4-30-2018

A Comparison of Dietary and Caloric Restriction Models on Measures of Body Composition and Physical Performance in Young Mice

Nicholas John Grayson Smith

A Comparison of Dietary and Caloric Restriction Models on Measures of Body Composition and Physical Performance in Young Mice

by

Nicholas John Grayson Smith

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

Major: Health Studies

The University of Memphis

May 2018

Abstract

Background: In recent years, time-restricted feeding, alternate day fasting, and the Daniel Fast have garnered attention as potential dietary interventions to combat obesity. *Objective:* To compare the effects of various dietary models on measures of body composition and physical performance in male C57BL/6 mice. *Methods:* 60 young C57BL/6 male mice were assigned a diet of time-restricted feeding, alternate day fasting, the Daniel Fast, caloric restriction, a high-fat rodent diet, or a standard rodent chow for 8 weeks. Body composition and run time to exhaustion were determined. *Results:* Compared to the high-fat *ad libitum* group, all groups displayed significantly less weight and fat mass gain and non-significant changes in fat-free mass. Additionally, although not statistically significant, all groups displayed greater run time to exhaustion, relative to the high-fat *ad libitum* group. *Conclusion:* The Daniel Fast, time-restricted feeding, and alternate day fasting may improve body composition and physical performance as compared to a high-fat diet.

Table of Contents

Introduction

The prevalence of obesity - a complex condition caused by a combination of genetic, metabolic, social, behavioral, and cultural factors - has risen substantially in the United States since the year 1976 (1). Recent estimates indicate 36.5% of adult Americans are classified as obese, defined as a body mass index [BMI] of ≥ 30 kg·m^{-2}) (2). This figure is important because obesity is associated with negative alterations to many markers of overall health, notably with unfavorable changes in body composition (i.e. increased fat mass [FM], abdominal FM, and ratio of FM to fat-free mass [FFM]) (1) and decreased physical fitness (3). Given that the prevalence of obesity is expected to increase in the coming decades, it is clear that novel interventions are needed to induce favorable changes in body composition and increases in physical fitness in obese individuals.

In recent years, three dietary modifications have been suggested as potential aids in preventing and treating obesity: time-restricted feeding (TRF), alternate day fasting (ADF), and the Daniel fast (DF). TRF regimes restrict daily feeding periods to designated hours of the day (i.e. only eating from 12:00 pm – 7:00 pm), extending the typical overnight fast by several hours. Recent studies have indicated that TRF is capable of producing positive alterations in markers of overall health in humans, including positively altering blood lipids (4, 5), blood glucose (5), and blood insulin (5). With regard to body composition, studies in both humans (4, 5) and animals (6-8) have suggested that TRF may be successful in inducing significant reductions in body mass and FM, while human studies have indicated that FFM can be maintained during this weight loss (4, 5). Few studies have reported measures of physical fitness following a TRF intervention. Studies in humans have indicated that physical fitness levels are maintained in normal weight individuals following short-term TRF (5, 9). Two TRF studies conducted using animal models (one using

C57BL/6J mice and one using *Drosophila*) have reported greater physical performance in TRF groups, relative to control groups (10, 11).

Traditional ADF protocols follow the practice of fasting for one day, followed by *ad libitum* consumption on the subsequent day. Recent research has also utilized a modified ADF, which permits the consumption of small meals on fast days, typically consisting of 25-30% of normal daily caloric consumption (12, 13). Studies suggest that ADF may improve measures of cardio-metabolic health in humans, including decreased cholesterol (14-16), decreased LDL levels (14-16), decreased triglyceride levels (14-16), and decreased blood pressure (14, 17, 18). Additionally, ADF has been shown to improve measures of body composition in humans, resulting in significant reductions in FM (13-15, 17, 19, 20), visceral FM (20), and waist circumference (17, 18) while maintaining FFM (13, 14, 17). To the author's knowledge, no studies have reported measuring physical fitness following ADF.

The DF is a religious motivated fast derived from the Biblical book of Daniel (21). Typically, individuals partake in the DF for a 21-day period. It is a strict vegan diet that generally leads to reduced caloric intake. Individuals participating in a DF may consume fruits, vegetables, whole grains, nuts, legumes, seeds, and healthy oils (such as olive oil) *ad libitum*. Products that contain additives or preservatives, as well as caffeine and alcohol, are prohibited. In humans, the DF has resulted in improvements of many markers of overall health, including blood lipids, blood pressure, insulin, inflammation, and oxidative stress (21-24). With regards to body composition, only one study has indicated that DF results in significant alterations in body composition in humans (23). In this study, body mass, FM, and FFM all decreased significantly. However, other DF studies have failed to produce significant alterations in body composition, likely the result of the short duration of the intervention used in the studies (21, 22, 24). However, weight loss is

typically 5-6 pounds over the course of a 21-day period. No DF studies have examined physical fitness in humans. However, one study in Long-Evans rats resulted in a significant increase in exercise capacity (run time to exhaustion) in mice consuming a DF-inspired chow and participating in regular exercise, relative to control groups (25). This indicates that the DF may result in increased physical fitness in rodent models.

Currently, no studies have compared these above dietary interventions within one protocol with respect to body composition and physical fitness. The proposed study aimed to accomplish this goal for the first time using a mouse model.

Methods

Mice and Dietary Protocol

Male C57BL/6 mice were used for this study, with 60 mice (7 groups of 8 mice, with 4 additional mice obtained to ensure statistical power was maintained in the event of the death of an animal) allocated to one of seven groups. Mice were obtained at 4 weeks of age and were co-housed (as described in Hatori, 2012) at the animal facility on the University of Memphis campus. They were entrained under a 12h light: 12h dark schedule for 2 weeks with standard rodent chow available *ad libitum*. During the 2-week entrainment period, mice began the reverse light-dark schedule, with lights off between the hours of 7am-7pm. This was done so that the feeding time was during the active phase ("lights off" phase) of the mice. Mice were housed in Life Sciences in an area that is used for studies of the circadian rhythm, and therefore, the light was well-regulated.

Following the 2-week entrainment period, all mice entered a 6-week lead-in phase. During this 6-week lead-in period, 8 mice continued consuming a standard chow diet. These mice served as the control group (referred to as CHOW). They maintained *ad libitum* consumption of standard rodent chow through the entire study. The remaining 52 mice consumed a high-fat diet, consisting

of 45% lard and 41% carbohydrate (20% sucrose, 9% corn starch, and 12% Maltodextrin). This 6-week period of *ad libitum* feeding of a high-fat diet allowed for significant weight gain. Following the 6-week period lead-in period, the 52 mice fed the high-fat diet were divided into 6 additional groups (see Tables 1-3 for dietary composition of each diet):

Group 1 (HF) had access to the high-fat diet *ad libitum*, 24 hours per day (n=8).

Group 2 (SWITCH) had access to a standard rodent chow *ad libitum*, 24 hours per day (n=8).

Group 3 (DF) had access to a purified, high-fiber, vegan-based diet *ad libitum*, 24 hours per day (n=10; 2 additional animals were assigned to this group following the death of 2 animals during baseline testing). The DF chow consumed by Group 3 (product: D13092801) was custom-made by Research Diets, Inc., based on the average macronutrient sources and quantities of the dietary intakes of human participants following the Daniel Fast in our previous studies (25).

Group 4 (CR) received 80% of *ad libitum* intake as determined during week 6 of the high-fat diet intake period (n=8).

Group 5 (TRF) had *ad libitum* access to the high-fat diet for 6 hours at the beginning of their active phase (8am-2pm) (n=9; an additional animal was assigned to this group *a priori* because it was deemed a high-risk group).

Group 6 (ADF) had *ad libitum* access to the Western diet every other day (n=9; an additional animal was assigned to this group *a priori* because it was deemed a high-risk group). That is, on day 1 they received as much food as desired during the entire 24-hour period. On day 2, they received no food. On day 3, they received *ad libitum* access to food, and so on.

The diets were purchased from Research Diets, Inc (New Brunswick, NJ), which has experience in producing the high-fat diet and purified vegan diets for rodent studies. The mice remained on their particular diets for 8 weeks and then post-testing began. Mice continued on their

diets until all testing was completed (~ middle of week 9). Water was provided *ad libitum* throughout the study period. The amount of food consumed was measured daily and the weights of the mice were taken on alternating days. Following the conclusion of post-testing, mice were euthanized with cervical dislocation (using isoflurane inhalation for anesthesia).

Measurements of Body Composition

Animals underwent a MRI for determination of body mass/body fat. This was done during the 6th week of the lead-in period (baseline) and 9th intervention week (post-intervention) using a small animal MRI unit (EchoMRI™) which uses a specialized NMR-MRI-based technology to rapidly measure lean and fatty tissue in small animals. Baseline and post-intervention scans were performed on the same days for all animals during the last hour of the animal's inactive (light) phase. With regard to the ADF group, baseline and post-intervention scans were performed following 24 hours of feeding. The total scan time for each animal was approximately 60 seconds. Animals simply remained in a stationary tubing while the scan was performed. There was no need to anesthetize the animals during the scan.

Measurements of Physical Fitness

Animals underwent a treadmill run-time-to-exhaustion test using a motorized treadmill with 5% incline. Animals ran at 20m/min for 30 min and 25m/min for the remaining time until they reach exhaustion. A warm-up was provided for 15min (5min at 5m/min, 5min at 10m/min, 5min at 15m/min). Exhaustion was defined as the time at which mice were no longer able to continue running and sat on the shock grid with all 4 paws on the grid for 5 seconds, despite gentle hand prodding. The very mild electric shock was only used when mice did not respond well to gentle hand prodding and at the end of the run-time-to-exhaustion test to determine the stopping

point. The frequency and amplitude of shock was as low as possible (3Hz) to motivate the animals to remain on the treadmill belt, without causing unnecessary distress.

The run-time-to-exhaustion testing was conducted twice in the mice, once prior to starting the intervention period (during the 6th week of the lead-in period) and once at the end of the 8th intervention week. The first run-time-to-exhaustion test was used to acclimate the mice to the treadmill and the run-time-to-exhaustion protocol. The second test was used as a primary dependent variable to characterize the aerobic capacity of each group after the 8-week intervention.

Statistical Analysis

Data collected for anthropometric variables and the run-time-to-exhaustion test were analyzed using GraphPad Prism (GraphPad, LaJolla, CA, USA). A one-way ANOVA was used to calculate main effects, and Tukey post-hoc tests were used for multiple comparisons. An alpha value of 0.05 was used for all statistical testing. Cohen's d was calculated for the run time to exhaustion data using Microsoft Excel (Microsoft, Redmond, WA, USA) to further explore the differences observed between the HF group and the DF, TRF, and ADF groups.

Results

Overview

Three animals died during the course of the study. One animal died during the 6th week of the lead-in period due to sepsis caused by an injury of unknown origin sustained to the left hind limb. Two animals (1 from the CHOW group and 1 from the DF group) died during the 6th week of the lead-in period while performing the run-time-to-exhaustion test. Both mice died instantly from injuries sustained as a result of falling between the shock grid and the treadmill belt. All remaining animals completed the 16-week study.

Caloric Consumption Data

Caloric consumption during the 8-week intervention period (Figure 1) displayed multiple significant group effects. Caloric consumption for HF was significantly greater than for CHOW ($p<0.005$), SWITCH ($p<0.0001$), CR ($p<0.005$), TRF ($p<0.0001$), and ADF ($p<0.0001$). Caloric consumption for DF was significantly greater than for CHOW ($p<0.005$), SWITCH ($p<0.0001$), CR ($p<0.005$), TRF ($p<0.0001$), and ADF ($p<0.0001$). Caloric consumption for CR was significantly greater than for ADF ($p<0.05$). No other effects were noted for caloric consumption.

Anthropometric Data

All anthropometric data are presented in Table 4. Change in body mass from baseline to post-intervention (Figure 2) displayed several group effects. Change in body mass for HF was significantly different from all other groups ($p<0.0001$). Change in body mass for CHOW differed significantly from DF ($p<0.0001$), TRF ($p<0.005$), and ADF ($p<0.0005$). Change in body mass for SWITCH was significantly different from CHOW ($p<0.0001$), CR ($p<0.0001$), TRF ($p<0.005$), and ADF ($p<0.05$).

The data for change in FM from baseline to post-intervention are presented in Table 4 and Figure 3. The SWITCH, DF, TRF, and ADF groups displayed decreased FM after the 8-week intervention, while the CHOW, HF, and CR groups displayed an increase in FM. As expected, the HF group gained more FM (6.76 ± 0.46g) than all other groups ($p<0.0001$). The greatest decrease in FM was observed in the SWITCH group, which lost significantly more FM (-6.275 ± 0.86g; $p<0.005$) than all other groups with the exception of DF (-4.10 ± 0.49g; $p>0.05$). Change in FM for CHOW was significantly different from SWITCH ($p<0.0001$), DF ($p<0.0001$), TRF ($p<0.005$), and ADF ($p<0.0005$). Change in FM for DF was significantly

different from CR ($p<0.0005$). No significant differences were observed with regard to change in FM between the DF, TRF (-1.89±0.55g), and ADF (-2.39±0.49g) groups.

Data for the change in FFM from baseline to post-intervention are displayed in Figure 4. No main effect or group effect was noted. However, the change in FFM between HF - which gained FFM - and CR - which lost FFM - trended towards significance ($p=0.068$).

Change in %FM from day 1 of the intervention to the final day of the intervention (Figure 5) displayed several significant group effects. Change in %FM for HF was significantly different from SWITCH, DF, CR, TRF, and ADF ($p<0.0001$). Change in %FM for CHOW was significantly different from SWITCH ($p<0.0001$), DF ($p<0.0001$), TRF ($p<0.0005$), and ADF ($p<0.0001$). Change in %FM for SWITCH was significantly different from CR ($p<0.0001$), TRF ($p<0.0001$), and ADF ($p<0.0001$). Change in %FM for DF was significantly different from CR ($p<0.0001$) and TRF ($p<0.05$). Change in %FM for CR was significantly different from ADF ($p<0.05$).

Run Time to Exhaustion Data

Data for the run time to exhaustion are presented in Figure 6. No significant effects were noted ($p>0.05$). However, large effect sizes were observed when comparing DF to HF ($d=1.10$), TRF to HF ($d=0.99$), and ADF to HF ($d=1.10$).

Discussion

To our knowledge, this is the first study to compare the effects of dietary protocols mimicking caloric restriction, the Daniel Fast, time-restricted feeding, and alternate day fasting on measures of body composition and physical performance in male C57BL/6 mice. Our results indicate that the CR, DF, ADF, and TRF protocols used in this study produce favorable alterations in body composition in male C57BL/6 mice. Additionally, though the differences

between groups were not significant, the DF, TRF, and ADF groups displayed greater run time to exhaustion when compared with the HF group, with large effect sizes noted.

Anthropometric Findings

As can be seen in Table 4 and Figure 2, the SWITCH, DF, CR, TRF, and ADF groups all displayed decreased body mass after the 8-week intervention. SWITCH displayed the greatest decrease in body mass, losing significantly more weight than all groups besides DF. No significant differences were observed with regard to change in body mass between the DF, CR, TRF, and ADF groups, indicating that all of these diet regimes are effective for inducing weight loss and/or preventing excessive weight gain following a period of high-fat feeding. As expected, the CHOW and HF groups exhibited increased body mass after the 8-week intervention. The increase in body mass displayed by the CHOW group is characteristic of the normal growth of C57BL/6 mice, and the increase in body mass displayed by the HF group is characteristic of the growth rate of C57BL/6 mice given *ad libitum* access to a calorically dense diet.

With regard to body composition, the SWITCH, DF, TRF, and ADF groups displayed decreased FM (Table 4 and Figure 3) after the 8-week intervention, while the CHOW, HF, and CR groups displayed an increase in FM. As noted above, the greatest decrease in FM was observed in the SWITCH group, which lost significantly more FM than all other groups with the exception of DF. No significant differences were observed with regard to change in FM between the DF, TRF, and ADF groups; although, it should be noted that the DF group displayed the lowest post-intervention FM despite consuming the most kilocalories during the 8-week intervention period (Table 4). These data indicate that the DF, TRF, and ADF protocols used in this study can effectively decrease FM following a period of high-fat feeding, albeit likely through different mechanisms. TRF and ADF likely reduced FM by inducing a form of caloric

restriction while DF likely modulated FM via the quality of the macro-and-micronutrients contained in the purified vegan rodent chow.

Figure 4 presents data respective to change in FFM after the 8-week intervention. No main or group effects were noted for this data, though the comparison between HF and CR trended towards significance ($p=0.068$). This lack of a significant main effect is likely due to the relatively small changes in FFM observed after the 8-week intervention, coupled with the small group sizes and the relatively large variation observed (SEM). However, it is worth noting that only the CR and ADF groups displayed decreased FFM after the 8-week intervention. Animals in the DF and TRF groups actually gained FFM over the course of the 8-week intervention. The gain in FFM observed in the DF group is particularly notable because the purified vegan rodent chow consumed by the DF group contains only soy protein; it does not contain any animal protein. These data demonstrate that the TRF and DF protocols used in this study can maintain FFM, even during periods of weight and fat loss.

Many of the findings discussed in this section are consistent with the anthropometric measures reported by other animal studies that utilized DF, TRF, and ADF dietary protocols. Studies have consistently reported significant reductions in body mass following the DF (25), TRF (6, 8, 26, 27), and ADF (28-30) protocols in animal models. With regard to body composition, the DF (25) and TRF (8, 26) protocols have often resulted in reductions in FM, while only one animal study (31) has indicated that ADF results in decreased FM, relative to control. However, it should be noted that many of these studies, and all of the ADF studies mentioned here, use epididymal FM as a measure of overall FM. The current study measured FM using a small animal MRI.

The available FFM data relevant to the DF compares well to the data reported in the current study. Notably, our previous work with the DF in male Long-Evans rats reported maintenance of FFM following 12 weeks of purified vegan rodent chow consumption, despite weight loss and decreased FM (25). Measures of FFM have not been reported following TRF or ADF protocols in animals. It should be noted, though, that a number of human studies have reported reductions in body weight and FM while maintaining FFM following TRF (4, 5) and ADF (13, 14, 17) protocols.

Taken together, the data from the current study and other studies mentioned here indicate that DF, TRF, and ADF protocols are capable of producing favorable alterations in body composition. The DF appears to produce the best results with regard to anthropometric measures of body composition in animal models, though more studies are needed to confirm these findings. Additionally, TRF and ADF protocols appear to be viable options for individuals seeking to decrease body mass and FM while consuming a Western diet that is high in fat and simple sugar. More work is needed to compare the effects of the DF, TRF, and ADF on measurements of body composition.

Run Time to Exhaustion Findings

As indicated by Figure 6, no main or group effects were observed for the run-time-to-exhaustion test. The lack of a significant main effect in these data is likely due to the small group sizes and the relatively large standard error observed. Additionally, the animals' genetic aerobic capacity likely affected run time to exhaustion. For example, during our run-time-to-exhaustion tests, many of the animals had difficulty running at the predetermined testing speed, even during the early stages of testing (20m/min). It may be that some of these animals simply possessed genetic qualities that predisposed them to poor physical performance.

Despite the lack of a significant main effect, however, it is valuable to highlight the mean values for the main intervention groups. The mean run time for the DF (39.00±2.90min), TRF (40.00±4.88min), and ADF (27.78±3.30min) groups was greater than the mean run time for the HF group (16.38±3.85min). Additionally, large effect sizes were observed when the mean run time for the DF (d=1.10), TRF (d=0.99), and ADF (d=1.10) groups were compared with the HF group. These data indicate that the DF, TRF, and ADF protocols had a large impact on run time to exhaustion performance, when compared with the HF group. This may be attributed to the relatively higher FM of the HF group. Further, because the mean run time for the DF and TRF groups closely resembled the mean run time for the CHOW group (39.86±7.87min), the DF and TRF dietary appear to optimize performance, relative to ADF.

