Increased Dietary Protein in Sedentary Vegans and Vegetarians and its Effect on Body Composition and Strength

by

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ABSTRACT

Chronic diseases, such as diabetes, heart disease, and cancer are leading causes of death in the United States. Although they result from a host of personal and environmental factors, diet remains a critical way to reduce the risk. Plant-based diets in particular are associated with reduction in risk for chronic disease due to an intake that closely mirrors the Dietary Guidelines for fruit and vegetable consumption, fiber, and fat intake. Additionally, plant-based diets offer a sustainable alternative in relation to food production as they often require fewer natural resources overall.

While there are many benefits to following a plant-based diet, potential concerns arise as well. Certain micronutrients can be lacking and protein intake can be inadequate without careful consideration of dietary intake. Protein is especially important for its role in maintaining lean body mass, which allows individuals to function in activities of daily living. Plant-based sources of protein are often less digestible; therefore, those consuming vegetarian and vegan diets may benefit from increased protein intake for preservation and perhaps improved lean body mass as well as strength changes.

Recent research has shown that vegetarians had significantly less muscle mass compared to omnivores despite similar amounts of protein intake in grams per day. Other research has shown that vegetarians do not necessarily see an increase in muscle mass when exposed to resistance exercise, whereas those following an omnivorous diet or lacto-ovo-vegetarian diet do. However, other studies have found that vegetarians can achieve increases in lean body mass comparable to omnivores if 30g/meal of plant-based protein is ingested consistently.

It remains unclear what effect protein supplementation might have on strength and muscle mass among sedentary plant-based eaters. As such, the present study assessed sedentary vegetarian and vegan individuals as to whether increases in dietary plant-based protein could elicit changes in body composition, hand grip and lower body strength independent of exercise. After an 8-week intervention, no significant differences for lean body mass or strength were noted. Results are discussed in the context of trial integrity and supplement consumption issues.

DEDICATION

I would like to dedicate this Master's thesis to my fiancé, Steven Goss, for being extremely supportive during the process. Thank you for supporting my dreams and moving across the country for me to pursue a dream career in nutrition while putting in countless hours at work and home in providing and making it possible for me to follow my dreams. In addition, I would like to thank my friends within the nutrition program I have met that continue to push me in this field and support my dreams as well. Finally, I would like to support my family back home who are always supporting and encouraging me to be the best version of myself.

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INTRODUCTION

Statement of the Problem

Based on a 2016 Harris Poll, an estimated 3.3% of the American population consumes a plant-based diet; as such, over 10 million people could be considered vegetarian or vegan.[1] Given the size of this population, ensuring adequate nutrient intake becomes important. At the moment, some nutrients have garnered attention from the United States Department of Agriculture (USDA) for their particular importance in a vegetarian population or even been assigned a vegetarian-specific recommended dietary allowance (RDA), such as iron and calcium.[16] Other nutrients, in particular protein, have received less attention as they relate to the healthfulness of vegetarian diets. This is especially important given the fact that protein bioavailability from plant-based sources is far lower compared to animal-based sources and inadequate intake may affect body composition.[6.8-10.12]

The current body of literature related to plant-based diets includes considerable work focused on health benefits of vegetarian and vegan diets as well as protein intake as it relates to strength training.[2-5,12-14] However, limited research exists examining aspects of plant-based protein intake and lean body mass in sedentary individuals, independent of exercise, which make up a larger proportion of the overall population. As such, important areas of research remain to be addressed to better evaluate protein intake and its health impacts in plant-based eaters who may not be physically active.

Background:

Plant-based eating focuses on nutrients coming from plant sources rather than animal-based sources. Plant-based diets are varied and can be defined based on the animal-based foods they include or exclude. For example, those that include dairy and eggs are lacto-ovo-vegetarian, those that include fish are pescatarian, and those abstaining from all animal-based sources and their by-products are vegan.[18] Individuals following plant-based diets have been studied for the potential the benefits of consuming less animal products and more plant products.[2-5]

Plant-based diets are known to be associated with lower body mass index (BMI) and reduced risk of chronic disease such as diabetes, but also increased risk of nutrient deficiencies including inadequate protein intake.[2-5] Ranges for BMI include: underweight ($<18.5 kg/m^2$), normal ($18.5-24.9 kg/m^2$), overweight ($25.0-29.9 kg/m^2$) and obese ($>30 kg/m^2$). Research has shown mean BMI to be lowest in vegans ($23.6 kg/m^2$) compared to mixed vegetarian diets ($25.7-27.3 kg/m^2$) and omnivorous diets ($28.8 kg/m^2$) ($p<0.0001$).[5] Additionally, associated with increased BMI in animal-based diets was increased prevalence of diabetes, which was significantly lower in vegans compared to all other groups ($p<0.0001$).[5] While plant-based eaters may be at risk for micronutrient deficiencies such as vitamin B_{12}, calcium, and Vitamin D due to inadequate intake, they also have increased levels of plasma ascorbic acid (vitamin C) that may have protective effects against cardiovascular disease and cancer.[2,3] And while protein intake fell into the acceptable macronutrient distribution range (AMDR) of 10-35% of total calories, for both male and female omnivores, vegetarians, and vegans, vegans had the lowest percentage of caloric intake at both 12.9% and 13.5% respectively for males and females.[3] While this may not seem alarming, this does not account for differences in bioavailability of protein sources between groups, adding to the possibility of inadequate protein intake.

