**Impact of Feeding Time and Duration on Body Mass and Composition in Young, Exercising Mice**

Richard J. Bloomer *, Marie van der Merwe, Melissa J. Puppa, Matthew Butawan

College of Health Sciences, University of Memphis, Memphis, TN 38152, USA; E-Mails: rbloomer@memphis.edu; mvndrmrw@memphis.edu; mpuppa@memphis.edu; mbutawan@cbu.edu

* Correspondence: Richard J. Bloomer; E-Mail: rbloomer@memphis.edu

**Academic Editor**: Jennifer Keogh

**Recent Progress in Nutrition**

2023, volume 3, issue 3

doi:10.21926/rpn.2303014

**Abstract**

Time-restricted feeding (TRF) has increased in popularity among various groups, including fitness enthusiasts. The ideal timing of TRF in relation to daily exercise is unknown. Most fitness enthusiasts consume meals immediately or soon after exercise to improve body composition (e.g., lean mass). We compared two different TRF approaches, as well as an *ad libitum* control diet, with regards to body mass and body composition in C57BL/6 mice. Young, healthy, male mice exercised five days per week and were assigned to consume food *ad libitum* (control), or to follow a 6-hour TRF that began immediately after exercise (TRF-I) or 5 hours after exercise (TRF-D); n = 12 mice per group. Body mass, lean mass, and fat mass were assessed weekly. Due to animal deaths, only 10 animals were included in the analysis for each TRF group, with 8 animals included for the control group. When computing the 8-week average, body mass varied between groups (p < 0.0001), with the TRF-I (25.4 ± 1.7 g) weighing less than the TRF-D (26.3 ± 2.3 g) and control (26.9 ± 2.3 g). Lean mass also differed (p < 0.0001), with control (22.8 ± 1.9 g) higher than TRF-D (21.4 ± 1.7 g) and TRF-D (21.7 ± 1.8 g). Additionally, fat mass differed between groups (p < 0.0001), with the TRF-D (2.7 ± 0.9 g) higher than the TRF-I (2.2 ± 0.9 g) and control (2.0 ± 1.2 g). Finally, percent body fat differed (p < 0.0001), with TRF-D (10.5 ± 3.3%) higher than TRF-I (8.6 ± 3.7%) and control (7.5 ± 4.3%). At the end of the 8-week intervention, TRF-I was lower in fat mass and percent body fat than TRF-D (p < 0.05),
while body mass and lean mass were higher for control as compared to both TRF groups (p < 0.05). These results indicate that when combined with regular exercise, *ad libitum* feeding may be more beneficial (greater overall and lean mass gain) than TRF, regardless of feeding timing.

**Keywords**
- Time-restricted feeding; fasting; exercise; body composition; lean mass

### 1. Introduction

Obesity has become an epidemic in the Western world [1], largely due to physical inactivity [2] and poor dietary habits [3]. Excess fatty tissue, the hallmark sign of obesity, negatively alters body composition—which has a direct impact on aesthetics and may also adversely impact physical performance. While multiple dietary programs exist to alter body composition, one that has received a great deal of attention recently is Time-Restricted Feeding (TRF) [4]. This program involves a block of time each day when feeding is allowed, typically 4-8 hours, with the remainder of the day involving food restraint. In our recent mouse studies using TRF protocols, a 6-hour feeding window has been met with very favorable outcomes with regards to health-specific parameters [5-7], in particular when we utilize a Western diet chow, which is very high in fat and processed carbohydrate.

While many prior studies have focused on TRF, few have included exercise training within the research design. With regards to humans, many exercise enthusiasts have expressed interest in utilizing a TRF protocol [8] typically with the goal of reducing body fat and maintaining lean body mass. The problem for many is the difficulty of scheduling the feeding block at a time of day that allows for the potential desired effects without greatly interfering with the social aspects of eating. That is, most exercise enthusiasts have been led to believe that they must consume nutrients within 30-60 minutes following acute exercise—for purposes of fueling muscle tissue [9]. Anecdotally, it has been observed that most working adults exercise either early morning (e.g., 5:00 am) or early evening (e.g., 5:00 pm). If attempting to start feeding 30-60 minutes post exercise and adopting a typical TRF regimen inclusive of 5-6 hours of feeding, the morning exerciser will close their feeding window by lunch time. The evening exerciser will need to go the entire workday without food. Both scenarios are not ideal for a variety of reasons and may severely hamper long-term compliance.