As mentioned above, literature describing the effects of DF, TRF, and ADF on physical performance is scant. Only one known study has reported any measure of physical performance following a DF intervention in animals (25). Conducted in male Long-Evans rats, this study indicated the DF combined with regular exercise results in significantly greater aerobic performance than a high-fat diet combined with regular exercise. However, run time between the DF and high-fat groups that did not participate in regular exercise did not differ significantly, findings that align well with the findings of the current study. With regard to TRF studies in animals, one study (11) has reported that C57BL/6J mice following TRF protocols display significantly greater run time to exhaustion, relative to a high-fat control, and another study has indicated that TRF improves physical performance (as measured by flight index) in *Drosophila* (10). Additionally, studies have indicated that TRF does not diminish physical performance in humans, relative to a control (5, 9). No animal model or human subject studies have reported any

measurement of physical fitness following an ADF intervention. More work is needed to investigate the impacts of DF, TRF, and ADF on measures of physical performance.

Conclusions

To our knowledge, this is the first study to compare the effects of dietary protocols mimicking caloric restriction, the Daniel Fast, time-restricted feeding, and alternate day fasting on measures of body composition and physical performance in male C57BL/6 mice. The findings presented here indicate that the Daniel Fast, time-restricted feeding, and alternate day fasting are viable option for improving anthropometric measures and physical performance, when compared with an *ad libitum* high-fat diet.

Future Directions

Future research using animal models and human participants are needed to more fully elucidate the mechanisms responsible for the improved anthropometric measures in response to the Daniel Fast, time-restricted feeding, and alternate day fasting. Specifically, research should focus on the caloric consumption of each group and relate it to changes in anthropometrics. For example, in the present study, the Daniel Fast group consumed the greatest number of kilocalories during the intervention period, yet the Daniel Fast group also had the lowest FM. Future research should determine the causes of the noted changes and seek to address these results more specifically.

Additionally, future studies should aim to better characterize the effects of the Daniel Fast, time-restricted feeding, and alternate day fasting on physical performance, both in an animal model and with human participants. These studies should use larger sample sizes than what was used in the present study to better mitigate the effects of within group variation in physical performance. Future studies should utilize baseline and post-intervention performance

tests to measure changes in physical performance after the completion of the dietary interventions. Additionally, future studies aiming to characterize physical performance following DF, TRF, and ADF should likely aim to control body mass between groups to better measure the effects of the dietary protocols on physical performance, independent of body mass.

References

1. Moyer VA. Screening for and management of obesity in adults: U.S. Preventive Services Task Force recommendation statement. *Annals of Internal Medicine*. 2012 Sep 4,;157(5):373.

2. Ogden CL, Carroll MD, Fryar CD, Flegal KM. Prevalence of Obesity Among Adults and Youth: United States, 2011-2014. *NCHS data brief*. 2015 Nov(219):1.

3. Häkkinen A, Rinne M, Vasankari T, Santtila M, Häkkinen K, Kyröläinen H. Association of physical fitness with health-related quality of life in Finnish young men. *Health and Quality of Life Outcomes*. 2010;8(1):15.

4. Stote KS, Baer DJ, Spears K, Paul DR. A controlled trial of reduced meal frequency without caloric restriction in healthy, normal-weight, middle-aged adults1,2,3. *The American Journal of Clinical Nutrition*. 2007 Apr 1,;85(4):981.

5. Moro T, Tinsley G, Bianco A, Marcolin G, Pacelli QF, Battaglia G, et al. Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in resistance-trained males. *Journal of Translational Medicine*. 2016 Jan 1,;14(1).

6. Arble DM, Bass J, Laposky AD, Vitaterna MH, Turek FW. Circadian Timing of Food Intake Contributes to Weight Gain. *Obesity*. 2009 Nov;17(11):2100-2.

7. Sherman H, Frumin I, Gutman R, Chapnik N, Lorentz A, Meylan J, et al. Long-term restricted feeding alters circadian expression and reduces the level of inflammatory and disease markers. *Journal of Cellular and Molecular Medicine*. 2011 Dec;15(12):2745-59.

8. Sherman H, Genzer Y, Cohen R, Chapnik N, Madar Z, Froy O. Timed high-fat diet resets circadian metabolism and prevents obesity. *FASEB Journal*. 2012 Aug;26(8):3493.

9. Tinsley GM, Forsse JS, Butler NK, Paoli A, Bane AA, La Bounty PM, et al. Time-restricted feeding in young men performing resistance training: A randomized controlled trial. *European Journal of Sport Science*. 2017 Feb 7,;17(2):200-7.

10. Gill S, Le HD, Melkani GC, Panda S. Time-restricted feeding attenuates age-related cardiac decline in Drosophila. *Science*. 2015 Mar 13,;347(6227):1265-9.

11. Chaix A, Zarrinpar A, Miu P, Panda S. Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. *Cell Metabolism*. 2014 Dec 2,;20(6):991-1005.

12. Hoddy KK, Gibbons C, Kroeger CM, Trepanowski JF, Barnosky A, Bhutani S, et al. Changes in hunger and fullness in relation to gut peptides before and after 8 weeks of alternate day fasting. *Clinical Nutrition*. 2016 Dec;35(6):1380-5.

13. Varady KA, Bhutani S, Church EC, Klempel MC. Short-term modified alternate-day fasting: a novel dietary strategy for weight loss and cardioprotection in obese adults. *The American Journal of Clinical Nutrition*. 2009 Nov;90(5):1138-43.

14. Varady KA, Bhutani S, Klempel MC, Kroeger CM, Trepanowski JF, Haus JM, et al. Alternate day fasting for weight loss in normal weight and overweight subjects: a randomized controlled trial. *Nutrition Journal*. 2013;12(1):146.

15. Catenacci VA, Pan Z, Ostendorf D, Brannon S, Gozansky WS, Mattson MP, et al. A randomized pilot study comparing zero-calorie alternate-day fasting to daily caloric restriction in adults with obesity. *Obesity*. 2016 Sep;24(9):1874-83.

16. Bhutani S, Klempel MC, Berger RA, Varady KA. Improvements in Coronary Heart Disease Risk Indicators by Alternate-Day Fasting Involve Adipose Tissue Modulations. *Obesity*. 2010 Nov;18(11):2152-9.

17. Bhutani S, Klempel MC, Kroeger CM, Trepanowski JF, Varady KA. Alternate day fasting and endurance exercise combine to reduce body weight and favorably alter plasma lipids in obese humans. *Obesity*. 2013 Jul;21(7):1370-9.

18. Eshghinia S, Mohammadzadeh F. The effects of modified alternate-day fasting diet on weight loss and CAD risk factors in overweight and obese women. *Journal of Diabetes and Metabolic Disorders*. 2013;12(1):4.

19. Heilbronn LK, Smith SR, Martin CK, Anton SD, Ravussin E. Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. *The American Journal of Clinical Nutrition*. 2005 Jan;81(1):69.

20. Hoddy KK, Kroeger CM, Trepanowski JF, Barnosky A, Bhutani S, Varady KA. Meal timing during alternate day fasting: Impact on body weight and cardiovascular disease risk in obese adults. *Obesity*. 2014 Dec;22(12):2524-31.

21. Bloomer RJ, Kabir MM, Canale RE, Trepanowski JF, Marshall KE, Farney TM, et al. Effect of a 21 day Daniel Fast on metabolic and cardiovascular disease risk factors in men and women. *Lipids in Health And Disease*. 2010;9(1):94.

22. Alleman J, Rick J, Harvey IC, Farney TM, Bloomer RJ. Both a traditional and modified Daniel Fast improve the cardio-metabolic profile in men and women. *Lipids in Health And Disease.* 2013;12(1):114.

23. Trepanowski JF, Kabir MM, Alleman J, Rick J, Bloomer RJ. A 21-day Daniel fast with or without krill oil supplementation improves anthropometric parameters and the cardiometabolic profile in men and women. *Nutrition & Metabolism*. 2012;9(1):82.

24. Bloomer RJ, Trepanowski JF, Kabir MM, Alleman J, Rick J, Dessoulavy ME. Impact of short-term dietary modification on postprandial oxidative stress. *Nutrition Journal*. 2012;11(1):16.

25. Bloomer RJ, Schriefer JM, Gunnels TA, Lee SR, Sable HJ, van der Merwe M, et al. Dietary composition significantly impacts physical performance, body composition, blood lipids, oxidative stress and inflammation in male rats. In Review.

26. Hatori M, Vollmers C, Zarrinpar A, DiTacchio L, Bushong EA, Gill S, et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metabolism*. 2012 Jun 6,;15(6):848-60.

27. Salgado-Delgado R, Angeles-Castellanos M, Saderi N, Buijs RM, Escobar C. Food Intake during the Normal Activity Phase Prevents Obesity and Circadian Desynchrony in a Rat Model of Night Work. *Endocrinology*. 2010 Mar;151(3):1019-29.

28. Wan R, Camandola S, Mattson MP. Intermittent fasting and dietary supplementation with 2-deoxy-D-glucose improve functional and metabolic cardiovascular risk factors in rats. *The FASEB Journal*. 2003.

29. Varady KA, Roohk DJ, Loe YC, McEvoy-Hein BK, Hellerstein MK. Effects of modified alternate-day fasting regimens on adipocyte size, triglyceride metabolism, and plasma adiponectin levels in mice. *Journal of Lipid Research*. 2007 Oct 1,;48(10):2212-9.

30. Ahmet I, Wan R, Mattson MP, Lakatta EG, Talan M. Cardioprotection by Intermittent Fasting in Rats. *Circulation*. 2005 Nov 15,;112(20):3115-21.

31. Dorighello GG, Rovani JC, Luhman CJF, Paim BA, Raposo HF, Vercesi AE, et al. Food restriction by intermittent fasting induces diabetes and obesity and aggravates spontaneous atherosclerosis development in hypercholesterolaemic mice. *The British Journal of Nutrition*. 2014 Mar 28,;111(6):979-86.

Appendix A

Tables and Figures

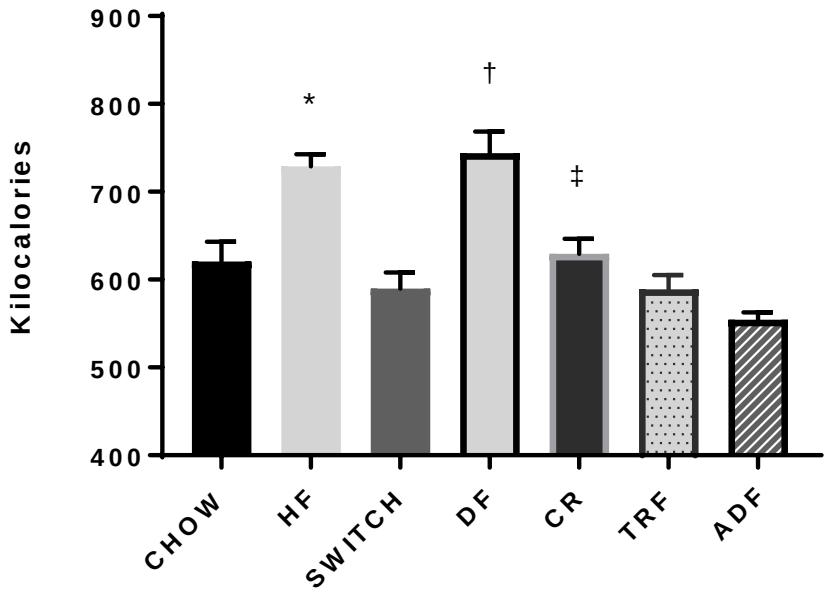

Figure 1. Total kilocalories consumed during the 8-week intervention period by male mice assigned to seven different dietary protocols.

Values are Mean ± SEM

* A group effect noted for caloric consumption: HF>CHOW, SWITCH, CR, TRF, & ADF ($p<0.005$)
† A group effect noted for caloric consumption: DF>CHOW, SWITCH, CR, TRF, & ADF ($p<0.0005$)
‡ A group effect noted for caloric consumption: CR>ADF ($p<0.05$)

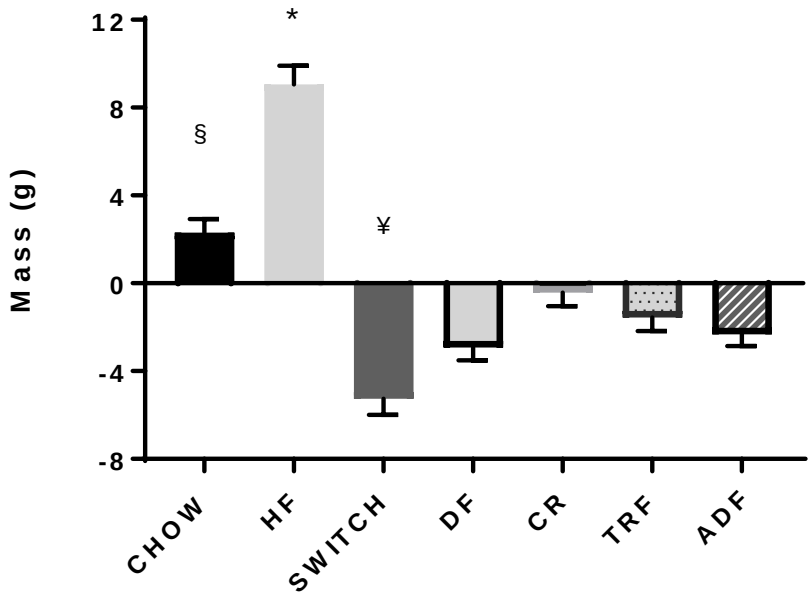

Figure 2. Change in total body mass (g) from baseline (start of the intervention) to the last day of the 8-week intervention of male mice assigned to seven different dietary protocols.

Values are Mean±SEM

§ A group effect noted for change in body mass: CHOW different from SWITCH, DF, TRF, & ADF ($p<0.005$)
* A group effect noted for change in body mass: HF different from all groups ($p<0.0001$)
¥ A group effect noted for change in body mass: SWITCH different from CR, TRF, & ADF ($p<0.05$)

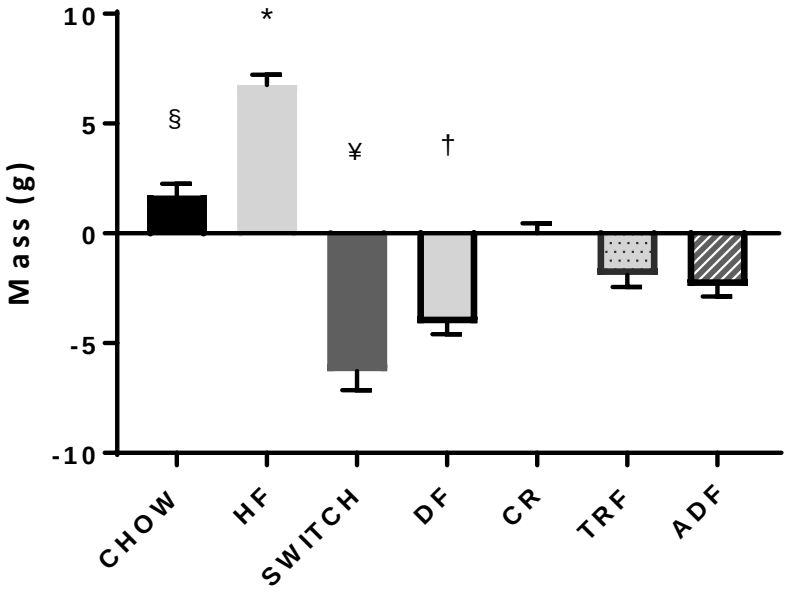

Figure 3. Change in FM (g) from baseline (start of intervention) to the last day of the 8-week intervention of male mice assigned to seven different dietary protocols.

Values are Mean±SEM

§ A group effect noted for change in FM: CHOW different from SWITCH, DF, TRF, & ADF ($p<0.005$)
* A group effect noted for change in FM: HF different from all groups ($p<0.0001$)
¥ A group effect noted for change in FM: SWITCH different from CR, TRF, & ADF ($p<0.0005$)
† A group effect noted for change in FM: DF different from CR ($p<0.0005$)

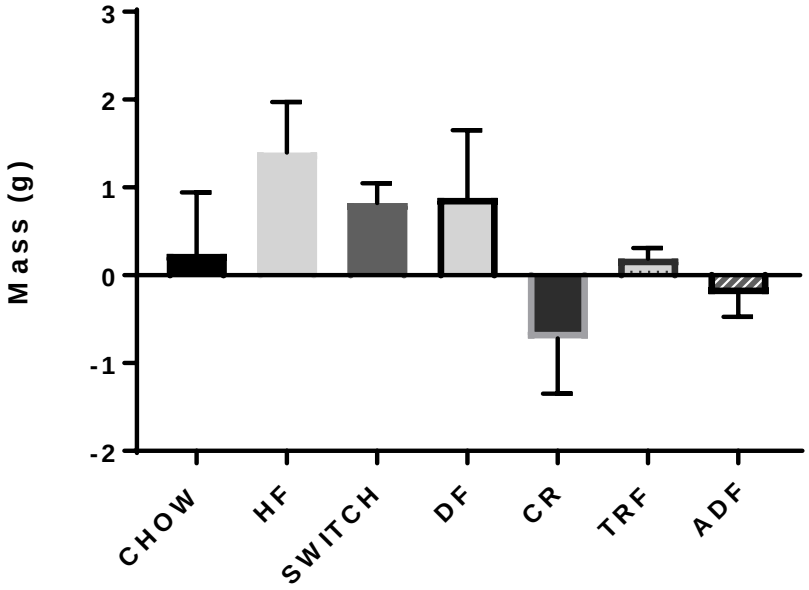

Figure 4. Change in FFM (g) from baseline (start of intervention) to the last day of the 8-week intervention of male mice assigned to seven different dietary protocols.

Values are Mean±SEM

No main or group effects observed ($p>0.05$)

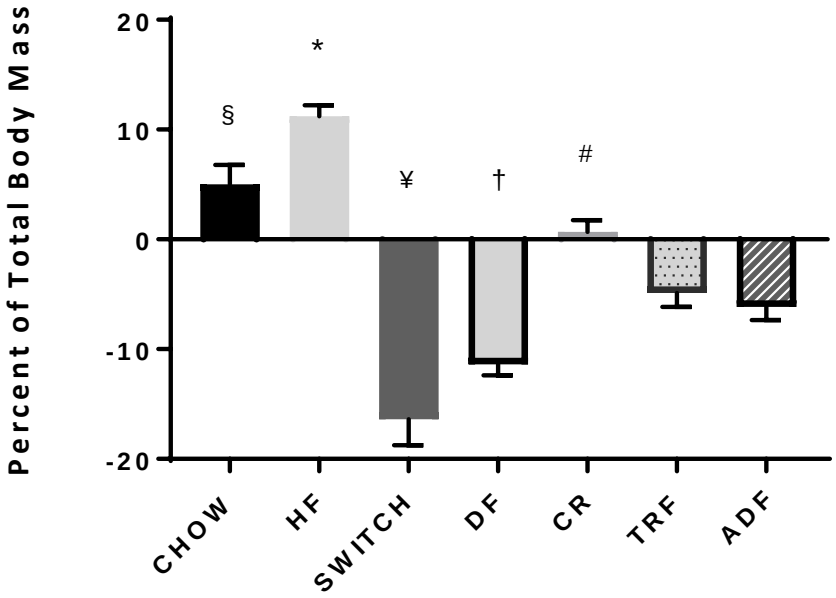

Figure 5. Change in percent fat mass (%FM) from baseline (start of intervention) to the last day of the 8-week intervention of male mice assigned to seven different dietary protocols.

