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The Effects of Time Restricted Feeding on Markers of Type 2 Diabetes

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The Effects of Time Restricted Feeding on Markers of Type 2 Diabetes

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Abstract

Type 2 diabetes is one of the fastest growing chronic diseases in the country, comprising 10% of the American population. Obesity rates are at an all-time high and greatly increase risk factors for developing diabetes. Insulin resistance is the main feature of type 2 diabetes and causes management of the disease to be extremely difficult. Currently there are different possible treatments for type 2 diabetes including medication and dieting. However, diets involving fasting, though high in popularity, do not have a lot of evidence regarding their ability to prevent the development of type 2 diabetes.

Time restricted feeding (TRF) is a daily fasting model in which eating only occurs in a specific 12-hour or less period, followed by a fasting period for the rest of the day. This model does not necessarily focus on calorie restriction or a change in the quality of food intake, but on the amount of time spent eating per day. Constant food ingestion is accompanied by worsened glucose tolerance and high insulin levels that lead to insulin resistance. A TRF model may be able to provide a feasible diet that people can incorporate into their daily lives to lower the risk of developing diabetes.

The current pharmacological approach for treating type 2 diabetes includes taking medications to keep insulin and glucose levels under control. Fasting has been hypothesized to improve insulin and glucose levels but has not yet been proven to reduce type 2 diabetes risk. Therefore, the **objective** of this study was to determine if TRF improves markers of type 2 diabetes. This research will make a positive contribution to society by suggesting a feasible diet to add into a daily lifestyle to prevent developing one of the fastest growing chronic diseases in the country.

Introduction

Type 2 diabetes is a chronic disease that affects the way the body responds to and produces insulin (Grajower, 2019). Insulin is a necessary hormone that regulates glucose movement into cells. Without it, normal glucose levels cannot be maintained, and the body cannot get the energy it needs. The current criteria for fasting blood glucose classify less than 100 mg/dL as normal, 100 to 125 mg/dL as prediabetes, and 126 mg/dL or greater as type 2 diabetes (Mayo Clinic, 2021). Previous studies have shown that as fasting plasma glucose levels increase the risk of developing type 2 diabetes increases, even increases in the currently accepted normal range (Nichols, 2008). Patients with diabetes mellitus comprise approximately 10% of the US population (Grajower, 2019). People with overweight and obese classifications are at a higher risk for developing diabetes. The large population increase in obesity in the last 50 years has been followed by a 7-fold increase in diabetes (Long, 2016). Body composition measures associated with obesity such as BMI, waist-to-hip ratio (WHR), and body fat percentage have been shown to be predictive of type 2 diabetes (Chen, 2020). Management of diabetes is extremely intricate and challenging, causing it to become one of the leading causes of death. Fasting could serve as a simpler and more convenient alternative treatment for managing diabetes.

Since the 1970's, a dietary switch has occurred where snacking has been added in between meals. With this constant feeding schedule comes constant, high insulin levels, leading to insulin resistance and weight gain (Fung, 2016). Time restricted feeding (TRF) is a daily form of intermittent fasting where eating occurs in a 12 hour or less time period, followed by fasting for the remainder of the day. This model does not necessarily focus

on calorie restriction or a change in the quality of food intake, but on the amount of time spent eating per day. This fasting ideology arose from the concept of circadian rhythm, a daily 24-hour metabolic and physical behavior rhythm based on the light/dark cycle (Longo, 2016). Eating out of line with these rhythms worsens glucose tolerance (Jamshed, 2019), therefore eating in line with these rhythms may improve metabolic health. During the feeding state, the insulin-pAKT-mTOR pathway is activated and promotes anabolic processes (Longo, 2016). These processes include the growth and increase of muscle mass through mTORC1. Stored energy is utilized a few hours into the fasting state and triggers the "fasting physiology" (Hatori, 2012). This state activates AMPK, which promotes catabolic processes (Longo, 2016). These processes contribute to the break-down aspect of metabolism. Both the catabolic and anabolic processes make up the circadian clock and impart pleiotropic benefits (Longo, 2016).

