Comparison of Dietary and Caloric Restriction Models on Insulin Sensitivity in Young Mice

Jade Leanna Caldwell

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COMPARISON OF DIETARY AND CALORIC RESTRICTION MODELS ON INSULIN SENSITIVITY IN YOUNG MICE

By

Jade Leanna Caldwell

A Thesis
Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science

Major: Health Studies

The University of Memphis
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ABSTRACT

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Comparison of dietary and caloric restriction models on insulin sensitivity in young mice
Major Professor: Dr. Richard J. Bloomer

Background: Various forms of fasting can result in improvements in insulin sensitivity. Objective: Compare the effects of various fasting models on measures of insulin sensitivity in young male C57BL/6 mice. Methods: 60 mice were assigned to the following groups: HF, CHOW, SWITCH, DF, CR, TRF, and ADF for 8 weeks, following a 6-week lead-in period of ad libitum HF consumption. Fasting blood glucose (mg/dL) and insulin (ng/mL) were measured and HOMA-IR, determined. Glucose tolerance tests were performed to determine glucose clearance pre- and post-intervention. Results: Compared to the HF ad libitum group, all groups displayed significantly lower fasting glucose, insulin, and HOMA-IR, as well as improved glucose tolerance. Additionally, ADF displayed the lowest fasting blood glucose and glucose AUC. Conclusion: All investigated fasting protocols may improve fasting glucose, insulin, and HOMA-IR. ADF may allow for ideal fasting blood glucose and clearance of glucose loads following a glucose challenge.
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INTRODUCTION

Obesity has become an epidemic in the United States over the past four decades [1]. Despite many available treatment options for obesity and its comorbidities, the prevalence continues to increase [2]. The development of obesity can be linked directly to the poor lifestyle choices of physical inactivity [3,4] and the consumption of a calorie dense, high-fat diet [5-10]. This type of lifestyle leads to the accumulation of adipose tissue and often to the development of type 2 diabetes [11] and cardiovascular disease [12]. With obesity being an underlying cause of these issues, it is important to have treatment and lifestyle options to prevent the growth of this epidemic.

Insulin sensitivity relates to how effectively insulin facilitates the uptake of glucose into cells. When the process of glucose uptake is compromised, an individual is said to have insulin resistance. The term insulin resistance is therefore used to describe the condition whereby insulin-induced glucose uptake is impaired, leading to hyperglycemia and hyperinsulinemia [13]. Although the exact reasons why the biological process of insulin signaling stops working properly remain to be fully elucidated, the potential mechanisms and associated conditions related to insulin resistance are many and commonly related to obesity [14] and the accumulation of adipose tissue [15].

Dietary manipulation is often used as a first line approach to target weight loss. A very commonly used approach is caloric restriction (CR), which is the practice of reducing one’s caloric intake without inducing malnutrition. It is well documented that CR improves overall health. Like CR, most dietary programs involve some form of restriction (e.g., calories, food groups, time in which food is consumed). Fasting is described as “abstinence from some or all food, drink, or both usually for a pre-determined period of time,” thereby, indicating CR as a form of fasting. Other commonly followed fasting plans include dietary restriction (DR), time restricted feeding (TRF), and alternate day fasting (ADF).
DR involves a form of partial fasting, typically eliminating certain food items. Such plans do not intend to restrict calories, but often this occurs by default. The Daniel Fast (DF) is one such popular DR model for which the individual eliminates all animal products, processed foods, caffeine, alcohol, additives and preservatives. The few human studies examining the effects of the DF on health-related outcomes have produced significant findings for several variables, including clinically significant decreases in plasma insulin levels and HOMA-IR. Also, it should be noted that in a recent animal study, improvements in insulin and glucose were reported.

TRF is a dietary change model implementing a time period, typically 4-8 hours, in which daily caloric needs are consumed. All daily food and drink, other than water, are completely restricted during the remaining hours of the day. Very few studies have investigated the role of TRF and markers of insulin sensitivity. One human study [16] and one animal study [17] have reported significant decreases in both fasting blood glucose and insulin.

ADF involves a 48-hour cycle: 24 hours of feasting followed by 24 hours of fasting. Ad libitum eating is allowed during the feasting days, while no food or very little (e.g., 500 calories in humans) is consumed during the fasting days. ADF in humans has shown to decrease fasting glucose [18, 19], fasting insulin [20], and improve HOMA-IR[20]. Animal studies involving ADF is abundant and repeatedly shows improvements to measures of insulin sensitivity [21-26].

Based on these findings and the fact that many dietary plans have been studied but few have been directly compared, the purpose of the present investigation was to directly compare various dietary interventions on measures of insulin sensitivity in young C57BL/6 male mice. The intervention included changes in dietary composition (Chow and DF) and dietary restriction (TRF, ADF and CR). To the author’s knowledge, no such study has been conducted in either animals or humans. It was hypothesized that the mice consuming the high fat “Western diet” ad libitum would
display the greatest degree of insulin resistance, as assessed by fasting glucose, fasting insulin, HOMA-IR, and the glucose tolerance test (GTT). Also, it was expected that at the conclusion the 8 week intervention, all dietary groups except for the Western diet would have improved insulin sensitivity and glucose homeostasis.

**METHODS**

*Mouse and Dietary Protocol*

Four week old C57BL/6 male mice (n=60; 8 allocated to one of seven groups; two intervention groups received a ninth mouse due to “higher risk of incidence”) were entrained under a reverse 12h light:12h dark schedule for two weeks with light off between 7am-7pm. This allowed feeding time to coincide with working hours during the active phase (“dark” phase) of the mice. During this time, mice were fed a standard rodent chow (Teklad Diets, 2018) available *ad libitum*. Mice were pair-housed in an area used for circadian rhythm studies and therefore, light was well-regulated. After two weeks of entrainment, all but 8 mice were switched to a Western diet (Research Diets, D12451), consisting of 45% lard, 41% carbohydrate (20% sucrose, 9% corn starch, and 12% maltodextrin) and 24% protein. This six-week lead-in period of *ad libitum* feeding allowed for significant weight gain. Eight mice continued a standard control chow diet (CHOW) during this lead-in period and for the remainder of the study, serving as the age match, gender match non-obese control group. Following the lead-in period, the mice fed the Western diet were divided into 6 groups:

Group 1 (HF) remained on the Western diet *ad libitum*. Group 2 (SWITCH) switched to a standard rodent chow with *ad libitum* access, 24 hours per day. Group 3 (DF) was supplied a purified, high-fiber, vegan-based diet (Research Diets, D13092801) *ad libitum*, 24 hours per day. Group 4 (CR) received 80% of *ad libitum* intake of the Western diet as determined during week 6
of the lead-in period. Group 5 (TRF) had *ad libitum* access to the Western diet for 6 hours at the beginning of their active phase (7am-1pm), while no food was supplied for the remaining 18 hours of the day. Group 6 (ADF) had *ad libitum* access to the Western diet every other day. That is, on day 1 ADF received as much food as desired during the entire 24-hour period. On day 2 ADF received no food. This 48-hour cycle was repeated for the entirety of the intervention period.

The diets were purchased from Research Diets, which has experience in producing the Western diet and purified vegan diets for rodent studies. An overview of the macro and micronutrient contents of each diet can be found in Tables 1, 2, and 3. The mice remained on their particular diets for eight weeks. Post testing began following the end of week eight. Mice remained on their diets until all testing was completed (~ end of week 9). Water was provided *ad libitum* throughout the study period. Food volume and kilocalorie consumption was recorded daily. Mice were weighed every other day. Mice were pair-housed. Based on data from our previous studies, we know that genetically similar mice eat relative volumes of food, consistently. Therefore, we were able to calculate an average of the kilocalories consumed per mouse, per cage based on food volume. Mice were euthanized via isoflurane inhalation followed by cervical dislocation.

*Glucose Tolerance Test Protocol*

A glucose tolerance test (GTT) was performed following the lead-in (pre-intervention) and intervention periods (post-intervention) at the end of week 2 and during week 9, respectively. For the pre-intervention GTT, a subgroup of twenty mice being fed the Western diet were chosen (based on total body weight ranges) to represent the HF population, while all CHOW mice were tested. All mice were subjected to a post-intervention GTT during week 9 of the intervention period.
All mice were fasted eight hours prior to pre and post-intervention testing, except TRF mice at post-intervention. Due to TRF protocol, on the day of post-intervention testing, animals in the TRF group had been fasting from 2:00 p.m. the day prior. This fasting period was standard for their dietary protocol. Post-testing occurred after a feeding day for ADF mice to ensure equal hours of fasting in all groups. Blood was collected via the tail vein. Fasting glucose levels were measured using a standard glucometer (OneTouch®, Ultra). Mice received 1g-glucose/kg total bodyweight via intraperitoneal injection and blood (~10ul) was collected at 30, 60, and 90 minutes after injection to measure glucose clearance. These measurements were used to calculate the following variables: fasting glucose and area under the glucose curve (AUC).

Facial Bleed Protocol

For fasting insulin and glucose levels, blood was collected from the sub-mandibular vein, a procedure colloquially known as “facial bleeding.” During the pretest period, this procedure was performed on all CHOW mice and a subgroup of 20 animals consuming the Western diet, (this group did not overlap with the GTT subgroup). These measurements were used to calculate the difference in pre-intervention fasting insulin levels between the HF and CHOW groups. A post-intervention facial bleed was performed on all mice immediately prior euthanasia. All mice were fasted eight hours prior to sacrifice, except TRF. Euthanasia began at 8:00 a.m. This allowed TRF to continue their fasting protocol the day prior to euthanasia. Euthanasia occurred after a feeding day for ADF mice to ensure equal hours of fasting in all groups. HOMA-IR was calculated using the methods as previously described, using the equation: (Plasma Glucose [mg/dL] × Plasma Insulin [ng/mL])/2,658^{26}.