Values are Mean±SEM

§ A group effect noted for change in %FM: CHOW different from SWITCH, DF, TRF, & ADF ($p<0.0005$)
* A group effect noted for change in %FM: HF different from SWITCH, DF, CR, TRF, & ADF ($p<0.0001$)
¥ A group effect noted for change in %FM: SWITCH different from CR, TRF, & ADF ($p<0.0001$)
† A group effect noted for change in %FM: DF different from CR & TRF ($p<0.05$)
# A group effect noted for change in %FM: CR different from ADF ($p<0.05$)

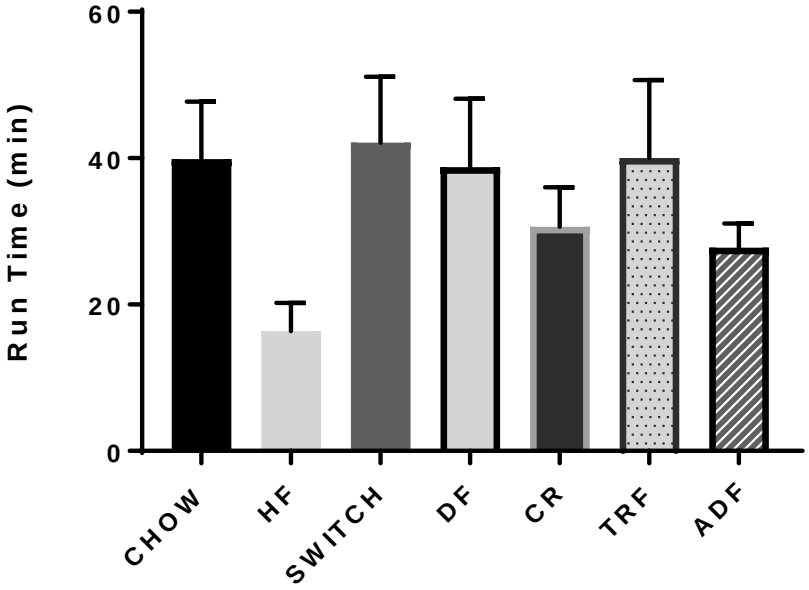

Figure 6. Run time to exhaustion (min) in male mice assigned to seven different dietary protocols for 8 weeks.

Values are Mean±SEM

While no main or group effects were observed ($p>0.05$), all groups demonstrated a higher mean run time to exhaustion, relative to HF.

Table 1. Macronutrient composition and Caloric Density of Experimental Diets.

Macronutrient	**DF**	**HF**	**CHOW**
	kcal%	**kcal%**	**kcal%**
Protein	15	20	24
Carbohydrate	59	35	58
Fat	25	45	18
kcal/gm	3.9	4.73	3.1

Table 2. Ingredient List of Daniel Fast and High-Fat Diets

	DF		**HF**	
	gm	**kcal**	**gm**	**kcal**
Casein	0	0	200	800
Soy Protein	170	680	0	0
DL-Methionine	3	12	0	0
Corn Starch	0	0	72.8	291
Corn Starch-Hi Maize 260	533.5	2134	0	0
(70% Amylose and 30% Amylopectin)				
Maltodextrin	150	600	100	400
Sucrose	0	0	172.8	691
L-Cystine	0	0	3	12
Cellulose, BW200	100	0	50	0
Inulin	50	50	0	0
Soybean Oil			25	225
Lard	0	0	177.5	1598
Flaxseed Oil	71	639	0	0
Safflower Oil, High Oleic	59	531	0	0
Ethoxyquin	0.04	0	0	0
DiCalcium Phosphate			13	0
Mineral Mix S10001	35	0	10	40
Calcium Carbonate	4	0	5.5	0
Mineral Mix S10026			10	0
Vitamin Mix V10001	10	40	0	0
Choline Bitartrate	2	0	2	0
Ascorbic Acid Phosphate, 33% active	0.41	0	0	0
Potassium Citrate, 1 H2O	0	0	16.5	0
Cholesterol	0	0	0	0
FD&C Red Dye #40	0.05	0	0.05	0

Table 3. Macronutrient and Micronutrient Composition of Standard Rodent Chow

Micronutrients			**Amino Acids**		
Calcium	%	1	Aspartic Acid	%	1.4
Phosphorous	%	0.7	Glutamic Acid	%	3.4
Non-Phytate Phosphorous	%	0.4	Alanine	%	1.1
Sodium	%	0.2	Glycine	%	0.8
Potassium	%	0.6	Threonine	%	0.7
Chloride	%	0.4	Proline	%	1.6
Magnesium	%	0.3	Serine	%	1.1
Zinc	mg/kg	70	Leucine	%	1.8
Manganese	mg/kg	100	Isoleucine	%	0.8
Copper	mg/kg	15	Valine	%	0.9
Iodine	mg/kg	6	Phenylalanine	%	1
Iron	mg/kg	200	Tyrosine	%	0.6
Selenium	mg/kg	0.23	Methionine	%	0.4
Vitamins			Cysteine	%	0.3
Vitamin A [e,f]	IU/g	15	Lysine	%	0.9
Vitamin D_3 [e,g]	IU/g	1.5	Histidine	%	0.4
Vitamin E	IU/kg	110	Arginine	%	1
Vitamin K_3 (menadione)	mg/kg	50	Tryptophan	%	0.2
Vitamin B_1 (thiamin)	mg/kg	17	**Fatty Acids**		
Vitamin B_2 (riboflavin)	mg/kg	15	C16:0 Palmitic	%	0.7
Niacin (nicotinic acid)	mg/kg	70	C18:0 Stearic	%	0.2
Vitamin B_6 (pyridoxine)	mg/kg	18	C18:1ω9 Oleic	%	1.2
Pantothenic Acid	mg/kg	33	C18:2ω6 Linoleic	%	3.1
Vitamin B_{12} (cyanocobalamin)	mg/kg	0.08	C18:3ω3 Linolenic	%	0.3
Biotin	mg/kg	0.4	Total Saturated	%	0.9
Folate	mg/kg	4	Total Monounsaturated	%	1.3
Choline	mg/kg	1200	Total Polyunsaturated	%	3.4

Table 4. Anthropometric and run time to exhaustion data for male mice assigned to seven different dietary protocols for 8 weeks.

	Body Mass (g)		FM (g)		FFM (g)		%FM		Run time to exhaustion (min)
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	POST
CHOW	25.49±0.66	27.79±1.03	2.48±0.38	4.21±0.71	21.05±0.48	21.30±0.39	0.10±.0.1	0.15±0.02	39.86±7.87
HF	30.54±1.01	39.60±1.71	7.96±0.72	14.72±0.92	20.64±0.37	22.03±0.85	0.26±0.02	0.37±0.01	16.38±3.85
SWITCH	33.90±0.79	28.65±0.83	10.71±0.68	4.43±0.89	21.20±0.34	22.02±0.34	0.31±0.01	0.15±0.03	42.13±9.01
DF	31.83±1.02	28.91±0.5	7.82±0.84	3.72±0.44	21.97±0.57	22.85±0.76	0.24±0.02	0.13±0.01	39.00±9.4
CR	34.12±1.05	33.69±1.01	10.55±0.82	10.56±0.62	21.50±0.30	20.78±0.86	0.31±0.02	0.31±0.01	30.63±5.38
TRF	30.03±1.06	28.47±0.63	7.80±0.89	5.91±0.51	20.31±0.31	20.50±0.26	0.25±0.02	0.21±0.01	40.00±10.68
ADF	30.96±0.56	28.64±0.40	7.75±0.63	5.36±0.35	21.13±0.23	20.91±0.38	0.25±0.02	0.19±0.01	27.78±3.30

Values are Mean±SEM

Appendix B

Literature Review

Introduction

The prevalence of obesity - a complex condition caused by a combination of genetic, metabolic, social, behavioral, and cultural factors - has risen substantially in the United States since the year 1976 (1). As of 2014, the WHO estimated that, worldwide, 35% of adults over the age of 20 years were overweight (body mass index [BMI] $\geq$ 25 $kg \cdot m^{-2}$), with 10% of men and 14% of women being classified as obese (BMI $\geq$ 30 $kg \cdot m^{-2}$) (2). The United States exhibits particularly high levels of overweight and obesity. More than one in three (36.5%) adult Americans is classified as obese (3). More than two in three (69.2%) adult Americans are classified as either overweight or obese. In Americans, obesity is more prevalent in non-Hispanic blacks (48.1%) and Hispanics (42.5%) when compared to both the national average and non-Hispanic whites (34.5%), and non-Hispanic Asians (11.7%). Additionally, obesity is more prevalent in older adults than in younger adults: the prevalence of obesity in Americans ages 40-59 (40.2%) and Americans age 60 and older (37.0%) is generally greater than the prevalence of obesity individuals age 20-39 (32.3%). Perhaps more troubling, these trends extend to the youth of America. The Centers for Disease Control and Prevention (CDC) estimate that 12.7 million (17%) American adolescents age 2-19 suffer from obesity (defined as BMI in $kg \cdot m^{-2}$ greater than or equal to the age- and sex-specific 95th percentile of the 2000 CDC growth charts).

Obesity places a major financial burden on the healthcare system of the U.S. In 2011, Tsai et al. estimated that overweight status and obesity accounted for $170.2 billion U.S. dollars (7.1%) of the total healthcare dollars spent (4). The authors noted that this obesity-related spending

doubled or tripled the percentage of healthcare dollars spent to combat obesity in several other developed nations, including Canada and nations in the European Union (4). Unfortunately, the future looks even bleaker. Experts have projected direct healthcare costs for preventing, diagnosing, and treating overweight and obesity will rise to 16-18% of total healthcare dollars spent in the U.S. by the year 2030.

In addition to causing a great national financial burden, obesity may substantially reduce an individual's quality of life, impacting physical, psychological, and financial functioning (5). Obese individuals often experience some degree of decreased physical and psychological function. Notably, aerobic and anaerobic capacity, ability to perform basic tasks, ability to perform at work, and ability to sleep (including the development of sleep apnea) may decline with obesity (6-10). These decreases in physical function may be accompanied by, or compounded by, some combination of feelings of depression, shame, guilt, and social isolation, as well as other potential psychological stressors (5, 11-13). Moreover, because obesity has been linked to cardiovascular disease (CVD) (14, 15), type II diabetes (16), and some forms of cancer (including colon, kidney, endometrial, and postmenopausal breast cancers) (17, 18), individuals may have no choice but to spend a substantial amount of money to assuage the negative healthcare outcomes associated with obesity. Studies have estimated that obese individuals spend as much as $1429-$1723 more per year on healthcare when compared to individuals of normal weight (BMI between 18 and 25 $kg \cdot m^{-2}$) (4, 19).

Weight Loss Strategies

The figures and statistics listed above make it clear that obesity is a major healthcare problem, and an intervention is needed. Several strategies exist that may help an individual lose weight. They exist on three tiers. The first tier of weight loss strategies, dietary intake and physical

activity/exercise, may be implemented by an individual at home. Dietary modification can effectively stimulate weight loss in obese individuals (discussed in further detail below). Exercise, of sufficient volume and intensity, has also been shown to decrease fat mass and, thus, BMI (20).

The second tier of weight loss strategies requires intervention by healthcare professionals. These methods should be employed after the first tier of intervention has been deemed unsuccessful with regards to obtaining the needed weight loss/health outcomes. These strategies include weight-loss counselling by a registered dietitian or a counselor, as well as consideration for the use of prescription weight-loss drugs. The Food and Drug Administration (FDA) has approved several drugs designed to induce weight loss, including lorcaserin and phentermine–topiramate, which have been shown to assist individuals with weight loss goals (21).

The third and final tier consists of bariatric surgeries. These surgeries, such the Laparoscopic Adjustable Gastric Banding (LAGB), Laparoscopic Sleeve Gastrectomy (LSG), and Roux-en-Y Gastric Bypass (RYGB), are typically reserved for individuals with extremely high BMIs (BMI > 40 $kg \cdot m^{-2}$). These surgeries should be considered as a last resort for those seeking weight loss and should be reserved for those who have medical conditions secondary to being obese. However, they have been shown to be extremely effective in individuals who have extreme trouble with losing weight.

The intervention that is most applicable to the average obese person include lifestyle modification, involving increased physical activity and diet modification. Perhaps the most recognizable form of diet modification is caloric restriction. Caloric restriction (CR) consists of restricting the number of calories consumed per day. Typically, CR attempts to reduce caloric intake by 20-40% of *ad libitum* consumption, achieved by consuming a reduced volume of food at feeding periods (22). CR has been shown to increase lifespan and improve myriad health

markers (22-24). Because of the low tolerability of CR in human populations (25), researchers and healthcare professionals have developed indirect methods to achieve caloric restriction that are better tolerated by humans. One such method is intermittent fasting (IF). IF is a general term that describes any dietary modification in which an individual intentionally alternates between periods of "feeding" and extended, self-induced "fasting" (26). Two notable methods of IF have emerged: time-restricted feeding (TRF) and alternate day fasting (ADF). TRF restricts daily feeding periods to designated hours of the day (i.e. only eating from 12:00 pm – 7:00 pm), extending the typical overnight fast by several hours. TRF often, but not always, reduces daily caloric intake by reducing feeding time. Alternate day fasting (ADF) is the practice of fasting for one day, followed by *ad libitum* consumption on the subsequent day. Although it has been documented that individuals practicing ADF typically increase caloric intake on the feeding days, net calorie ingestion is typically lowered over the total duration of the regime (27).

For many individuals, CR is intolerable for extended periods of time. Dietary restriction (DR) may be more tolerable to these individuals. DR is a dietary modification in which an individual reduces or eliminates the consumption of specific dietary components (28). DR commonly involves the reduction of a specific macronutrient (carbohydrates, lipids, or proteins). Vegetarian diets, ketogenic diets, and the Daniel Fast (DF) are examples of DR. The DF is a religiously motivated DR, derived from the Bible. As described in the book of Daniel, the DF is a strict vegan diet, consisting of only of pulse (food grown from seeds) (29). Today, a typical DF consists of the *ad libitum* consumption of vegetables, whole grains, nuts, legumes, seeds, and healthy oils. It prohibits the consumption of processed foods, coffee, and alcohol. Individuals typically partake in the DF for 21-day periods.

Time-restricted feeding, alternate day fasting, and the Daniel Fast have been shown to improve many markers of health and longevity (29-31). However, relative to caloric restriction, time-restricted feeding, alternate day fasting, and the Daniel Fast have not been studied in great detail, especially with regard to changes in body composition and physical fitness (e.g., endurance capacity). Because body composition and physical fitness are important markers of overall health, it is important to compare TRF, ADF, and DF in a single study to determine the relative effectiveness of each dietary modification these outcomes. To the author's knowledge, no such study has been conducted.

This review will discuss the relevant literature regarding TRF, ADF, and DF and the health benefits associated with each. It will also discuss measures of body composition and physical fitness and their relation to overall health and TRF, ADF, and DF.

Body Composition

The term "body composition" describes the chemical composition of the body. The human body is composed of muscle mass, connective tissue, organs, bone, adipose tissue, and water. Because it is difficult to measure the contribution of each of these components to overall body composition through traditional methods, conventional measurements of body composition often group the components of the human body into two gross categories: fat mass (FM) and fat-free mass (FFM). Muscle mass, connective tissue mass, organ mass, and bone mass are collectively termed FFM, while all adipose tissue is termed FM. Assessments of body composition attempt to measure the relative percentages of FFM and FM and are often expressed simply in terms of percent fat-free mass (%FFM) versus percent fat mass (%FM).

Body composition can either be directly measured or estimated. Cadaver analysis and diagnostic imaging (Computerized Axial Tomography [CAT scan], Magnetic Resonance Imaging

[MRI], and dual-energy x-ray absorptiometry [DXA]) are the only methods currently available to directly measure total body composition. Cadaver analysis is considered the gold standard of body composition assessment, which is obviously restricted only to use in animal models (32). Thus, diagnostic imaging is the best method for assessing body composition in living humans. Currently, many investigators use DXA due to its validity and reliability (33). Body composition may also be estimated through various methods, including bioelectrical impedance analysis (BIA), densitometry (hydrostatic weighing, water displacement, air plethysmography), and ^{40}pattasium counting. Moreover, anthropometric measurements such as waist circumference and skinfold thickness measurements are often used in large populations to estimate body composition, as these assessments are quick to perform and inexpensive. However, estimates of body composition are typically, but not always, less reliable than diagnostic imagery (34).

Body Composition and Overall Health

Body composition an important indicator of overall health. A high percentage of FM (% FM) is closely correlated to elevated BMI (1). In turn, elevated BMI is associated with a number of negative health outcomes. Obese individuals (BMI $\geq$ 30 kg·m^{-2}) present with increased CVD risk factors, including hypertension, dyslipidemia, insulin resistance, and type II diabetes mellitus (14). Ultimately, an increase in CVD risk factors leads to increased incidence of CVD-related events in obese individuals, such as myocardial infarction, heart failure, sudden death, coronary heart disease, and ischemic stroke (14, 35, 36).

Abdominal obesity, or visceral obesity, is another important measurement of body composition. Measured by DXA and CT scan, or estimated via waist circumference, abdominal obesity has been linked to a number of adverse outcomes, including increased risk of mortality,

myocardial infarction, and heart failure (37). Weight loss strategies that reduce abdominal obesity should be prioritized.

FFM can also be an important indicator of overall health because FFM is the single best predictor of resting metabolic rate (38). Resting metabolic rate (RMR) - a measurement of resting energy expenditure - accounts for 60-70% of total daily caloric expenditure, depending on physical activity (39). Because energy expenditure must exceed energy intake to induce weight loss, RMR and its influence on energy homeostasis should be considered in models of weight loss. Indeed, researchers have hypothesized that abnormally low RMR is a strong indicator of future weight gain (40). It has been known for some time that FFM is the single best predictor of RMR (38). In fact, FFM explains between 60-80% of the variance in measured RMR (41). Additional adjustments for specific components of FFM such as organ volume and density can push this R^2 value to as high as 85% (42, 43). Thus, dietary interventions that result in weight loss but maintain FFM should be prioritized.

Body Composition and Physical Fitness

Body composition is an independent predictor of physical fitness. Tests of physical fitness often involve moving one's body (i.e. running) as long or as fast as possible. FFM (skeletal muscle) performs the work during such tests. Underweight individuals (BMI < 18 $kg \cdot m^{-2}$) with low levels of FFM may display worse fitness than normal weight individuals (6). Because FM is not responsible for performing human work, FM may be viewed as "dead weight" during physical activity. Individuals must simply carry this extra weight during physical fitness tasks and examinations, similar to wearing a backpack while running. For obvious reasons, it is logical that increased FM will have a detrimental impact on physical performance/fitness. Indeed, physical fitness is negatively correlated with BMI in adolescents aged 12-18 years (6), as well as in young

adults (7, 8). Additionally, studies have shown a negative correlation between body fatness and performance in activities requiring running or jumping (44, 45). Therefore, generally, it can be said that individuals with abnormally high or low BMIs may display lower fitness than individuals of normal BMI.

Body Composition and Dietary Modification

Body composition can be altered significantly through dietary modification. Certainly, many dietary modifications have been created for the sole purpose of decreasing total body mass and FM. Caloric restriction has been shown to positively change body composition by decreasing total body mass and FM (46, 47). Additionally, studies have reported that CR decreases abdominal subcutaneous (48), visceral (48, 49), and intermuscular fat (48, 49). However, studies have also indicated that CR significantly decreases FFM (49). For this reason, CR interventions may recommend consumption of a high-protein diet. CR with a high protein diet shows promise in maintaining FFM during CR-induced weight loss (50), although it should be understood that reducing caloric intake will almost certainly lead to some loss in FFM.