Inadequate protein intake is a major concern of vegetarians and vegans because of protein's important functions in the body and reduced bioavailability from plant sources. Proteins act as messengers, maintain cells, assist in fluid balance, and contribute to increased lean muscle mass.[6] Animal-based protein sources are typically considered complete proteins because they contain all twenty essential amino acids, including those that cannot be synthesized within the body, and can be bioavailable upwards of 90%, making the use of protein in the body more accessible for omnivores.[6] Many plant-based proteins do not contain all twenty essential amino acids and their bioavailability can be as low as 60%.[6] Some plant-based sources are considered complete proteins such as soy products and other plant-based sources that are lacking certain essential amino acids can be combined to create complete proteins such as when combining legumes and grains.[6] Additionally, the bioavailability or digestibility of proteins is important for vegetarians and vegans as it affects how much protein is absorbed for utilization. The protein digestibility corrected amino acid score (PDCAAS) calculates a value for protein based on its digestibility with higher digestibility or bioavailability correlating with more complete sources.[6] Therefore, since many plant-based sources are incomplete, apart from soy and a few others, their bioavailability or PDCAAS is significantly lower than animal-based sources.

The Academy of Nutrition and Dietetics (AND) promotes vegetarian and vegan diets for its associated health benefits and indicate these diets meet or exceed the recommended dietary protein intake for individuals; however, it does not take into account protein bioavailability or sources.[7] A report created in 2005 by the Institute of Medicine for Dietary References Intakes suggested vegetarians adjust micronutrient status of iron, zinc, and calcium due to bioavailability but had no mentions of protein.[7] This same report made assumptions using meta-analysis of nitrogen balance studies, stating vegetarians consume 49% of their protein from animal-based source.[18] Kniskern and colleagues challenged this notion by examining vegetarian women's diets to compare to the DRI report of current protein source intake in this population.[7] It was found that vegetarians may consume two and a half times less protein from animal-based sources as the DRI reports , whereas cereals, nuts, and other plant foods contribute the majority of vegetarians protein intake.[8] Protein bioavailability from these sources can be between 54-77%, suggesting similar examination in increased need for this macronutrient.[8-10]

Decreased bioavailability of plant protein may lead to inadequate lean body mass and in turn decreased strength. For example, animal protein intake is significantly correlated with muscle mass index (p=0.001) and significant muscle mass differences exist between omnivorous and vegetarian women (18.2 vs. 22.6 kg respectively).[12,19] Although most studies have been performed in both vegetarian and omnivorous athletes, vegetarians consuming plant-based proteins have smaller increases in lean body mass as those supplementing with the same amounts of animal-based proteins.[13.14] Other research has shown that, vegetarians can achieve similar lean body mass increases if plant-based protein intake was increased to more than 30g/meal.[13,14]

The current RDA guidelines for protein are based on nitrogen balance studies that define quality sources as those from animal-based origin and do not distinguish the needs of those abstaining from or ingesting less than an adequate amount of these sources.[11]

Due to the decreased bioavailability of protein in plant sources, it may be possible that increasing protein intake from plant sources could elicit an increase in lean body mass and in turn increase strength as well as support the need for a separate RDA for vegetarians and vegans.

There is limited research examining the effect of plant-based protein intake and changes in lean body mass and strength independent of exercise. The proposed study will compare high and low plant-based protein intake and its effect on lean body mass and strength in vegetarian and vegan populations.

Deficiency of Literature

Although a body of literature exists examining the differences between lean body mass and strength in vegetarians and omnivores involved in athletics or as part of training regimens, limited research has been done solely in sedentary vegetarians or vegans. Additionally, less research exists examining the impact of protein intake in plant-based dieters independent of exercise.[12-14,17] As such, a considerable gap in the literature exists regarding the impact of increased plant protein intake in and of itself in vegetarians and vegans in terms of strength and body composition.

Purpose and Hypotheses

The purpose of the study was to examine the acute effect of increased plant-based protein intake (21g/day) on body composition, specifically lean body mass, and strength in healthy, sedentary adult vegetarians and vegans who do not currently supplement with additional protein sources. An iso-caloric commercially available low protein bar was chosen as the control treatment to minimize participant bias.