During the fasting period, a "metabolic switch" occurs when glycogen stores are drained, and fatty-acid derived ketones are then utilized (Mattson, 2014). Ketones are the preferred fuel for the body (Grajower, 2019). Studies have shown that after a fasting period, insulin sensitivity increases, and insulin levels fall (Jeff, 2014). Insulin resistance is also associated with an increased inflammatory state, which can be improved through the fasting state (Grajower 2019, Jeff 2014). Excessive energy intake promotes inflammation (Mattson, 2014).

The current pharmacological approach to treating type 2 diabetes involves replacing a biochemical agent (insulin), exerting a pleiotropic effect (Jeff, 2014). Because both fasting and medicine exert pleiotropic effects, fasting may be an effective treatment for chronic diseases. Therefore, the objective of this study was to examine the effects of a

time restricted feeding model on reducing the risk of developing type 2 diabetes. I hypothesized that incorporating an eight-hour fasting schedule will improve markers of type 2 diabetes.

Materials and Methods

Subject Recruitment

The study was approved by the International Review Board at the University of Arkansas (protocol # 1912236045A006). The recruitment process consisted of three phases. In Phase 1, subjects were recruited through advertisements in the University digital newspaper, which is distributed to faculty, staff, and students daily. Advertisements were also placed on the Center for Human Nutrition website, Food Science department website, social media (e.g., Facebook, Instagram, and Twitter), and through word-of-mouth. Phase 2 involved a phone screening. All information was kept confidential. In order to participate in the study, participants had to be overweight or obese (BMI $\geq$ 25kg/m^2) men and women between the ages of 25-50 years with no preexisting health conditions. Those individuals who had food allergies, restricted eating (e.g., vegan), were taking medications that interfered with metabolism, were smoking, consumed alcohol 3 times per week or more, or had dieted in the last 6 months were excluded at this time. Participants who were pregnant or breastfeeding were also excluded from the study at this time. In Phase 3, an in-person screening took place at the Center of Human Nutrition at the University of Arkansas. The consent forms were explained and signed at this time. A total of 16 participants, 4 males and 13 females, were qualified to participate in the study. Participants were randomly assigned to an intervention group.

Intervention

All research took place at the Center for Human Nutrition at the University of Arkansas. A total of 20 participants were recruited to take part in this 12-week, randomized control study. Four participants dropped out of the study at various stages for different reasons. Participants were randomly assigned to one of two TRF groups: 1) the control group or 2) the treatment group. The dietary interventions were as follows: 1) control- follow current dietary pattern within the time restricted feeding eating window, and 2) whey protein supplementation at 20g per day at the beginning of the eating period of time restricted feeding. Protein was consumed with the first meal of the day and was provided to the participants. The dietary interventions were conducted using a time restricted feeding model, in which participants ate during a defined 8-hour period of their choosing during the day and then fasted for 16 hours. Participants came to the Center for Human Nutrition every 4 weeks (baseline, 4 weeks, 8 weeks, 12 weeks) for sample collection and metabolic measurements. To ensure compliance, participants were provided with detailed educational guides, met monthly with study personnel, and could request meetings with study personnel in between study visits.

Anthropometrics

Body height and weight were measured at baseline, 4, 8, and 12 weeks. Body height was measured to the nearest 0.1 cm using a stadiometer (Detecto, St. Louis MO), while participants were barefoot, in free standing position. Body weight was measured in the fasted state without shoes to the nearest 0.05 kg using calibrated scales (Detecto, St. Louis MO). Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Waist measurements were taken using a soft tape measure, with the belly button

serving as the standard position of the waist and rounding to the nearest 0.1 cm. Hip measurements were taken at the widest point below the waist using a soft tape measure and rounding to the nearest 0.1 cm. Waist-to-hip ratio was calculated by diving the waist measurement (cm) by the hip measurement (cm). Pulse and O_2 saturation levels were also measured using a pulse oximeter (Zacurate). Body composition was measured using x-ray dual absorptiometry (DXA; Lunar iDXA, GE Healthcare).

