Title: Time-Restricted Feeding Improves Markers of Cardiometabolic Health in Physically Active College-Age Men: A 4-week randomized pre-post pilot study

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Abbreviation List

AMPK  AMP Activated Protein Kinase
CRP   C-Reactive Protein
DBP   Diastolic Blood Pressure
FFM   Free Fat Mass
GSH   Glutathione
HDL-c High Density Lipoprotein Cholesterol
HGH   Human Growth Hormone
LDL-c Low Density Lipoprotein Cholesterol
NOx   Total Nitrate/Nitrite
PAR-Q Physical Activity Readiness Questionnaire
RMR   Resting Metabolic Rate
SBP   Systolic Blood Pressure
SD    Standard Deviation
SE    Standard Error
SOD   Superoxide Dismutase
TAG   Triglycerides
TC    Total Cholesterol
TRF   Time Restricted Feeding
VAS   Visual Analog Scale
Abstract

Time restricted feeding (TRF) has been shown to improve body composition, blood lipids, and reduce markers of inflammation and oxidative stress. However, most of these studies come from rodent models and small human samples, and it is not clear if the benefits are dependent upon a caloric deficit, or the time restriction nature of TRF. Based off of previous research, we hypothesized that humans following an ad libitum TRF protocol would reduce caloric intake and this caloric deficit would be associated with greater improvements in cardiometabolic health including blood pressure, body composition, blood lipids, and markers of inflammation and antioxidant status compared to an isocaloric TRF protocol. The purpose of this study was to: 1) examine the impact of TRF on markers of cardio-metabolic health and antioxidant status and 2) determine if the adaptations from TRF would differ under ad libitum compared to isocaloric conditions. Twenty three healthy men were randomized to either an ad libitum or isocaloric 16:8 (fasting: feeding) TRF protocol. A total of 22 men completed the 28-day TRF protocol (mean ± SD; age: 22 ± 2.5 yrs; height: 178.4 ± 6.9 cm; weight: 90.3 ± 24 kg; BMI: 28.5 ± 8.3 kg/m²).

Fasting blood samples were analyzed for glucose, lipids, as well as adiponectin, human growth hormone, insulin, cortisol, c-reactive protein, superoxide dismutase, total nitrate/nitrite, and glutathione. Time restricted feeding in both groups was associated with significant (p < 0.05) reductions in body fat, blood pressure, and significant increases in adiponectin and HDL-c. No changes in caloric intake were detected. In summary, the results from this pilot study in metabolically healthy, active young men, suggest that TRF can improve markers of cardiometabolic health.

Keywords: diet; intermittent fasting; inflammation; oxidative stress; antioxidants, weight loss
1. Introduction

Dietary interventions such as caloric restriction, ketogenic and/or carbohydrate restricted diets and high protein diets have been extensively practiced and researched in relation to their potential impact on body composition [1] and other markers of cardiometabolic health. These diets either directly or indirectly aim at reducing total calorie intake for the purpose of creating a negative energy balance and thus, creating a calorie deficit. Several studies have reported health benefits associated with dietary caloric restriction including improved apoptosis, improved hormone sensitivity/secretion, and reduced oxidative stress [2-7]. An energy deficit increases activity of AMP activated protein kinase (AMPK) as well as various sirtuin proteins which upregulate beneficial metabolic effects including (but not limited to) enhanced lipolysis and glucose metabolism, and reduced inflammation [8].

Fasting may be one method to achieve dietary caloric restriction [2, 3]. Intermittent fasting involves periods of abstinence from calorie intake from food or beverages [9] which occur daily or weekly for a few to an extended number (i.e., 36) of hours [9-14]. Alternate day fasting, religious fasting and time restricted feeding (TRF) may all be used as methods of intermittent fasting [9]. A recent review by Patterson & Sears [9] summarizes the findings from human trials of various forms of intermittent fasting, with many studies reporting cardiometabolic benefits such as reduced insulin, and reduced inflammatory markers. Most of the human trials that were reviewed involved a form of alternate day or modified fasting where fasting or extreme calorie restriction was practiced intermittently typically on one or two days per week. The majority of data from alternate day fasting studies come from animal or rodent models and have demonstrated reduced cholesterol and triglyceride concentrations [15, 16]. It should be noted that most human trials incorporating alternate day fasting utilized diet
interventions which involve consumption of \( \leq 20-25\% \) of habitual dietary intake [17-21]. However, as outlined by Patterson & Sears [9], three trials involved fasting of 20-28 hours which were associated with reduced leptin, increased adiponectin [14], increased HDL-c, and reduced TAG levels [13]. Based on these findings, it appears that alternate day fasting is as effective as traditional moderate caloric restriction (i.e., 10-30\% reduction of ad libitum dietary intake) at improving some markers of cardio-metabolic health [9]. While more human trials are needed, it also important to consider that prolonged periods of fasting (~20 hours) may be difficult to adhere to. Thus, TRF may be a more practical alternative for the general population.