Biochemical Analyses
Glucose samples other than those analyzed via glucometer were analyzed using a Vet Axcel clinical chemistry system (Alfa Wasserman, Diagnostic Technologies LLC, West Caldwell, NJ, USA) with a glucose reagent (SA1014, Alfa Wasserman, Diagnostic Technologies LLC, West Caldwell, NJ, USA). Fasting insulin was analyzed using a Mouse Ultrasensitive Insulin ELISA kit (Alpco, Salem, NH, USA) using a PowerWave 340 microplate reader (BioTek, Winooski, VT, USA). Samples were analyzed in duplicate on first thaw.

Measurements of Body Composition

For analysis of post-intervention body composition, animals underwent a MRI during the 9th intervention week. This was done using a small animal MRI unit (EchoMRI™). Animals were placed in a stationary tube designed for insertion into the MRI unit. Total scan time for each mouse was approximated 60 seconds; therefore, anesthetizing the animals was unnecessary.

Data Analyses

Data were analyzed using a one-way Analysis of Variance, with Tukey post hoc testing as needed. Single degree of freedom contrasts were used to further compare groups. Data were analyzed using JMP software (version 4.0.3 SAS Institute, Cary, NC, USA). Statistical significance was set at p<0.05 and data are presented as mean ± SEM.

RESULTS

Overview

57 animals completed the study without incident: TRF (n=9), ADF (n=9), HF (n=8), DF (8), Switch (n=8), CR (n=8) and CHOW (n=7). Three mice died during the course of the study during the 6th week of the lead-in period. One animal became ill with sepsis caused by injury to the left hind limb and was euthanized via CO₂ inhalation. Although not certain, it was assumed the injury was sustained from repetitive biting from the animal’s cage mate. One animal from both the
DF and CHOW group died during the pre-intervention run to exhaustion test (data presented in separate paper) as a result of falling into the gap between the treadmill belt and shock grid. Both animals died instantly.

**Fasting Blood Glucose**

Pre-intervention fasting blood glucose levels are presented in Table 4 and Figure 1. As can be seen, CHOW fasting glucose was significantly lower than the HF (p<0.0054). Post-intervention fasting blood glucose levels are presented in Table 5 and Figure 2. A significant main effect on fasting blood glucose was noted (p=0.0009). Fasting blood glucose of HF was significantly higher than all other groups (p<0.05). Fasting blood glucose of ADF was significantly lower than SWITCH (p<0.05), DF (p<0.05), and CR (p<0.05).

**Fasting Blood Insulin**

Pre-intervention fasting blood insulin levels are presented in Table 6 and Figure 3. No statistical differences were present between HF and CHOW groups following the lead in period (p<0.0672). This is likely due to the small sample size (n=6) of successful facial bleeds in the CHOW group. Infection caused by various skin punctures can lead to animal death; therefore, multiple attempts at blood collection were avoided. Post-intervention fasting insulin levels are presented in Table 7 and Figure 4. A significant main effect on fasting blood insulin was noted (p=0.0001). Fasting blood insulin of HF was significantly higher than all other groups (p<0.05). There were no statistical differences noted between any other groups.

**HOMA-IR**

Post-intervention HOMA-IR for each intervention group are presented in Table 8 and Figure 5. A significant main effect on HOMA-IR was noted (p=0.0002). HOMA-IR of HF group was significantly different from all other groups (p<0.05). No statistical differences were observed
between intervention groups. However, it should be noted that 9 samples were excluded from the
data set due to invalid test results or missing blood: 1 from HF, CHOW, and SWITCH groups and
2 from CR, ADF, and TRF.

*Glucose Area Under the Curve*

Post-intervention glucose AUC for each intervention group are presented in Table 9, Figure
6, and Figure 7. A significant main effect on glucose AUC was noted (p<0.0001). Glucose AUC
of HF was significantly different from all other groups (p<0.05), except that of TRF. Glucose AUC
of TRF was significantly different from DF (p<0.05) and ADF (p<0.05). Glucose AUC of ADF
was significantly different from CR (p<0.05). Contrasts revealed glucose AUC of DF was
significantly different from CR (p<0.0176) and TRF (p<0.0012). Additionally, a table
summarizing the outcomes from all measures of insulin sensitivity can be found in Table 10.

*Body Composition*

Post-intervention body composition for each intervention group are presented in Table 11.
Significant main effects on total fat mass (g) (p<0.001) and % fat mass (p<0.001) were noted. Fat
mass of HF was significantly different from all other groups (p<0.0001). Fat mass of CR was
significantly different from all other groups (p<0.0001). Percent fat mass of HF was different from
all other groups (p<0.001), except CR. Percent fat mass of CR was significantly different from
CHOW (p<0.0001), SWITCH (p<0.0001), TRF (p<0.0001), and ADF (p<0.0001). Percent fat
mass of DF was significantly different from TRF (p<0.0001). No significant effects were noted
(p>0.05) for fat free mass (g).

**DISCUSSION**

The results of the current study show that positive changes to measures of insulin
sensitivity can be associated with adhering to dietary fasting protocols. It was hypothesized that
the HF group would display the greatest degree of insulin resistance. Additionally, it was hypothesized that all intervention groups’ measures of insulin sensitivity would be significantly improved from that of the HF group, while producing results comparable to CR. Prior research involving different models of dietary fasting has shown improvements to several measures of insulin sensitivity in both animals and humans, and while many methods of fasting have been independently investigated, to our knowledge, no study has sought to directly compare the methods used in the present work. Although not all groups produced the same results, each intervention group did improve at least one measured variable.

Our results indicate that animals following the DF, TRF, ADF, and CR protocols used in this study can reverse the negative effects on insulin sensitivity induced by high fat feeding. Following the 6-week lead in period of HF feeding, all dietary conditions appeared to result in a positive impact on fasting glucose, insulin, and HOMA-IR (see Table 5, Table 7, and Table 8, respectively). Of all investigated dietary fasting models, ADF appears to have the greatest positive impact on fasting blood glucose and ability to clear glucose loads (see Table 5 and Table 9, respectively). Additionally, CR, DF, and ADF may have a more positive effect on the ability to clear glucose loads than TRF (see Table 9).

As can be seen in Table 5 and Figure 2, our data revealed significantly lower glucose levels in all groups relative to the HF group (194.570±7.97mg/dL). These results support the outcomes of previous research investigating dietary fasting models in both humans and animals. In the present study, SWITCH (162.000±7.97mg/dL), DF (164.375±7.97), and CR (163.875±7.97) groups displayed fasting glucose levels significantly higher than the ADF group (138.444 ± 7.51mg/dL). Thus, suggesting ADF may be more impactful on lowering fasting blood glucose than other fasting methods, although similar values were noted between ADF and the DF for glucose
AUC. Much research supports the association between improvements in blood glucose levels and ADF, but no other research compares ADF to other dietary models. More investigation is needed to elaborate on these results.

As expected, fasting blood insulin of the HF group (1.91±0.24ng/mL) was significantly higher than that of all other groups (Table 7 and Figure 4). These results have been repeatedly shown in prior studies, indicating that dietary change from the consumption of the Western diet can be beneficial. Additionally, no significant differences between intervention groups were noted in regards to fasting blood insulin (p<0.05). However, despite not reaching statistical significance, it should be noted that CR (0.568±0.24ng/mL) and TRF (0.561±0.23ng/mL) displayed levels double that of SWITCH (0.255±0.24ng/mL) and DF (0.281±0.24ng/mL). These results suggest that a purified diet in the form of either standard chow or the DF is likely better than simply modifying the amount or timing of ingestion of a Western diet. Furthermore, SWITCH levels were not only similar to the DF, but were unexpectedly better than CHOW (0.635±0.61ng/mL). These results suggest the negative effects of the Western diet on fasting blood insulin can be reversed to levels comparative to the healthy population.

As can be seen in Table 8 and Figure 5, post-intervention HOMA-IR of all groups was significantly lower than the HF (4.315±1.42) group, with no statistical differences observed between groups (p<0.05). However, similar to the fasting blood insulin levels, CR (1.084±0.24) and TRF (1.048±0.15) groups produced magnitudes twice that of the SWITCH (0.485±0.07) and DF (0.458±0.09) groups. Considering HOMA-IR is the relationship between fasting glucose and fasting insulin, this is not surprising. Despite fasting glucose levels of CR, TRF, SWITCH, and DF being similar, CR and TRF resulted in much greater fasting insulin levels; thus, indicating a
greater degree of insulin resistance in the CR and TRF groups as compared to SWITCH and DF groups.

The fourth outcome variable investigated in the current study, and perhaps the most important due to its functional nature, was glucose AUC. Glucose AUC of all intervention groups was significantly lower than the HF (1134±52.97), except for TRF (927.17±49.94). These results contradict Moro et. al. (2016) who noted significant improvements in HOMA-IR in a human model, following a TRF regimen consisting of an eight hour feeding window over the course of eight weeks. However, in the study by Moro and colleagues, subjects also participated in a resistance training protocol, which certainly could have contributed to the improvements in HOMA-IR. Additionally, in the current study, DF (677.69±52.97) and ADF (630±49.94) groups exhibited significantly lower glucose AUC than the TRF group, characterizing these groups with a greater ability to clear glucose loads. Also, ADF glucose AUC was significantly lower than that of CR (861.63±52.97), suggesting ADF to be an alternative dietary approach to CR, which has been suggested and is being investigated in other research [28-31]. Considering it has been reported by some patients that complying to ADF is easier than daily CR [27], our results may further support ADF as an alternative to CR, for individuals who find it difficult to reduce caloric intake seven days per week as part of a lifestyle intervention.

It is well known that accumulation of adipose tissue, specifically visceral, correlates with the development of insulin resistance [32-34]. It has been proposed that for every standard deviation increase in visceral adipose tissue, an 80% increase in risk of developing insulin resistance occurs [33]. Additionally, due to the accumulation of visceral fat, inflammation of the liver and muscle occurs. This inflammation drives an increase in the secretion of adipokines and cytokines by adipose tissue; thus; further exacerbating insulin resistance [35]. Therefore, despite not being a
direct outcome measure of the current investigation, body composition was analyzed prior to euthanasia for each animal. As can be seen in Table 7, fat mass of all intervention groups was significantly lower than that of the HF group (14.72±0.92g). However, fat mass of the CR group (10.56±0.62g) was significantly higher than all other intervention groups, while no changes in fat free mass were noted. Also, (though not reaching significance) fat masses of the TRF (5.91±0.51) and ADF (5.36±0.35) groups were greater than SWITCH and DF.