Markers of Type 2 Diabetes

At baseline, 4-, 8-, and 12-weeks participants underwent blood draw in the fasted state by a licensed phlebotomist. Markers of metabolism were measured that included blood glucose, total cholesterol, LDL, HDL, and triglycerides. The blood samples were placed in a cassette and analyzed using Alere Cholestech LDX Analyzer. Blood pressure was measured at each visit using a digital Paramed monitor.

Statistical Analysis

At the end of the 12-week study, the data was analyzed using two-way ANOVA to compare differences between dietary treatments and one-way ANOVA to compare changes within each dietary treatment. GraphPad Prism version 9.0 was used for data analysis and significance was set at $p < 0.05$.

Results

Participant Characteristics

At the time of completion of this thesis, 16 participants have finished the study. **Table 1** shows the baseline characteristics of participants in the study including: age, height, weight, body mass index (BMI), waist-to-hip ratio (WHR), pulse, and oxygen levels. All baseline characteristics are reported in **Table 1** as the mean ± standard deviation.

Table 1. Participant Characteristics

Baseline Characteristics	Control (n= 7)	Protein (n= 9)
Age (Years)	37.14 ± 7.82	36.22 ± 7.33
Sex:	-	-
Male	2	2
Female	5	7
Anthropometrics:	-	-
Weight (kg)	85.4 ± 10.75	107.2 ± 26.46
Height (cm)	167.3 ± 4.74	168.7 ± 8.99
BMI (kg/m^2)	30.51 ± 3.74	37.10 ± 7.22
WHR (W/H)	0.93 ± 0.05	0.96 ± 0.06
Pulse (bpm)	69.0 ± 9.11	77.78 ± 13.91
O_2 (%)	97.17 ± 0.98	92.89 ± 5.80

Anthropometric Assessment

At each visit, all participants had their weight, height, and waist-to-hip ratio measured. The body mass index of each subject was calculated from the weight and height measurements. **Figure 1a** shows the average body weight of each treatment group over the course of the study. A two-way repeated measures ANOVA demonstrated significance for time ($p < 0.05$). **Figure 1b** displays the bodyweight of all individuals at each time point of the study.

The line graph in **Figure 2a** shows the average BMI for each treatment group over the course of the study. A two-way repeated measures ANOVA demonstrated significance for time. **Figure 2b** shows the average change in BMI from baseline to week 12 of the study for each treatment group. An unpaired t-test did not demonstrate significance.

Figure 3a displays the waist-to-hip ratio of all individuals at each time point of the study. A two-way ANOVA did not demonstrate significance. **Figure 3b** shows the average change in waist-to-hip ratio from baseline to week 12 of the study. An unpaired t-test did not demonstrate significance.