Time restricted feeding involves fasting for a set period of time each day, typically ranging from 12 to 21 hours [9]. This form of fasting results in a restriction of food intake timing, such that all daily calories are consumed within a certain amount of hours each day. Recent findings have led to the suggestion that TRF, when properly aligned with the central circadian clock, may enhance synchronization of a metabolic “clock” that is located in the liver that is controlled by transcriptional and translational feedback and is responsible for regulating metabolism of lipids, carbohydrates, and proteins [22]. This circadian clock is often disrupted by weight gain, age, shift work, and calorie dense diets [23-26] and may be a potential cause of cardiometabolic disease [27]. It is important to note that TRF does not promote caloric restriction per se, in fact it is generally encouraged to maintain habitual dietary intake, and simply restrict the timing of caloric intake [28]. Most of the data showing cardiometabolic benefits of TRF are coming from rodent models utilizing isocaloric TRF [23, 29-31]; however, mice following ad libitum high fat, energy dense diets are protected against obesity and metabolic disease when following a TRF protocol [31]. Improved glucose tolerance, reduced levels of blood lipids, and inflammatory markers have also been reported [29-31]. One recent study in humans reported
improved body composition and cardiometabolic health in resistance trained men following a TRF protocol for 8 weeks [28] however, more human trials are needed to further examine the potential benefits.

While intermittent fasting has produced changes in markers of cardiometabolic health in healthy and obese populations, such as body composition [17, 19-21, 32-35], inflammatory markers (leptin, c-reactive protein) [9], cholesterol [17, 18, 20, 21, 35, 36] and glucose concentrations [14, 35, 37, 38], it is not clear if a caloric deficit is needed to achieve these changes. One recent study reported increased adiponectin, and decreased fat mass in trained men following an isocaloric TRF for a period of 8 weeks [28] however it is not clear if the same findings would be produced in an ad libitum TRF protocol. While a recent study reported that ad libitum TRF resulted in reduced energy intake [39], more data are needed to compare the potential benefits of ad libitum and isocaloric TRF. In addition, a recent report showed cardiometabolic benefits with TRF when food intake is controlled [40], however, it is also unclear if nutrient intake would be changed when food intake is not controlled. Therefore, the primary objectives of this pilot study was to utilize a two-arm, between subjects design with repeated measures to: 1) examine the impact of TRF on markers of cardio-metabolic health and antioxidant status in humans and 2) determine if adaptations from TRF would differ between groups (i.e., in ad libitum (eating to satiation) compared to isocaloric (defined as < ±300kcal difference from baseline)) conditions. Secondary outcomes include the impact of TRF on energy intake in ad libitum and isocaloric conditions. We hypothesized that humans following an ad libitum TRF protocol would reduce caloric intake and this caloric deficit would be associated with greater improvements in cardiometabolic health including blood pressure, body composition, blood lipids, and markers of inflammation and antioxidant status compared to an
isocaloric TRF protocol. This hypothesis was made based on findings from a previous ad libitum TRF protocol resulting in reductions in caloric intake [39].

2. Methods and materials

2.1 Subjects

A total of 26 participants were initially recruited with flyers and via word of mouth to participate in this study. However, three participants failed to attend the initial baseline testing session. One participant experienced a seizure during the duration of the study—the cause of which is not known and was also excluded from the study at that time. A CONSORT diagram is included as Figure 1. Therefore, a total of 22 participants (mean ± SD; age: 22 ± 2.5 yrs; height: 178.4 ± 6.9 cm; weight: 90.3 ± 24 kg; BMI: 28.5 ± 8.3) completed all testing sessions, adhered to the diet, and were included for analysis. These participants were randomized into either the isocaloric (n = 10) or ad libitum (n = 12) condition. All participants completed a health history questionnaire, physical activity readiness questionnaire (PAR-Q), and provided written informed consent prior to the initiation of testing. The experimental procedures and dietary protocols were and reviewed and approved by the University’s Institutional Review Board. Participants were excluded from the study if they were current cigarette smokers, currently taking any prescription drugs, or symptomatic of any cardiometabolic, neurological, or musculoskeletal disorders, or if they had practiced intermittent fasting in the last 6 months. Baseline fasting interval and food intake timing was not collected and therefore not considered for eligibility. All participants were asked to refrain from strenuous exercise, the use of any alcohol, caffeine, and nicotine consumption 24 h prior to all trials. All participants reported engaging in regular physical activity, averaging at over 150 min/week of regular activity.

2.2 Experimental Design
This study utilized a between-subjects, randomized design where each subject was randomly assigned to either an ad libitum or isocaloric TRF group. Upon recruitment, each subject was provided with a subject number. A random number generator (random.org) was used to generate even and odd numbers which was coded by a researcher and used to assign treatments in a counterbalanced manner. Treatment assignments were made at the baseline testing session. Both groups followed a 16:8 protocol which consisted of 16 hours of fasting, and 8 hours of eating each day for a period of 28-days. Blood samples were collected once before and once after the end of the 28-day dietary protocol. Blood pressure and body composition were also measured concurrently with blood samples. Visual analog scales (VAS) to measure perceived hunger, satiety, mood, energy, focus, and alertness were collected every 7 days for the duration of the 28-day protocol. Blinding was not conducted at any part of the study.