These data, in supplement with our biochemical data, indicate that the consumption of a Western diet, in any capacity, may contribute to an increase in fat mass relative to the consumption of purified vegan and standard chow diets in mice; thus, contributing to an increase in insulin resistance. However, in the case of ADF, the lowest levels of fasting glucose and smallest glucose AUC were observed, while simultaneously having increased levels of fasting insulin. These results indicate ADF produces results allowing the body to efficiently clear glucose loads, but that the insulin-signaling cascade may be altered. This could possibly be due to the effects of consuming the Western diet prior to intervention, or from the ADF regimen itself.

**CONCLUSION**

In conclusion, the results of the study indicate SWITCH, DF, CR, TRF, and ADF appear to significantly improve the following measures: fasting glucose and insulin, HOMA-IR, and glucose AUC, compared to a HF control. ADF showed to impact the measures of fasting blood glucose significantly more than DF and CR, but not TRF. Although not of statistical significance, DF was shown to best most impactful to fasting blood insulin and HOMA-IR, resulting in levels half that of the other intervention groups. Additionally, DF and ADF groups resulted in AUC statistically lower than TRF, while ADF glucose AUC was statistically lower than CR. These results indicate that multiple strategies involving dietary fasting to improve measures of insulin
sensitivity may exist, and one form may not be the solution for all individuals. To the researcher’s knowledge, this is the first and only study comparing these methods of dietary fasting. Therefore, more research is needed to assess the long-term effects of these fasting models and their impact on the human population.

**FUTURE DIRECTIONS**

Future studies involving both animal and human models are needed to fully elucidate the improvements in measures of insulin sensitivity reported in this study. Specifically, larger sample sizes should be used to interpret the clinical relevance observed in fasting blood insulin and HOMA-IR produced by the Daniel Fast, which trended towards statistical significance. Also, outcome measures of SWITCH were reversed to results similar to CHOW after only eight weeks of intervention; supporting future investigation of how quickly this dietary change impacts measures of insulin sensitivity. Additionally, the impact of these dietary fasting models on other biochemical measures of health should be investigated. While our study shows improvements to measures of insulin sensitivity, it is unknown the simultaneous effects on other health markers.

Additionally, future studies should investigate the addition of a re-feeding period and a cycling period to the dietary protocol, in which all intervention groups return to *ad libitum* intake. This addition would allow exploration of the question concerning the need to adopt these dietary fasting methods as lifestyle changes or simply temporary dietary changes in order to maintain improvements long-term. Also, through the incorporation of a cycling protocol, it would be interesting to learn how variation in diet type could impact both long-term success and metabolic outcomes—as we noted in the current study that the SWITCH group actually presented with slightly more favorable values than the CHOW group which was never exposed to the HF diet.
References


Appendix

A. TABLES AND FIGURES

Table 1. Composition of experimental diets.

<table>
<thead>
<tr>
<th>Macronutrient</th>
<th>DF kcal%</th>
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Table 2. Ingredient list of Daniel fast and high-fat diet

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<td>Mineral Mix S10001</td>
<td>35</td>
<td>0</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Calcium Carbonate</td>
<td>4</td>
<td>0</td>
<td>5.5</td>
<td>0</td>
</tr>
<tr>
<td>Mineral Mix S10026</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin Mix V10001</td>
<td>10</td>
<td>40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Choline Bitartrate</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Ascorbic Acid Phosphate, 33% active</td>
<td>0.41</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Potassium Citrate, 1 H2O</td>
<td>0</td>
<td>0</td>
<td>16.5</td>
<td>0</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FD&amp;C Red Dye #40</td>
<td>0.05</td>
<td>0</td>
<td>0.05</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3. Nutrient list of standard rodent chow

<table>
<thead>
<tr>
<th></th>
<th>Amino Acids</th>
<th></th>
<th>Amino Acids</th>
<th></th>
<th>Amino Acids</th>
<th></th>
<th>Amino Acids</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>% 1</td>
<td>Aspartic Acid</td>
<td>% 1.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorous</td>
<td>% 0.7</td>
<td>Glutamic Acid</td>
<td>% 3.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Phytate Phosphorous</td>
<td>% 0.4</td>
<td>Alanine</td>
<td>% 1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>% 0.2</td>
<td>Glycine</td>
<td>% 0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>% 0.6</td>
<td>Threonine</td>
<td>% 0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloride</td>
<td>% 0.4</td>
<td>Proline</td>
<td>% 1.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>% 0.3</td>
<td>Serine</td>
<td>% 1.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>mg/kg 70</td>
<td>Leucine</td>
<td>% 1.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mangenese</td>
<td>mg/kg 100</td>
<td>Isoleucine</td>
<td>% 0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>mg/kg 15</td>
<td>Valine</td>
<td>% 0.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodine</td>
<td>mg/kg 6</td>
<td>Phenylalanine</td>
<td>% 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>mg/kg 200</td>
<td>Tyrosine</td>
<td>% 0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenium</td>
<td>mg/kg 0.23</td>
<td>Methionine</td>
<td>% 0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td>IU/g 15</td>
<td>Lysine</td>
<td>% 0.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D$_3$</td>
<td>IU/g 1.5</td>
<td>Histidine</td>
<td>% 0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>IU/kg 110</td>
<td>Arginine</td>
<td>% 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin K$_3$ (menadione)</td>
<td>mg/kg 50</td>
<td>Tryptophan</td>
<td>% 0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B$_1$ (thiamin)</td>
<td>mg/kg 17</td>
<td>Fat Acid</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B$_2$ (riboflavin)</td>
<td>mg/kg 15</td>
<td>C16:0 Palmitic</td>
<td>% 0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Niacin (nicotinic acid)</td>
<td>mg/kg 70</td>
<td>C18:0 Stearic</td>
<td>% 0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B$_6$ (pyridoxine)</td>
<td>mg/kg 18</td>
<td>C18:1ω9 Oleic</td>
<td>% 1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pantothenic Acid</td>
<td>mg/kg 33</td>
<td>C18:2ω6 Linoleic</td>
<td>% 3.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B$_{12}$ (cyanocobalamin)</td>
<td>mg/kg 0.08</td>
<td>C18:3ω3 Linolenic</td>
<td>% 0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biotin</td>
<td>mg/kg 0.4</td>
<td>Total Saturated</td>
<td>% 0.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td>mg/kg 4</td>
<td>Total Monounsaturated</td>
<td>% 1.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline</td>
<td>mg/kg 1200</td>
<td>Total Polyunsaturated</td>
<td>% 3.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Pre-intervention fasting glucose (mg/dL) of male mice prior to being assigned to seven different dietary protocols for eight weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF pre-intervention</td>
<td>195.4 ± 4.75</td>
</tr>
<tr>
<td>CHOW pre-intervention</td>
<td>169.125 ± 6.78</td>
</tr>
</tbody>
</table>

Values are mean±SEM

Figure 1. Pre-intervention fasting glucose (mg/dL) of male mice prior to being assigned to seven different dietary protocols for eight weeks.

Values are mean±SEM

* denotes significant difference from HF (p <0.0054)
Table 5. Fasting glucose (mg/dL) collected during the 9th week of intervention of male mice assigned to seven different dietary protocols for eight weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF (n=8)</td>
<td>194.570 ± 7.97</td>
</tr>
<tr>
<td>CHOW* (n=7)</td>
<td>158.429 ± 8.52</td>
</tr>
<tr>
<td>SWITCH *† (n=8)</td>
<td>162.000 ± 7.97</td>
</tr>
<tr>
<td>DF*† (n=8)</td>
<td>164.375 ± 7.97</td>
</tr>
<tr>
<td>CR*† (n=8)</td>
<td>163.875 ± 7.97</td>
</tr>
<tr>
<td>TRF* (n=9)</td>
<td>157.444 ± 7.51</td>
</tr>
<tr>
<td>ADF* (n=9)</td>
<td>138.444 ± 7.51</td>
</tr>
</tbody>
</table>

Values are mean±SEM
*denotes significant difference from HF (p<0.05)
† denotes significant difference from ADF (p<0.05)

Figure 2. Fasting glucose (mg/dL) collected during the 9th week of intervention of male mice assigned to seven different dietary protocols for eight weeks.

Values are mean±SEM
*denotes significant difference from HF (p<0.05)
† denotes significant difference from ADF (p<0.05)
Table 6. Pre-intervention fasting insulin (ng/mL) of male mice prior to being assigned to seven different dietary protocols for eight weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Insulin (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>0.867 ± 0.253</td>
</tr>
<tr>
<td>CHOW</td>
<td>0.19 ± 0.023</td>
</tr>
</tbody>
</table>

Values are mean±SEM

Figure 3. Pre-intervention fasting insulin (ng/mL) of male mice prior to being assigned to seven different dietary protocols for eight weeks.
Values are mean±SEM
Table 7. Fasting insulin (ng/mL) collected immediately prior to euthanasia (via facial vein) of male mice assigned to seven different dietary protocols for eight weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Insulin (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF (n=8)</td>
<td>1.91 ± 0.24</td>
</tr>
<tr>
<td>CHOW* (n=7)</td>
<td>0.308±0.26</td>
</tr>
<tr>
<td>SWITCH *† (n=8)</td>
<td>0.255±0.24</td>
</tr>
<tr>
<td>DF*† (n=8)</td>
<td>0.281±0.24</td>
</tr>
<tr>
<td>CR*† (n=8)</td>
<td>0.568±0.24</td>
</tr>
<tr>
<td>TRF* (n=9)</td>
<td>0.561±0.23</td>
</tr>
<tr>
<td>ADF* (n=9)</td>
<td>0.430±0.23</td>
</tr>
</tbody>
</table>