TRF also displays the potential to reduce total body mass and FM while maintaining FFM. To the author's knowledge, three studies have examined the relationship between TRF and body composition in humans. One study failed to report significant differences in total body mass, FM, or FFM following a TRF intervention (51), while the other two studies reported extremely favorable body composition outcomes (52, 53). The most successful study found that participants in the TRF group lost significantly more FM compared to the control group when measured at the conclusion of the 8-week intervention (−16.4 in TRF vs −2.8 %FM in control). Moreover, despite losing significantly more FM, the TRF group did not differ from baseline or between groups for FFM (+0.86 in TRF vs +0.64 %FFM in control) (53). It should be noted that, though existing

evidence suggests that TRF is successful in maintaining FFM during weight loss, no studies have measured body composition in overweight or obese individuals following TRF.

Relative to simple CR, ADF also seems to have the potential to induce favorable changes in body composition in humans. Similar to simple CR, ADF has been shown to result in significant reductions in FM (54-59), visceral FM (58), and waist circumference (57, 60). However, whereas simple CR often results in loss of FFM, modified ADFs have demonstrated the ability to maintain FFM during weight loss. Three out of four studies that have measured FFM have reported no significant reductions in FFM relative to baseline (55, 57) or relative to a control group (56) following the completion of a modified ADF protocol. It should be noted that while individuals appear to retain FFM relatively well during modified ADF, traditional ADF has resulted in significant reductions in FFM during ADF (54, 59).

Alternatively, DF has not resulted in favorable body composition alterations in humans. Only one study has displayed significant reductions in total body mass and/or FM (61). Following a 21-day DF intervention with krill oil supplementation, body weight (74.1 ± 2.4 versus 71.5 ± 2.3 kg), fat mass (21.9 ± 1.5 versus 20.8 ± 1.5 kg), and fat-free mass (52.2 ± 2.0 versus 50.8 ± 1.9) all decreased significantly, relative to baseline. However, neither a traditional DF or a modified DF (inclusion of one serving of meat and one serving of dairy per day) has resulted in significant reductions in any measure of body composition (29, 62, 63). The lack of findings of significant weight loss in these studies is likely due to the short duration of the intervention (21 days).

Physical Fitness

Generally, physical fitness is defined as the ability to perform physical activity (64). It is characterized by a number of subcategories, including cardiorespiratory fitness, muscular fitness, flexibility, balance, and speed. Physical fitness exists on a spectrum, with individuals being

classified anywhere from low fitness to highest fitness based on performance in a number of physical performance examinations (65). Many intrinsic factors such as age, sex, race, motivation, and genetic expression influence physical fitness. Extrinsic factors also influence physical fitness, most notably regular physical exercise (66).

Cardiorespiratory fitness and muscular fitness are the most important components of health-related physical fitness. Cardiorespiratory fitness (CRF) is broadly defined as the overall combined ability of the body's cardiovascular and respiratory systems to uptake, transport, and use oxygen (67). The most accurate measure of CRF is maximal oxygen uptake during an exercise performance test (VO_{2max}) (68). Measurements of VO_{2max} can either be obtained directly or indirectly. Direct measurement of VO_{2max} involves the collection of an individual's expired gases using the Douglas Bag method or automated gas-collection equipment such as a metabolic cart. The composition of the expired gas is analyzed to determine total oxygen uptake at the point of volitional failure. Indirect measurement of VO_{2max} involves the estimation of VO_{2max} using an exercise test. Graded exercise tests, such as a step-up test or a submaximal walk test, are frequently used in clinical settings. A variable (i.e. time or heart rate) is measured upon the completion of the exercise test. The value of this variable is then plugged into an adjusted equation to *estimate* VO_{2max}. Other exercise tests, such as the 1.5 mile run test and the run-time-to-exhaustion test, provide meaningful descriptive data related to CRF.

Muscular fitness is characterized by the strength and endurance of skeletal muscle. A variety of tests have been used to assess muscular fitness. These tests include maximal pushups achieved during a given time frame, maximal sit-ups achieved during a given time frame, maximal grip strength as measured by a hand dynamometer, and maximal weight achieved during resistance

exercise (i.e. bench press). Studies have not determined a single test of muscular capacity that best estimates overall muscular fitness.

Physical Fitness and Overall Health

Studies have routinely reported CRF to be an important predictor in overall health for a multitude of populations. Low CRF leads to decreased ability to perform seemingly-simple daily activities. Additionally, low CRF appears to be a powerful independent risk factor for all-cause mortality in both healthy and non-healthy populations (65, 69-71). Studies have also shown an inverse relationship between CRF and the risk of developing sudden cardiac death (72), cancer mortality (73), childhood obesity (74, 75), type 2 diabetes mellitus (76, 77), and cardiovascular disease (78). Similarly, studies have reported a positive relationship between CRF and cognition (79) and health-related quality of life (80).

Because low CRF is associated with adverse outcomes and high CRF is associated with positive outcomes, improving CRF is perhaps the most important fitness goal for the average individual. Fortunately, studies have repeatedly demonstrated that regular exercise improves CRF (66, 81). Moreover, drastic improvements in CRF may not be required to induce clinically meaningful improvements in health-related outcomes, as indicated by Kokkinos et al. In one study conducted by Kokkinos et al., they examined the relationship between CRF and mortality risk in 6,749 black and 8,911 white men. CRF was measured in metabolic equivalents (METs) (65). One MET was defined as the energy expended at rest by an average individual (estimated to be 3.5 $mL \cdot kg^{-1} \cdot min^{-1}$). The study reported that a 1-MET increase in exercise capacity resulted in a 13% reduction in risk of mortality for individuals with and without cardiovascular disease. Small improvements such as these may also lead to increased ability to perform basic daily tasks, especially in older adults.

Compared to CRF, research on the role of muscular fitness and overall health is limited. Muscular fitness is important for maintaining individual autonomy (82). Simple tests of muscular fitness such as the sit-stand provide valuable indicators of an individual's functional muscular fitness. Individuals that perform extremely poorly on these functional fitness exams will likely experience difficulty performing everyday tasks, a general characteristic of extreme obesity. Moreover, muscular fitness may be an important indicator of general health. Muscular fitness has been inversely linked to several adverse outcomes, including all-cause mortality (83, 84) and premature death (85).Therefore, improving muscular fitness should also be a goal for all individuals.

As mentioned above, physical fitness is closely associated with body composition which is also associated with overall health. Underweight individuals (BMI < 18 kg·m-2) may be less fit than normal weight individuals (6, 86). On the other end of the spectrum, high BMI has been associated with poor physical fitness (6-8). To maximize most measures of physical performance, individuals should strive to maintain normal body weight and health levels of FM.

Physical Fitness and Dietary Modification

Diet plays a major role in physical fitness. Relative to *ad libitum* feeding, CR appears to improve physical performance in mice (87, 88), likely because weight gain is inversely related to physical performance (6). High-carbohydrate diets have been recommended for prime physical performance because they are said to keep glycogen stores saturated and blood concentrations of triacylglycerides and free-fatty acids low (89, 90). Two recent reviews indicated that consumption of a high-fat diet may result in diminished physical performance, especially at high intensities and in anaerobic conditions (91, 92). Therefore, it appears that a high-carbohydrate diet augments performance when compared to a high-fat diet.

Few studies have examined the effects of DF, ADF, or TRF on physical performance. To date, only one study has examined the relationship of the DF and physical fitness. Following the conclusion of a 3-month DF and exercise intervention in male rats, cardiorespiratory fitness was measured (run-time-to-exhaustion) and compared to baseline fitness (Bloomer, In Publication). Exercise capacity increased significantly more (99% increase) in the DF + exercise group when compared to the WD + exercise group (51% increase), indicating that the DF accentuated exercise gains. To this author's knowledge, only 3 studies (excluding studies that focused on Ramadan) have examined the relationship of TRF and physical fitness. Two studies have tested the effects of TRF in humans. Both of these studies utilized 8-week interventions and similar intervention groups (TRF + resistance training 3 x weekly) and control groups (normal diet + resistance training 3 x weekly) (51, 53). Neither study reported significant differences between groups in physical performance as measured by muscular strength or endurance (51, 53). One study examined the effects of TRF on physical performance in animals. This study reported that *Drosophila* subjected to TRF displayed significantly better flying performance, relative to the control group (93). No publications have studied ADF and physical fitness. More research is needed in humans and animals to determine whether TRF, ADF, and DF will enhance physical fitness.

Time-Restricted Feeding

Intermittent fasting (IF) is a general term that describes any dietary modification in which an individual intentionally alternates between periods of "feeding" and extended, self-induced "fasting" (26). During the feeding period, individuals are often permitted to consume foods *ad libitum* (51, 59, 94), although some IF interventions have restricted caloric intake during the feeding period (53, 95). During the fasting period, individuals either abstain from caloric intake all together (51, 52, 96) or dramatically reduce caloric intake by 70-80% (56, 97, 98). Fasting

periods can last anywhere from 12 hours (99) to 48 hours (97) or longer, and the duration of this period typically demarcates the classification of each IF regime. Recently, two types of IF have recently garnered noteworthy interest from the scientific community.

In recent decades, alternate day fasting (ADF) and time-restricted feeding (TRF), two specific types of IF, have gained scientific momentum. ADF involves the cycling of 24-hour *ad libitum* feeding with 24-hour fasting or severe caloric restriction (26). Alternatively, TRF – the topic of this section - simply involves the daily reduction of the feeding period. Typical TRF interventions limit the daily feeding period to a 4-12 hour window in the afternoon/evening, eliminating any caloric consumption during the morning and night hours. This results in a lengthened fasting period (12-20 hours per day). However, during feeding periods, individuals are allowed *ad libitum* caloric consumption. Additionally, TRF does not necessarily mandate that individuals "improve" their diet, only alter their meal timing. For these reasons, TRF is viewed as a very user-friendly alternative to individuals who find it difficult to adhere to the guidelines of simple CR.

Time-Restricted Feeding: Overview of Current Literature

Human Trials

Few publications have examined the effects of controlled TRF interventions in a human model. However, despite utilizing short-term TRF interventions lasting between 2-8 weeks and some conflicting results, these studies have displayed promise. Many benefits have been associated with TRF, including - but not limited to – reduction of caloric intake (51), weight loss (52, 53), decreased FM (52, 53). Positive alterations in blood lipids following TRF such as increased HDL (52) and decreased triglycerides (53) have been noted. Additionally, TRF has been associated with decreased blood glucose and decreased blood insulin (53). Studies examining the effects of

Ramadan-fasting-induced TRF have reported similar positive results (100-104). Furthermore, studies indicate that TRF, especially 16/8 (16 hour fasting period/8 hour feeding period) TRF, is well tolerated by participants (53).

Currently, few negative or conflicting results have been reported in regards to TRF. Carlson and colleagues stated that an 8-week TRF intervention (1 iso-caloric [relative to control group] meal per day consumed between 5:00pm – 9:00pm) resulted in increased morning blood glucose concentrations and decreased glucose tolerance (105). The authors suggested that the increase in blood glucose levels could be a result of the study's design, stating that early-morning blood glucose levels were likely impacted by the consumption of extremely calorically dense intervention meal the during the preceding night. The duration of period may have also influenced this outcome. Longer feeding periods (6-8 hours) may improve measures of glucoregulation, as displayed by the findings of Moro et al. (53). Additionally, Tinsley et al. indicated that an 8-week TRF intervention (4-hour *ad libitum* feeding period between 4:00pm – 8:00pm) may lead to decreased hypertrophic gains in untrained males relative to untrained males following the same resistance training protocol but consuming food *ad libitum* (51). Thus, TRF may not be appropriate in the early stages of resistance training when hypertrophy is the main goal. However, it should be noted that TRF has been reported to maintain FFM during weight loss and resistance training (51-53).

Animal Trials

Animal trials have largely corroborated many of the beneficial findings of human trials discussed above. TRF in animals appears to be an effective method for preventing weight gain and/or inducing weight loss in a number of species, including *Drosophila* (93), mice (99, 106-110), rats (111) and geese (112). Notably, evidence suggests that weight loss and/or prevention of

weight gain can be achieved through TRF despite consumption of at high-fat diet (99, 107). Studies have indicated that TRF is successful in improving important lipid factors, such as lowering total cholesterol (107, 108, 113) and triglycerides (107, 109). TRF has also been noted to improve glucoregulatory factors by lowering plasma insulin levels and increasing insulin sensitivity (107, 108). Studies have also reported diminished levels of inflammatory markers following TRF (107, 108).

It should be noted that not all animal trials have yielded exclusively positive results. Some studies have failed to report differences in weight gain post-TRF, relative to a control group (114, 115). Additionally, not all studies have demonstrated significant improvements in glucoregulatory factors following TRF (115). In fact, Park et al. stated that TRF exacerbated hepatic insulin resistance, despite loss of FM, in hormone-sensitive lipase knockout mice (115). These discrepant results may be the result of a number of factors. In some cases, small sample sizes may have limited statistical power, diminishing the opportunity to find significant differences between groups (114). In other cases, differences in reported outcomes may be attributed to differences is protocols, as studies have used various animal models (i.e. hormone-sensitive lipase knockout mice vs wild-type mice), interventions of differing lengths (i.e. a 4-week intervention v. a 12-week intervention), and daily feeding periods of differing lengths (i.e. a 4-hour feeding period versus a 12-hour feeding period).

Time-Restricted Feeding and Physical Fitness

Human Trials

Two studies have examined the effects of TRF on physical fitness in humans, both utilizing tests of muscular fitness. Tinsley and colleagues utilized an 8-week TRF in combination with resistance training in young, normal-weight, untrained males (n = 18) (51). The intervention group

consumed a modified TRF diet, while the control group continued to consume their normal diet throughout the duration of the study. Both the control group and the intervention group completed resistance training (8-12 repetitions to failure) on non-consecutive days 3 x weekly, alternating between lower-body and upper-body sessions. Maximal muscular output – classified by maximal muscular strength and maximal muscular endurance – was measured at base-line and post-intervention. Maximal strength was defined by maximal weight achieved during 1-repetition (1RM) during the hip sled and barbell bench press exercises. Muscular endurance was defined by maximal repetitions using a standardized weight during the hip sled and barbell bench press exercises. Relative to baseline, hip-sled endurance, bench-press endurance, and hip sled strength increased, post-study. However, no differences existed between groups.

A second study focused on TRF in resistance-trained men (n = 34). Moro and colleagues implemented a 16/8 TRF intervention (16 fasting period; 8 hour feeding period between 1:00pm-9:00pm; meals consumed at 1:00pm, 4:00pm, and 8:00pm) in combination with 3 x weekly resistance training (split routine; 6-8 repetitions to failure) (53). The control group was instructed to consume meals at standard times (1:00pm, 4:00pm, and 8:00pm) in combination with 3 x weekly resistance training (split routine; 6-8 repetitions to failure). Muscular strength was measured at baseline and post-intervention. Muscular strength was defined as 1RM achieved during leg press and bench press exercises. Moro et al. reported that weight achieved during 1RM for leg press increased in both groups, relative to baseline. However, no differences existed between groups. 1RM achieved during bench press did not differ from baseline or between groups.

Taken together, these studies indicate that short-term TRF interventions do not inhibit anaerobic muscular performance during resistance training. These data are generally supported by similar studies that have examined the relationship between performance and TRF during

Ramadan (116). More research is needed in this area to verify that TRF does not inhibit, or preferably improves, physical performance. Additional studies should also focus on measures of CRF.

Animal Trials

To the author's knowledge, only one animal study has included any physical performance measure following TRF. Gill et al. indicated that 5 weeks of 12-hour TRF in wild type *Drosophila* significantly improved ability to fly, as measured by flight index (93). However, the study failed to specify how or when flight performance was measured. Additionally, the researchers did not mention the specifics of the flight index that was used. Because of the lack of detail provided in the paper of Gill and coworkers and the fact that no other studies are available in this area, more studies are needed to better understand the potential influence of TRF on physical performance in animals.

TRF and Body Composition

Human Trials

To the author's knowledge, three studies have examined the relationship between TRF and body composition. One study reported moderately favorable body composition outcomes, while the other two studies reported extremely favorable body composition outcomes. The two studies described above in the TRF section reported measures of body composition in addition to the aforementioned measures of muscular performance. Tinsley et al. measured body composition via DXA (51). No significant differences existed relative to baseline or between groups for total body mass, FFM, or FM. However, the study did state that, though the difference was not significant, FFM increased in the control group (+2.3 kg FFM) and decreased in the TRF group (-0.2 kg FFM). The researchers mentioned that the non-significant difference in FFM gains between groups could

most likely be attributed to the training status of the participants (i.e. untrained) and the caloric demand associated with hypertrophy during the onset of training.

Alternatively, Moro and colleagues reported very promising results. They used DXA to measure body composition at baseline and post-intervention (53). The study indicated that participants in the TRF group (10.90 ± 3.51 kg at baseline versus 9.28 ± 2.47 kg post-intervention) lost significantly more FM compared to the control group (11.36 ± 4.5 kg at baseline versus 11.05 ± 4.27 kg post-intervention). The TRF group also experienced greater reductions in %FM relative to the control group (−16.4 % FM in TRF vs −2.8 % in control). However, despite losing significantly more FM than individuals in the control group, individuals in the TRF group (73.08 ± 3.88 kg at baseline versus 73.72 ± 4.27 kg post-intervention) did not exhibit significantly different levels of FFM post-intervention when compared to baseline or the control group (73.93 ± 3.9 kg at baseline versus 74.41 ± 3.59 kg post-intervention). Additionally, measurements of mid-arm circumference and mid-thigh circumference did not differ between groups or from baseline for either condition.

A third study examined the effects of an 8-week 20/4 (20-hour feeding period, 4-hour fasting period) TRF intervention on body composition in normal-weight, middle-aged adults (n = 15) (52). All meals were provided by the lab staff, and caloric intake did not differ between the control condition (3 meals daily) and the intervention condition (all food consumed between 5:00pm – 9:00pm). Body composition was assessed via BIA at baseline and post-intervention. Favorable changes in body composition were exhibited post-intervention. Relative to the control condition, the TRF condition exhibited significantly greater weight loss (1.4 kg) and reduction of FM (2.1 kg). No differences were observed between-groups for FFM. Additionally, none of the body-composition measures differed significantly relative to baseline.

No studies have measured body composition in overweight or obese individuals following TRF. However, the evidence discussed in this section shows that similar individuals can achieve loss of FM and retention of FFM through TRF. Further studies should apply these findings to an obese sample to ascertain if similar alterations in body composition are attainable through TRF.

Animal Trials

A majority of animal trials that have reported body composition have indicated that TRF has a major impact on body mass and FM. Studies show that mice fed during the dark phase (active phase) display significantly lower total body mass and lower total FM and %FM when compared with mice fed *ad libitum* or during the light (inactive) phase (99, 108, 117, 118). Notably, this reduction appears to occur independent of diet composition. Studies have demonstrated that mice fed a high fat diet during the dark phase display lower %FM relative to controls (107, 108). No studies have described alterations in FFM or %FFM following TRF. More research is needed to determine how TRF affects FFM in animals.

Alternate Day Fasting

Alternate day fasting (ADF) is a type of IF protocol that has received increased attention from members of the scientific community over the past decade. ADF regimes consist of alternating “feed” and “fast” days, often grouped in 14-day cycles. For example, ADF protocols should be arranged such that individuals experience 7 fast days and 7 feed days over a given 14-day period. Feed days typically consist of *ad libitum* caloric consumption (27), although at least one study has implemented a caloric limit on feed days (60). Fast days involve either total or severe caloric restriction. ADF interventions that eliminate caloric intake for the entire 24-hour period are considered traditional ADFs (54, 59). Traditional ADFs may permit consumption of calorie free beverages and/or calorie-negligible options such as bouillon/stock cube soup (59). ADF

interventions that allow for consumption of a small meal on fast days are termed modified ADFs. (56, 57). Modified ADFs typically permit consumption of 1 small meal on fast days in an effort to depress feelings of hunger on fast days and, ultimately, attrition rates. These meals typically consist of 25-30% of normal daily caloric consumption and are typically consumed in the early afternoon (55, 94), though evidence has suggested that meal timing on fast days does not impact outcomes (58).

