamins/supplements
8. Vacation/travel schedule
9. Inconvenience of making several trips to the Clinical Research Center (CRC) each week
10. Restriction of alcohol/drug/tobacco use
11. Compensation was insufficient.

For the following questions, please provide your thoughts. You may leave any question blank that you do not feel comfortable answering. You may write as much or as little as you wish. If you need additional space, feel free to write on the back or on additional sheets of paper [for paper version only].

Please list any other factors that may have been important in your decision to withdraw.

Could you think of any changes to the intermittent fasting diet such that it might be more feasible for people to practice?

Any other comments?

References

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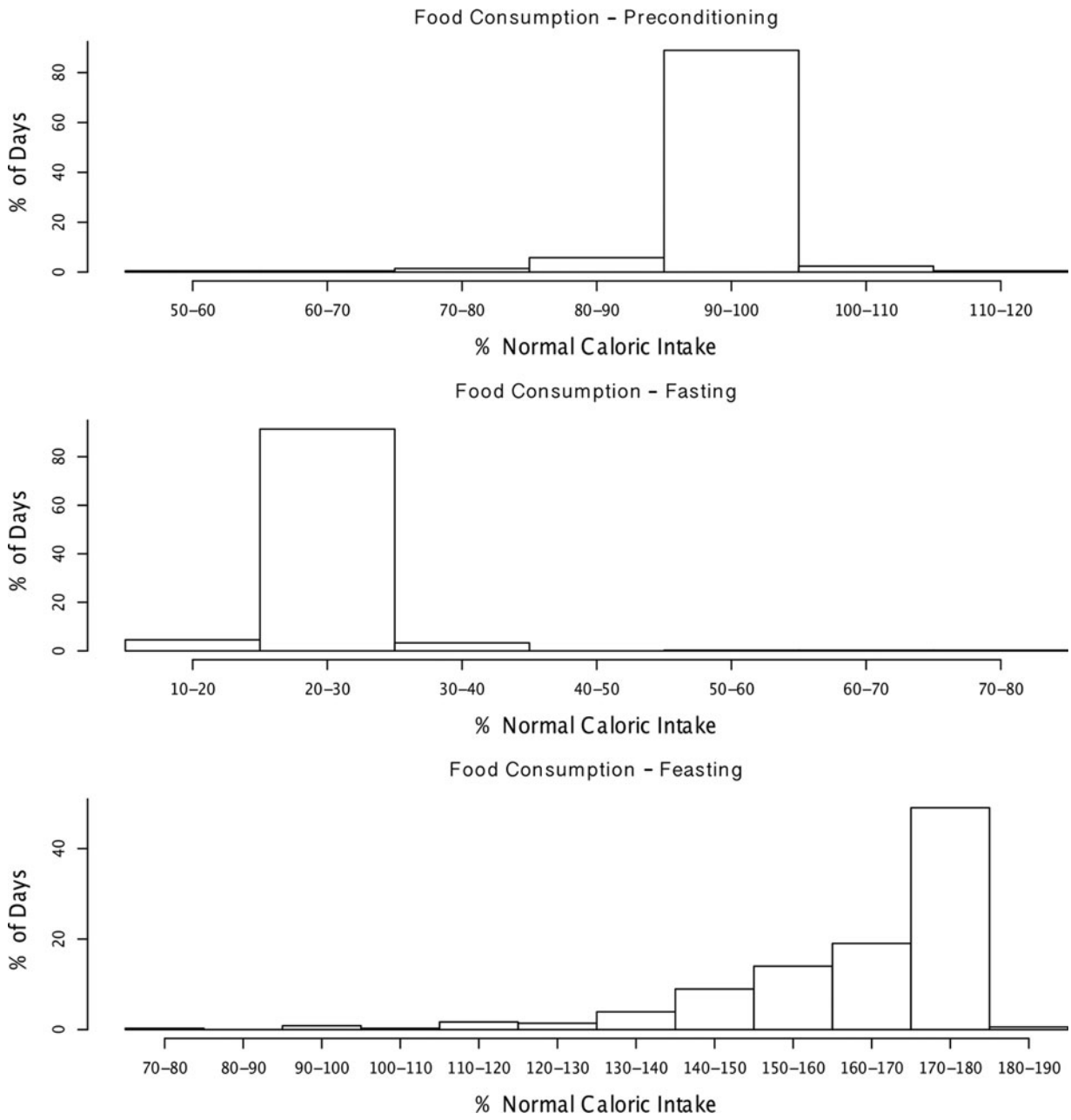
3. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG, Research electronic data capture (REDCap)—A metadata-driven methodology and workflow process for providing translational research informatics support, J Biomed Inform 2009;42:377–381.