Values are mean±SEM
*denotes significant difference from HF (p<0.05)

Figure 4. Fasting insulin (ng/mL) collected immediately prior to euthanasia (via facial vein) of male mice assigned to seven different dietary protocols for eight weeks.
Values are mean±SEM
*denotes significant difference from HF (p<0.05)
Table 8. Post-intervention HOMA-IR of male mice assigned to seven different dietary protocols for eight weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF (n=7)</td>
<td>4.315±1.42</td>
</tr>
<tr>
<td>CHOW* (n=6)</td>
<td>0.635±0.61</td>
</tr>
<tr>
<td>SWITCH *† (n=7)</td>
<td>0.485±0.07</td>
</tr>
<tr>
<td>DF*† (n=8)</td>
<td>0.458±0.09</td>
</tr>
<tr>
<td>CR*† (n=6)</td>
<td>1.084±0.24</td>
</tr>
<tr>
<td>TRF* (n=7)</td>
<td>1.048±0.15</td>
</tr>
<tr>
<td>ADF* (n=7)</td>
<td>0.659±0.34</td>
</tr>
</tbody>
</table>

Values are mean±SEM
*denotes significant difference from HF (p<0.05)

Figure 5. Post-intervention HOMA-IR of male mice assigned to seven different dietary protocols for eight weeks.
Table 9. Post-intervention area under the glucose curve (AUC) of male mice assigned to seven different dietary protocols for eight weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF (n=8)</td>
<td>1134±52.97</td>
</tr>
<tr>
<td>CHOW* (n=7)</td>
<td>735±56.63</td>
</tr>
<tr>
<td>SWITCH *† (n=8)</td>
<td>768.44±52.97</td>
</tr>
<tr>
<td>DF*† (n=8)</td>
<td>677.69±52.97</td>
</tr>
<tr>
<td>CR*† (n=8)</td>
<td>861.63±52.97</td>
</tr>
<tr>
<td>TRF* (n=9)</td>
<td>927.17±49.94</td>
</tr>
<tr>
<td>ADF* (n=9)</td>
<td>630±49.94</td>
</tr>
</tbody>
</table>

Values are mean±SEM
* denotes significant difference from HF (p<0.05)
† denotes significant difference from TRF (p<0.05)
‡ denotes significant differences from ADF (p<0.05)
Figure 7. Post-intervention area under the glucose curve (AUC) of male mice assigned to seven different dietary protocols for eight weeks.

Values are mean±SEM

Table 10. Post-intervention measures of insulin sensitivity of male mice assigned to seven different dietary protocols for eight weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Fasting Blood Glucose (mg/dL)</th>
<th>Fasting Blood Insulin (ng/dL)</th>
<th>HOMA-IR</th>
<th>Glucose AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>194.570 ± 7.97</td>
<td>1.91 ± 0.24</td>
<td>4.315±1.42</td>
<td>1134±52.97</td>
</tr>
<tr>
<td>CHOW</td>
<td>158.429 ± 8.52</td>
<td>0.308±0.26</td>
<td>0.635±0.61</td>
<td>735±56.63</td>
</tr>
<tr>
<td>SWITCH</td>
<td>162.000 ± 7.97</td>
<td>0.255±0.24</td>
<td>0.485±0.07</td>
<td>768.44±52.97</td>
</tr>
<tr>
<td>DF</td>
<td>164.375 ± 7.97</td>
<td>0.281±0.24</td>
<td>0.458±0.09</td>
<td>677.69±52.97</td>
</tr>
<tr>
<td>CR</td>
<td>163.875 ± 7.97</td>
<td>0.568±0.24</td>
<td>1.084±0.24</td>
<td>861.63±52.97</td>
</tr>
<tr>
<td>TRF</td>
<td>157.444 ± 7.51</td>
<td>0.561±0.23</td>
<td>1.048±0.15</td>
<td>927.17±49.94</td>
</tr>
<tr>
<td>ADF</td>
<td>138.444 ± 7.51</td>
<td>0.430±0.23</td>
<td>0.659±0.34</td>
<td>630±49.94</td>
</tr>
</tbody>
</table>

Values are mean±SEM
Table 11. Post-intervention body composition of male mice assigned to seven different dietary protocols for eight weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total BM (g)</th>
<th>Fat Mass (g)</th>
<th>Fat-Free Mass (g)</th>
<th>%Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>39.60±1.71</td>
<td>14.72±0.92</td>
<td>22.03±0.85</td>
<td>0.37±0.01</td>
</tr>
<tr>
<td>CHOW</td>
<td>27.79±1.03</td>
<td>4.21±0.71*†</td>
<td>21.30±0.39</td>
<td>0.15±0.02*†</td>
</tr>
<tr>
<td>SWITCH</td>
<td>28.65±0.83</td>
<td>4.43±0.89*†</td>
<td>22.02±0.34</td>
<td>0.15±0.03*†</td>
</tr>
<tr>
<td>DF</td>
<td>28.91±0.5</td>
<td>3.72±0.44*†</td>
<td>22.85±0.76</td>
<td>0.13±0.01*†‡</td>
</tr>
<tr>
<td>CR</td>
<td>33.69±1.01</td>
<td>10.56±0.62*</td>
<td>20.78±0.86</td>
<td>0.31±0.01</td>
</tr>
<tr>
<td>TRF</td>
<td>28.47±0.63</td>
<td>5.91±0.51*†</td>
<td>20.50±0.26</td>
<td>0.21±0.01*†</td>
</tr>
<tr>
<td>ADF</td>
<td>28.64±0.40</td>
<td>5.36±0.35*†</td>
<td>20.91±0.38</td>
<td>0.19±0.01*†</td>
</tr>
</tbody>
</table>

Key: BM (body mass)  
Values are mean±SEM  
No main or group effects were observed for fat-free mass (p<0.05)  
* denotes significant difference from HF  
† denotes significant difference from CR  
‡ denotes significant difference from TRF
B. EXTENDED LITERATURE REVIEW

Introduction

The rate of overweight individuals and those diagnosed with obesity has become an epidemic in the United States[1]. With trends of a gradual increase over the past four decades in all populations, the need for intervention has never been more important. The 2016 National Health and Nutritional Examination Survey (NHANES) results show the number of those classified as overweight or obese is 68.8%. Therefore, more than two out of three adults in the nation are classified as obese or at risk of becoming obese[2]. These numbers are disturbing and strike concern for the future population of the United States. 33% of the nation’s youth, ages 6-19, meet the qualifications to be classified as obese (18.2%) or overweight (14.8%)[2]. With the prevalence of obesity more than doubling since the 1960’s (13.4% to 35.7%)[2], it has been predicted that over the next two decades, the obesity prevalence rate will increase by 33%[3]. This prediction estimates the prevalence of obesity to be 51% by 2030.

Obesity is directly related to what is referred to as “energy imbalance.” Calories are the measurement for energy in food. A person begins to gain weight when their caloric (energy) intake is greater than their output/expenditure. The accumulation of adipose tissue leading to obesity can be linked to an individual’s poor lifestyle choices, such as physical inactivity[4,5] and consumption of a high-fat diet[6-11]. However, there are many contributors to the escalating rates of overweight status and obesity. Genes, family history, coexisting health conditions, medication use, emotional factors, smoking, age, and lack of sleep have all been shown to be causative factors in gaining weight[12].

The increase in adipose tissue that is centrally located within the abdominal region and associated with obesity[13] elevates plasma free fatty acids. This is then associated with the onset
of hyperglycemia, hyperinsulinemia, impaired glucose tolerance, and commonly the development of type 2 diabetes\textsuperscript{[14]}. Moreover, hyperglycemia and hyperinsulinemia are widely believed to contribute to an increase in blood pressure in obese individuals\textsuperscript{[14]}. Also, obese individuals typically have an abnormal lipid panel including elevated total and low-density lipoprotein cholesterol and triglycerides, along with decreased high-density lipoprotein levels\textsuperscript{[14]}. Therefore, it should not be surprising that obese individuals also have a higher risk for the development of cardiovascular diseases. Additionally, obesity has been shown to be a major risk factor for the development of nonalcoholic fatty liver disease\textsuperscript{[15]} and certain forms of cancer\textsuperscript{[16]}. Indeed, obesity is a major health problem that needs to be addressed aggressively.

**Treatment Options for Obesity**

Obesity is a lifestyle disease and therefore, is almost always a preventable disease. Multiple treatment options exist, including the performance of physical activity and structured exercise, the use of pharmaceuticals and dietary supplements targeting weight loss, bariatric surgery, and dietary change. An increase in physical activity may be the easiest and most cost effective way to address the obesity problem. “Physical activity” for the inactive persons is simply adding 150 minutes per week of activity, such as brisk walking, which an individual can incorporate into their daily routine\textsuperscript{[17]}. In combination with physical activity, several medications have been shown to help treat obesity. Those approved by the FDA and currently being prescribed include: orlistat, lorcaserin, phentermine, topiramate, buproprion, naltrexone, and liraglutide\textsuperscript{[18]}.

Bariatric surgery is typically considered the last option for treatment of obesity. Those who qualify must have been unsuccessful through other means of weight loss, with a BMI that remains above 40 (kg·m\textsuperscript{-2}), or greater than 35 (kg·m\textsuperscript{-2}) when combined with a high-risk medical condition\textsuperscript{[19]}. The four most commonly used bariatric surgeries are the adjustable gastric banding,
commonly know as LAP band, a vertical sleeve gastrectomy, Rou-en-Y gastric bypass, and the biliopancreatic diversion with duodenal switch. The Roux-en-Y bypass has resulted in more long-term weight loss and is most well-accepted\[20\].

Dietary manipulation in the treatment of obesity

Like physical activity, dietary change is typically viewed as a first line approach to target weight loss and arguably yields the most benefit with regards to non-medically-induced weight loss. Moreover, incorporating appropriate dietary strategies prior to the development of an obese state is the ideal approach, in an attempt to prevent obesity. A very common type of dietary change is caloric restriction (CR), which is the practice of reducing one’s caloric intake, without inducing malnutrition, for the purpose of weight loss\[21\]. Countless studies have reported the use of CR as a lifestyle change or habit, which improves overall health in both humans\[22\] and animals\[22\]. However, many individuals find the processes of CR (counting calories, measuring food, etc.) to be difficult to adhere to for extended periods, making the adoption of this dietary change problematic.