Alternate Day Fasting: Overview of Current Literature

Human Trials

The benefits of ADF in humans have been well-documented. Thus far, studies have primarily focused on ADF in obese populations (56), although some trials have included normal-weight and over-weight participants (54, 56). Many of these publications have utilized a modified ADF intervention (55, 58, 60, 119, 120), while relatively few studies have implemented a traditional ADF in humans (54, 59). Additionally, although most studies have utilized short-term (2-12 week) ADF interventions, the results have been encouraging.

Generally, human trials have reported improved health-related outcomes following ADF. Studies have repeatedly shown that ADF is effective for inducing weight loss in both obese (55, 57-60, 121, 122) and non-obese individuals (54-56), while resulting in favorable changes in body composition (these variables will be discussed in further detail below) (57, 59). Additionally, ADF has been associated with improved measures of cardio-metabolic health, including decreased cholesterol (56, 59, 98), decreased LDL levels (56, 59, 98), decreased triglyceride levels (56, 59, 98), and decreased blood pressure (56, 57, 60). These cardiovascular-health-related benefits of ADF may be further enhanced when combined with an exercise intervention (57). Furthermore,

one study has also shown that markers of inflammation and oxidative stress may be improved following ADF in obese individuals with asthma (122).

Some null or negative findings have been associated with ADF. One study failed to report reductions in total body mass, likely due to the short duration of the 2-week intervention used (96). Another study has indicated that short-term ADF can lead to unfavorable reductions in HDL levels (59). Still others have indicated that ADF results in the loss of FFM (54, 59). Interestingly, all of these undesirable outcomes have been reported following traditional ADF. Potentially, the total elimination of any caloric intake on fast days of a traditional ADF leads to the development of these adverse outcomes. Indeed, Heilbronn et al. address this notion in the discussion section of their paper, hypothesizing that a modified ADF that allows for 10-20% of caloric needs on fast days may lead to better outcomes (54). Thus, it is recommended that individuals undergo a modified ADF for a period of at least 3 weeks for best clinical results.

Animal Trials

ADF interventions conducted in animal models have yielded positive results. Similar to simple CR, ADF has been reported to protect against the development of several severe negative health outcomes, such as diabetes, cancers, heart disease, and neurodegeneration (123). ADF has also been associated with significant cardio-protection (124). Studies have indicated that ADF favorably alters measures of cardiovascular health by lowing heart rate, blood pressure, blood insulin, and blood glucose (125, 126). Moreover, ADF has been shown to induce weight loss and prevent weight gain (125, 127-129), as well as decrease visceral fat mass (129, 130). Indeed, weight loss appears to be achievable through ADF despite the consumption of a high fat diet (131). Ultimately, these protective effects of ADF may result in increased longevity (132, 133).

ADF and Physical Fitness

To the author's knowledge, no studies have reported any measure of physical fitness or physical performance with respect to ADF in humans or animals. However, because high FM and low FFM are associated with decreased physical fitness and performance (7, 8), it is expected that ADF would improve physical fitness and performance simply by improving body composition in overweight and obese individuals and animals.

ADF and Body Composition

Human Trials

Relative to simple CR, ADF seems to have the potential to induce favorable changes in body composition. Similar to simple CR, ADF has been shown to result in significant reductions in FM (54-59), visceral FM (58), and waist circumference (57, 60). However, whereas simple CR often results in loss of FFM, modified ADFs have demonstrated the ability to maintain FFM during weight loss. Three out of four studies that have measured FFM have reported no significant reductions in FFM relative to baseline (55, 57) or relative to a control group (56) following the completion of a modified ADF protocol.

It should be noted that while individuals appear to retain FFM relatively well during modified ADF, traditional ADF may result in significant reductions in FFM. The only two traditional ADF trials in humans have reported significant decreases in FFM following interventions of 3 (54) and 8 weeks, respectively (59). For this reason, modified ADFs are recommended for optimizing loss of FM and retention of FFM during ADF.

Animal Trials

Interestingly, though many animal studies have reported ADF-induced weight loss, few studies have investigated body composition following ADF, and these results are conflicting. Two studies have found that ADF does not result in decreased fat mass. Varady et al. reported no

significant difference in inguinal adipose mass or epididymal adipose mass following a 4-week traditional ADF (32). A subsequent study also failed to report significant differences in fat mass between control and ADF groups after 4 weeks of ADF (130). Only one study, conducted by Dorighello et al., has reported successful reductions in total fat mass. This study indicated that a 12-week traditional ADF was successful in reducing epididymal fat pad mass in wild-type mice (134). No studies have reported measures of total FFM.

While the literature has failed to support unequivocally the claim that ADF reduces fat mass in animals, it should be noted that preliminary evidence has suggested that ADF can alter the size and distribution of adipose cells in clinically meaningful ways. ADF has been shown to favorably alter adipose tissue distribution by decreasing visceral fat mass and increasing subcutaneous fat mass (130). Because visceral obesity has been linked to increased incidence of insulin resistance, cardiovascular events and premature death, decreases in visceral fat mass are a significant finding (130). Additionally, another study has indicated that ADF results in decreased inguinal adipose cell size (32). This finding is important because large adipose cells have been reported to increase the risk of developing chronic disease (130). So, while the evidence does not yet fully support the notion that ADF leads to decreased total FM in animals, studies do suggest that ADF favorably alters the composition and distribution of FM, a worthwhile outcome.

Dietary Restriction

Dietary restriction (DR) is a dietary modification in which an individual reduces or eliminates the consumption of one or more specific dietary components (28). DR commonly involves the reduction of a specific macronutrient (carbohydrates, lipids, or proteins), but other dietary components may be the subject of DR. One such example is methionine (an essential amino acid) restriction, which has been shown to lower the incidence of cancer and extend life spans in

animal models (135). Though the primary goal of DR may not be calorie restriction, it typically results in reduced caloric intake by default. The Daniel Fast is an example of a DR model that typically leads to decrease in caloric consumption, relative to normal *ad libitum* calorie intake.

The Daniel Fast (DF) is a religious fast, derived from the Bible (29). Typically, individuals partake in the DF for a 21-day period, as described in the book of Daniel. It is a strict vegan diet that inherently leads to reduced caloric intake. Individuals participating in a DF may consume fruits, vegetables, whole grains, nuts, legumes, seeds, and healthy oils (such as olive oil) *ad libitum*. Products that contain additives or preservatives, as well as coffee and alcohol, are prohibited. Because the DF involves *ad libitum* intake of the permitted foods, individuals may find it an easier model to follow than other caloric restriction models.

Daniel Fast: Overview of Current Literature

Human Trials

Several studies have shown the Daniel Fast, followed for as few as 21 days, to improve markers of health. All have been conducted at The University of Memphis. In the first such study conducted, Bloomer and colleagues implemented a 21-day DF in 43 human participants (13 men; 30 women; 35 ± 1 yrs; range: 20-62 yrs) to assess its effects on metabolic and cardiovascular risk factors (29). Participants were not excluded based on body mass. Thus, individuals with a variety of BMIs enrolled in the study: 21 participants were considered to have a "normal" BMI (BMI < 25 $kg \cdot m^{-2}$); 9 participants were considered overweight (BMI 25-29.9 $kg \cdot m^{-2}$); and 13 participants were considered obese (BMI > 30 $kg \cdot m^{-2}$Participants were generally considered to be healthy individuals. Thirty-four of 43 participants were considered exercise trained (participate in 3 hours of anaerobic and aerobic exercise per week). A pretest was conducted for all tested variables before the 21-day intervention was initiated. Post-intervention testing revealed that many important

metabolic and cardiovascular markers of health had improved following the 21-day fast. Amongst other variables, total cholesterol (171.07 ± 4.57 vs. 138.69 ± 4.39 mg·dL^{-1}), systolic blood pressure (SBP; 114.65 ± 2.34 vs. 105.93 ± 2.12 mmHg), and diastolic blood pressure (DBP; 72.23 ± 1.59 vs. 67.00 ± 1.43 mmHg) were significantly lowered when compared to baseline values ($p < .05$). Additionally, insulin blood-concentrations and HOMA-IR scores were lowered ($p > .05$) by clinically meaningful values. Moreover, total caloric intake was significantly reduced during the intervention period when compared to baseline (2185 ± 94 vs. 1722 ± 85 kcals). The only noted downside of the DF was a significant decrease in HDL-C. Thus, Bloomer et al. concluded that a 21-day DF can result in key metabolic changes that may improve overall health.

A year later, a subsequent study was published on the same data set which assessed antioxidant status and markers of oxidative stress following a 21-day DF (136). Results showed that a 21-day DF positively effects antioxidant status and markers of oxidative stress. Concentrations of certain variables increased significantly ($p < .05$) post-intervention, including Nitrate/Nitrite (NOx) and Trolox Equivalent Antioxidant Capacity (TEAC). Markers of oxidative stress were reduced, post-intervention. Malondialdehyde (MDA) levels were significantly lowered ($p = .004$) by 15%. H_2O_2 levels were also meaningfully lowered ($p = .074$) by 14%. Together, these data indicate that a 21-day DF can lower levels of oxidative stress while raising levels of antioxidants.

Subsequent studies have validated the majority of these results. In 2012, Bloomer et al. conducted a 21-day DF on 22 participants to examine post-prandial oxidative stress. Ten men and 12 women (aged 35 ± 3 years) completed the study (62). Participants had a wide variety of BMIs (13 normal, 4 overweight, and 5 obese). Bloomer and colleagues noted no significant decrease in post-prandial oxidative stress. However, several health benefits were reported. Both DBP and SBP

were lowered post-intervention. NOx increased following the DF (TEAC was not significantly higher). Total caloric intake was lowered by 16% during the DF.

A modified DF has also been shown to produce meaningful health benefits (61, 63). A modified DF is inclusive of dietary components that are excluded in a traditional DF. In a study published in 2012, Trepanowski and colleagues instructed participants in the experimental group to consume krill oil capsules (2 g/day in 2 daily dosages of 1 g) daily in an effort to maintain HDL-C levels (61). While HDL-C levels were not significantly different between the control and experimental groups, the litany of health benefits reported in the original study conducted by Bloomer et al. in 2010 were observed. Similarly, Alleman and colleagues reported a significant decrease in HDL-C following a 21-day DF modified to include one serving of lean meat (3 oz.) and dairy (8 oz. skim milk) per day (63).

Animal Trials

To date, two studies have implemented a DF intervention in an animal model. Bloomer et al. utilized a 3-month DF intervention in male Long-Evans rats (n=60) to determine the effects of the DF on physical performance, body composition, blood lipids, oxidative stress and inflammation (Bloomer, unpublished). Following a 2-week acclimation period, animals were assigned to an exercise group and a diet group. Two exercise groups existed, with animals either being assigned to the exercise-trained group (E), which exercised 3 times weekly on a treadmill, or the sedentary group, which was only permitted normal daily activity. Two diet groups were used, with animals either assigned to an *ad libitum* WD group or an *ad libitum* DF group. At the conclusion of the study, DF rats displayed relatively lower body mass, cholesterol, triglycerides, lipid peroxidation, and protein oxidation than WD rats. Additionally, rats in the DF+E group

displayed greater physical performance increases, relative to the WD+E group. Markers of inflammation did not differ between groups.

In a separate publication, Daniels et al. detailed how the intervention described above affected the rats' viscera, particularly the small intestine. Daniels et al. reported several key findings. Following the 13-week intervention, WD rats had heavier livers than DF rats (25.9 ± 2.0 kg versus 17.1 ± 0.6 kg). The intestines of the WD rats were shorter when compared to the DF rats' intestines. The weight of the WD rats' intestines represented a smaller percentage of total body weight ($g \cdot kg^{-1}$ body weight) than the intestines of the DF mice. The WD rats intestines displayed shallower crypts and narrower villi, compared to the DF rats' intestines. These results are likely the result of the low fat, high fiber composition of the DF diet and indicate that the small intestines of DF rats adapted to become more absorptive per kg of body weight compared to the WD rats' small intestines.

Daniel Fast and Physical Performance

Human Trials

No human trials have tested physical fitness following DF. However, many subjects have commented that they "feel" better physically when following the DF eating plan. Of course, quantitative data are needed to confirm improvements in performance.

Animal Trials

To date, only one study has examined the relationship of the DF and physical fitness. This study utilized a 3-month DF intervention in male Long-Evans rats to determine the effects of the DF on physical performance (Bloomer, unpublished). Baseline cardiorespiratory capacity was measured for all animals as run time to exhaustion. Following the conclusion 3-month intervention, cardiorespiratory fitness was measured again. Exercise capacity significantly increased for both

exercise groups, relative to baseline. However, exercise capacity increased significantly more (99% increase) in the DF + E when compared to the WD + E group (51% increase), indicating that the DF increased exercise gains independent of body mass.

Daniel Fast and Body Composition

Human Trials

To date, the DF has not resulted in consistent and favorable body composition alterations in humans but has resulted in a mean weight loss of approximately 4-6 pounds over a three week intervention period. Only one study has displayed significant reductions in total body mass and/or FM (61). Following a 21-day DF intervention with krill oil supplementation, body weight (74.1 ± 2.4 versus 71.5 ± 2.3 kg), fat mass (21.9 ± 1.5 versus 20.8 ± 1.5 kg), and fat-free mass (52.2 ± 2.0 versus 50.8 ± 1.9) all decreased significantly, relative to baseline. However, other studies, using either a traditional DF or a modified DF (inclusion of one serving of meat and one serving of dairy per day), have not reported significant improvements in any measure of body composition (29, 62, 63). Two traditional DF studies have reported measures of body composition. The first traditional DF study indicated that total body mass (77.5 ± 3.0 versus 74.7 ± 2.7 kg), %FM (30.2 ± 1.6 versus 29.9 ± 1.6%), %FFM (53.8 ± 2.0 versus 52.1 ± 1.9%), and waist circumference (92.2 ± 2.0 versus 90.4 ± 2.0 cm) remained unchanged at the conclusion of the 21-day intervention (29). Similarly, the second traditional DF study reported that body mass (77.8 ± 3.8 versus 75.1 ± 3.5 kg), FM (21.1 ± 2.2 versus 19.9 ± 2.1 kg), %FM (26.7 ± 2.3 versus 26.1 ± 2.1%), FFM (56.7 ± 2.8 versus 55.2 ± 2.7 kg), and waist circumference (88.7 ± 2.9 versus 87.5 ± 2.9 cm) remained unchanged at the conclusion of the 21-day intervention (62) . A DF modified to include meat and dairy also reported no changes in body composition following 21 days of DF, including no changes in total body mass in the traditional DF group (81.7 ± 4.8 versus 79.3 ± 4.9 kg) or in the modified DF

group (74.5 ± 6.1 versus 72.4 ± 5.7 kg); no changes in %FM in the traditional DF group (35.7 ± 2.4 versus 35.5 ± 2.5%) or in the modified DF group (33.8 ± 3.5 versus 33.4 ± 3.6%); and no changes in %FFM in the traditional DF or the modified DF group (63). The lack of findings of statistically significant weight loss in these studies is likely due to the short duration of the intervention (21 days) and the variability in subjects' response.

Animal Trials

The animal trial discussed above in *Daniel Fast and Physical Fitness* also reported measures of body composition. The data indicated that DF is effective in preventing weight gain in rats, relative to *ad libitum* feeding (Bloomer, unpublished). Rats in the WD group (571.1 ± 14.7 kg) weighed significantly more post-intervention than all other groups (516.8 ± 10.7 kg WD + E; 478.7 ± 11.3 kg DF + E; 496.8 ± 13.5 kg DF). Additionally, rats in the DF groups weighed significantly less than rats in the WD groups (data not given). Bloomer et al. also reported a group effect for %FM. Both DF groups (20.3 ± 1.3% DF + E; 24.6 ± 1.4% DF) displayed lower %FM than both WD groups (30.6 ± 1.3% WD + E; 33.5 ± 1.0% WD). Furthermore, no significant between-group differences were noted for FFM (366.0 ± 9.2 kg WD + E; 386.8 ± 6.7 kg WD; 391.4 ± 8.8 kg DF +E; 376.5 ± 7.8 kg DF), indicating that the DF may be effective in attenuating FFM loss in animals.

Conclusion

Obesity is a multifactorial disease that presents myriad problems, both in the United States and worldwide. It is associated with increased risk of chronic, and potentially fatal, disease, such as cardiovascular disease, heart attack, and suddent death (14). Additionally, obesity places a major financial burden on the individual and the United States healthcare system (4). Thus, attenuating

the rise of obesity worldwide is a primary goal of many healthcare organizations, such as the World Health Organization. This goal may be achieved through dietary modification.

Though more research is needed, three novel dietary interventions – time-restricted feeding, alternate day fasting, and the Daniel fast – appear able to successfully induce weight loss, while simultaneously generating additional positive health outcomes. However, each one of these dietary modifications currently displays unique benefits. TRF causes the most favorable changes in body composition. While studies have linked DF and ADF to insignificant or unfavorable alterations in body composition, studies generally show that TRF leads to significant reductions in FM (52, 53, 99) without reducing FFM (52, 53). Additionally, because TRF permits the daily consumption of a high-fat diet, it has an added psychological benefit, relative to ADF (which severely restricts caloric intake every other day) and DF (which eliminates processed foods). Conversely, ADF is the only dietary modification out of the three that has been shown to consistently reduce total body mass in a significant manner. Varady et al. and others have repeatedly demonstrated that ADF results in significant reductions of total body mass (54-56, 59). TRF and DF have yet to consistently demonstrate large improvements in weight, though TRF has not been utilized in an obese sample, and DF studies have yet to span longer than 3 weeks in humans. DF appears to be the most comprehensive dietary modification, compared to TRF and ADF. DF studies have shown improvements in virtually every health-related factor, despite short-term interventions (29, 61, 63, 136). Additionally, DF is the only dietary modification that has been shown to increase physical performance (Bloomer, unpublished).

Despite the promising results of the literature detailed above, much still needs to be learned about these dietary modifications. Little literature has been published on DF and TRF, and few of these studies have reported measures of body composition or physical performance, essential

indicators of overall health. Moreover, no studies have implemented TRF, ADF, and DF in the same study to compare outcomes of each dietary modification. Thus, a single study comparing TRF, ADF, and DF is needed to determine the relative effectiveness of these dietary modifications on body composition and physical performance.

References

1. Moyer VA. Screening for and management of obesity in adults: U.S. Preventive Services Task Force recommendation statement. Annals of internal medicine. 2012 Sep 4,;157(5):373.

2. World Health Organization. Overweight and obesity. . 2014.

3. Ogden CL, Carroll MD, Fryar CD, Flegal KM. Prevalence of Obesity Among Adults and Youth: United States, 2011-2014. NCHS data brief. 2015 Nov(219):1.

4. Tsai AG, Williamson DF, Glick HA. Direct medical cost of overweight and obesity in the United States: a quantitative systematic review. Obes Rev. 2011 -1;12(1):50-61.

5. Kolotkin RL, Meter K, Williams GR. Quality of life and obesity. Obesity Reviews. 2001 November 1,;2(4):219-29.

6. Mak K, Ho S, Lo W, Thomas GN, McManus AM, Day JR, et al. Health-related physical fitness and weight status in Hong Kong adolescents. BMC public health. 2010;10(1):88.

7. Häkkinen A, Rinne M, Vasankari T, Santtila M, Häkkinen K, Kyröläinen H. Association of physical fitness with health-related quality of life in Finnish young men. Health and quality of life outcomes. 2010;8(1):15.

8. Leyk D, Rohde U, Gorges W, Ridder D, Wunderlich M, Dinklage C, et al. Physical Performance, Body Weight and BMI of Young Adults in Germany 2000 - 2004: Results of the Physical-Fitness-Test Study. International Journal of Sports Medicine. 2006 /08;27(08):642-7.

9. Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP. Pathophysiology of Sleep Apnea. Physiological Reviews. 2010 Jan 1,;90(1):47-112.