2.3 Baseline Measures

After the participants provided consent to participate in the research, body mass was measured using a digital scale (Defender 5000, OHAUS, Parsippany, NJ, USA), and height was measured using a wall mount, digital stadiometer (235D; QuickMedical, Issaquah, WA, USA). After collection of height and mass, the participants rested in a supine position for 15 min. Resting heart rate was collected by a Polar heart rate chest strap and watch (FT1; Polar Electro Ltd., Kempele, Finland) and blood pressure was measured with an electronic blood pressure cuff (Carescape V100, GE Healthcare, Milwaukee WI, USA). One blood sample (14 mL) was also collected via venipuncture using a 21 G butterfly needle (BD Vacutainer, Franklin Lakes, NJ, USA). Another blood sample was collected by finger prick and whole blood from this sample was analyzed for blood glucose and lipids (Cholestech LDX® Analyzer, Orlando, FL, USA). In order to quantify baseline caloric intake for each participant, a three-day food log was recorded
for each subject using MyFitnessPal (MyFitnessPal, Calorie Counter, 2018, Baltimore, MD, USA). The average of the three days was used to assign target caloric loads for the isocaloric group and to determine changes from baseline caloric intake for the ad libitum group. Finally, body composition was measured via 7-site skinfold and air displacement plethysmography (BodPod, COSMED USA, Concord, CA, USA)[41]. A summary of each group’s baseline data are reported in Table 1.

2.4 Dietary Protocol

Each subject was randomly assigned to either an isocaloric or ad libitum group where both groups were asked to follow a TRF protocol. The TRF protocol involved 16 hours of fasting followed by 8 hours of eating each day. Therefore, each subject was asked to record the time of the first and last meal on a data sheet that was provided to them before the diet began. Only water was allowed during the fasting time. These sheets were collected each week to measure compliance. Participants were not asked to fast for specific hours each day, however, they were asked to attempt to keep the fasting times consistent through the trial. Beverages containing calories were consumed during the feeding hours each day. Adherence was monitored by data sheets, on which each subject recorded the time of their first and time of last meal each day. In addition, all food was logged in the MyFitnessPal app for the duration of the study. This approach has been previously used and shown to be a valid tool for tracking short term dietary data [42, 43]. Three-day averages (2 week days, one weekend day) for caloric and macronutrient intake was recorded once each week. Both groups were instructed to follow normal dietary habits with the exception of the timing of calorie intake. The ad libitum group was instructed to eat as many calories as desired for satiation, as long as the calories were ingested during the feeding window. The isocaloric group was asked to observe their daily caloric intake from
MyFitnessPal and stay within 300 kilocalories of habitual dietary intake (based off preliminary baseline caloric intake). Compliance was also monitored via weekly analyzed macronutrients.

2.5 Blood Sampling & Analysis

Blood samples were collected between 0500 and 0900 following at least an 8 hr fast via venipuncture and finger prick. Regarding the former, 14 mL were collected from an antecubital vein using a 21 G butterfly needle (BD Vacutainer, Franklin Lakes, NJ, USA). Samples were collected into two seven mL sealed sodium heparin vacutainers. After collection, a deproteination step was followed per kit instructions in which 500µL of whole blood were mixed with an equal volume of a 5% 5-sulfosalicylic acid dehydrate solution (Sigma Aldrich, St Louis, MO, USA). Aliquots were then centrifuged at 10,000 x g for 10 min. The supernatant was aliquoted and stored at -80 ºC for later analysis of glutathione. Vacutainers containing whole blood samples were separately centrifuged at 1000 x g for 10 min at 4ºC. Plasma was aliquoted and stored at -80 ºC.

Regarding the finger prick method, samples of approximately 40 uL were collected into capillary tubes from the lateral side of the index or middle finger using a 1.8mm, 26 G Dynarex self with-drawing lancet (Orangeberg, NY, USA). Whole blood samples were immediately placed in a whole blood analyzer (Cholestech LDX® Analyzer, Orlando, FL, USA) and analyzed for glucose, triglycerides, HDL-c, LDL-c, total cholesterol (TC), and TC:HDL ratios.

Plasma was analyzed in duplicate samples for total adiponectin, cortisol, c-reactive protein (CRP), human growth hormone, insulin, total nitrate/nitrite (NOx), superoxide dismutase (SOD), and whole blood was analyzed for glutathione levels. Adiponectin and human growth hormone were analyzed using a sandwich enzyme immunoassay according to assay instructions (R&D Systems, Inc. Minneapolis, MS, USA). Insulin and CRP were analyzed in duplicate
samples using ultrasensitive and high sensitivity sandwich assays using commercially available kits (Alpco, Salem, NH, USA). Plasma was diluted 1:100 before assaying for CRP levels. Plasma samples were diluted 1:5 prior to assaying for SOD activity using a commercially available assay kit (Cayman Chemical, Ann Arbor, MI, USA). This assay uses tetrazolium salt to facilitate identification of all three types of SOD: Cu/Zn, Mn, and FeSOD. Total plasma NOx levels were analyzed according to kit instructions (Cayman Chemical, Ann Arbor, MI, USA). Prior to assaying for total plasma NOx levels, thawed plasma samples were ultra filtered at 17000 x g through a 10 kDa spin column (Sigma-Aldrich, St. Louis, MO, USA) prior to assaying per assay instructions. Regarding the glutathione analysis, following the deproteination step with 5% sulfosalicylic dehydrate (as mentioned above) samples were thawed and analyzed in duplicate for whole blood concentrations of glutathione according to assay instructions (Arbor Assays, Ann Arbor, MI, USA). Absorbance values for total adiponectin, cortisol, CRP, human growth hormone, insulin, SOD, and glutathione were determined using a colorimetric plate reader (BioTek Epoch 2, Winooski, VT, USA). For assays that required wash steps (cortisol, adiponectin, CRP, and human growth hormone), the plates were washed with an automatic plate washer (Biotek, Winooski, VT, USA).