Most dietary programs involve some form of restriction, either in calories, types of food, or time in which food is consumed. Fasting has been described as “abstinence from some or all food, drink, or both usually for a pre-determined period of time”\[23\]. Related to caloric intake, restricting calories, by definition, is a type of fasting. Fasting is often motivated by religious beliefs (e.g., Christian, Muslim) and followed for spiritual purposes. However, after observing populations following religious fasts, in addition to the desired spiritual and mental benefits, numerous studies noted multiple associated physical health benefits\[24,25\]. Fasting is not always religiously motivated. Commonly studied non-religious fasting plans include dietary restriction
DR, intermittent fasting (IF), and alternate day fasting (ADF)—all noting associated health benefits.

DR models are partial fasts and are not designed specifically to reduce caloric intake. However, it is common for caloric intake to be lower when following these plans, due to the increased degree of satiety associated with the consumption of a more nutrient dense diet. DR involves the limitation or elimination of one or more food items, such as dairy, sugar-sweetened beverages, or meat[26]. The calories and nutrients not consumed from the specifically restricted food are then typically consumed through other food sources. People adhere to DR for many different reasons, such as to avoid food allergies, achieve presumed health benefits, and/or for religious beliefs. The Daniel Fast (DF) is one such popular DR model, commonly referred to as a purified vegan fast[25], which eliminates all animal products, processed foods, caffeine, alcohol, additives and preservatives[25]. It is a religious fast developed from the writings of the Christian prophet Daniel (Daniel 1:8-14).

IF is used to describe several forms of periodic breaks from eating[28]. The most common patterns practiced are time-restricted feeding (TRF), total fasting 1-2 days of the week, and ADF. TRF involves a period, typically ranging from four to eight hours, in which daily caloric needs are consumed[29]. All food and drink, other than water, are completely restricted during the remaining time of the day. ADF is repeated over the course of two days, alternating from a so-called “feast day” in which food is consumed ad libitum and a “fast” day in which no food or only a very small amount of food is consumed (e.g., 500 calories)[29].

Overview of Research Designs Involving DF, TRF, and ADF

With diet plans, many variables are altered in hopes of yielding desired results, producing the numerous diet plans people follow today. Even within each category of dietary change model,
study designs differ from one to the next, including models of DF, TRF, and ADF. In comparison to ADF, research studies involving DF and TRF are minimal. However, the few studies examining the effects of DF on health related outcomes have produced significant findings for several variables\cite{23-25,30-32}. To date, studies have implemented both traditional Daniel Fast interventions (TDF) and modified Daniel Fast interventions (MDF)\cite{23}. Other studies have added krill oil\cite{31} and exercise (unpublished findings) to DF. Most DF studies involve a 21-day intervention, but long-term compliance\cite{32} to the dietary model has been examined. While research involving DF may be somewhat limited, in comparison to the number of studies involving other dietary change models, the dietary plan has shown promise to help combat obesity in a similar way as CR\cite{23-25,30-32}. Comparable to DF, research involving TRF as an intervention is fairly limited. In addition, variability exists within the research designs. Windows of feeding times have been researched ranging from 3 to 12 hours. In animals, diet composition has been manipulated and compared, typically between a normal chow diet\cite{33-35}, high fat diet\cite{35,36}, and low fat diet\cite{37}. Different populations including overweight, obese, healthy, male and female subjects have been observed, and exercise regimens have been included in some studies.

In alternate day fasting (ADF), published research is abundant in both humans and animals. Many different research designs, variables, and outcomes have been measured. Populations, percentages of caloric restriction on fasting days, intervention duration, and activity levels have varied. Most studies have indicated benefits with ADF protocols. Of note, as is also the case for DR and TRF, measures of insulin\cite{38-42} and insulin sensitivity improve\cite{38-42}. This is important, as obesity is strongly linked to insulin resistance and this topic has received a great deal of attention in recent years.
Insulin Sensitivity

The insulin-signaling cascade is very complex and not completely understood\[43\]. Many of the biochemical reactions have, however, been explored and explained in depth. Insulin is a polypeptide hormone\[44\]. The hormone is initially synthesized as preproinsulin\[45\], an inactive precursor, which is cleaved and turned into proinsulin\[44\], which is later turned into insulin\[44\]. Proinsulin is released specifically from the Beta cells of the islets of Langerhans\[46\] of the pancreas before being cleaved\[44\] to create insulin. Insulin acts on liver, muscle, and adipose cells to decrease blood glucose by increasing glycogen synthesis\[44\], fatty acid synthesis\[44\], and the translocation of glucose transport protein\[44\] (GLUT4 – receptor that allows glucose to enter into the cell) to the plasma membrane.

These actions happen via the insulin receptor tyrosine kinase\[44\]. This receptor is a heterotetrameric structure\[47\], composed of four subunits: two extracellular alpha subunits\[47\] and two transmembrane beta subunits\[47\]. Once the insulin binds to the extracellular alpha subunits, the beta subunits become activated and auto phosphorylate on tyrosine residues\[44\]. Once the beta subunits are activated they lead to the phosphorylation and activation of insulin receptor substrate 1 (IRS-1) and 2 (IRS-2)\[48\]. The phosphorylation and activation of IRS-1/2 is regulated by the protein phosphatase and tensin homolog (PTEN) by dephosphorylating IRS\[48\]. Once PTEN regulates and allows the activation of IRS-1/2\[49\], PI3K binds to IRS1/2 and then phosphorylates phosphatidylinositol (4,5)-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3)\[49\]. When PIP3 concentrations increase enough, phosphoinositide-dependent kinase 1 (PDK1)\[50\] and AKT (also know as protein kinase B)\[50\] are recruited toward the plasma membrane. This activates PDK1, which phosphorylates AKT\[50\].
Insulin sensitive cells normally possess vesicles containing GLUT4\textsuperscript{[51]}, which is the glucose transporter that is primarily found in adipose tissue and striated muscle. The protein AS160 inhibits the translocation of GLUT4 to the plasma membrane of the cell\textsuperscript{[52]}. However, due to the nature of the insulin cascade, PDK1 phosphorylates AKT, which in turn, inactivates AS160 and allows the translocation of GLUT4\textsuperscript{[52]}. This allows glucose to enter the cell. This is the normal cascade of how insulin stimulates the uptake of glucose into the cell.

Unfortunately, the process of insulin facilitating glucose uptake is faulty in some individuals. Insulin sensitivity is the measure of how effectively the hormone insulin binds to IRS1/2\textsuperscript{[49]}, signaling its downstream of effects to allow for glucose to be taken from the blood stream into tissues. Insulin sensitivity is calculated using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)\textsuperscript{[53]}, a mathematical model calculated using fasting blood glucose and fasting insulin. When the process of glucose uptake into the tissue is compromised, an individual is said to have insulin resistance. The term insulin resistance is used to describe the condition whereby insulin-induced glucose uptake is impaired\textsuperscript{[54]}. Although the exact reasons why the biological process of insulin signaling stops working properly remains to be fully elucidated, the potential mechanisms and associated conditions related to insulin resistance are many: obesity\textsuperscript{[54]}, inflammation\textsuperscript{[54]}, mitochondrial dysfunction\textsuperscript{[54]}, hyperinsulinemia\textsuperscript{[54]}, hyperlipidemia\textsuperscript{[54]}, genetics\textsuperscript{[54]}, endoplasmic reticulum\textsuperscript{[54]} and oxidative stress\textsuperscript{[54]}, aging\textsuperscript{[54]}, fatty liver disease\textsuperscript{[54]}, hypoxia\textsuperscript{[54]}, lipodystrophy\textsuperscript{[54]}, and pregnancy\textsuperscript{[54]}. Many of these suggested contributing factors are related to obesity and the accumulation of adipose tissue.

Obesity is prevalent in ninety percent of individuals who are insulin resistant\textsuperscript{[55]}. It has been proposed that with each standard deviation increase in visceral adipose tissue an 80% increase in risk of developing insulin resistance occurs\textsuperscript{[56]}. Several possibilities of this correlation have been
explored. One possibility of this correlation is visceral fat itself being diabetogenic\textsuperscript{[57]}. Visceral fat secretes adipokines which impair insulin sensitivity in tissues such as the liver and muscles\textsuperscript{[57]}. Secondly, the accumulation of visceral fat causes the development of ectopic lipid accumulation and lipotoxicity in the liver and muscles\textsuperscript{[57]}, leading to further insulin resistance. Additionally, the production of cytokines by visceral fat due to inflammation has been shown to contribute to insulin resistance\textsuperscript{[57]}. The precise physiological events initiating the inflammatory response in obesity remains somewhat of a mystery, but theories have been proposed. The theory of accumulation of adipose tissue leading to hypertrophy of adipocytes, activating cellular stress pathways and thereby causing the release of cytokines is widely accepted\textsuperscript{[58]}.

\textit{Common Measures of Insulin Sensitivity}

There are several measurement tools to assess insulin sensitivity. The most reliable measurement tool is the hyperinsulinemic euglycemic clamp (HEC). It is a direct measure of insulin sensitivity and is considered the “gold standard”\textsuperscript{[59]}. However, HEC must be performed under clinical guidance and is expensive and invasive\textsuperscript{[59]}. Common indirect measures of insulin sensitivity include: the minimal model analysis of frequently sampled intravenous glucose tolerance test (IVGTT) and the oral glucose tolerance test (OGTT)\textsuperscript{[59]}.