SUPPLEMENTARY FIG. S1. Distributions of daily caloric intake during pre-conditioning, fasting, and feasting arms of the study.

Supplementary Table S1. Reasons for Study Withdrawal

	Reason	*Important (%)*	*Not important (%)*
Initermittent fasting diet related	Fasting days were too difficult	14	57
	Feasting days were too difficult	14	57
Trial-related restrictions	Restricted my travel plans	71	29
	Too little meal flavor	57	43
	Too little meal variety	57	43
	Meals differed from my normal diet	57	43
	Restricted my exercise	57	43
	Trips to hospital inconvenient	57	43
	Restricted my alcohol, tobacco, or drug use	29	71
	Restricted my vitamin intake	29	71
	Compensation was insufficient	0	100

The percentage of study of participants who withdraw from the study ranking the listed reasons for withdrawal as important or not important. Trial related restrictions represent unique requirements of trial participation that are distinct from the prescribed intermittent fasting dieting regimen.

Supplementary Table S2. Vital Sign Measurements

	IF treatment arms						*IFAO treatment arms*					
	Week 1		*Week 2*		*Week 3*		*Week 1*		*Week 2*		*Week 3*	
	Average	*Range*	*Average*	*Range*	*Average*	*Range*	*Average*	*Range*	*Average*	*Range*	*Average*	*Range*
Blood pressure (systolic)	116.0	96–129	118.6	92–136	118.7	96–140	118.2	92–138	120.6	93–138	118.1	91–140
Blood pressure (diastolic)	70.5	53–88	69.5	58–78	70.5	63–80	70.6	56–84	70.3	55–87	69.1	57–87
Respiration rate (RR)	17.0	14–20	16.6	12–20	17.7	14–24	17.0	16–20	17.4	14–20	16.3	12–20
Pulse (bpm)	71.3	51–90	71.6	53–86	75.9	54–95	73.7	64–85	77.9	60–100	71.7	59–81
Temp (°C)	36.4	36.0–37.0	36.5	36.1–37.1	36.6	36.0–37.0	36.4	35.9–36.8	36.5	36.0–37.2	36.6	36.0–37.3

Vital signs (blood pressure, RR, pulse, and temperature) are shown for enrolled participants throughout each of the treatment arms in the study. Vital signs were obtained once per week during these treatment arms.

IF, intermittent fasting; IFAO, IF with anti-oxidant supplementation.

Supplementary Table S3. Comprehensive Metabolic Profile of Participants on the Initermittent Fasting Diet with and without Anti-Oxidants, before and after Treatment for Each Treatment Arm