2.6 Body Composition

At the same time point in which the blood samples were taken, blood pressure and resting heart rate measures which were collected before the start and upon completion of the diet intervention, body composition was measured via air displacement plethysmography [41] and 7-site skinfold methods. Regarding the skinfold method, two measures were taken using a Langue® skinfold caliper (California, USA) from the chest, abdomen, axilla, subscapularis, suprailliac, thigh and triceps in non-consecutive steps. A third measure was taken if the first two
differed by more than 2mm. The two closest measures were averaged and body density and % body fat were calculated using Jackson & Pollock equations [44]. In attempt to maintain consistent results, the same researcher administered skinfold measures each time, for every participant. In addition, pre-and post-fat mass, fat-free mass, resting metabolic rate, and % body fat were determined via air displacement plethysmography (BodPod, COSMED USA, Concord, CA, USA) [41].

2.7 Visual Analog Scales

Every seven days over the 28-day study, the participants reported to the lab and completed a visual analog scale (VAS) to measure perceived alertness, energy, focus, hunger, mood, and satiety. The VAS consisted of a line that was numbered one to ten. The participants were asked to draw a vertical line, perpendicular to the numbered line, to indicate the extent to which they perceived their level of all of these variables. Averages for each variable were calculated from the following timepoints: day 0 (pre-diet), as well as 7, 14, 21, 28 days after the initiation of the TRF protocol. VAS data were collected in the early morning during the hours of 0500 and 0900 and were collected after at least an 8 hr fast.

2.8 Statistical Analyses

All statistical procedures were conducted with SAS 9.4 (Cary, NC, USA). For blood pressure (systolic and diastolic), body mass, body fat %, fat free mass, fat mass, resting metabolic rate, resting heart rate, as well as blood glucose, triglycerides, HDL-c, LDL-c, TC, and TC/HDL-c ratios, adiponectin, cortisol, CRP, human growth hormone, insulin, SOD, and glutathione, 2 x 2 (treatment x time) ANOVAs were conducted with repeat measures on time point (i.e., pre-post diet). Dietary data including total caloric intake (kilocalories), carbohydrate, fat, and protein intake, in addition to VAS data were analyzed with 2 x 5 (treatment x time)
ANOVA with repeat measures on time (i.e., days 0, 7, 14, 21, and 28). In the instance of a significant interaction or main effect (p < 0.05), Fisher’s least significant difference post hoc test was utilized to further determine differences. However, in situations where only two comparisons are being made (i.e., pre and post diet), paired t tests were used as post hoc analysis. Baseline characteristics between groups were compared with two sample tests. This was a pilot study and power calculations were not done. Sample size was chosen based off a similar pilot study by Gabel et al., [45] in which they had 23 participants. In instances were missing data points existed, a “.” was entered into the SAS command file in attempt to ensure the missing point was properly recognized by the SAS software.

3. Results

3.1 Dietary Results

All data are reported as means ± standard deviation (SD) unless otherwise noted. It is first important to note that several between group differences were present at baseline that remained consistent at the conclusion of the study as well. Baseline comparisons between groups are shown in Table 1. The average eating time was 7.2 ± 0.7 hours with participants on average starting their eating window at 1135 ± 0125 and ending their eating window at 1851 ± 0124. The average fasting time was 16.7 ± 0.8 hours. In terms of mean total caloric intake (kilocalories), there was no significant treatment x time interaction (p = 0.88), and no effect for time (p = 0.37) indicating no change in total caloric intake from TRF. The main effect for treatment approached significance (F = 3.81, p = 0.054) which indicated greater, but not significant, overall caloric intake in the ad libitum group compared to the isocaloric group. There was no treatment x time interaction for carbohydrate intake (p = 0.19), and no main effect for treatment (p = 0.15) or time (p = 0.86). There was also no treatment x time interaction for protein (p = 0.65) and no main
effects for treatment (p = 0.94) or time (p = 0.12). Regarding dietary fat intake, there was no
treatment x time interaction (p = 0.30) and no main effect for treatment (p = 0.81), and no
significant main effect for time (p = 0.07). However a decrease in fat intake from pre to post 28
day TRF intervention (90.7 ± 41.0g; 70.8 ± 38.9g) was noted for both groups. This decrease of
approximately 22% in fat intake did not reach statistical significance by the main effect test (p =
0.07). Dietary data are presented in Table 2.