IVGTT in humans requires an individual’s body to properly produce insulin in response to high levels of glucose through intravenous administration\textsuperscript{[60]}. Considering that the individuals receiving IVGTT characteristically lack the ability to produce adequate amounts of insulin, the technique has recently been modified to include the use of bolus administered insulin\textsuperscript{[60]}. IVGTT is commonly used in animal research. In rodents, the test involves an injection of glucose and sampling blood from the tip of the tail\textsuperscript{[61]}. The tail is snipped 1-2 mm and baseline glucose and insulin levels are measured\textsuperscript{[61]}. The tail is then massaged gently to continue blood flow at different
times over the course of two hours\cite{61}. Additional blood collections typically occur after 15, 30, 60, 90, and 120 minutes\cite{61}. The OGTT is similar in design to IVGTT, only instead of administering an injection of glucose, a designated amount of glucose (typically 75g) is orally ingested\cite{62}. OGTT is commonly used in humans, both clinically and in research, due to being less invasive in nature. Like IVGTT, blood samples are typically retrieved after 15, 30, 60, 90, and 120 minutes to measure plasma glucose and insulin concentrations\cite{62}. The glucose/insulin ratio (G:I) is calculated, with lower levels indicating higher degrees of insulin resistance.

Fasting methods for assessing insulin sensitivity are commonly used because they are inexpensive and uncomplicated to perform. The fasting blood insulin test is a test performed by drawing a blood sample after the individual has fasted for at least 8 hours\cite{59}. In the healthy individual, a fasting insulin level of 18-90 pmol/L\cite{63} is expected. Levels exceeding this may indicate insulin resistance. Fasting glucose levels are measured in the same way. Clinically, if one’s fasting blood glucose level exceeds 100mg/dL, impaired insulin sensitivity is a concern\cite{64}. Fasting glucose and insulin levels are used to calculate insulin resistance using the HOMA-IR. HOMA-IR is calculated by multiplying the fasting plasma insulin (FPI) by the fasting plasma glucose (FPG), then this calculation is divided by the constant 22.5 (HOMA-IR=(FPIxFPG)/22.5)\cite{65,66}. HOMA-IR has been shown to correlate strongly with HEC\cite{67}, and is widely used in research to estimate insulin resistance. The quantitative insulin sensitivity check index (QUICKI) also uses fasting plasma glucose and insulin levels to calculate insulin resistance but has been reported to have a higher linear correlation to HEC\cite{66,68}. QUICKI is nearly a duplication of HOMA-IR, except that a log transform of the fasting plasma and glucose measurements are used in the calculation (QUICKI = 1/(logI0 + logG0))\cite{59}. 

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Dietary Impact on Insulin Sensitivity

Maintaining proper glycemic control is best done through proper dietary intake\[^{69}\]. Dietary treatment of insulin resistance has traditionally involved regulating the amount and type of carbohydrates consumed. This treatment process developed based on the correlation between carbohydrate intake and blood glucose levels\[^{70}\]. The common theory suggests if an individual consistently consumes small amounts of carbohydrates throughout the day (as opposed to large bolus dosages), the pancreas by default will not be repeatedly over-stimulated to produce insulin. Instead, the pancreas would produce insulin at a steadier rate, reducing stress to the organ. Therefore, it has been generally accepted that a steady release of insulin is ideal and may help to possibly slow down or prevent loss in the ability of the pancreas to produce insulin\[^{71}\]. Recent research involving different DR and fasting methods has shown a beneficial impact on plasma glucose and insulin levels, as well as decreased HOMA-IR.

While the volume of carbohydrate consumed has traditionally been the focus\[^{72}\], insulin sensitivity is being shown to change not only due to alterations in energy content, but by dietary composition modification as well. For example, a fiber rich diet has been shown to improve measures of insulin sensitivity\[^{73,74}\]. In addition, a diet consisting of low carbohydrate intake but high fat intake has also been reported to improve insulin sensitivity\[^{75}\]. This is likely due to the fact that the carbohydrate load is reduced significantly. Typically individuals consuming a high-fat diet also consume high amounts of carbohydrates and calories. High fat diets are known to be linked to obesity and associated health problems, including insulin resistance\[^{76,77}\]—in particular when combined with excess carbohydrate and calorie intake. This information suggests that the macronutrient composition of the diet affects insulin sensitivity, as well as the macronutrient source and type.
Impact of Dietary Change Models on Insulin Sensitivity

DF, ADF, and TRF protocols have all resulted in multiple health associated outcomes, including measurements of insulin sensitivity. Each dietary change model has shown significant decreases in fasting blood glucose and insulin levels, as well as HOMA-IR, all of which are associated with improved insulin sensitivity. Relating to DF, even though results did not reach statistical significance, clinically significant decreases in plasma insulin levels and HOMA-IR have been observed in three studies involving humans following TDF\cite{23,31,78}, as well as in one study involving MDF\cite{23}. Favorable outcomes have also been noted in one animal study involving the DF (unpublished results). In human TRF studies, results have shown significant decreases in fasting glucose\cite{79} and insulin levels\cite{79}, along with significant improvements in HOMA-IR\cite{79}. Unfortunately little has been reported from animal studies regarding measures of insulin sensitivity and TRF. Either changes in glucose, insulin, and HOMA-IR were not measured or no significant changes have been seen in most studies. However, one study\cite{80} involving diabetic mice did compare fasting glucose and insulin levels of TRF and CR mice. The results showed decreases in both fasting insulin and glucose but not as great as CR mice.

Fasting glucose levels have been shown to decrease in humans after an intervention of ADF\cite{81} and in combination with exercise\cite{82}. Additionally, studies have produced clinically significant decreases in fasting insulin\cite{83} and HOMA-IR\cite{83} in those with severe insulin resistance. Related to animal research, ADF has resulted in much evidence as a way to improve insulin sensitivity. Fasting blood glucose levels have been shown to be significantly lower than *ad libitum* fed rodents\cite{84,85}, and levels have decreased similarly to those of CR rodents\cite{86,87}. Additionally, after the administration of an oral glucose tolerance test, ADF rodents presented the smallest area under the glucose curve\cite{88}.
**Dietary Restriction**

Dietary restriction is the limitation or elimination of one or more food items or food groups from the diet for either a short period of time or as a lifestyle change. Examples include a vegetarian diet, the purified DF, and a ketogenic diet. While most of these plans are not purposely designed to restrict calories, only manipulate nutrient intake, the elimination of certain foods and the incorporation of nutrient dense foods (as is the case for the DF), will often lead to a lower caloric intake.

**Daniel Fast Overview**

The DF is a traditional fast followed by many Christians each year for spiritual purposes, although has recently been adopted as a dietary approach by many individuals. It is based on the Biblical story of the prophet Daniel, noted in the book of Daniel, chapter 1 verses 8-14. For ten days Daniel asked the chief official who was appointed over himself and his three friends, Hananaiah, Mishael, and Azariah, to test them with presentation of only vegetables (pulse: food grown from seed) to eat and water to drink. At the end of the ten days it was said the men looked healthier and better nourished than any of the other young men who had partaken in the consumption of the royal food of the king, Nebuchadnezzar. Later in chapter 10 verse 2, Daniel writes “at that time I, Daniel, mourned for three weeks. I ate no choice food; no meat or wine touched my lips; and I used no lotions at all until the three weeks were over.” Based on these passages, a modern day Daniel Fast resembles a vegan diet of no animal products, but is much more stringent, in that no animal products, processed foods, preservatives, additives, sweeteners, flavorings, caffeine, or alcohol\(^{[31]}\) are included.
Published research on the DF and its potential health benefits is limited to a series of studies performed by investigators at The University of Memphis, although other studies are ongoing and being performed elsewhere. To our knowledge, the first study involving the DF was published in 2010\cite{24}. This study consisted of forty-three subjects, thirteen men and thirty women, following a twenty-one day DF, with ages and BMI ranging from 20-62 (kg·m\(^{-2}\)) and 18-40.6 (kg·m\(^{-2}\)) respectively. At the end of the three-week intervention no statistically significant changes in weight, BMI, waist circumference, percent total body fat, fat free mass, heart rate, total HDL, or triglycerides were observed. However, many changes were of clinical relevance. Moreover, systolic and diastolic blood pressure, total cholesterol, and LDL decreased significantly, while C-reactive protein, insulin and HOMA-IR also followed this trend, but without reaching statistical significance.

In a second study involving humans, Trepanowski and colleagues\cite{31}, the effect of krill oil supplementation on the maintenance of HDL in individuals adhering to the DF was investigated\cite{31}. Their findings show that HDL decreased similarly in the supplementation and placebo groups. These results, along with several other krill oil investigations\cite{89,90} contradict the work of Bunea and colleagues\cite{91} who reported 43-60% increases in HDL. However, the collective data of all participants shows promise for the DF producing multiple positive health outcomes\cite{31}. In thirty-nine subject, male and female, the DF showed to improve cardiovascular health as evidence by significant decreases in systolic blood pressure, body weight, fat mass, LDL, blood glucose levels, plasma insulin levels, and HOMA-IR. No significant differences were observed in diastolic blood pressure, C-reactive protein, or triglycerides, and unwanted decreases in HDL and fat free mass occurred\cite{31}. 

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Two studies have been conducted by Alleman and Bloomer with other colleagues\cite{23,32} comparing a traditional Daniel Fast (TDF) to a modified Daniel Fast (MDF). In the modified version of the Daniel Fast, participants were instructed to consume three ounces of lean meat and eight ounces of skimmed milk per day. The first study\cite{23} involved a 21-day intervention and no significant decreases in body weight, fat mass, total body fat, heart rate, or blood pressure from pre to post intervention occurred in either group. Total cholesterol decreased significantly in both groups similarly\cite{23}, but plasma glucose levels were significantly higher in the TDF individuals\cite{23}. Although not of statistical significance, insulin, HOMA-IR, LDL, and triglycerides decreased on a clinically significant level in both groups\cite{23}. Seeing the possible trends of beneficial health outcomes from both a TDF and MDF, Bloomer and colleagues\cite{32} chose to extend the intervention length to six months to explore compliance with the strict dietary restriction. After six months, both the TDF and MDF groups saw a clinically significant decrease in body weight (4-6 kilograms), approximately five percent and eight percent, respectively\cite{32}. No other outcome measures were observed other than compliance. In both groups, compliance after 6 months (80.0% ± 1.1 in TDF and 82.6% ± 3.2 in MFD) was very good and no significant differences between the groups occurred\cite{32}.