Figure 1

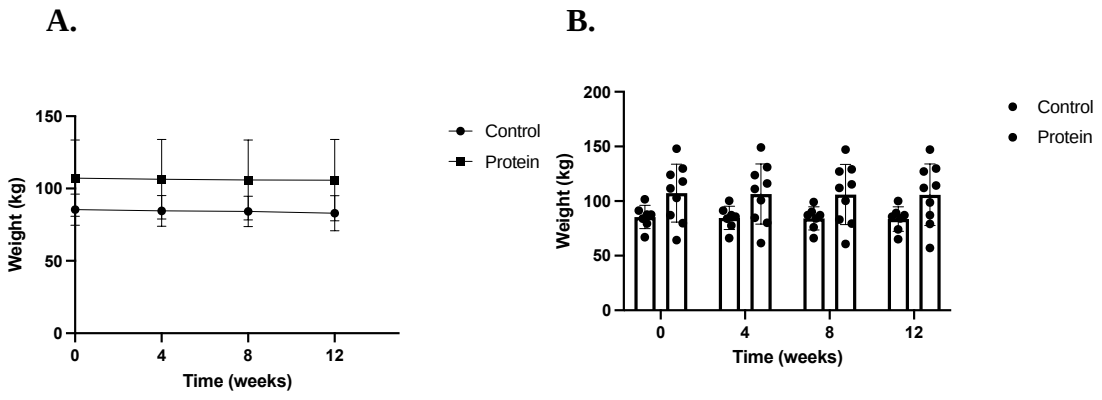

Figure 1. (A) The average bodyweight (kg) measurements of each treatment group calculated throughout the study. The average bodyweights are expressed with the standard deviations. Significance was found for time (p <0.0001). **(B)** The individual bodyweight (kg) measurements of each treatment recorded at each timepoint throughout the study. The bodyweight measurements are expressed as means ± the standard deviations.

Figure 2

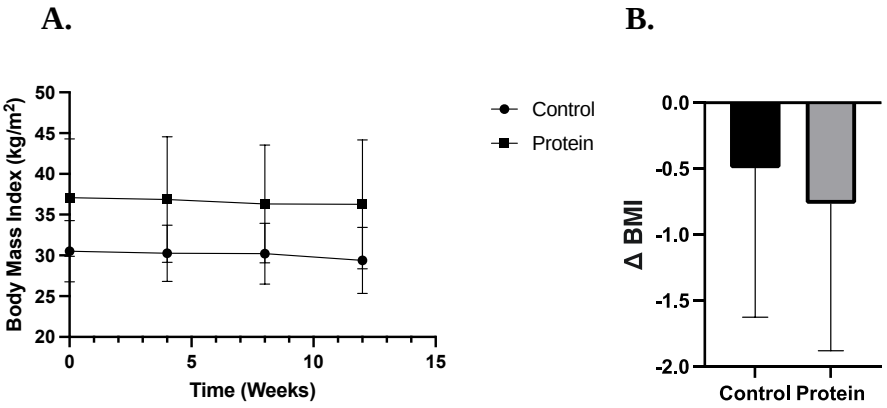

Figure 2. (A) The average BMI (kg/m^2) measurements of each treatment group calculated throughout the study. The average BMI are expressed with the standard deviation. Significance was found for time (p < 0.0001). **(B)** The average change in BMI from baseline to 12 weeks for each treatment group. The average changes in BMI are expressed means ± standard deviation. No statistical significance was found.