3.2 Blood Markers

3.2.1 Blood Glucose and Lipids

Data from all blood results that were not significantly impacted by the TRF intervention
can be found in Table 3. It should be noted that only data for 20 subjects were available for
cortisol, adiponectin, CRP, insulin, human growth hormone and SOD. This is due to inability to
obtain a blood sample at a particular point of collection. Further, due to inadequate volume
and/or quality of sample available, data for 19 subjects were available for GSH, and data for 17
subjects were available for analysis of NOx levels. In terms of blood glucose levels, there was no
significant treatment x time interaction (p = 0.79), and no main effect for treatment (p = 0.26) or
time (p = 0.16). Regarding HDL-c levels, there was no significant treatment x time interaction
(0.58), and no main effect for treatment (p = 0.90). However, there was a significant increase
from pre to 28 days post TRF protocol (p = 0.005, ηp² = 0.37) in both groups (95% CI: 40.8 -
44.0; 44.3 - 47.6). The average changes in adlibitum and isocaloric groups were 2.9 and
4.8mg/dL respectively. Mean HDL-c levels are reported in Figure 2. In regards to mean LDL-c
levels, there was no significant treatment x time interaction (p = 0.12), and no main effect for
time (p = 0.64). However, the ad libitum group demonstrated significantly higher LDL-c levels
when compared to the isocaloric group shown by a significant treatment effect (F = 8.78, p =
There was no significant treatment \( x \) time interaction for mean triglyceride levels (\( p = 0.50 \)) and no main effect for treatment (\( p = 0.09 \)) or time (\( p = 0.71 \)). No significant treatment \( x \) time interaction was found for mean TC levels (\( p = 0.30 \)) and no main effect for time (\( p = 0.56 \)) but the ad libitum group overall, demonstrated higher TC levels (\( p = 0.006 \)). Regarding TC: HDL-c ratios, there was no treatment \( x \) time interaction (\( p = 0.22 \)), and no main effect for time (\( p = 0.24 \)). However, the ad libitum group demonstrated significantly higher TC:HDL-c ratios (\( p = 0.01 \)).

### 3.2.2 Oxidative Stress and Inflammatory Markers

There was no significant treatment \( x \) time interaction for mean plasma adiponectin levels (\( p = 0.78 \)). However, there was a main effect for treatment (\( F = 32.92, p < 0.001, \eta_p^2 = 0.64 \)) and time (\( F = 5.66, p = 0.02, \eta_p^2 = 0.23 \)) with significantly lower adiponectin levels in the ad libitum group (\( p < 0.001, 95\% \text{ CI: 3344.2 - 3943.5; 4570.8 - 53304.8} \)) and a significant increase from pre to post TRF protocol (\( p = 0.02, 95\% \text{ CI: 3687.5 – 4357.6; 4224.0 – 4894.1} \)) in adiponectin in both groups. Mean adiponectin levels are shown in Figure 3. Regarding mean plasma CRP levels, there was no significant treatment \( x \) time interaction (\( p = 0.69 \)) and no main effect for time (\( p = 0.37 \)). However, the ad libitum group demonstrate significantly higher levels of CRP (\( p = 0.02 \)). No significant treatment \( x \) time interaction was found for mean SOD levels (\( p = 0.41 \)) and there was no main effect for treatment (\( p = 0.69 \)) or time (\( p = 0.29 \)). In terms of mean whole blood glutathione levels, there was no significant treatment \( x \) time interaction (\( p = 0.45 \)) and no main effect for time (\( p = 0.22 \)). The main effect for treatment approached significance (\( p = 0.08 \)) with lower levels of glutathione in the ad libitum compared to the isocaloric group (\( 1109.7 \pm 297\mu\text{M; 1341.3 \pm 413.8}\mu\text{M} \)). Finally, there was no significant treatment \( x \) time interaction for mean NOx levels (\( p = 0.52 \)), and no main effect for time (\( p = 0.57 \)). However, there was a main
effect for treatment ($F = 10.95, p = 0.005$) which revealed significantly higher NOx levels in the ad libitum group.

3.2.3 Metabolic Hormones

Regarding mean plasma cortisol levels, the treatment $\times$ time interaction was not significant ($p = 0.08$). There was no main effect for time ($p = 0.76$). However, there was an effect for treatment which demonstrated significantly higher mean cortisol levels in the ad libitum group ($p = 0.006$). No significant treatment $\times$ time interaction was found for mean plasma human growth hormone levels ($p = 0.45$) and there was no main effect for treatment ($p = 0.51$) or time ($p = 0.26$). Finally, there was no significant treatment $\times$ time interaction for plasma insulin levels ($p = 0.88$) and no main effect for time ($p = 0.70$). However, the ad libitum group overall, demonstrated significantly higher plasma insulin levels ($p = 0.01$).

3.3 Anthropometric Data and Metabolic Rate

A summary of body composition data is provided in Table 4. In terms of mean body mass (kg), there was no significant treatment $\times$ time interaction ($p = 0.76$). However, there was a main effect for treatment ($F = 3918.8, p < 0.001, \eta_p^2 = 0.99$) with higher body mass in the ad libitum compared to the isocaloric group (97.7 ± 28.8kg; 80.25 ± 11.8kg, 95% CI: 89.1 – 89.9; 88.0 – 88.9). In addition, a significant decrease in body mass was found in both groups from the TRF intervention ($p < 0.001, 95\% \text{ CI: } 89.1 – 89.9; 88.0 – 88.8$).