**Daniel Fast in Animals**

One study involving the DF has been conducted in animals, with data currently under review. In this study, Bloomer and colleagues explore the effect of dietary composition and physical activity on different health related outcomes. Fifty-six rats were divided into four groups: western diet (WD), western diet plus exercise (WD+E), Daniel Fast (DF), and Daniel Fast plus exercise (DF+E). All animals had access to food and water at all times throughout the three-month study. After three months, the DF+E rats had the greatest improvement of treadmill run time to
exhaustion, lowest percentage of weight gain, and lowest body fat percentage. It should be noted, that the average body mass of DF rats (496.8 ± 13.5) was lower than that of the WD+E rats (516.8 ± 10.7 g) post intervention. Also, triglycerides and cholesterol were 6-fold and 2-3-fold higher in the WD groups than DF groups. Other parameters were positively impacted, including those related to oxidative stress and insulin sensitivity. These results indicate that the DF produces positive changes in multiple health related parameters.

**Intermittent Fasting**

Intermittent fasting is an umbrella term used to describe multiple patterns of restraining from food and calorie containing beverages. The most common of these patterns are TRF and ADF. These practices have become popular in recent years as a form of weight loss and as a method to improve health. A key point to these methods is that total calorie intake is not necessarily meant to be lowered but rather, the time in which an individual is allowed to eat the food is restricted. These types of intermittent fasting plans have been researched in humans and different animal species using various patterns and over different periods of time, resulting in many positive health outcomes.

*Time Restricted Feeding in Humans*

Time restricted feeding (TRF) has a period, usually ranging from four to eight hours, in which the individual consumes their total daily energy needs and then withholds from consuming any energy-containing food and beverage the remaining hours of the day. Research regarding TRF is limited and results are inconsistent. Studies on time restricted feeding involving humans have shown different changes, both positive and negative, in weight, fasting glucose, plasma insulin levels, insulin response, but similar decreases in fat mass. This is probably due to the difference of the feeding time frames, populations observed, and the study lengths.
In a study performed by Tinsley and colleagues\textsuperscript{[79]}, healthy men participated in a TRF regimen combined with a resistance-training program. Four days out of the week, the participants consumed their daily caloric needs in a four-hour window of their choice between the hours of 4:00 p.m. and 12:00 a.m. on the other three days of the week they took part in resistance training of either the upper or lower extremities and consumed food and beverages \textit{ad libitum}. This study design is novel in the fact that instead of restricting the time window of eating every day of the week reflective of other studies involving TRF, the time window is restricted on alternate days only. Despite an eight-week intervention and an average daily decrease of 667 calories on the fasted days in comparison to the non-fasted days, the authors reported no significant changes in body weight\textsuperscript{[79]}. These results are conflicting with other investigators reporting significant weight loss following TRF regimens\textsuperscript{[79,93,94]}. This could be due to the addition of resistance training to the intervention, the participants eating \textit{ad libitum} three days per week, or both.

Two studies reported changes in plasma glucose levels\textsuperscript{[79,93]}, plasma insulin levels, and insulin response following a TRF regimen. In one study, Stote and colleagues\textsuperscript{[93]} observed poorer glucose tolerance as indicated by significantly higher levels of morning fasting plasma glucose concentrations in individuals consuming one meal per day (95.9 \( \pm \) 1.7 mg/dl) than those consuming three meals (85.4 \( \pm \) 1.7 m/dl). Also, more prolonged levels of morning fasting plasma glucose was observed in the one meal per day group. Interestingly, no significant differences in plasma insulin levels or measures of insulin sensitivity between TRF (5.0 \( \pm \) 0.7 ng/ml) and control groups (5.8 \( \pm \) 0.7 ng/ml) in response to the OGTT were observed. Moro et al\textsuperscript{[79]}. reported a decrease in fasting plasma glucose (96.64 \( \pm \) 5.1 mg/dl at baseline to 85.92 \( \pm \) 7.13 mg/dl post-intervention) and insulin (2.78 \( \pm \) 0.6 ng/ml at baseline to 1.77 \( \pm \) 0.9 ng/ml post-intervention) inherently resulting in a significant improvement in HOMA-IR.
The contradictory results from these two studies are likely due to the differences in research design. Stote et al.\textsuperscript{[93]} observed fifteen healthy males and females for eight weeks. The experimental group ate one meal during a four-hour feeding window (between the hours of 4:00 pm and 8:00 pm) while the control group ate three meals per day. Meals were provided to ensure equal consumption of calories across groups. In the study performed by Moro and colleagues\textsuperscript{[79]}, thirty-four resistance-trained males were observed for eight weeks. Both the control and TRF groups consumed three meals (not provided by the researchers). Meal times for the control group were 8:00 am, 1:00 pm, and 8:00 pm. Meal times for the TRF group were 1:00 pm, 4:00 pm, and 8:00 pm thus providing an eight-hour feeding window. The participants in this study also completed resistance training three days per week during the course of the study. This addition of the resistance training could have been a contributing factor to the decrease in fasting plasma glucose levels (96.64 ± 5.1 mg/dl at baseline to 85.92 ± 7.13 mg/dl post-intervention) reported by Moro et al.\textsuperscript{[79]}, considering that much research has supported a link between physical activity and improvement in fasting glucose\textsuperscript{[96,97]}.

\textit{Time Restricted Feeding in Animals}

Many of the studies on time restricted feeding (TRF) using animals are designed similar to the traditions followed during Ramadan, allowing the animals to either eat for twelve hours during their normal active (dark phase) or sleeping times (light phase)\textsuperscript{[98-102]}. These studies mimic humans eating between the hours of 9:00 am to 9:00 pm, a typical time frame of caloric consumption in the human population. The results from these studies are interesting. Despite consuming equivalent calories, animals fed during the dark phase weighed considerably less when compared to the light phase fed animals\textsuperscript{[98,99,101]}. Also, it has been shown that rats working while food was available \textit{ad libitum} and rats working while feeding was restricted to the light phase weighed significantly more
than both non-working *ad libitum* fed rats and all rats restricted to dark phase feeding (working or not)[101].

Other studies investigated the influence of diet composition and/or the differences in feeding times[99,102,103]. Mostly comparisons involve a normal rodent chow diet (NC), a high fat diet (HF), and a low fat diet (LF). Bray et al.[99] in two separate study designs noted no significant differences in body weight between mice fed a HF diet or a NC diet in either the light or dark phases. However, in a third study design by Bray[99] and colleagues, when the mice were fed a HF “meal” during the first four hours of the dark phase followed by eight hours of *ad libitum* NC, they weighed significantly less than mice fed a HF “meal” during the last four hours of the dark phase. The same results were observed in a fourth study design[99] in which the mice were fed one HF “meal” during the first four hours of the dark phase, followed by a four hour fasting period, then fed one NC “meal” during the last four hours, compared to the NC “meal” and HF “meal” being provided during the first and last four hours of the dark phase, respectively. These mice also exhibited lower body fat composition and increased glucose tolerance. The overall results by Bray and colleagues[99] suggests that eating a high fat meal upon awakening increases the body’s ability to appropriately respond to carbohydrates throughout the day, while eating a high fat meal towards the end of the day could promote the development of cardio-metabolic syndrome.

Other studies using the time windows of nine[80], three[104], and four hours[103] observed decreases of 11.6-15%, 9%, and 17.5% respectively. Belkacemi et al.[80] reported that fasting diabetic rats lost 15% of their body weight while non-fasting diabetic rats gained 21.7%. The same trend was observed in non-diabetic rats in the same study. Also, it is interesting that mice fed a high fat diet for four hours per day weighed less than mice fed an *ad libitum* low fat diet, despite equivalent calories consumed[80]. The trend of weight loss is reflective in body fat composition in
many studies\(^{99,101,103}\). With research supporting a link between high amounts of adipose tissue to an increase in insulin resistance\(^{56,58}\), it is no surprise that studies showed decreases in fasting plasma glucose\(^{99,80}\), fasting plasma insulin levels\(^{80,103}\), and increased insulin sensitivity\(^{80,103}\) in animals that were exposed to time restricted feeding.

Alternate Day Fasting

Alternate day fasting (ADF), a subcategory of intermittent fasting, is commonly defined as a type of dietary change consisting of alternating twenty-four hour periods of fasting and *ad libitum* eating\(^{105}\). ADF is considered a “true” alternate-day fast when the individual restrains from consuming any energy containing foods and beverages during the fasting days\(^{106}\). A “modified” alternate day fast allows the person to eat anywhere from 20-60% of their baseline caloric needs during the fasting days\(^{28}\). An important point about this specific diet approach is that frequency of food consumption is the only alteration. Total caloric intake or type of foods consumed within the forty-eight hour cycle is not limited.

Alternate day fasting has been proposed as an alternative to calorie restriction (CR), based on the thought that adhering to a lifestyle in which one has to limit their caloric intake *everyday* is too difficult for most individuals to follow long-term. Research is exploring the possibilities of ADF having the same positive effects on longevity\(^{107}\), blood pressure\(^{108}\), heart rate\(^{108}\), glucose\(^{109}\), and insulin levels\(^{110}\) as seen in CR protocols. If so, ADF may be a viable alternative to CR with regards to improving health outcomes. To date, more studies have been conducted in animals, but more human studies are becoming available each year.

Alternate Day Fasting in Humans

To date, relatively few publications have examined ADF’s effect on chronic diseases using human subjects. Variables related to diabetes, cardiovascular health, and obesity, such as changes
in weight, fat mass, plasma levels of glucose and insulin, plasma lipids, fat oxidation, BMI, oxidative stress, inflammation, blood pressure, heart rate, resting metabolic rate (RMR), and insulin sensitivity, have been assessed in these studies.

In the majority of the studies, significant decreases in weight\cite{29,40,41,82,111-114} and fat mass\cite{29,41,82,112-114} were seen. Only two reported otherwise, and these observed no change\cite{42,105}. This is most likely due to the duration of those studies only being two weeks, possibly not allowing significant differences to develop.