	IF				*IFAO*			
	Pre-treatment		*Post-treatment*		*Pre-treatment*		*Post-treatment*	
	Average	*Range*	*Average*	*Range*	*Average*	*Range*	*Average*	*Range*
Glucose, serum (mg/dL)	87.4	65–101	85.4	76–113	87.6	74–109	85.1	66–110
Albumin, serum (g/dL)	4.4	3.8–5.2	4.3	3.6–5.5	4.3	4–4.7	4.3	3.7–4.7
Protein, total, serum (g/dL)	7.1	6.7–7.2	7.0	6.2–7.9	7.1	6.6–7.7	7.0	6.3–7.9
Calcium, serum (mg/dL)	9.4	8.6–10	9.0	8.8–9.8	9.3	8.8–9.9	9.3	8.7–9.8
Sodium, serum (mEq/L)	139.1	136–141	138.2	135–141	138.8	135–144	138.0	135–141
Potassium, serum (mEq/L)	4.7	3.9–5.3	4.0	3.8–4.5	4.1	3.9–4.5	4.0	3.7–4.7
Carbon dioxide, total (mmol/L)	26.8	23–31	27.3	23–30	26.5	22–32	26.7	24–29
Chloride, serum (mmol/L)	102.4	100–106	102.7	99–105	102.2	99–106	102.6	101–104
Blood urea nitrogen (mg/dL)	14.2	9–19	12.8	8–18	14.4	8–19	12.3	7–17
Creatinine, serum (mg/dL)	0.8	0.5–1.2	0.8	0.5–1.1	0.8	0.5–1.0	0.8	0.5–1.1
Alkaline phosphatase (IU/L)	70.1	46–106	69.5	47–121	71.3	52–125	67.7	43–110
Alanine aminotransferase (IU/L)	18.2	6–41	18.9	7–52	15.0	6–30	13.7	8–26
Aspartate aminotransferase (IU/L)	19.2	10–29	22.3	11–68	19.4	15–29	20.1	12–34
Bilirubin, total (mg/dL)	0.6	0.2–1.8	0.7	0.2–2.3	0.6	0.2–1.3	0.7	0.3–1.8

Supplementary Table S4. Pill Compliance Was Assessed by Counting the Number of Remaining Pills Returned by Participants at the End of Each Treatment Arm

Pill compliance (% of participants)			
Compliance level	*Vitamin C*	*Vitamin E*	*Placebo*
100%	41.2	47.1	29.4
90%–99%	23.5	29.4	29.4
80%–89%	11.8	5.9	2.9
<80%	0.0	0.0	2.9
Not reported	23.5	17.6	35.3

Pill bottles were returned approximately 80% of the time.

Supplementary Table S5. Full Quality-Of-Life Survey Results For Participants Who Completed The Study (Table 5A) And Participants Who Withdrew (Table 5B)

Table S5A. Perceived Challenges of Intermittent Fasting Diet and Effects on Quality of Life ($n=17$)

	Disagree (%)	*Neutral (%)*	*Agree (%)*
General Assessment of IF Diet			
Feasting days were more difficult than fasting days	55	12	29
Feasting days became more difficult with time	47	12	41
Unable to finish food on feasting days	12	12	76
Fasting days were difficult because of the limited amount of food	24	12	65
Consumed all food in one sitting between 2:00–6:00 pm during fasting days	24	6	71
If recommended by a physician, people would follow the IF diet	41	35	18
Would recommend the IF diet to a friend	18	12	71
IF diet was more difficult than previous diets tried	24	6	71
Perceived effects on activities of daily living			
Quality of life improved on the IF diet	41	24	35
Felt less energy on the IF diet (overall)	41	24	35
During feasting days	82	18	0
During fasting days	41	12	47
Feasting made ADLs difficult	59	6	35
Fasting made ADLs difficult	35	6	59
Feasting interfered with sleep	82	18	0
Fasting interfered with sleep	64	18	12
Self-reported diet and exercise changes			
Exercised more in the 2 months prior to the study	41	18	41
Changed exercise habits because of the diet	59	24	18
Exercised more in the first half of the study	82	12	6
Trial-related considerations			
It was inconvenient coming to the hospital for food	47	12	41
I enjoyed having meals prepared	12	0	88
I wish meals had more flavors	6	6	88
I wish there were more meal options	0	0	100

Table S5B. Reasons for Withdrawal from Study ($n=6$)

	Not Important (%)	*Important (%)*
IF diet related		
Fasting days were too difficult	57	14
Feasting days were too difficult	57	14
Trial-related restrictions		
There was not enough meal variety	43	57
There was not enough flavor in the meals	43	57
The meals differed from my normal diet	43	57
The diet plan restricted my exercise	43	57
The diet plan restricted my vitamin intake	71	29
The diet plan restricted my travel plans	29	71
The diet plan restricted my alcohol, tobacco, or drug use	71	29
Trips to the hospital were too inconvenient	43	57
Compensation was insufficient	100	0

IF, intermittent fasting; ADLs, activities of daily living.