In regards to the results from the air displacement plethysmography assessment, no significant treatment $\times$ time interaction was found for mean resting metabolic rate ($p = 0.87$). There was no main effect for time ($p = 0.37$); however, the ad libitum group demonstrated
significantly higher resting metabolic rate (p < 0.001) compared to the isocaloric group. Further, there was no significant treatment x time interaction for mean body fat % (p = 0.73). A significant main effect for treatment (F = 46.93, p < 0.0001, $\eta^2_p = 0.51$) and time (F = 8.78, p = 0.007, $\eta^2_p = 0.30$) were noted. The ad libitum group had significantly higher body fat % (p < 0.001, 95% CI: 22.8 - 23.7; 20.6 – 21.6) and both groups demonstrated significant reductions in body fat from pre to post diet (p = 0.007, 95% CI: 22.2 – 23.1; 21.3 – 22.2. In regards to mean fat mass (kg) there was no significant treatment x time interaction (p = 0.94). However, there was a significant main effect for treatment (F = 852.19, p < 0.001, $\eta^2_p = 0.97$) and time (F = 6.96, p = 0.01, $\eta^2_p = 0.25$). The ad libitum group had significantly higher fat mass (25.7 ± 19.3kg) compared to the isocaloric group (16.4 ± 8.2kg) 95% CI: 25.3 – 26.1; 15.9 – 16.9. Both groups experienced a significant decrease in fat mass (p = 0.01, 95% CI: 21.0 – 21.9; 20.2 – 21.1). In terms of fat free mass, there was no significant treatment x time interaction (p = 0.90) and no main effect for time (p = 0.22); however, there was a main effect for treatment (F = 906.54, p < 0.001) with significantly higher fat free mass in the ad libitum group.

In terms of body fat percentage measured by skinfolds, there was no treatment x time interaction (p = 0.25) and no main effect for treatment (p = 0.20). There was a main effect for time (F = 4.80, p = 0.04, $\eta^2_p = 0.19$) with a significant decrease in body fat percentage in both groups from pre to post TRF intervention (14.5 ± 4.3%; 13.6 ± 4.2%, 95% CI: 13.8 – 15.2; 12.8 – 14.2). Further, there was no significant treatment x time interaction (p = 0.24) for mean body density from the skinfold assessment. There was also no main effect for treatment (p = 0.20). There was a main effect for time (F = 4.86, p = 0.03, $\eta^2_p = 0.19$) with a significant increase in body density in both groups from pre to post TRF (1.066 ± 0.01; 1.068 ± 0.01, 95% CI: 1.064 – 1.067; 1.066 – 1.07).
3.4 Blood Pressure and Heart Rate

In regards to mean resting heart rate, no significant treatment \( \times \) time interaction was noted (\( p = 0.41 \)). There was no main effect for treatment (\( p = 0.25 \)). The main effect for time was not statistically significant (\( p = 0.07 \)) but a decrease in mean heart rate from pre (70 ± 9 bpm) to post TRF intervention (65 ± 14 bpm) was noted. Regarding mean systolic blood pressure, no significant treatment \( \times \) time interaction was noted (\( p = 0.37 \)). However, there was a main effect for time (\( F = 5.72, \ p = 0.02, \eta^2_p = 0.23 \)) and treatment (\( F = 4.8, \ p = 0.04, \eta^2_p = 0.20 \)). Overall, the ad libitum group had significantly higher systolic blood pressure (118 ± 12 mmHg) compared to the isocaloric group (114 ± 10 mmHg) 95% CI: 115.5 – 120.8; 110.8 – 117.0. In addition, both groups experienced a significant reduction in systolic blood pressure from pre to post TRF intervention (119 ± 11 mmHg; 114 ± 10 mmHg) 95% CI: 115.4 – 121.3; 110.9 – 116.5. Finally, in terms of mean diastolic blood pressure, no significant treatment \( \times \) time interaction was noted (\( p = 0.61 \)). There was no main effect for treatment (\( p = 0.75 \)). However, there was a main effect for time (\( F = 24.6, \ p < 0.001, \eta^2_p = 0.56 \)) indicating a significant reduction in both groups from pre intervention (75 ± 10 mmHg) to post intervention (65 ± 8 mmHg) 95% CI: 72.5 – 79.1; 61.9 – 68.2.