Figure 3

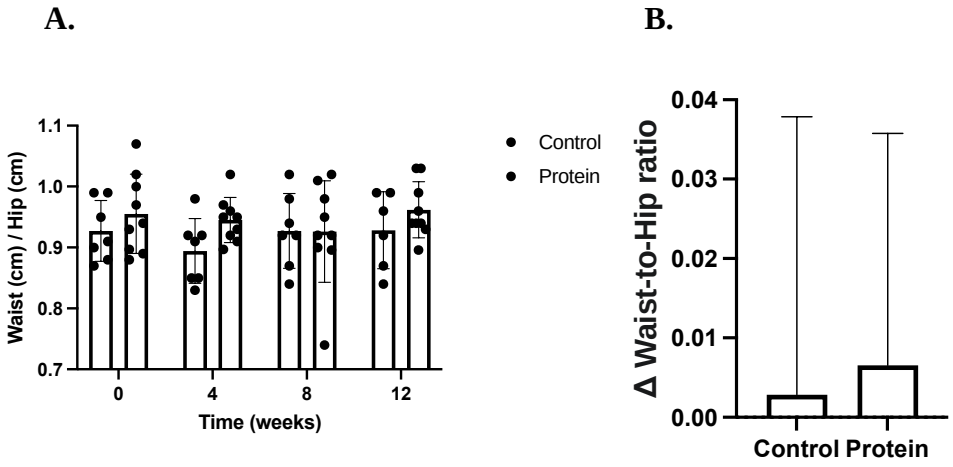

Figure 3. (A) The individual waist-to-hip ratio (W/H) of each treatment recorded at each time point thorughout the study. The waist-to-hip ratios are expressed with standard deviations. No statistical significance was found. **(B)** The average changes in waist-to-hip ratio from baseline to 12 weeks for each treatment group. The average changes in waist-to-hip ratio are expressed with standard deviations. No statistical significance was found.

Markers of Metabolism

Glucose levels in blood samples obtained through a finger prick were measured using the Alere Cholestech LDX Analyzer. **Figure 4** shows the change in fasting blood glucose levels for individuals throughout the study. A repeated measures two-way ANOVA demonstrated significance for time.

Figure 4

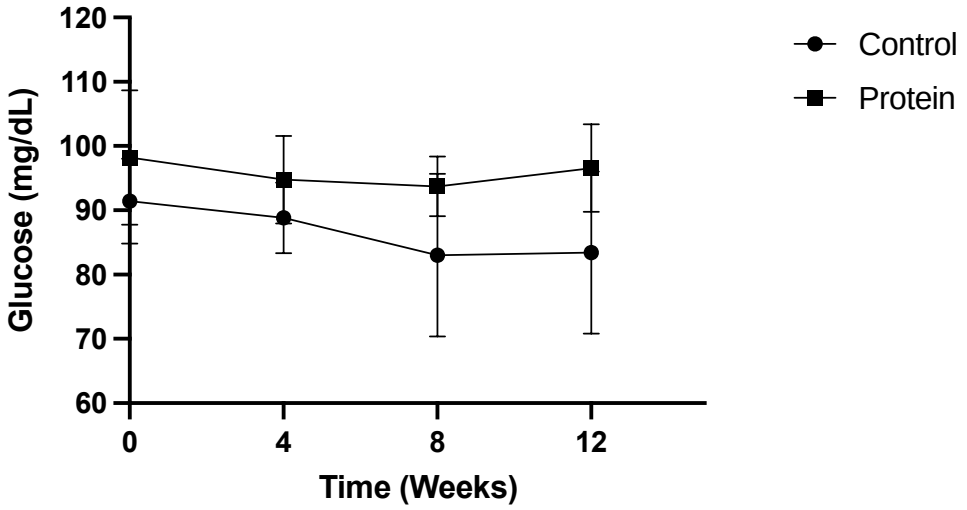

Figure 4. The mean and standard deviation of fasting blood glucose levels for participants throughout the study. Significance for time was found ($p < 0.0003$).

Body Composition

Body Composition was measured for all participants at baseline (Week 0) and at the end of the study (Week 12). Body composition was measured using x-ray dual absorptiometry (DXA). **Figure 5a** shows the average fat free mass (kg) of all participants at the beginning and end of the study. A repeated measures two-way ANOVA demonstrated significance for time. **Figure 5b** shows the average change in fat free mass from baseline to week 12 for each treatment group. An unpaired t-test did not demonstrate significance.

Figure 6a shows the average total body fat (%) of all participants at the beginning and end of the study. A repeated measures two-way ANOVA did not demonstrate significance. **Figure 6b** shows the average change in total body fat from baseline to 12 weeks for each treatment group. An unpaired t-test did not demonstrate significance.