It is possible that the ingestion of food within the one-hour post exercise is unnecessary for purposes of maintaining or increasing muscle mass. If so, exercise enthusiasts would be able to begin their TRF regimen whenever they desired, making this much more manageable for the majority of those who exercise and are interested in utilizing this approach.

The goal of the present study was to compare two different TRF approaches with regards to body mass and body composition in C57BL/6 young, male mice. An *ad libitum* control condition was also included in the design, as many individuals move from this feeding protocol to that of a TRF protocol. Rather than using a high fat diet (e.g., Western diet), which had typically been used for TRF studies, we chose to use a diet more similar in quality to what most healthy exercise enthusiasts consume, in this case the *Growing Rodent Diet*, consisting of 21% protein, 15% fat, 64% carbohydrate.
2. Materials and Methods

2.1 Animals, Food, and Experimental Design

This study was approved by the University of Memphis Institutional Animal Care and Use Committee (protocol #0833). Based on body composition findings from our prior studies [5, 7], a total of 36, six-week-old C57BL/6 male mice were purchased from Envigo (Indianapolis, Indiana). Mice were pair-housed in the animal facility on the University of Memphis campus. Animals were entrained under a 12 hour light:12 hour dark schedule for three weeks with 24-hour access to food (Growing Rodent Diet [21% protein, 15% fat, 64% carbohydrate]; AIN-93G; Research Diets, New Brunswick, New Jersey). During the three-week entrainment period, mice began the reverse light-dark schedule, with lights off between the hours of 6:00 am-6:00 pm. This was done so that the exercise and feeding times occurred during the active phase (“lights off” phase) of the mice. During the three weeks, mice were also acclimated to the treadmill to be used for daily exercise.

At nine weeks of age, mice were assigned to one of the three groups (n = 12 animals per group). The total of 36 animals was chosen based on our prior work of a similar nature, in which we see relatively low variability and adequate power to detect changes in the noted outcome measures. The three dietary groups included: control, ad libitum access to food 24 hours per day (with the exception of the exercise time); TRF immediate phase (TRF-I), start feeding immediately post exercise on each day; TRF delayed phase (TRF-D), start feeding at 5 hours post exercise on each day. The TRF-D group, which also was the first group to run each day, had food access from 12:00 pm until 6:00 pm. The TRF-I group, which ran second, had food access from 8:00 am until 2:00 pm. The control group, which ran third, had ad libitum access to food throughout the day. It should be noted that on “off-days” (Saturday and Sunday) feeding times remained the same as during the week. In both TRF groups, food was provided for 6 hours each day, with 18 hours of fasting. Food intake was measured daily and recorded. Water was provided ad libitum throughout the study period for all three groups. Mice continued on their diets until all testing was completed (during early week 9).

2.2 Exercise

All mice exercised 5 days a week for 8 weeks of intervention using a motorized treadmill (Columbus Instruments: Exer 3/6, Columbus OH). During the first week of exercise all mice underwent a familiarization protocol. This included a standard warm-up consisting of 5 minutes at 5 meters/minute, 5 minutes at 10 meters/minute and 5 minutes at 15 meters/minute at a 10% incline, followed by 15 minutes at 20 meters/minute (30 minutes total). Starting in week 2 and through the end of the study, all mice followed a protocol that consisted of the same 15-minute warm-up, followed by 45 minutes of running at 20 meters/minute (60 minutes total). All mice completed the exercise training within the first 3 hours of the active phase. The TRF-D group began running at approximately 6:00 am, the TRF-I group began at 7:00 am, and the control group began at 8:00 am.

2.3 Magnetic Resonance Imaging (MRI) Protocol

Once per week, starting one week before initiation of the exercise and dietary protocols, mice underwent an MRI. All mice were fasted at least 8 hours prior to the MRI. The two TRF groups
underwent their usual fasting. The ad libitum group had their food removed at 10:00 pm the night prior to testing. Mice were weighed using a laboratory balance and then placed in a holding tube, without anesthesia or sedation. The tubes were then placed into the MRI machine (EchoMRI-100, EchoMRI, Houston, Texas) and scans for each animal were completed in approximately 40 seconds. The results from the machine were recorded and included lean mass, fat mass, and percent body fat. Mice were then removed from the tubes and placed back in their cages. MRI testing was conducted prior to daily exercise. Body mass was also recorded twice weekly using a laboratory balance.

2.4 Muscle Removal and Weighing

Following the 8-week intervention and all MRI testing, animals were euthanized via cervical dislocation. Hindlimb muscles, including the gastrocnemius, soleus, plantaris, tibialis anterior, and extensor digitorum longus were dissected from the right and left limbs and weighed. Hindlimb muscle mass was calculated for each mouse as the average sum of the hindlimb muscles taken from the right and left limbs.