3.5 Visual Analog Scales

In relation to the VAS data, no significant treatment \( \times \) time interaction was found for mean alertness (\( p = 0.63 \)), and there was no main effect for time (\( p = 0.94 \)) but there was a main effect for treatment (\( F = 41.1, \ p < 0.001 \)) with significantly higher perceived alertness in the ad libitum (7.0 ± 1.9) compared to the isocaloric group (5.6 ± 1.2) which is perhaps attributed to the elevated caloric intake. No significant treatment \( \times \) time interaction was found for perceived
energy levels (p = 0.39). There was no main effect for time (p = 0.43) but there was a main effect for treatment (F = 39.18, p < 0.001) with significantly higher perceived energy levels in the ad libitum (6.3 ± 1.9) compared to the isocaloric (5.0 ± 1.5) group. Regarding perceived focus, there was no treatment x time interaction (p = 0.13), and no main effect for time (p = 0.92). There was a main effect for treatment (F = 65.79, p < 0.001) with significantly higher perceived focus in the ad libitum group (6.6 ± 1.9) compared to the isocaloric group (5.1 ± 1.3). In terms of mean hunger, there was no significant treatment x time interaction (p = 0.82) and no main effect for treatment (p = 0.15) or time (p = 0.63). Regarding perceived mood, no significant treatment x time interaction was noted (p = 0.32). There was no main effect for time (p = 0.44); however, there was a main effect for treatment (F = 35.08, p < 0.001) with significantly higher perceived mood in the ad libitum group (6.9 ± 1.9) compared to the isocaloric group (5.7 ± 1.2). Finally, in regards to mean perceived satiety, there was no treatment x time interaction (F = 1.07, p = 0.37) and no main effect for time (p = 0.12). The main effect for treatment was not significant (p = 0.07) but higher levels in the ad libitum group (4.8 ± 2.5) compared to the isocaloric group (5.3 ± 1.4) were noted.

4. Discussion

The main findings of this study are that a 28-day TRF intervention results in significant reductions in systolic and diastolic blood pressure, increases in HDL-c and adiponectin levels and reductions in body mass and body fat percentage. The findings do not support the original hypothesis that the changes would be more significant in the ad libitum when compared to the isocaloric conditions. These findings are especially meaningful since various forms of dietary modification promote caloric restriction, including some types of intermittent fasting including alternate day fasting [9]. Dietary macronutrient analysis showed considerable decreases in fat
intake (approximately 22%) among both groups. However, a recent report also similarly reported beneficial cardiometabolic responses from a 16:8 TRF protocol without a change in caloric intake [28].

Changes in plasma adiponectin levels from TRF has been similarly shown previously [28]. It is possible that the increase in adiponectin levels is due to the loss in fat mass since there is an inverse relationship between adipocyte size and adiponectin secretion [46]. However, given the role of adiponectin in the promotion of glucose and lipid metabolism [47] it is also possible that the increase in adiponectin may have accelerated weight loss since adiponectin can increase energy expenditure and may be responsible for loss in body mass [48]. Adiponectin has been shown to increase activity of AMPK which is a kinase enzyme known to promote lipolysis, glucose metabolism, and insulin sensitivity [49, 50]. Adiponectin knockout mice also have lower AMPK activity and are more susceptible to insulin resistance, further suggesting a role that adiponectin increases AMPK activity [47]. Given the role adiponectin plays in the potential treatment of insulin resistance [51], TRF may be an effective tool to incorporate among insulin resistant populations.

Intermittent fasting has been previously shown to improve body composition [28]. While most findings have shown creating a negative energy balance is required to achieve a loss in fat mass [52, 53], our study suggests a energy deficit is not needed to reduce fat mass. Our findings are similar to a 16:8 TRF intervention conducted by Moro et al., [28] where the participants lost significant amounts of fat mass with no change in caloric intake, and no measurable change in resting energy expenditure. While increased adiponectin levels may increase energy expenditure and facilitate weight loss [48], our findings are similar in that we did not find a significant change in resting metabolic rate.
The current findings do not fully support findings from previous studies that intermittent fasting promotes beneficial changes in blood lipids [17, 19, 20, 54-57]. However, our results in terms of the impact on blood lipids are also similar to a previous study [28], which is likely attributed to the participants involved, as both participants in the current study as well as those involved in Moro et al., [28] were both considered healthy and within normal ranges for lipids and glucose levels. With that said, the 28-day TRF resulted in significant increases in HDL-c which is a negative risk factor for coronary heart disease. The changes in HDL-c may be attributed to the changes in adiponectin levels as adiponectin has been shown to facilitate cholesterol metabolism and increase HDL-c activity [58]. Since HDL-c and adiponectin are strongly associated with improved cardiometabolic health, these findings suggest potential implications for clinical populations.

Fasting and caloric restriction are dietary approaches extensively shown to increase antioxidant potential and reduce susceptibility to oxidative stress [2, 59] likely by means of the hormesis hypothesis, such that acute exposure to moderate stressors can elicit beneficial redox adaptations. It is well documented that an increase in body mass is associated with reduced antioxidant potential and increased oxidative stress susceptibility [60-62]. Considering the aforementioned, and that TRF has been shown to be an effective tool to decrease body mass[28], we aimed to investigate the impact of TRF on antioxidant levels. A recent study [40] reported improved β-cell function, reduced blood pressure, and oxidative stress from TRF—changes that were also not dependent upon of weight loss. Based on the current findings that we did not detect changes in endogenous antioxidants glutathione and SOD with TRF, it can be concluded that TRF does not cause oxidative stress, and may actually reduce oxidative stress based on the findings from Sutton et al., [40]. Further, our findings provide additional support to those from
Sutton et al., [40] showing decreased blood pressure from TRF. While extensive literature suggests that body mass may be a main predictor of blood pressure [63-66], TRF appears to be effective at reducing blood pressure, independent of both energy deficit and weight loss [40].