10. Pronk N, Martinson B, Kessler R, Beck A, Simon G, Wang P. The Association Between Work Performance and Physical Activity, Cardiorespiratory Fitness, and Obesity. Journal of Occupational and Environmental Medicine. 2004 Jan;46(1):19-25.

11. Luppino FS, de Wit LM, Bouvy PF, Stijnen T, Cuijpers P, Penninx, Brenda W. J. H, et al. Overweight, Obesity, and Depression: A Systematic Review and Meta-analysis of Longitudinal Studies. Archives of General Psychiatry. 2010 Mar 1,;67(3):220-9.

12. Sjoberg RL, Nilsson KW, Leppert J. Obesity, Shame, and Depression in School-Aged Children: A Population-Based Study. Pediatrics. 2005 Sep 1,;116(3):e392.

13. Pratt LA, Brody DJ. Depression and Obesity in the U.S. Adult Household Population, 2005–2010. National Center for Health and Statistics Data Brief. 2014.

14. Wormser DK, Stephen. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. The Lancet. 2011 April 1,;377(9771):1085-95.

15. Bastien M, Poirier P, Lemieux I, Després J. Overview of Epidemiology and Contribution of Obesity to Cardiovascular Disease. Progress in Cardiovascular Diseases. 2014 January;56(4):369-81.

16. Subhashini Y. Obesity and type 2 diabetes. Journal of Diabetes Mellitus. 2011 -11-30;2011.

17. De Pergola G, Silvestris F. Obesity as a Major Risk Factor for Cancer. Journal of Obesity. 2013 Jan 1,;2013:1-11.

18. Paredis-Gonzalez X, Fuentes F. Obesity-Cancer Relationship: Emerging Challenges and Opportunities in Healthcare. Pharmaceutical Science. 2015 June 25,;1.3:151-2.

19. Finkelstein EA, Trogdon JG, Cohen JW, Dietz W. Annual Medical Spending Attributable To Obesity: Payer-And Service-Specific Estimates. Health Affairs. 2009 Sep 1,;28(5):w822.

20. Goedecke JH, Micklesfield LK. The Effect of Exercise on Obesity, Body Fat Distribution and Risk for Type 2 Diabetes. Diabetes and Physical Activity. 2014;60:82-93.

21. Kushner RF. Weight Loss Strategies for Treatment of Obesity. Progress in Cardiovascular Diseases. 2014 /01/01;56(4):465-72.

22. Cantó C, Auwerx J. Caloric restriction, SIRT1 and longevity. Trends in Endocrinology & Metabolism. 2009 September;20(7):325-31.

23. Bales CW, Kraus WE. Caloric Restriction. J Cardiopulm Rehabil Prev. 2013;33(4):201-8.

24. Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, et al. Caloric restriction delays disease onset and mortality in rhesus monkeys. Science. 2009 -7-10;325(5937):201-4.

25. Grossi E, Dalle Grave R, Mannucci E, Molinari E, Compare A, Cuzzolaro M, et al. Complexity of attrition in the treatment of obesity: clues from a structured telephone interview. Int J Obes. 2006 January 24,;30(7):1132-7.

26. Adela Caramoci, Brandusa Mitoiu, Mirela Pop, Virgil Mazilu, Mirela Vasilescu, Anca Mirela Ionescu, et al. Is intermittent fasting a scientifically-based dietary method? Medicina Sportiva : Journal of Romanian Sports Medicine Society. 2016 Apr 1,;12(2):2747.

27. Johnstone A. Fasting for weight loss: an effective strategy or latest dieting trend? International Journal of Obesity. 2015 -05-01;39(5):727-33.

28. Trepanowski JF, Bloomer RJ. The impact of religious fasting on human health. Nutrition journal. 2010;9(1):57.

29. Bloomer RJ, Kabir MM, Canale RE, Trepanowski JF, Marshall KE, Farney TM, et al. Effect of a 21 day Daniel Fast on metabolic and cardiovascular disease risk factors in men and women. Lipids in health and disease. 2010;9(1):94.

30. Rothschild J, Hoddy KK, Jambazian P, Varady KA. Time-restricted feeding and risk of metabolic disease: a review of human and animal studies. Nutrition Reviews. 2014 May;72(5):308-18.

31. Tinsley GM, La Bounty PM. Effects of intermittent fasting on body composition and clinical health markers in humans. Nutrition reviews. 2015 Oct;73(10):661-74.

32. Varady KA, Roohk DJ, Loe YC, McEvoy-Hein BK, Hellerstein MK. Effects of modified alternate-day fasting regimens on adipocyte size, triglyceride metabolism, and plasma adiponectin levels in mice. Journal of Lipid Research. 2007 Oct 1,;48(10):2212-9.

33. Toombs RJ, Ducher G, Shepherd JA, De Souza MJ. The Impact of Recent Technological Advances on the Trueness and Precision of DXA to Assess Body Composition. Obesity. 2012 January 1,;20(1):30-9.

34. Dehghan M, Merchant AT. Is bioelectrical impedance accurate for use in large epidemiological studies? Nutrition journal. 2008;7(1):26.

35. Yusuf S, Hawken S, Ôunpuu S, Bautista L, Franzosi MG, Commerford P, et al. Obesity and the risk of myocardial infarction in 27 000 participants from 52 countries: a case-control study. The Lancet. 2005 November 11,;366(9497):1640-9.

36. Kurth T, Gaziano JM, Berger K, Kase CS, Rexrode KM, Cook NR, et al. Body Mass Index and the Risk of Stroke in Men. Arch Intern Med. 2002 /12/09;162(22):2557-62.

37. Dagenais GR, Yi Q, Mann JFE, Bosch J, Pogue J, Yusuf S. Prognostic impact of body weight and abdominal obesity in women and men with cardiovascular disease. American Heart Journal. 2005 January;149(1):54-60.

38. Ravussin E, Lillioja S, Anderson TE, Christin L, Bogardus C. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. The Journal of clinical investigation. 1986 Dec;78(6):1568-78.

39. Donahoo W, Levine J, Melanson E. Variability in energy expenditure and its components. Current Opinion in Clinical Nutrition and Metabolic Care. 2004 Nov;7(6):599-605.

40. Ravussin E, Lillioja S, Knowler WC, Christin L, Freymond D, Abbott WGH, et al. Reduced Rate of Energy Expenditure as a Risk Factor for Body-Weight Gain. The New England Journal of Medicine. 1988 Feb 25,;318(8):467-72.

41. Korth O, Bosy-Westphal A, Zschoche P, Glüer CC, Heller M, Müller MJ. Influence of methods used in body composition analysis on the prediction of resting energy expenditure. European Journal of Clinical Nutrition. 2007 May;61(5):582-9.

42. Kirsten Illner, Gisbert Brinkmann, Martin Heller, Anja Bosy-Westphal, Manfred J. Müller. Metabolically active components of fat free mass and resting energy expenditure in nonobese adults. American Journal of Physiology - Endocrinology And Metabolism. 2000 Feb 1,;278(2):308-15.

43. Bosy-Westphal A, Reinecke U, Schlörke T, Illner K, Kutzner D, Heller M, et al. Effect of organ and tissue masses on resting energy expenditure in underweight, normal weight and obese adults. Int J Obes Relat Metab Disord. 2003 November 25,;28(1):72-9.

44. Wilmore JH. Body Composition and Sports Medicine: Research Considerations. Body Composition Assessments in Youth and Adults. Columbus, OH: Ross Laboratories. 1985.

45. Wilmore JH. Body composition in sport and exercise: directions for future research. Med Sci Sports Exerc. 1983;15(1):21-31.

46. Davoodi SH, Ajami M, Ayatollahi SA, Dowlatshahi K, Javedan G, Pazoki-Toroudi HR. Calorie Shifting Diet Versus Calorie Restriction Diet: A Comparative Clinical Trial Study. Int J Prev Med. 2014 -4;5(4):447-56.

47. Racette SB, Weiss EP, Villareal DT, Arif H, Steger-May K, Schechtman KB, et al. One Year of Caloric Restriction in Humans: Feasibility and Effects on Body Composition and Abdominal Adipose Tissue. J Gerontol A Biol Sci Med Sci. 2006 /09/01;61(9):943-50.

48. Ian Janssen, Anne Fortier, Robert Hudson, Robert Ross. Effects of an Energy-Restrictive Diet With or Without Exercise on Abdominal Fat, Intermuscular Fat, and Metabolic Risk Factors in Obese Women. Diabetes Care. 2002 Mar 1,;25(3):431-8.

49. Redman LM, Heilbronn LK, Martin CK, Alfonso A, Smith SR, Ravussin E. Effect of Calorie Restriction with or without Exercise on Body Composition and Fat Distribution. The Journal of Clinical Endocrinology & Metabolism. 2007 Mar;92(3):865-72.

50. Layman DK, Evans E, Baum JI, Seyler J, Erickson DJ, Boileau RA. Dietary Protein and Exercise Have Additive Effects on Body Composition during Weight Loss in Adult Women. J Nutr. 2005 08/01/;135(8):1903-10.

51. Tinsley GM, Forsse JS, Butler NK, Paoli A, Bane AA, La Bounty PM, et al. Time-restricted feeding in young men performing resistance training: A randomized controlled trial. European Journal of Sport Science. 2017 Feb 7,;17(2):200-7.

52. Stote KS, Baer DJ, Spears K, Paul DR, Harris GK, Rumpler WV, et al. A controlled trial of reduced meal frequency without caloric restriction in healthy, normal-weight, middle-aged adults. Am J Clin Nutr. 2007 -4;85(4):981-8.

53. Tatiana Moro, Grant Tinsley, Antonino Bianco, Giuseppe Marcolin, Quirico Francesco Pacelli, Giuseppe Battaglia, et al. Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in resistance-trained males. Journal of Translational Medicine. 2016 Jan 1,;14(1).

54. Heilbronn LK, Smith SR, Martin CK, Anton SD, Ravussin E. Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. Am J Clin Nutr. 2005 Jan;81(1):69-73.

55. Varady KA, Bhutani S, Church EC, Klempel MC. Short-term modified alternate-day fasting: a novel dietary strategy for weight loss and cardioprotection in obese adults. The American journal of clinical nutrition. 2009 Nov;90(5):1138-43.

56. Varady KA, Bhutani S, Klempel MC, Kroeger CM, Trepanowski JF, Haus JM, et al. Alternate day fasting for weight loss in normal weight and overweight subjects: a randomized controlled trial. Nutrition journal. 2013;12(1):146.

57. Bhutani S, Klempel MC, Kroeger CM, Trepanowski JF, Varady KA. Alternate day fasting and endurance exercise combine to reduce body weight and favorably alter plasma lipids in obese humans. Obesity. 2013 Jul;21(7):1370-9.

58. Hoddy KK, Kroeger CM, Trepanowski JF, Barnosky A, Bhutani S, Varady KA. Meal timing during alternate day fasting: Impact on body weight and cardiovascular disease risk in obese adults. Obesity. 2014 Dec;22(12):2524-31.

59. Catenacci VA, Pan Z, Ostendorf D, Brannon S, Gozansky WS, Mattson MP, et al. A randomized pilot study comparing zero-calorie alternate-day fasting to daily caloric restriction in adults with obesity. Obesity. 2016 Sep;24(9):1874-83.

60. Eshghinia S, Mohammadzadeh F. The effects of modified alternate-day fasting diet on weight loss and CAD risk factors in overweight and obese women. Journal of diabetes and metabolic disorders. 2013;12(1):4.

61. Trepanowski JF, Kabir MM, Alleman J, Rick J, Bloomer RJ. A 21-day Daniel fast with or without krill oil supplementation improves anthropometric parameters and the cardiometabolic profile in men and women. Nutrition & metabolism. 2012;9(1):82.

62. Bloomer RJ, Trepanowski JF, Kabir MM, Alleman J, Rick J, Dessoulavy ME. Impact of short-term dietary modification on postprandial oxidative stress. Nutrition journal. 2012;11(1):16.

63. Alleman J, Rick J, Harvey IC, Farney TM, Bloomer RJ. Both a traditional and modified Daniel Fast improve the cardio-metabolic profile in men and women. Lipids in health and disease. 2013;12(1):114.

64. Ortega FB, Ruiz JR, Castillo MJ, Sjöström M. Physical fitness in childhood and adolescence: a powerful marker of health. International Journal of Obesity. 2008 Jan;32(1):1-11.

65. Kokkinos P, Myers J, Kokkinos JP, Pittaras A, Narayan P, Manolis A, et al. Exercise Capacity and Mortality in Black and White Men. Circulation. 2008 Feb 5,;117(5):614-22.

66. Lin X, Zhang X, Guo J, Roberts CK, McKenzie S, Wu W, et al. Effects of Exercise Training on Cardiorespiratory Fitness and Biomarkers of Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Journal of the American Heart Association. 2015 Jul;4(7).

67. Sven Plein, John Greenwood, John Phillip Ridgway. Cardiovascular Fitness Procedures Manual. 2 ed. ed. Cham: Springer Verlag; 2005.

68. Shephard RJ, Allen C, Benade AJ, Davies CT, Di Prampero PE, Hedman R, et al. The maximum oxygen intake. An international reference standard of cardiorespiratory fitness. Bulletin of the World Health Organization. 1968;38(5):757.

69. Lee CD, Blair SN, Jackson AS. Cardiorespiratory fitness, body composition, and all-cause and cardiovascular disease mortality in men. The American journal of clinical nutrition. 1999 Mar;69(3):373.

70. Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE. Exercise Capacity and Mortality among Men Referred for Exercise Testing. New England Journal of Medicine. 2002 March 14,;346(11):793-801.

71. Kokkinos P, Myers J, Faselis C, Panagiotakos DB, Doumas M, Pittaras A, et al. Exercise capacity and mortality in older men: a 20-year follow-up study. Circulation. 2010 Aug 24,;122(8):790-7.

72. Laukkanen JA, Mäkikallio TH, Rauramaa R, Kiviniemi V, Ronkainen K, Kurl S. Cardiorespiratory Fitness Is Related to the Risk of Sudden Cardiac Death. Journal of the American College of Cardiology. 2010;56(18):1476-83.

73. Schmid D, Leitzmann MF. Cardiorespiratory fitness as predictor of cancer mortality: a systematic review and meta-analysis. Annals of Oncology. 2015 Feb;26(2):272-8.

74. Lee SJ, Arslanian SA. Cardiorespiratory fitness and abdominal adiposity in youth. European Journal of Clinical Nutrition. 2007 Apr;61(4):561-5.

75. Ara I, Moreno LA, Leiva MT, Gutin B, Casajús JA. Adiposity, Physical Activity, and Physical Fitness Among Children From Aragón, Spain. Obesity. 2007 Aug;15(8):1918-24.

76. Katzmarzyk P, Craig C, Gauvin L. Adiposity, physical fitness and incident diabetes: the physical activity longitudinal study. Diabetologia. 2007 Mar;50(3):538-44.

77. Duck-chul Lee, Xuemei Sui, Timothy S. Church, I.-Min Lee, Steven N. Blair. Associations of Cardiorespiratory Fitness and Obesity With Risks of Impaired Fasting Glucose and Type 2 Diabetes in Men. Diabetes Care. 2009 Feb 1,;32(2):257-62.

78. Carnethon MR, Gulati M, Greenland P. Prevalence and Cardiovascular Disease Correlates of Low Cardiorespiratory Fitness in Adolescents and Adults. JAMA. 2005 Dec 21,;294(23):2981-8.

79. Voelcker-Rehage C, Godde B, Staudinger UM. Physical and motor fitness are both related to cognition in old age. The European journal of neuroscience. 2010 Jan;31(1):167-76.

80. Sloan RA, Sawada SS, Martin CK, Church T, Blair SN. Associations between cardiorespiratory fitness and health-related quality of life. Health and quality of life outcomes. 2009;7(1):47.

81. Bouchard C, An P, Rice T, Skinner JS, Wilmore JH, Gagnon J, et al. Familial aggregation of VO2 max response to exercise training: results from the HERITAGE Family Study. Journal of Applied Physiology. 1999 Sep 1,;87(3):1003.

82. Ferrucci L, Guralnik JM, Buchner D, Kasper J, Lamb SE, Simonsick EM, et al. Departures from linearity in the relationship between measures of muscular strength and physical performance of the lower extremities: the Women's Health and Aging Study. The journals of gerontology. Series A, Biological sciences and medical sciences. 1997 Sep;52(5):M285.

83. Rantanen T, Guralnik JM, Foley D, Masaki K, Leveille S, Curb JD, et al. Midlife Hand Grip Strength as a Predictor of Old Age Disability. JAMA. 1999 Feb 10,;281(6):558-60.

84. Newman AB, Kupelian V, Visser M, Simonsick EM, Goodpaster BH, Kritchevsky SB, et al. Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. The journals of gerontology. Series A, Biological sciences and medical sciences. 2006 Jan;61(1):72-7.

85. Ortega FB, Silventoinen K, Tynelius P, Rasmussen F. Muscular strength in male adolescents and premature death: cohort study of one million participants. . 2012.

86. Ferreira FS. Relationship between physical fitness and nutritional status in a Portuguese sample of school adolescents. Journal of Obesity and Weight Loss Therapy. 2013;5(3):1-6.

87. Minor RK, Villarreal J, McGraw M, Percival SS, Ingram DK, de Cabo R. Calorie restriction alters physical performance but not cognition in two models of altered neuroendocrine signaling. Behavioural Brain Research. 2008;189(1):202-11.

88. Ishihara H, WenYing F, Kouda K, Nakamura H, Kohno H, Nishio N, et al. Effects of Dietary Restriction on Physical Performance in Mice. Journal of PHYSIOLOGICAL ANTHROPOLOGY and Applied Human Science. 2005;24(3):209-13.

89. E. F. Coyle, A. R. Coggan, M. K. Hemmert, J. L. Ivy. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. Journal of Applied Physiology. 1986 Jul 1,;61(1):165-72.

90. Foskett A, Williams C, Boobis L, Tsintzas K. Carbohydrate availability and muscle energy metabolism during intermittent running. Medicine and science in sports and exercise. 2008 Jan;40(1):96.

91. Burke L. Re-Examining High-Fat Diets for Sports Performance: Did We Call the 'Nail in the Coffin' Too Soon? Sports Med. 2015 Nov;45(S1):33-49.

92. Evan E Schick. The role of the ketogenic diet in exercise performance. Medicina Sportiva : Journal of Romanian Sports Medicine Society. 2016 Apr 1,;12(2):2756.

93. Shubhroz Gill, Hiep D Le, Girish C Melkani, Satchidananda Panda. Time-restricted feeding attenuates age-related cardiac decline in Drosophila. Science. 2015 Mar 13,;347(6227):1265-9.

94. Hoddy KK, Gibbons C, Kroeger CM, Trepanowski JF, Barnosky A, Bhutani S, et al. Changes in hunger and fullness in relation to gut peptides before and after 8 weeks of alternate day fasting. Clinical Nutrition. 2016 Dec;35(6):1380-5.

95. Klempel MC, Kroeger CM, Bhutani S, Trepanowski JF, Varady KA. Intermittent fasting combined with calorie restriction is effective for weight loss and cardio-protection in obese women. Nutrition journal. 2012;11(1):98.

96. Halberg N, Henriksen M, Söderhamn N, Stallknecht B, Ploug T, Schjerling P, et al. Effect of intermittent fasting and refeeding on insulin action in healthy men. J Appl Physiol. 2005 Dec;99(6):2128-36.

97. Harvie MN, Pegington M, Mattson MP, Frystyk J, Dillon B, Evans G, et al. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomised trial in young overweight women. Int J Obes (Lond). 2011 -5;35(5):714-27.

98. Bhutani S, Klempel MC, Berger RA, Varady KA. Improvements in Coronary Heart Disease Risk Indicators by Alternate-Day Fasting Involve Adipose Tissue Modulations. Obesity. 2010 Nov;18(11):2152-9.