Figure 5

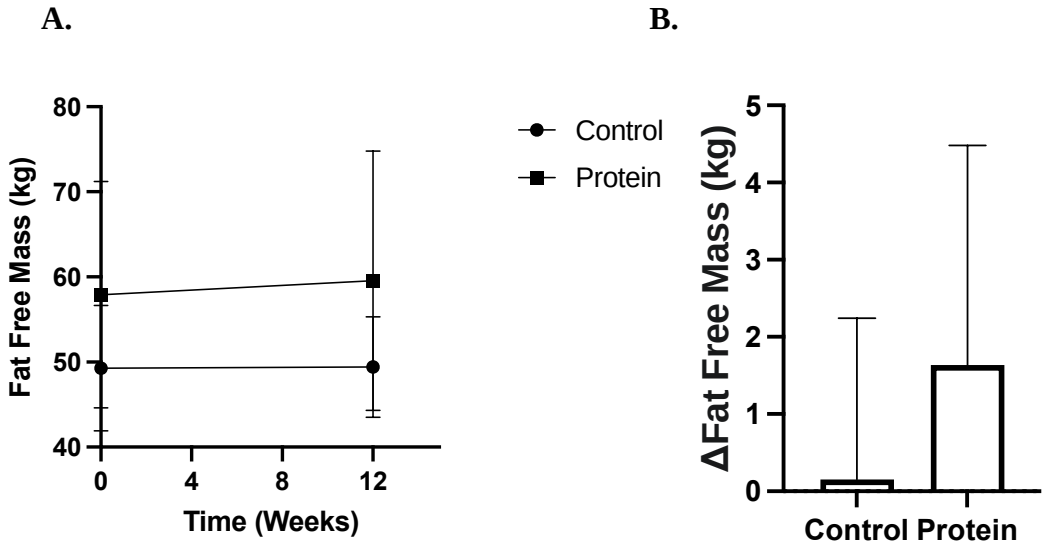

Figure 5. (A) The mean and standard deviation of fat free mass (kg) for participants at baseline and at the end of the study. Significance was found for time (p <0.05). **(B)** The average changes in fat free mass from baseline to 12 weeks for each treatment group. The average changess in fat free mass are expressed with standard deviations. No statistical significance was found.

Figure 6

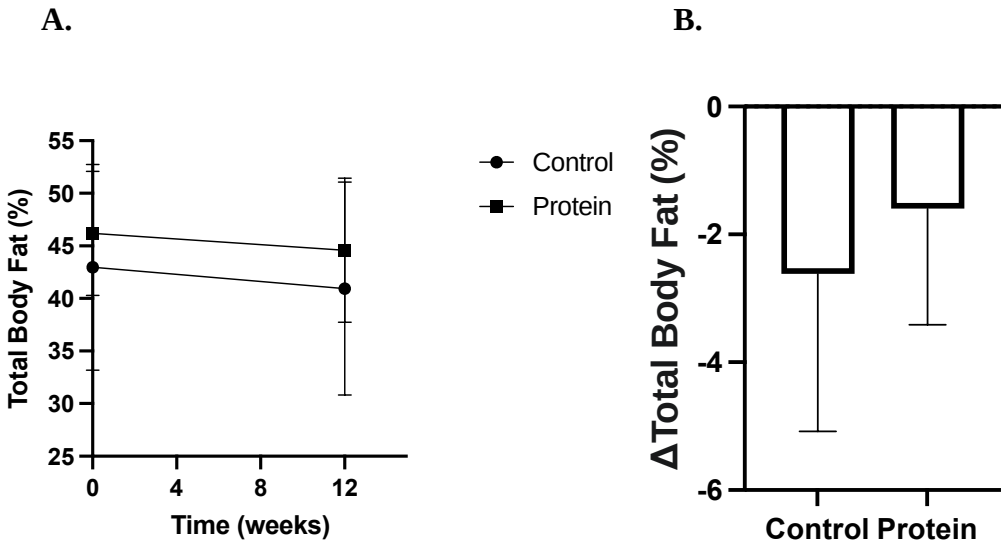

Figure 6. (A) The mean and standard deviation of percent total body fat at baseline and at the end of the study. No statistical significance was found. **(B)** The average changes in percent body fat for each treatment group from baseline to 12 weeks. The average changes in percent body fat are expressed with standard deviations. No statistical significance was found.