It is important to note that between group differences did exist for several variables including adiponectin, body composition, HDL-c, cortisol, total caloric intake, resting metabolic rate, blood pressure, NOx, and CRP. However, since there were no significant interactions, these differences were consistent throughout the entire study and changes from the diet were not different between groups. This should be viewed as a limitations since the two groups were not balanced in terms of these markers. However, there were no significant interactions therefore the two groups did not differ in their response to the diet. In addition, the current study is limited by only obtaining a three day food log to record baseline data and define isocaloric intake based on these data. Thus, the researchers are not fully aware of actual caloric requirements for the subjects and therefore we cannot be sure if the participants were actually in a caloric deficit or not during the duration of the study. However, it is important to note that there was no change in caloric intake from baseline ad libitum energy intake. Finally, while physical activity was not monitored throughout the duration of the study, participants were asked to attempt to maintain habitual physical activity throughout the trial. Additionally, being a small exploratory pilot study, confidence in results is limited due to small sample size and lack of non-TRF group. Furthermore, the population of healthy, active men could also potentially be viewed as a limitation as these findings may not be extrapolated to general population. However, this could also be viewed as a strength as the findings of this pilot study demonstrated significant improvements in multiple biomarkers in an already healthy population.
Other meaningful findings are that the ad libitum group demonstrated significantly higher fat mass as well as blood levels of CRP, insulin, LDL-c, cortisol, TC, TC:HDL-c ratios, NOx, and lower levels of adiponectin, and glutathione. These findings are also significant as they demonstrate not only a relationship between reduced antioxidant status and elevated markers of inflammation and blood lipids, but also that these characteristics of cardiometabolic health are favorably altered with a TRF intervention. With that said, it is also important to note that given these differences between groups, both ad libitum and isocaloric groups were within desirable ranges for blood markers of cardiometabolic health and thus considered healthy participants. Therefore, since both groups benefitted from TRF in terms of blood pressure, fat mass, HDL-c and adiponectin levels, it can be assumed that individuals with chronic hyperinsulinemia and inflammation would likely demonstrate more pronounced changes.

The current findings suggest beneficial cardiometabolic adaptations from a 28-day TRF intervention. While a caloric deficit may result from ad libitum TRF providing cardiometabolic benefits [39], our data are in line with a previous study showing improvements in body composition and metabolic hormones when following an isocaloric TRF protocol [28]. Thus, based on the results of this pilot study, TRF may be an effective strategy to improve body composition, increase adiponectin and HDL-c. Further, both ad libitum and isocaloric conditions appear to be effective at inducing such changes. It should be noted that the time of day for TRF can significantly impact the results [67] therefore larger scale trials are needed to further elucidate longitudinal impact of TRF especially when practiced at varying hours within the day.

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**Disclaimer:** None
Figure 1. CONSORT diagram.

Figure 2. Changes in whole blood high density lipoprotein cholesterol (HDL-c) levels from pre to post time restricted feeding intervention. Data are shown as means ± SE. * indicates a significant increase from pre to post diet in both the ad libitum (n = 12) and isocaloric group (n=10).

Figure 3. Changes in plasma adiponectin levels from pre to post time restricted feeding intervention. Data are shown as means ± SE. * indicates a significant increase from pre to post diet in both the isocaloric (n = 10) and ad libitum (n=12) groups.

Table 1. Baseline body composition, blood markers, and caloric intake data for ad libitum (n = 12) and isocaloric (n = 10) conditions. Data are shown as means ± SD. No significant differences were noted between groups at baseline as indicated by two sample t tests. BMI, Body Mass Index; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TAG, triglyceride; TC, total cholesterol; HS CRP, high sensitivity c-reactive protein; GSH, glutathione; SOD, superoxide dismutase; NOx, total nitrite/nitrate; HGH, human growth hormone; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2. Total caloric intake for both groups. On average, total caloric intake (kcal/day) was higher in the ad libitum (n = 12) group compared to the isocaloric (n = 10) but this difference did not reach statistical significance (p = 0.054) demonstrated by the main effect test for treatment. There were no changes from the diet intervention. Data are shown as means ± SD.

Table 3. Changes in blood markers from pre to post time restricted feeding intervention between the ad libitum (n = 12) group and the isocaloric (n = 10) group. These blood markers were not significantly impacted by the time restricted feeding intervention. However, * denotes a significant main effect for treatment indicating a difference overall between ad libitum and
isocaloric groups. Data are shown as means ± SD. HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TAG, triglyceride; TC, total cholesterol; HS CRP, high sensitivity c-reactive protein; GSH, glutathione; SOD, superoxide dismutase; NOx, total nitrite/nitrate; HGH, human growth hormone

**Table 4.** Changes in body composition from pre to post time restricted feeding intervention. * indicates a significant main effect for treatment difference between ad libitum (n = 12) and isocaloric (n = 10) groups. † indicates a significant difference between groups as well as a significant reduction from the time restricted feeding intervention. ϒ indicates a significant change in both groups from pre to post time restricted feeding intervention. Data are shown as means ± SD. RMR, resting metabolic rate; FFM, fat free mass
References