99. Arble DM, Bass J, Laposky AD, Vitaterna MH, Turek FW. Circadian Timing of Food Intake Contributes to Weight Gain. Obesity. 2009 Nov;17(11):2100-2.

100. Adlouni A, Ghalim N, Benslimane A, Lecerf JM, Saïle R. Fasting during Ramadan Induces a Marked Increase in High-Density Lipoprotein Cholesterol and Decrease in Low-Density Lipoprotein Cholesterol. Annals of Nutrition and Metabolism. 1997;41(4):242-9.

101. Temizhan A, Tandogan I, Dönderici Ö, Demirbas B. The effects of ramadan fasting on blood lipid levels. The American Journal of Medicine. 2000;109(4):341.

102. Ziaee V, Razaei M, Ahmadinejad Z, Shaikh H, Yousefi R, Yarmohammadi L, et al. The changes of metabolic profile and weight during Ramadan fasting. Singapore medical journal. 2006 May;47(5):409.

103. Nematy M, Alinezhad-Namaghi M, Rashed MM, Mozhdehifard M, Sajjadi SS, Akhlaghi S, et al. Effects of Ramadan fasting on cardiovascular risk factors: a prospective observational study. Nutrition journal. 2012;11(1):69.

104. Kiyani M, Memon A, Amjad M, Ameer M, Sadiq M, Mahmood T. Study of Human Biochemical Parameters During and After Ramadan. J Relig Health. 2017 Feb;56(1):55-62.

105. Carlson O, Martin B, Stote KS, Golden E, Maudsley S, Najjar SS, et al. Impact of reduced meal frequency without caloric restriction on glucose regulation in healthy, normal-weight middle-aged men and women. Metabolism. 2007 December;56(12):1729-34.

106. Sherman H, Frumin I, Gutman R, Chapnik N, Lorentz A, Meylan J, et al. Long-term restricted feeding alters circadian expression and reduces the level of inflammatory and disease markers. Journal of Cellular and Molecular Medicine. 2011 Dec;15(12):2745-59.

107. Sherman H, Genzer Y, Cohen R, Chapnik N, Madar Z, Froy O. Timed high-fat diet resets circadian metabolism and prevents obesity. FASEB J. 2012 08/01/;26(8):3493-502.

108. Hatori M, Vollmers C, Zarrinpar A, DiTacchio L, Bushong EA, Gill S, et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell metabolism. 2012 Jun 6,;15(6):848-60.

109. Tsai J, Villegas-Montoya C, Boland BB, Blasier Z, Egbejimi O, Gonzalez R, et al. Influence of dark phase restricted high fat feeding on myocardial adaptation in mice. Journal of molecular and cellular cardiology. 2013 Feb;55:147-55.

110. Chaix A, Zarrinpar A. The effects of time-restricted feeding on lipid metabolism and adiposity. Adipocyte. 2015 -4-20;4(4):319-24.

111. Belkacemi L, Selselet-Attou G, Louchami K, Sener A, Malaisse W. Intermittent fasting modulation of the diabetic syndrome in sand rats. II. In vivo investigations. International Journal of Molecular Medicine. 2010 Nov;26(5):759-65.

112. Lui Z, Chu H, Wu Y, Yang S. Effect of Two-step Time-restricted Feeding on the Fattening Traits in Geese. Asian-Australasian journal of animal sciences. 2014 Jun;27(6):841-6.

113. Farooq N, Priyamvada S, Arivarasu NA, Salim S, Khan F, Yusufi ANK. Influence of Ramadan-type fasting on enzymes of carbohydrate metabolism and brush border membrane in small intestine and liver of rat used as a model. British Journal of Nutrition. 2006 Dec 1,;96(6):1087-94.

114. Belkacemi L, Selselet-Attou G, Hupkens E, Nguidjoe E, Louchami K, Sener A, et al. Intermittent Fasting Modulation of the Diabetic Syndrome in Streptozotocin-Injected Rats. International Journal of Endocrinology. 2012;2012:1-12.

115. Park S, Yoo KM, Hyun JS, Kang S. Intermittent fasting reduces body fat but exacerbates hepatic insulin resistance in young rats regardless of high protein and fat diets. The Journal of Nutritional Biochemistry. 2017 Feb;40:14-22.

116. Chaouachi A, Coutts A, Chamari K, Wong D, Chaouachi M, Chtara M, et al. Effect of Ramadan Intermittent Fasting on Aerobic and Anaerobic Performance and Perception of Fatigue in Male Elite Judo Athletes. Journal of Strength and Conditioning Research. 2009 Dec;23(9):2702-9.

117. Salgado-Delgado R, Angeles-Castellanos M, Saderi N, Buijs RM, Escobar C. Food Intake during the Normal Activity Phase Prevents Obesity and Circadian Desynchrony in a Rat Model of Night Work. Endocrinology. 2010 Mar;151(3):1019-29.

118. Sherman H, Genzer Y, Cohen R, Chapnik N, Madar Z, Froy O. Timed high-fat diet resets circadian metabolism and prevents obesity. FASEB Journal. 2012 Aug;26(8):3493.

119. Varady KA. Intermittent versus daily calorie restriction: which diet regimen is more effective for weight loss? Obesity Reviews. 2011 Jul;12(7):e601.

120. Klempel MC, Bhutani S, Fitzgibbon M, Freels S, Varady KA. Dietary and physical activity adaptations to alternate day modified fasting: implications for optimal weight loss. Nutrition journal. 2010;9(1):35.

121. Hoddy KK, Kroeger CM, Trepanowski JF, Barnosky AR, Bhutani S, Varady KA. Safety of alternate day fasting and effect on disordered eating behaviors. Nutrition journal. 2015;14(1):44.

122. Johnson JB, Summer W, Cutler RG, Martin B, Hyun D, Dixit VD, et al. Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. Free Radical Biology and Medicine. 2007 March 1,;42(5):665-74.

123. Longo VD, Mattson MP. Fasting: molecular mechanisms and clinical applications. Cell metabolism. 2014 Feb 4,;19(2):181.

124. Beigy M, Vakili S, Berijani S, Aminizade M, Ahmadi-Dastgerdi M, Meshkani R. Alternate-day fasting diet improves fructose-induced insulin resistance in mice. Journal of Animal Physiology and Animal Nutrition. 2013 Dec;97(6):1125-31.

125. Wan R, Camandola S, Mattson MP. Intermittent fasting and dietary supplementation with 2-
deoxy-D-glucose improve functional and metabolic
cardiovascular risk factors in rats The FASEB Journal. 2003.

126. Mager DE, Wan R, Brown M, Cheng A, Wareski P, Abernethy DR, et al. Caloric restriction and intermittent fasting alter spectral measures of heart rate and blood pressure variability in rats. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2006 Apr;20(6):631-7.

127. Ahmet I, Wan R, Mattson MP, Lakatta EG, Talan M. Cardioprotection by Intermittent Fasting in Rats. Circulation. 2005 Nov 15,;112(20):3115-21.

128. Elaine A. Hsieh, Christine M. Chai, Marc K. Hellerstein. Effects of caloric restriction on cell proliferation in several tissues in mice: role of intermittent feeding. American Journal of Physiology - Endocrinology And Metabolism. 2005 May 1,;288(5):965-72.

129. Higashida K, Fujimoto E, Higuchi M, Terada S. Effects of alternate-day fasting on high-fat diet-induced insulin resistance in rat skeletal muscle. Life sciences. 2013 Aug 14,;93(5-6):208-13.

130. Varady KA, Allister CA, Roohk DJ, Hellerstein MK. Improvements in body fat distribution and circulating adiponectin by alternate-day fasting versus calorie restriction. The Journal of Nutritional Biochemistry. 2010;21(3):188-95.

131. Joslin PMN, Bell RK, Swoap SJ. Obese mice on a high-fat alternate-day fasting regimen lose weight and improve glucose tolerance. J Anim Physiol Anim Nutr (Berl). 2016 Jun 8,.

132. Goodrick CL, Ingram DK, Reynolds MA, Freeman JR, Cider N. Effects of intermittent feeding upon body weight and lifespan in inbred mice: interaction of genotype and age. Mechanisms of Ageing and Development. 1990;55(1):69-87.

133. Descamps O, Riondel J, Ducros V, Roussel A. Mitochondrial production of reactive oxygen species and incidence of age-associated lymphoma in OF1 mice: Effect of alternate-day fasting. Mechanisms of Ageing and Development. 2005;126(11):1185-91.

134. Gabriel G Dorighello, Juliana C Rovani, Christopher J F Luhman, Bruno A Paim, Helena F Raposo, Anibal E Vercesi, et al. Food restriction by intermittent fasting induces diabetes and obesity and aggravates spontaneous atherosclerosis development in hypercholesterolaemic mice. The British Journal of Nutrition. 2014 Mar 28,;111(6):979-86.

135. Cavuoto P, Fenech MF. A review of methionine dependency and the role of methionine restriction in cancer growth control and life-span extension. Cancer Treatment Reviews. 2012 Oct;38(6):726.

136. Bloomer RJ, Kabir MM, Trepanowski JF, Canale RE, Farney TM. A 21 day Daniel Fast improves selected biomarkers of antioxidant status and oxidative stress in men and women. Nutrition & metabolism. 2011;8(1):17.

Appendix C

IACUC Protocol Action Form

IACUC PROTOCOL ACTION FORM

To:	Richard Bloomer
From	Institutional Animal Care and Use Committee
Subject	Animal Research Protocol
Date	June 21, 2017

The institutional Animal Care and Use Committee (IACUC) has taken the following action concerning your Animal Research Protocol No. **0806** Impact of dietary and caloric restriction models on metabolic health and physical function in male mice

☒ Your protocol is approved for the following period:

From: July 1, 2017 To: June 30, 2020

☐ Your protocol is not approved for the following reasons (see attached memo).

☐ Your protocol is renewed without changes for the following period:

From: _____ To: _____

☐ Your protocol is renewed with the changes described in your IACUC Animal Research Protocol Update/Amendment Memorandum dated _____ for the following period:

From: _____ To: _____

☐ Your protocol is not renewed and the animals have been properly disposed of as described in your IACUC Animal Research Protocol Update/Amendment Memorandum dated _____

Amy L. de Jongh Curry

Amy L. de Jongh Curry, PhD, Chair of the IACUC

Karyl K Buddington

Dr. Karyl Buddington, University Veterinarian and Director of the Animal Care Facilities

IACUC PROTOCOL

FOR USE OF LIVE VERTEBRATES FOR RESEARCH, TEACHING OR DEMONSTRATION

UNIVERSITY OF MEMPHIS

5/3/17

Date submitted to Attending Veterinarian for pre-review:

IACUC Protocol # 0806 Date Submitted to IACUC 5/30/17

Dates Protocol will be in effect: from 7/1/17 to 6/30/20

(not to exceed three years including two yearly renewals)

Is this protocol related to an external grant or contract application? Yes ☐ No ☒

If yes, complete the following:

Agency: _____ Date Submitted _____

Grant # _____

University account for Animal Care Facility per diem charge: _____

If the protocol is not related to an external grant or contract application, complete the following:

University account for Animal Care Facility per diem charge: 211700

Project Title: (If project relates to a grant or contract application, give that title; if multiple protocols relate to one grant, give unique titles for each protocol; if the project is related to a class, give the course name and number):

Impact of dietary and caloric restriction models on metabolic health and physical function in male mice

I. Personnel

Investigator/Instructor: Marie van der Merwe and Richard Bloomer

Department: Health Studies

Academic Rank: Assistant Professor and Professor

Campus phone**:** 678 3476 and 678 5638 Emergency phone: 901 406 7458 / 901 267 3514

Attending Veterinarian: Karyl Buddington

Phone: 901-678-2359 Emergency phone: 901-258-1232

List all individuals that will handle animals using this protocol, their affiliation, and their level of expertise (e.g. relevant qualifications). If the protocol applies to a class then so specify.

Marie van der Merwe, PhD (Molecular Pharmacology), Postdoctoral Fellowship (Bone Marrow Transplantation): More than 10 years of experience using mice as a research model.

Richard Bloomer, PhD: 2 years of prior experience using rodents in research.

Melissa Puppa, PhD: 8+ years of experience working with mice including breeding, exercise training/testing, injections, surgery, dietary interventions, gavage, GTT, electroporation, blood collection, and dissection/necropsy.

Matt Butawan: 2 years of rodent handling experience.

Harold Lee: 1 year of rodent handling experience, including exercise testing (treadmill running).

Sunita Sharma: 6 months animal handling experience; has been to Laboratory Animal Training.

Nick Smith: No experience with animals, but has been to Laboratory Animal Training; training will be provided during experiment.

Jade Caldwell: No experience with animals, but has been to Laboratory Animal Training; training will be provided during experiment.

Kyle Truska: No experience with animals and will attend Laboratory Animal Training; training will be provided during experiment

If additional personnel become involved in handling animals used in this protocol, it is the responsibility of the principal investigator to notify the Animal Care Facility in writing before they start.

Has the investigator/instructor and all personnel listed above received the appropriate vaccinations (tetanus, rabies)? Yes ☒ No ☐ Not Applicable ☐

Will be done by the time the study is initiated.

Is it necessary for personnel listed on this protocol to be tested for TB?

Yes ☐ No ☒

If you have questions about the kind of vaccination or about TB, call the Animal Care Facility at 678 2359.

All U of M personnel involved in this protocol must complete the animal care and use training program before animals can be procured or before experiments/teaching or demonstration. In submitting this protocol, I, as Principal Investigator/Instructor accept the responsibility for compliance with this requirement.

In addition, the Principal Investigator/Instructor must be willing to provide appropriate supervision for all persons working on this protocol. In the case of a class, the Instructor must be responsible for training any students in classes involved prior to using animals.

II. Project Description

A. Summary **(Enter a brief description below of your project, using lay terminology)**:

Obesity has become an epidemic in the Western world, largely due to poor dietary habits. Multiple dietary programs have been studied in recent years, showing promise with regards to weight loss and improvement in multiple health related outcomes, including glucose control, inflammation, and oxidative stress. More recently, a great deal of attention has been placed on the microbiome and the influence of dietary intake on this very important component of overall health. What is unknown is the degree of improvement noted when following each of the popular dietary regimens as compared to simple caloric restriction. That is, are certain protocols more beneficial than others and if so, in regards to which specific outcome(s)? Much debate centers on these questions and no study has sought to make direct comparisons between the well-investigated protocols.

In fact, most dietary protocols have simply been compared to a typical high fat Western diet. Very few comparison studies have been conducted inclusive of the four most common dietary approaches: Caloric Restriction (CR), Dietary Restriction (DR), Time Restricted Feeding (TRF), and Alternate Day Fasting (ADF). The goal of the present study is to directly compare the above four dietary plans to a Western Diet, with regards to body mass/body fat, physical performance, insulin sensitivity, inflammation, oxidative stress, and the microbiome. A diet of standard rodent chow will be used as a control. Outcome measures will be determined after 8 weeks of assignment to the dietary programs, with a total of 56 animals assigned to one of 7 diet groups (n=8 per group).

4 week old C57BL/6 male mice will be entrained under a 12h light: 12h dark schedule for two weeks with standard rodent chow available ad libitum. During the entrainment period, mice will begin the reverse light-dark schedule, with lights off between the hours of 7am-7pm. This will be done so that the feeding time will be during the active phase ("light off" phase) of the mice. Mice will be housed in Life Sciences in an area that is currently used for studies of the circadian rhythm and therefore the light is well-regulated. After two weeks of entrainment, all but 8 mice will be switched to a Western diet for four weeks, consisting of 45% fat with lard as the fat source. This additional four week period of ad libitum feeding should allow for significant weight gain. Eight mice will continue following a standard chow diet during this 4-week period and serve as a low-fat control diet group. They will maintain this same diet throughout the entire study period. Following the 4-week period, the mice fed the Western diet will be divided into 6 additional groups: Western, CR, DR, TRF, ADF, and chow.

A MRI for the determination of body mass/fat and treadmill run to exhaustion will occur at baseline (prior to starting the specific diet plan) and following eight weeks on the specific diet assignment. Before and after the 4-week period of Western diet feeding and at the end of the intervention period, a glucose tolerance test will be performed. Mice will be fasted for 7 hours and glucose levels measured in 10 ul of blood collected via the tail vein. Glucose will be administered intraperitoneally and blood glucose measured every 30 minutes for 90 minutes from 10 ul of blood collected from the tail vein. At the end of the dietary intervention, prior to sacrifice, blood will be collected from the facial vein for the determination of lipids, glucose, insulin,

oxidative stress markers, cytokines, immune cell populations, and other variables. All mice will be sacrificed by CO2 inhalation. Tissues will be harvested immediately. This form of euthanasia does not affect the outcome measures as determined by our previous studies. Organs to be harvested are liver, spleen, intestine, lymph nodes, adipose tissue, skeletal muscle, and heart.

B. Describe IN DETAIL the procedures you will follow. Include accompanying documentation and reference to previously published work in the box below. Provide a complete bibliographic citation and describe any variations from the published technique. The bibliography may be included in the box below or appended to this protocol.

Mice: 56, 4 week old C57BL/6 male mice will be purchased from Envigo or another vendor. After arrival, mice will be co-housed (as done in reference 1) at the animal facility on the University of Memphis campus.

After arrival at the University of Memphis, mice will be entrained under a 12h light: 12h dark schedule for two weeks with standard rodent chow available ad libitum. During the entrainment period, the light-dark cycle will be reversed with lights off between the hours of 7am-7pm. This will be done so that the feeding time will be during the active phase ("light off" phase) of the mice. Mice will be housed in Life Sciences in an area that is currently used for studies of the circadian rhythm and therefore the light is well-regulated. After two weeks of entrainment, all but 8 mice will be switched to a Western diet, consisting of 45% lard and 41% carbohydrate (20% sucrose, 9% corn starch, and 12% Maltodextrin). This additional four week period of ad libitum feeding should allow for significant weight gain. Following this period,

mice will be divided into 6 additional groups and will consume their respective diets for an additional 8 weeks.

Group 1 will have access to a standard rodent chow ad libitum, 24 hours per day.

Group 2 will have access to a purified, high-fiber, vegan-based diet ad libitum, 24 hours per day. We have used this same customized diet in past studies and it results in normal weight/muscle mass gain, while minimizing fat accumulation. This will be the DR arm of the trial.

Group 3 will have access to the Western diet ad libitum, 24 hours per day.

Group 4 will have ad libitum access to the Western diet for 6 hours at the beginning of their active phase (7am-1pm). This will be the TRF arm of the trial. We have used this exact feeding protocol in a recent investigation of mice. No negative health consequences were observed using this protocol.

Group 5 will be on the alternate day fasting (ADF) protocol and have ad libitum access to the Western diet every other day. That is, on day 1 they will have unrestricted access to food during the entire 24 hour period. On day 2 they will receive no food. On day 3, they will have unrestricted access to food, and so on. This same protocol has been used in mice in several studies without incident. Please see references 2-10. It should be noted that if the ADF protocol leads to significant weight loss beyond what would be expected for mice following such a plan (≥20% body mass as compared to their body mass at the start of the intervention period [after 4 weeks of following the ad libitum Wester diet]) and/or mice show signs of stress or impaired health (e.g., scruffy coat, hunched posture, excessively aggressive behavior), the attending veterinarian will be notified and a rescue protocol will be set in place and will consist of the following:

instead of receiving no food on the fasting days, mice will receive 20% of the daily calories in the form of the Western diet during a one hour period at the mid-point of the animals' active phase (12-1pm).

It should be noted that animals in all groups will be monitored daily for signs of stress and impaired health, with particular attention given to animals in the ADF group. Monitoring body weight three times weekly should be more than adequate and should not cause undue stress to the animals due to frequency of handling.

Group 6 will receive 80% of ad libitum intake of the Western diet as determined during the prior 4 week period. This will be the CR arm of the trial. Multiple rodent studies have reduced caloric intake by 20-40% without incident. See references 5 and 11-17.