The changes in fasting glucose levels reported have been inconsistent. Several publications have observed ADF subjects having no significant differences in fasting plasma glucose levels than baseline levels or control groups post intervention\cite{41,42,83,113,115}; while in a true ADF, obese individuals showed significant decreases in fasting glucose\cite{39}. This average decrease was greater than the other experimental group following a calorie restriction (CR) of 400 calories\cite{39}. Heilbronn and colleagues\cite{40} observed healthy, non-obese individuals after sixteen weeks of a true ADF fast. Interestingly, men showed no significant difference in glucose tolerance, but the area under the glucose curve increased in women\cite{40}. In another study\cite{82}, ADF alone and in combination with exercise was explored and measures of insulin sensitivity were reported. In this study\cite{82} (n = 64) 25% of the ad libitum caloric intake was consumed during fasting days. Fasting plasma glucose levels were significantly lower that the control group (111 ± 6 mg/dl) in the ADF group (95 ± 5) as well individuals who performed moderately intense exercise three times weekly while adhering to a modified ADF (92 ± 3 mg/dl). This further supports the evidence exercise use as an intervention for hyperglycemia and insulin resistance\cite{96,97}.

All of the studies measuring a change in fasting plasma insulin\cite{42,82,115} reported no significant differences after the intervention of ADF except for one\cite{83}. Hoddy and colleagues\cite{83}
conducted a study on individuals with different pre-existing degrees of insulin resistance. The participants were divided into three tertiles based on their level of insulin resistance at baseline determined by HOMA-IR; tertile 1 had the lowest degree of fasting glucose and insulin resistance, while tertile 3 had the highest. After eight weeks of an ADF allowing consumption of 25% baseline caloric needs on fasting days, tertile 3 had as significantly greater percentage of change (decrease) in fasting insulin than tertile 1\[83\]. Tertile 3 also had a greater percent change in HOMA-IR than tertile 1\[83\]. These results suggest that ADF could possibly be more effective for decreasing insulin resistance in individuals who are severely insulin resistant\[83\].

Overall, ADF in humans has shown to have possible benefits to health in many different aspects: decreases in triacylglycerol\[^{40,114}\], total cholesterol\[^{39,41,105,112,114}\], triglycerides\[^{39,41,111,112}\], asthma symptoms\[^{111}\], oxidative stress\[^{111}\], inflammation\[^{111}\], BMI\[^{41,112-114}\], LDL\[^{39,41,82,112}\], blood pressure\[^{41,82,112,113}\], heart rate\[^{41,112}\], REE\[^{42}\], waist circumference\[^{113,114}\], and RMR\[^{39,41}\] in addition to the glucoregulatory functions reviewed in detail. The differences in outcomes show that ADF could possibly benefit healthy, overweight, and obese individuals, while much more research is needed in each of these parameters to understand further its extent.

*Alternate Day Fasting in Animals*

In human research involving a dietary intervention the researcher has limited control over the dietary intake of the subject. This limited control is a probable contributor to the more conclusive outcome measures reported in animals after following ADF interventions, considering the animal subject only has access to the food it is provided. The positive effect of ADF in rodents on weight loss and rate of weight gain is demonstrated repeatedly in the majority of studies comparing ADF to *ad libitum* feeding. However, when ADF rodents were compared to CR rodents, ADF rodents weighed significantly more (approximately 10-14 grams)\[^{86,106,108}\]. Two studies have
measured the effect of ADF on body fat in healthy, non-diseased rodents\textsuperscript{[105,106]}. These results are conclusive on the possible benefit of ADF on body fat, exhibiting improvements in body fat distribution\textsuperscript{[106]}, fat cell size\textsuperscript{[105]}, and intra-abdominal fat deposits\textsuperscript{[87]}. In 2007, Varady et al\textsuperscript{[105]}. observed net lipolysis to be 76\% higher (0.56 ± 0.08 g/day in inguinal fat) in the ADF-100\% group and 69\% higher (0.54 ± 0.04 g/day). In the ADF-50\% relative to the control (0.32 ± 0.06 g/day). This study also observed 47\% (p <0.0001) smaller inguinal fat cells in the ADF-50\% group and 56\% (p < 0.0001) smaller cells in the ADF-100\% than the control group. In 2010 Varady et al\textsuperscript{[106]}. observed similar results in all ADF rodents when compared to \textit{ad libitum} fed mice.

ADF has shown to improve fasting plasma insulin levels\textsuperscript{[85,86]} in all studies and insulin resistance\textsuperscript{[83,85,116]} in most when HOMA-IR has been reported. Higashida and colleagues\textsuperscript{[117]} were concerned with the possible ability of ADF to reverse diet-induced obesity and its negative effects on health markers. After a six-week intervention of ADF with a high fat diet, the rats’ body weights were significantly less than the rats fed normal chow \textit{ad libitum}\textsuperscript{[117]}. Despite reversing the high fat diet-induced increase in total intra-abdominal fat mass, ADF did not reverse diet-induced insulin resistance\textsuperscript{[87]}. More research is needed to explore the explanation of this finding.

Unfortunately, overall mixed findings were reported on the effect of ADF on fasting plasma glucose levels. Levels have been reported to either significantly decrease\textsuperscript{[83-87,116]}, not differ from the control\textsuperscript{[108]}, and even impairment in blood glucose response\textsuperscript{[117]} has been seen. However, the majority consensus is an improvement in blood glucose levels after an intervention period of ADF\textsuperscript{[83-87,116]}.

Collectively, ADF in rodents has shown to positively affect health outcomes. A difference in the incident of diabetes during life span of 27\% less than \textit{ad libitum} fed rodents\textsuperscript{[84]} was observed. Significant decreases in heart rate\textsuperscript{[85,108]}, blood pressure\textsuperscript{[85,108]}, body temperature\textsuperscript{[85]}, and an
increase in fat oxidation\cite{118} have been reported in addition to the positive effects on glucoregulatory functions reviewed in detail. The repeated positive outcomes in animals show that ADF could possibly benefit healthy, overweight, and obese individuals.

**Comparison of Dietary Change Models and Effects on Insulin Sensitivity**

Overall results regarding insulin sensitivity after an intervention involving the DF, TRF, and ADF are similar. DF has resulted in significant decreases in plasma glucose and a trend for decreases in plasma insulin and HOMA-IR. TRF and ADF studies have shown decreases in fasting plasma glucose and insulin levels, and HOMA-IR, indicating improvements to insulin sensitivity in both humans and animals. To the author’s knowledge, no studies have been published in either humans or animals comparing the DF, TRF, and ADF. While this review has revealed the many gaps in research published on each individual dietary change, it is apparent that more research on each diet is needed. Without a comparison of the different dietary change models, it cannot be said which diet could best improve insulin sensitivity.

**Conclusion**

The epidemic of overweight and obesity among Americans is getting worse with each year. The disease is leading to comorbidities in more than half of the population, causing millions of preventable deaths, and costing the healthcare system billions of dollars annually. Many lines of treatment for the disease are currently available including an increase in physical activity, pharmacological therapy, bariatric surgery, and dietary change.

If treatment of obesity is not sought (or preferably, proactive measures to minimize the development of the disease in the first place), individuals are at a very high risk for becoming insulin resistant with the common outcome being the development of type 2 diabetes. Dietary intake is widely accepted as the first line in preventing and treating an obese state, as well as
controlling blood glucose and insulin, while preventing the impairment in insulin sensitivity. Although many dietary plans exist, the DF, TRF, and ADF protocols have been investigated in controlled settings in both humans and animals with success. All three of these programs have the ability to result in lower fasting glucose and insulin, while improving HOMA-IR. What is unknown presently is whether or not one regimen yields more favorable results as compared to the others, as well as in comparison to a traditional CR approach. This is a research question that requires investigation.
References


64. Diagnosis and classification of diabetes mellitus. (2014). *Diabetes Care, 37 Suppl 1*(1), S90. doi:10.2337/dc14-S081


University of Memphis
Institutional Animal Care and Use Committee

Protocol Annual Update/Amendment form

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

IACUC Approval #: 0806

Primary Investigator: Rick Bloomer & Marie van der Merwe

Additional Personnel Involved in the Project:

1. Check the following appropriate category:
   ___ Annual Update
   ___ Protocol Modification (change in existing project)/ Amendment
   ___ Protocol Addition (addition of new project to existing protocol but significant changes may result in the IACUC requesting a new protocol to be submitted)
   ___ Extension of Protocol termination date (6 month minimum)
   ___ Termination of Protocol

2. Review the original protocol and explain changes in the categories marked below:
   ___ There have been no changes.
   ___ Changes in personnel:
     Persons deleted: _________________________________
     _________________________________
     Persons added: _________________________________
     _________________________________
     For each person added, please answer the following questions:
     Name: _________________________________
a. Has this person completed the introduction orientation training? ___
b. What species will this person be handling? ________________
c. What procedure(s) will this person be performing? ________________
d. How was this person trained to do the procedure?
       ________________________________
e. Who provided the training? ________________________________
f. How much experience (time or intensity) has this person had?
   ___ Changes in experimental procedures performed on animals: An additional facial bleed is added at the beginning of intervention to be able to compare glucose and insulin changes induced by the intervention, in addition to between-group comparisons. The facial bleed will be performed in a similar manner to the blood collection at the end of the study that is already approved in the protocol.
___ Changes in drugs or methods used to produce analgesia, anesthesia, or Euthanasia. Give reasons for changes.

___ Changes in numbers of animals used and why. (Note that an increase in total number exceeding 50% of the original total may require submission of a new protocol rather than an amendment.)

___ Changes in funding agency. Describe:

___ Any changes that alter the used and care of animal in your study. Explain:

In submitting this statement to the Chair of the IACUC, I attest that the animals covered by this protocol have not experienced more discomfort or pain than that which was stated in the original protocol.

Name of Primary Investigator: Rick Bloomer & Marie van der Merwe

Date: 6/29/17