Discussion

Type 2 diabetes is one of the fastest growing chronic diseases in the country, with obesity being one of the biggest risk factors for developing the disease (Grajower, 2019). With obesity rates at an all-time high and current management strategies for type 2 diabetes being extremely challenging and intricate, more feasible preventative measures are needed that people can incorporate in their daily lives to lower obesity rates and prevent type 2 diabetes development. Fasting has been hypothesized to improve certain markers of type 2 diabetes including blood glucose levels and body weight (Grajower, 2019). In this particular study, bodyweight, BMI, waist-to-hip ratio, blood glucose, fat free mass, and total percent body fat measurements were utilized to determine the effect of time restricted feeding on the prevention of type 2 diabetes.

A statistically significant difference in bodyweight measurements of all participants was found over time but not between treatment groups. This supports the results of a previous study that looked at the effects of an 8-hour time restricted feeding window on obese participants. They found a significant decrease in bodyweight over the course of 12 weeks in participants who followed the time restricted feeding model, but not in participants who were in the control group (Gabel, 2018). Seeing how a decrease in

bodyweight lowers the risk of type 2 diabetes development raises the question if lowering bodyweight is the most effective factor in decreasing development compared to other factors. The Diabetes Prevention Program Group focused on seeing if lifestyle intervention, where participants were to reduce body weight by 7% of their initial bodyweight, or the administration of medication was a better means of reducing the development of type 2 diabetes. They found that both the lifestyle intervention group and treatment with metformin lowered the incidence of diabetes but by 58% and 31% respectively. (Diabetes Prevention Program Research Group, 2002). This shows that bodyweight is a better factor in reducing type 2 diabetes development than the administration of medication. A statistically significant difference in Body Mass Index (BMI) was found over time but not between treatment groups. Any increase in BMI increases the risk of developing type 2 diabetes (Gray, 2015). The preliminary findings of this study showing a reduction in bodyweight and BMI over the course of 12 weeks supports my hypothesis that a time restricted feeding model can improve markers of type 2 diabetes.

No significance was found for the change in waist-to-hip ratio for participants over the course of the study. This does not support my hypothesis that a time restricted feeding model can improve markers of type 2 diabetes. Waist-to-hip ratio has a strong predictive effect on type 2 diabetes and has even been suggested to be a better indicator than BMI (Cheng, 2010, Freemantle, 2008). The lack of statistical significance may be due to a small sample size and may change as more data is added from the participants in the ongoing study.

A significant difference was found in fasting blood glucose levels for participants over time but not between treatment groups. This supports my hypothesis that a time restricted feeding model can improve markers of type 2 diabetes. Studies have shown that as fasting plasma glucose increase, the risk of type 2 diabetes development also increases, even if those increases are still within the normal range (Nichols, 2008). When blood glucose levels are constantly high, the body must produce excessive insulin, leading to insulin resistance (Choi, 2010). Insulin resistance is the main feature of type 2 diabetes. When switching from the feeding to fasting state, blood glucose levels fall early on and subsequently cause insulin levels to fall as well. This is called the postabsorptive phase and is characterized by the body now having an increase in glucagon to break down glycogen in the liver (Kerdnt, 1982). These results support the results of studies in the current literature. Longo found a decrease in blood glucose levels and increase in insulin sensitivity in obese subjects using an intermittent fasting schedule (Longo, 2014). Che saw a decrease in blood glucose levels by 15% in type 2 diabetes patients using a 10-hour time restricted feeding window (Che, 2021). This is the first study to look at a fasting model in participants already diagnosed with type 2 diabetes and suggest that time restricted feeding could possibly serve not only as a preventative measure but as a treatment for type 2 diabetes. Despite these two studies having similar results to my study, another study did not find these same results. Gabel did not see a statistically significant difference in blood glucose levels in obese participants on an 8-hour time restricted feeding window over 12 weeks (Gabel, 2018). Due to these conflicting conclusions from different studies, more research needs to be done in order to verify the results.