2.5 Data Analysis

The data are presented as mean ± SD. Data were analyzed using a three-group analysis of variance (ANOVA), considering average values across the entire 8-week intervention. Tukey post hoc testing was used to determine differences between groups. Paired contrasts were performed between groups to determine baseline differences and differences at the 8-week time point for each variable. Analyses were performed using JMP software (SAS, Cary, NC) and statistical significance was set at p ≤ 0.05.

3. Results

3.1 Animals

While numerous precautions were taken to care for the animals as best possible, unforeseen challenges were encountered. Two mice were fatally injured during the treadmill exercise, as they slipped through the treadmill belt and were caught between the belt and the shock grid (which was not used at all during testing). One mouse died during body composition testing, and five mice died following a presumed illness that swept through approximately 6 weeks into the intervention. Therefore, the complete data for 10 animals were included in the analysis for each TRF group, while data for only 8 animals were included for the control group. Based on the relatively low variability in response between animals in each group, we do not believe that the loss of these 8 animals altered the overall findings but this is certainly a possibility.

3.2 Body Mass and Composition

No baseline differences were noted between groups for any variable (p > 0.05). When considering the average values across 8 weeks, a condition effect was noted for body mass (p < 0.0001; Figure 1), with the TRF-I (25.4 ± 1.7 g) weighing less than the TRF-D (26.3 ± 2.3 g) and control (26.9 ± 2.3 g). A condition effect was noted for lean mass (p < 0.0001; Figure 2), with control (22.8
± 1.9 g) higher than TRF-I (21.4 ± 1.7 g) and TRF-D (21.7 ± 1.8 g). A condition effect was noted for fat mass (p < 0.0001; Figure 3), with the TRF-D (2.7 ± 0.9 g) higher than the TRF-I (2.2 ± 0.9 g) and control (2.0 ± 1.2 g). A condition effect was noted for percent body fat (p < 0.0001; Figure 4), with TRF-D (10.5 ± 3.3%) higher than TRF-I (8.6 ± 3.7%) and control (7.5 ± 4.3%).

![Figure 1](image)

**Figure 1** Body mass in mice over time (A) and as an average during an 8-week protocol (B) of *ad libitum* intake or a TRF regimen. Values are mean ± SD. A * Control significantly higher than TRF-I (p < 0.001) and TRF-D (p = 0.01); B * Significantly less than Control and TRF-D (p < 0.05). Due to animal deaths, only 10 animals were included in the analysis for each TRF group, with 8 animals included for the control group.
Figure 2 Lean mass in mice over time (A) and as an average during an 8-week protocol (B) of *ad libitum* intake or a TRF regimen. Values are mean ± SD. A * Control significantly higher than TRF-I (p = 0.006) and TRF-D (p = 0.003); B * Significantly greater than TRF-I and TRF-D (p < 0.05). Due to animal deaths, only 10 animals were included in the analysis for each TRF group, with 8 animals included for the control group.
Figure 3 Fat mass in mice over time (A) and as an average during an 8-week protocol (B) of *ad libitum* intake or a TRF regimen. Values are mean ± SD. A * Significantly lower fat mass for TRF-I compared to TRF-D (p = 0.03); B * Significantly greater than Control and TRF-I (p < 0.05). Due to animal deaths, only 10 animals were included in the analysis for each TRF group, with 8 animals included for the control group.
Figure 4 Body fat percentage in mice over time (A) and as an average during an 8-week protocol (B) of *ad libitum* intake or a TRF regimen. Values are mean ± SD. A * Significantly lower percent body fat for TRF-I compared to TRF-D (p = 0.05); B * Significantly greater than Control and TRF-I (p < 0.05). Due to animal deaths, only 10 animals were included in the analysis for each TRF group, with 8 animals included for the control group.

When only considering the values at the end of the 8-week intervention, TRF-I was lower in fat mass (p = 0.03) and percent body fat (p = 0.05) than TRF-D. In addition, body mass was higher for control as compared to TRF-I (p < 0.001) and TRF-D (p = 0.01). Lean mass was also higher for control as compared to TRF-I (p = 0.006) and TRF-D (p = 0.003). Finally, a condition effect was noted for hindlimb muscle mass (p < 0.0001; Figure 5), with control (0.33 ± 0.2 g) higher than TRF-I (0.30 ± 0.03 g) and TRF-D (0.29 ± 0.02 g). When considering these results, it should be noted that overall food intake was not different between groups (p > 0.05, Figure 6).
Figure 5 Hindlimb muscle mass normalized to tibia length at the conclusion of an 8-week protocol of *ad libitum* intake or a TRF regimen. Values are mean ± SD. * Significantly greater than TRF-I and TRF-D (p < 0.05). Due to animal deaths, only 10 animals were included in the analysis for each TRF group, with 8 animals included for the control group.