Al mice will be monitored daily for sign of malnutrition and stress.

The diets will be purchased from Research Diets, which has experience in producing the Western diet and purified vegan diets for rodent studies. The mice will remain on their particular diets for eight weeks and then post-testing will begin. Mice will continue on their diets until all testing is completed (~ end of week 9). Water will be provided ad libitum throughout the study period. The amount of food consumed will be measured daily and the weights of the mice will be taken three times per week at the same time of day. There will be a total of 56 mice assigned in this study.

There will be two mice housed per cage. From our previous studies we know that genetically similar mice eat basically a constant volume of food. We can therefore pair-house the mice and determine an average amount of food consumed. Previous studies have used a similar set up where 3-5 mice were co-housed (Hatori et al.). If there are any signs of fighting or it appears that one the mice is consuming the majority of the

food, those specific mice will be separated into individual cages. Food will be weighed daily. Mice will be weighed 3 times per week at the same time of day. A glucose tolerance test (GTT) will be performed at the beginning of the experiment (when mice are put on their respective diets) and following 8 weeks after the start of the dietary intervention. The final GTT will be performed 48h prior to euthanasia. For the glucose tolerance test, mice will be fasted for a minimum of 7 hours and blood glucose levels determined by blood from tail vein. Mice will be given a 1g glucose/kg body weight intraperitoneally and blood (10ul) collected every 30 minutes for 90 minutes to measure glucose levels. For blood collection, mice will be placed on a flat surface and restraint by gently holding onto the tail without pulling. The tip of the tail will be snipped – 1mm region. This part of the tail has little nerve innervation and does not cause the animal any distress. By "milking" the tail, blood can be collected at multiple time points without having to cut again. Isoflurane cannot be used as it increases blood glucose levels independent of treatment. We have used this exact procedure in a recent study.

In addition to the GTT, prior to commencing the specific diet plans (after the initial 4 week period on the Western diet), animals will undergo a MRI for determination of body mass/body fat. Body composition will be determined using an EchoMRI™ 1100. The MRI is housed in room 115 in Life Sciences, the same location that our mice are housed in. For scanning, animal are placed with cylindrical tube holders and movement restricted to the bottom 7.5 cm as stated in the instrument manual and the study by Jones et al. (Validation of quantitative resonance for the determination of body composition of mice. *Int. J Body Compos Res*. 2009; 7(2):68-72). The animals are scanned without anesthesia, sedation or restraint and are free to move within the holder. The smallest possible holder is used to limit the movement of the mouse (without constraining them) in order to reduce measurement errors induced by motion. Scanning time is approximately 40 seconds. There is no prior training required for the animal. We have prior experience using this system in mice.

Finally, animals will undergo a treadmill run test to exhaustion using a motorized treadmill without incline. Specifically, mice will be acclimated to the treadmill prior to testing. Run to fatigue test will be performed twice in the mice; once prior to starting the 8 week intervention and at the end of the 8 week intervention. Animals will run using a 5% grade at 20m/min for 30 min and 25m/min for the remaining time until they reach exhaustion. A warm up phase will be provided for 15min (5min at 5m/min, 5min at 10m/min, 5min at 15m/min). Fatigue will be defined as the time at which mice are no longer able or willing to keep up with the speed of the treadmill despite gentle hand prodding for a period of 30 seconds. Very mild electric shock will only be used if mice do not respond well to gentle hand prodding. Our past and current work using running

protocols demonstrates that mild shocking is preferable to obtain the best running performance. The frequency and amplitude of shock will be as low as possible to motivate the animals to remain on the treadmill belt, without causing unnecessary distress. We have used small electric shock in prior studies and this is well-accepted in rodent running studies. Equipment will be cleaned upon the completion of testing with ethanol solution. All urine and feces will be cleaned off of the device and the surrounding area.

After 8-9 weeks of dietary intervention, mice will be euthanized (CO_2 inhalation) and with cervical dislocation. Tissues will be harvested immediately. This form of euthanasia does not affect the outcome measures as determined by our previous studies. Organs to be harvested are liver, spleen, intestine, lymph nodes, adipose tissue, skeletal muscle, and heart. Immediately prior to euthanasia, blood will be collected via the facial vein to measure lipids, glucose, insulin, oxidative stress markers, cytokines, immune cell populations, and other variables as needed. Cecum and intestinal contents will be collected for microbiome analysis.

1. Hatori M, Vollmers C, Zarrinpar A, et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell Metab. 2012.

2. R. Michael Anson, Zhihong Guo, Rafael de Cabo, et al. Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. Proceedings of the National Academy of Sciences of the United States of America. 2003.

3. Krista A. Varady, D. J. Roohk, & Marc K. Hellerstein. Dose effects of modified alternate-day fasting regimens on in vivo cell proliferation and plasma insulin-like growth factor-1 in mice. Journal of Applied Physiology. 2007.

4. Varady, K. A., Roohk, D. J., Loe, et al. Effects of modified alternate-day fasting regimens on adipocyte size, triglyceride metabolism, and plasma adiponectin levels in mice. Journal of Lipid Research. 2007.

5. Varady, K., Allister, C., Roohk, d., & Hellerstein, M. Improvements in body fat distribution and circulating adioponectin by alternate-day fasting versus calorie restriction. The Journal of Nutritional Biochemistry. 2010.

6. Lu, J., E, L., Wang, W., Frontera, et al. Alternate day fasting impacts the brain insulin-signaling pathway of young adult male C57BL/6 mice. Journal of Neurochemistry. 2011.

7. Dorighello, G. G., Rovani, J. C., Luhman, C. J. F., Paim, B. A., Raposo, H. F., Vercesi, A. E., & Oliveira, H. C. F. Food restriction by intermittent fasting induces diabetes and obesity and aggravates spontaneous atherosclerosis development in hypercholesterolaemic mice. The British Journal of Nutrition. 2014.

8. Beigy, M., Vakili, S., Berijani, S., et al. Alternate-day fasting diet improves fructose-induced insulin resistance in mice. Journal of Animal Physiology and Animal Nutrition. 2013.

9. Joslin, P. M. N., Bell, R. K., & Swoap, S. J. Obese mice on a high-fat alternate-day fasting regimen lose weight and improve glucose tolerance. Journal of Animal Physiology and Animal Nutrition. 2016.

10. Descamps O, Riondel J, Ducros V, Roussel A. Mitochondrial production of reactive oxygen species and incidence of age-associated lymphoma in OF1 mice: effect of alternate-day fasting. Mech Ageing Dev. 2005.

11. Mager, D. E., Wan, R., Brown, et al. Caloric restriction and intermittent fasting alter spectral measures of heart rate and blood pressure variability in rats. FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology. 2006.

12. Lusseau, D., Mitchell, S. E., Barros, et al. The effects of graded levels of calorie restriction: IV. non-linear change in behavioural phenotype of mice in response to short-term calorie restriction. Scientific Reports. 2015.

13. Sharma, N., Wang, H., Arias, E. B., et al. Mechanisms for independent and combined effects of calorie restriction and acute exercise on insulin-stimulated glucose uptake by skeletal muscle of old rats. American Journal of Physiology. Endocrinology and Metabolism. 2015.

14. Ingram, D. K., Weindruch, R., Spangler, E. L., et al. Dietary restriction benefits learning and motor performance of aged mice. Journal of Gerontology. 1987.

15. Means, L. W., Higgins, J. L., & Fernandez, T. J. Mid-life onset of dietary restriction extends life and prolongs cognitive functioning. Physiology & Behavior. 1993.

16. Yu, B. P., Masoro, E. J., Murata, et al. Life span study of SPF fischer 344 male rats fed AdLibitum or restricted diets: Longevity, growth, lean body mass and disease. Journal of Gerontology. 1982.

Sohal, R. S., Agarwal, S., Candas, M., et al. Effect of age and caloric restriction on DNA oxidative damage in different tissues of C57BL/6 mice. Mechanisms of Ageing and Development. 1994.

C. Rationale for Involving Animals and the Appropriateness of Species and Number Used. Indicate (**here**) briefly the short and/or long-term benefits (to humans and/or other animals) of this use of animals for research, teaching or demonstration. Provide rational for and the number

of animals to be used. In addition, state briefly why living animals are required for this study, rather than some alternative model.

The goal of this experiment is to study common feeding patterns in a controlled environment over a moderate period of time to determine the cardio-metabolic health effects of these dietary plans. Results will provide evidence for or against certain models that can then be used by human subjects in an attempt to combat obesity and related co-morbidities. We know from our prior work in rodents that the TRF and DR models are favorable as compared to a WD. However, we are uncertain as to how CR and ADF plans compare. Moreover, we are unaware of studies focused on comparing these plans with regards to the microbiome or physical performance. These are important areas of interest to scientists and humans who are focused on which dietary plan may be "best." As obesity is becoming more of a problem in the Western world, determining which dietary approaches may be best to combat this disease is of great importance.

The C57BL/6 diet induced obesity mouse model has been used previously to study the effect of excess weight on various organ systems. As we are interested in the interaction between the immune system, oxidative stress, physical performance, and other organs, we cannot use isolated cell lines or model organisms such as yeast. Additionally, many reagents have been developed for the use of mouse tissues, especially antibodies that will be used to identify certain outcome measures. As we are focusing on the effect of the different dietary programs on multiple organ systems, it is only feasible in an animal study.

There will be 8 mice per group for a total of 56 mice. This number is the norm for similar studies of dietary-induced changes in our health-specific parameters. This number should be sufficient to determine statistical significance for the tests planned during this study.

D. Do the procedures described in B above, have the potential to inflict more than momentary pain or distress (this does not include pain caused by injections or other minor procedures)? Yes ☐ No ☒

I have considered alternatives to procedures that might cause more than momentary or slight pain/distress, and I have not found such alternatives. As such, I have used one or more of the following methods and sources to search for such alternatives: **(check below each method used)**

☐ Agricola Data Base ☐ Medline Data Base ☐ CAB Abstracts

☐ TOXLINE ☐ BIOSIS ☐ Lab. Animal Sci. Journal

☐ Lab. Animals Journal ☐ Lab Animal ☐ Animal Welfare Info Center

☐ ATLA (Alternatives to Laboratory Animal Journal) ☐ Quick Biblio. Series

☐ Lab Animal Welfare Bibliography (QL55L27311988) ☐ "Benchmarks"

☐ "Alternatives to Animal Use in Research, Testing and Education"

☐ Current Contents

☐ CARL

☐ Direct contact with colleagues **(if selected, you MUST document this below)**

List search words for the literature search:

Daniel Fast, dietary restriction, vegan fasting, fasting, time restricted feeding, intermittent fasting, caloric restriction, alternate day fasting, chow, obesity, inflammation, oxidative stress, microbiome, treadmill test, EchoMRI, insulin resistance, insulin sensitivity, fatty acids, body

composition, glucose, insulin, blood sugar (words used in isolated and in combination in PubMed and Google Scholar).

What is the length of time that the literature search covers?

1960-2017

III. Animal Use

A. List all animal species to be used (example below).

Species	Number[1]	Age[2]	Sex[2]	Weight[2]	Where Housed (Bldg./Rm#)

e.g. Hooded Wistar rats	45	2 months	male	250-350 gm	Psychology Bld./422I
C57Bl/6 mice	56	4 weeks	Male	15-20 gm	Life Sciences/115

[1]Individuals using ectotherms need to only approximate numbers.

[2]Individuals using fish or other ectotherms need not answer this question.

Is any species threatened or endangered? Yes ☐ No ☒

B. Source of animals

☒ Commercial vendor (Source___ Envigo

Labs_____)

☐ Bred at The University of Memphis

☐ Captured from wild. Identify method of capture: _____

☐ Transferred from another study (IACUC Protocol Number _____

☐ Donated (Source _____)

☐ Tennessee Wildlife Resources Agency

Is the supplier a USDA approved source? Yes ☒ No

☐

If not, explain why:

☐ Animals are already in residence at U of M

C. Will surgery be conducted on animals? Yes ☐ No

☒

If yes, complete this section:

☐ Non Recovery Surgery ☐ Recovery Surgery

☐ Multiple Survival Surgery (**if the latter is checked, complete section F)**

Surgeon(s) (Name/Job/Title/Academic Rank) Location of Surgery (Bldg. & Room #)

D. Will Anesthetic(s), Analgesic(s), or

Tranquilizing agents be administered? Yes No x☐

If yes, complete this section (example below).

Species & Sex Agent Dose Route Performed by

(Name/Title/Academic Rank)

e.g. male Hooded Wistar rats	sodium pentobarbitol	50 mg/kg	i.p.	Mr. Smith/Research Technician/B.S.

E. Will euthanasia be carried out? Yes ☒ No ☐

If yes, complete this section (example below).

Species & Sex Agent Dose Route Performed by (Name/Title/Academic Rank)

e.g. male Hooded Wistar rats	sodium pentobarbitol	150 mg/kg	i.p.	Mr. Smith/Research Technician/B.S.
C57BL/6 mice	CO_2	3L/min	Inhalation	Marie van der Merwe/ Assistant Professor Matt Butawan/Research Associate Sunita Sharma/Master's Student (Will be trained by Dr. Karyl Buddington)

If no, describe disposition of animal(s) at conclusion of this study in box below.

F. Will special housing, conditioning, diets or other conditions be required? Yes ☒ No ☐

If yes, please explain in box below.

Mice will be on high fat, chow, or purified diets. Some mice will have restricted access to food, either 7am-1pm or every other day. Some mice will have access to only 80% of their daily ad libitum intake.

G. Will animals be removed from the U of M campus at any time? Yes ☐ No ☒

If yes, please indicate to where and for how long in box below.

H. If they are to be housed for more than 24 hours outside approved facilities at U of M, provide a scientific justification in box below.

IV. Toxic and Hazardous Substances

A. Check off any of the following below that will be used in these experiments?

☐ Infectious agents (Fill out a, b)

☐ Radioisotopes (Fill out a, b, e)

☐ Toxic chemicals or carcinogens (Fill out a, b)

☐ Recombinant DNA (Fill out a)

☐ Experimental drugs (Fill out a)

☐ Malignant cells or hybridomas (Fill out a, c)

☐ Adjuvants (Fill out a)

☐ Controlled substances (Fill out a, d, e)

For each checked off category, answer the questions indicated below:

a. Identify the substance(s) and completely describe their use, including how will be injected or given to the animal(s):

b. Describe all procedures necessary for personnel and animal safety including biohazardous waste, carcass disposal and cage decontamination:

c. If transplantable tumors or hybridoma cells are to be injected into the animals, have the tissues/cells been tested for inadvertent contamination by viruses or mycoplasma? Yes ☐ No

☐

If yes, what was the result (indicate in box below).

d. In the box below, provide a complete list of these substances, and if their use is not explicitly explained in the materials already provided, explain their use and role in the research.

Provide DEA license # covering the use of these substances:

To whom (or what entity) is the license issued?

e. Provide Radioisotope License Number: _____

To whom is the license issued? _____

V. Categories of Animal Experimentation Based Upon Level of Manipulation and Pain: (check off each category that is applicable to this application)

☐ A. Animals will be involved in teaching, research, experiments or tests involving no pain, distress, or use of pain-relieving drugs.

☐ B. Animals will be subject to mild stress only (e.g., food or water deprivation of less than 24 hours for use in behavioral studies such as operant conditioning; physical restraint for less than 30 minutes), and will not be subject to surgery, painful stimuli, or any of the other conditions described below. Procedures described in this protocol have the potential to inflict no more than momentary or slight pain or distress on the animal(s)----that is, no pain in excess of that caused by injections or other minor procedures such as blood sampling.

☐ C. Animals will have minor procedures performed, blood sampling, etc. while anesthetized.

☐ D. Live animals will be humanely killed without any treatments, manipulations, etc. but will be used to obtain tissue, cells, sera, etc.

☐ E. Live animals will have significant manipulations, surgery, etc. performed while anesthetized. The animals will be humanely killed at experiment termination without regaining consciousness.

☒ F. Live animals will receive a painful stimulus of short duration without anesthesia (behavior experiments with flight or avoidance reactions--e.g., shock/reward) resulting in a short-term traumatic response. Other examples in this category are, blood sampling, injections of adjuvants, or drugs, etc.

Injection for glucose tolerance test; possible low grade shock while on treadmill.

☐ G. Live animals will have significant manipulations performed, such as surgery, while anesthetized and allowed to recover. Such procedures cause post-anesthetic pain/discomfort resulting from the experiment protocol (e.g., chronic catheters. surgical wounds, implants) which cause a minimum of pain and/or distress. Also included are mild toxic drugs or chemicals, tumor implants (including hybridomas). tethered animals, short-termed physically restrained animals (up to 1 hour), mother/infant separations.

☐ H. Live animals will have significant manipulations or severe discomfort, etc. without benefit of anesthesia, analgesics or tranquilizers. Examples to be included in this category are: toxicity testing, radiation sickness, irritants, burns, trauma, biologic toxins, virulence challenge, prolonged: restrictions of food or water intake, cold exposure, physical restraint or drug addiction. All use of paralytic agents (curare-like drugs) must be included in this category. Describe any abnormal environmental conditions that may be imposed. Describe and justify the use of any physical restrain devices employed longer then 1 hour.

VI. Justifications for Category G Studies and Deviations from Standard Techniques

Describe in the box below any steps to be taken to monitor potential or overt pain and/or distress during the course of this study and how such pain or distress will be alleviated. Be as detailed as necessary to justify your procedure.

VII. Certifications

(By submitting this protocol, I am acknowledging that I comply with the certifications included in Section VII.) *(check one)*

☒ Animal Use for Research. I certify that the above statements are true and the protocol stands as the original or is essentially the same as found in the grant application or program/project. The IACUC will be notified of any changes in the proposed project, or personnel, relative to this application, prior to proceeding with any animal experimentation. I will not purchase animals nor proceed with animal experimentation until approval by the IACUC is granted.

☐ Animal Use for Teaching/Demonstration. I certify that the information in this application is essentially the same as contained in the course outline and a copy of the laboratory exercises using animals is on file in the IACUC office. The IACUC will be notified of any changes in the proposed project, or personnel, relative to this application, prior to proceeding with any animal experimentation. I will not proceed with animal experimentation until approval by the IACUC is granted.

Estimate the cost of maintaining animals used in this protocol based on current per diem charge at University of Memphis.

$7.20/day ($0.24/cage/day)

Please specify cost per unit of time:

Specify anticipated total costs for project duration: $756 (15 weeks)

As supervisor of this project it is required that you inform your department chair concerning any animal per diem costs related to this project that are to be paid by the department.

By submitting this protocol, the Principal Investigator/Course Director indicates that the following have been considered:

1. Alternatives to use of animals.
2. Reduction of pain and stress in animals to the lowest level possible.
3. The proper needs of the animals with respect to housing and care.
4. The lowest number of animals used that will give the appropriate experimental results.
5. Use of the most primitive species that will give the appropriate experimental results.
6. Proper training of all personnel in the care and handling of the species used and in the procedures called for in this protocol before beginning the experiment/teaching or demonstration.
7. That this protocol is not an unnecessary repeat of results already in the literature or in the case of teaching/demonstrations, results that can be demonstrated using models or video material.

Marie van der Merwe/Richard Bloomer

Principal Investigator/Course Director (Type Name)

mvndrmrw@memphis.edu / rbloomer@memphis.edu
e-mail address

5/3/2017
Date

Federal Law requires that members of the IACUC be given adequate time to read and review protocols including any changes or revisions in them.

Pre-review of protocols by the Attending Veterinarian is required before submission to the IACUC. New protocols or modifications or renewals to protocols must be submitted to the IACUC Chair by the 1st business day of the month to be considered for review during that month. Incomplete protocols will be returned to the principal investigator.

E-mail the completed protocol to the IACUC Chair, Dr. Amy de Jongh Curry, adejongh@memphis.edu

February, 2015