Although no significance was found for the change in fat free mass throughout the study, an upward trend was displayed for both treatment groups. The protein group had roughly a 20% increase in fat free mass while the control group had roughly a 5% increase. This suggests that protein supplementation to break the fasting period could lead to an increase in muscle mass. Current literature supports the findings of this study. Moon found that a time restricted feeding selectively decreased fat mass while preserving muscle mass (Moon, 2020). Studies have shown that there is an inverse relationship between skeletal muscle mass and insulin resistance (Preethi, 2011). High muscle mass might also stabilize glucose levels, since insulin-induced glucose uptake occurs in skeletal muscle (Kim, 2018).

Although there was no significant change in total percent body fat in participants throughout the study, a downward trend was demonstrated. Once glycogen stores have been drained during the fasting period, the body then enters ketosis (Mattson, 2014). During ketosis, fat is now used for energy and triglycerides are broken down to glycerol and three fatty acids. Studies have shown that there is an increased risk of type 2 diabetes development with a high body fat percentage, even if BMI is in the normal range (Gómez, 2011). This suggests that body fat percentage could be a better indicator of type 2 diabetes than BMI alone, as BMI does not distinguish between lean or fat mass. The literature supports these findings. These studies found a significant change in percent body fat in participants on a time restricted feeding model (Antoni, 2018, Schroder, 2021).

This study focused on overweight and obese adults who had no pre-existing health conditions. Fasting is not for everyone and there are some people that should not

fast and can be harmed by it. Those who are pregnant, breastfeeding, or are severely malnourished or underweight should not fast (Fung, 2016). This study only included adults in the age range of 25-50. More research is needed to see how fasting affects children and the older population. It can be dangerous for young children to fast as adequate nutrition is needed for growth and development and possibly dangerous for the elderly population to participate in fasting due to complications from aging. Those with a chronic disease such as type 2 diabetes should not fast without consulting with their physician first. For those already diagnosed with type 2 diabetes, hypoglycemia and other complications due to antidiabetic medications can occur (Grajower, 2019). More research is needed to test the safety of fasting as a treatment measure for people with chronic diseases and not just as a preventative factor.

A big question that can arise from this study is why fasting over any other weight loss strategy to reduce the risk of type 2 diabetes? Studies have shown that although a low-carbohydrate diet can lower glucose levels, fasting lowered glucose levels by almost 50% more (Barnosky, 2014). Compared to a calorie restriction diet, fasting improved more indicators of type 2 diabetes such as blood glucose levels and insulin sensitivity in addition to weight loss, where as a calorie restriction diet usually only promotes weight loss. Fasting is also much more efficient and easier to incorporate into daily life than diets that require calorie counting or macronutrient tracking.

There are limitations to this study that need to be addressed. First, there is a small sample size of only 16 participants, which is not representative of adults in the United States. The study is still ongoing and actively taking new participants. Second, the study was very intricate with travel and time commitments, self-discipline to follow the fasting

schedule, as well as other forms and surveys for the participants to remember to complete. This caused 4 participants to drop out at different time points within the study. There was no system put in place to verify that participants were following the time restricted feeding model correctly. Other limitations of this study include anthropometric human error, researcher error when collecting data, and possible error when performing calculations.

This study showed significant changes in bodyweight, BMI, fasting blood glucose, and fat free mass over time. None of the measurements showed significance between treatment groups, indicating that protein supplementation does not improve type 2 diabetes markers more than the control group in a fasting model. Although other measurements did not have significant changes, there were trends towards positive changes in the markers. The current results of the study are promising in determining if a time restricted feeding model can reduce the risk of developing type 2 diabetes. More research is needed in order to verify the results of this study and other studies that have coinciding and conflicting outcomes.

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