Figure 6 Food intake of mice assigned to an 8-week protocol of *ad libitum* intake or a TRF regimen. Values are mean ± SD. No significant differences noted (p > 0.05). Due to animal deaths, only 10 animals were included in the analysis for each TRF group, with 8 animals included for the control group.

4. Discussion

Findings from the current study indicate that 1) the degree of total and lean mass gain is greatest when animals are provided *ad libitum* access to quality food throughout the day; 2) fat mass is lower
across time when adhering to an ad libitum food access plan, although fat mass may be lower at the end of the 8 week plan if adopting a TRF-I approach; 3) delaying feeding for 5 hours following acute exercise does not negatively impact lean mass gain as compared to feeding immediately post exercise but does result in greater fat mass accumulation; 4) adhering to a TRF regimen immediately following acute exercise results in lower overall body mass gain in young mice.

4.1 Total and Lean Mass

Total body mass and the amount of lean body mass are two important variables for those engaged in regular exercise. Many fitness enthusiasts have adopted a TRF regimen in the hopes of maintaining lean mass and shedding excess body fat. When consuming a Western diet, which contains a significant amount of dietary fat (~40%) and processed carbohydrate, the TRF protocol may yield success [4, 10], as reported previously. However, in the present study we incorporated a healthy diet consisting of a macronutrient breakdown similar to what many health and fitness enthusiasts might employ—based on personal observation over many years of consultation with such individuals. Our results demonstrate that when consuming such a diet, ad libitum intake results in the best overall success as related to total and lean mass gain.

In terms of total body mass, animals in the control and TRF-D groups appeared to gain mass at a nearly identical rate, with ending values very similar. Animals in the TRF-I group started gaining less mass at approximately week 2 and remained lower throughout the remainder of the study period (Figure 1). In terms of lean mass, animals in the control group experienced the greatest gain, ahead of both TRF groups starting at week 1 and continuing this trend throughout the study period to end with approximately 6% more lean mass than the TRF groups. Animals in both TRF groups appeared nearly identical throughout the study period, as shown in Figure 2A. Beyond total body mass, when considering only hindlimb muscle mass, the control group ended with approximately 10% more mass as compared to either TRF group (Figure 5). The exercised muscles indeed responded more favorably to the ad libitum food intake, as demonstrated by this significant gain in total hindlimb mass [11].

Consider this finding, if muscle mass is the main goal, ad libitum intake of good quality food appears to be the best option based on the present data. Following an ad libitum regimen also does not appear to be problematic in terms of fat mass accumulation, as overall fat mass and percent body fat across the intervention period was lowest in the control group (Figure 4). These findings for the most favorable results with the control diet are in contrast to many other TRF studies but it should be noted that in nearly all such studies, the diet used is a high-fat, high sugar Western diet. The diet used in the present study was of high quality, with 21% protein, only 15% fat, and 64% carbohydrate, which is similar to the macronutrient breakdown of many resistance trained athletes seeking optimal muscle mass, while desiring to remain relatively lean.

4.2 Fat Mass

If our findings translate to human subjects, it appears that for those individuals seeking reduced overall body mass, and potentially reduced fat mass at the conclusion of an intervention period, adhering to a program of TRF-I may be best. As shown in Figure 6, while not statistically different, overall food intake was lower in the TRF-I group as compared to the control and TRF-D, which may help to explain the findings for lower body mass. Other studies in both animals and humans have
noted lower overall body mass when participants adhere to a TRF regimen outside of the context of exercise [4, 10, 12, 13]. We noted lower overall body mass in the TRF-I group, which seems to persist from approximately week 2 and throughout the study period, with a mean approximately 5% lower than control and 4% lower than TRF-D (Figure 1). Fat mass across the study period was similar between TRF-I and control, while both groups were approximately 30% lower than TRF-D (Figure 2). That said, fat mass was initially higher for the TRF-I (weeks 1-3) and TRF-D (weeks 1-6), as compared to control, while lean mass was higher for control (yielding a similar trend in the body weight gain over time for all groups).

From a practical perspective, individuals need to consider what is most important—increasing lean mass or decreasing fat mass. Most prior studies of dietary manipulation indicate that dietary restriction almost always results in a decrease in fat mass at the expense of lean mass [14, 15], and even muscle damage [16]. This loss in lean mass appears true in the present study with regards to the TRF-I group, as body weight and fat mass at the conclusion of the study period were lower than control but the lean mass was also lower than control. While this study was not focused on weight/body fat loss in an overweight population, it cannot be directly compared to studies involving weight loss. Rather, findings from this study may be considered more specific for those who are embarking on an exercise program with the intention of gaining lean mass and attempting to minimize fat mass. With this in mind, it may be most appropriate to adopt a diet of healthy food consumed in smaller, frequent feedings throughout the day (as shown to have benefit previously [17], without restriction on the amount or timing of food ingestion.

Many exercise enthusiasts believe that food must be consumed very soon after the cessation of exercise in order to foster muscle growth and development [9, 18, 19]. A review of the findings for the TRF-D group indicate that delaying feeding for a period of 5 hours post exercise does not negatively impact lean mass, as values for TRF-D are nearly identical for those of TRF-I, but slightly less than those of control (Figure 2B). However, it should be noted that despite not having any negative impact on lean mass, delaying feeding resulted in significantly more fat mass as compared to ad libitum consumption, as well as TRF-I (Figure 3 and Figure 5). Based on these findings, it appears that when consuming high quality food, it may be best to consume calories for several hours throughout the day, rather than just for a period of time relative to a single exercise session. The reasons as to why the delayed feeding led to the greater increase in fat mass is unknown but may be associated with the hormone cortisol. Prior research indicates that eating later during the active cycle corresponds with lower metabolic activity and higher body fat [20, 21]. In a human study, fasting led to a shift of peak cortisol levels to the afternoon [22]. This would coincide with the time of delayed feeding and coupled with heightened cortisol levels from fasting [23], could explain the increase in body fat for the TRF-D. A noted limitation of the present work is a failure to measure circulating cortisol.

When reviewing Figure 6 we see that the overall amount of food consumed was near identical between the control and TRF-D groups. However, animals in the control group had approximately 6% more lean mass, 10% more hindlimb muscle mass, and 36% less fat mass as compared to TRF-D. A similar pattern was observed between control and TRF-I for lean and hindlimb mass, albeit not as pronounced. The regular influx of nutrient-dense calories may have allowed for the heightened lean mass gain, while possibly altering the metabolism in such a way as to result in less fat accumulation.

When considering the above results, it is important to note that our findings are specific to young animals. It is possible that results may have been different if we included older animals who were...
far beyond the initial growth period. Additionally, our study was the first to our knowledge to assess the impact of a TRF protocol while using a healthy, growing rodent diet. It is certainly possible that the use of a high fat diet alternative could have differently influenced the results. For example, including a Western Diet group in any future study may be considered for comparison purposes. Finally, while we included aerobic exercise in the design, future studies may determine the impact of anaerobic exercise in conjunction with TRF, as this has yet to be studied and is a popular form of exercise used by many fitness enthusiasts. Differing results may be observed when participating in a program of anaerobic/resistance exercise.

5. Conclusions

When consuming a good quality diet in conjunction with a moderate volume exercise program, *ad libitum* food intake throughout the day results in greater overall mass and lean mass gain in young, male mice as compared to a regimen of TRF. That said, a program of TRF commenced immediately following acute exercise does result in lower overall body mass gain in young mice, while also resulting in less overall body fat at the conclusion of an eight-week intervention period.

Acknowledgments

Funding for this work was provided by The University of Memphis. Appreciation is extended to Dr. Jackie Pence for constructive comments and assistance with manuscript preparation and formatting. Appreciation is also extended to Aaron Persinger, MS and Martina Faietti, MS for assistance with animal training.

Author Contributions

Conceptualization: Richard Bloomer, Marie van der Merwe and Melissa Puppa; Data curation: Melissa Puppa, Matthew Butawan; Formal analysis and manuscript writing, Richard Bloomer; Investigation, Marie van der Merwe, Melissa Puppa, Matthew Butawan; Methodology, Richard Bloomer, Marie van der Merwe and Melissa Puppa; Project administration, Richard Bloomer, Marie van der Merwe and Matthew Butawan.

Competing Interests

The authors declare no competing interests related to this work.

References


22. Bergendahl M, Vance ML, Iranmanesh A, Thorner MO, Veldhuis JD. Fasting as a metabolic stress paradigm selectively amplifies cortisol secretory burst mass and delays the time of maximal