Given the above, two hypotheses were tested:

1. Hypothesis 1: Increasing protein intake (21g/day) in comparison to an isocaloric low protein supplement will increase lean body mass in sedentary vegetarians and vegans over an 8-week trial.
2. Hypothesis 2: Increasing protein intake (21g/day) in comparison to an isocaloric low protein supplement will increase strength in sedentary vegetarians and vegans over an 8-week trial.

Delimitations

- Sedentary vegetarians and vegans aged 18-50 years free of chronic disease as well as any injury disallowing grip and/or leg strength testing
- Participants who are willing to travel to Arizona State University downtown campus for pre-and-post visits
- Participants without food allergies, and who do not diet and/or supplement with protein powder
- Medication use only if steady for the past 3 months

Limitations

- Use of 24-hour recalls and FFQ as sources of self-reported mean dietary intake
- Small sample size (n=37)
- Self-reported consumption of supplements
- Self-reported physical activity level
- Taste of experimental product

CHAPTER 2

REVIEW OF LITERATURE

Dietary Patterns

Americans are bombarded daily with dietary information including varied definitions and opinions as to what is considered optimal for health. However, reliable dietary guidelines have been created through years of extensive research and published by the United States Department of Agriculture (USDA). The USDA has published guidelines in various forms since the early 1900's. The earliest guidelines were based on the "Basic Seven" food groups, including leafy green vegetables, citrus, tomatoes, and raw cabbage; potatoes and other fruits and vegetables; dairy products including milk, cheese, and ice cream; meat and legumes; grain products; and butter and margarine; but this was considered too complex and became the "Basic Four".[18] In 1956, the "Basic Four" categorized foods into milk, meat, vegetable and fruit, and cereal and bread, but lacked guidance in caloric range intakes.[18] Again, a shift occurred in 1984 when the Food Wheel was introduced as a graphic display for dietary advice which included goals for servings of food groups along with new subcategories of foods, eventually leading to the adoption of the Food Guide Pyramid in 1992.[18] The Food Guide Pyramid introduced a new idea of variety, moderation, and portion size and was adapted further in 2005 to include physical activity.[18] Finally, in 2011, the USDA published MyPlate, the current iteration of the government's Dietary Guidelines which included new illustrations of a plate with recommended portions of food groups to make the graphic more user friendly.[18] This new guideline focuses on achieving a balanced diet using five main food groups, eating less saturated fat, sodium and sugar, and focusing on smaller changes to create lasting eating patterns.[18]

A trend over the years is the focus on increased fruit and vegetable consumption with varied amounts of grains, dairy, and meat products. MyPlate recommends one quarter of each meal, daily, to encompass a protein source as well as one serving of milk.[18] In addition, half of all meals should include a mixture of fruits and vegetables, which represents a problem when compared to the current consumption trends Americans exhibit.[18] According to a 2015 report by the Centers for Disease Control (CDC), only 1 in 10 people are consuming adequate amounts of fruits and vegetables based on to the 2015-2020 Dietary Guidelines and when considering financial hardship even fewer people may be adequately nourished in nutrients.[19] Conversely, roughly 80% of the United States population may meet or exceed the recommended dietary intakes for protein sources.[20] Each of these statistics is alarming considering the health benefits of increasing plant-based food sources and decreasing animal products in the diet.

Extensive research in the form of national surveys has been conducted through various organizations to assess health as it relates to diet including the National Health and Nutrition Examination Survey (NHANES), the Nurse’s Health Study (NHS), and the Healthy Professional Follow-Up Study (HPFS).[21,22] Research using NHANES data has shown individuals utilizing the Dietary Guidelines set forth by the USDA has enhanced healthy eating patterns in higher consumption of fruit and vegetable intake, lower saturated fat and added sugar, and interestingly significantly lower meat consumption.[21] Similarly, research utilizing information gathered in the NHS and HPFS examining heart health as it relates to diet showed individuals consuming more fruits and vegetables per day, and less red and processed meat, had a lower BMI as well as the lowest estimated risk of cardiovascular disease.[22] These research findings align closely to observed dietary patterns for those living a plant-based lifestyle or considered vegetarian and vegan.

Plant-based diets encompass various definitions and are perceived in various ways depending on individual opinion. However, Pilis and colleagues categorize different types of plant-based eating into various groups including semi-vegetarians, who are transitioning away from animal products; lacto-vegetarians, who consume dairy products; lacto-ovo-vegetarians, who include both dairy and eggs; and strict vegans, who exclude all animal products and their by-products.[27]

Vegetarian dietary patterns have been studied extensively in terms of positive and negative health implications and sustainability for the environment.[2-5,7-10,25,26] Enough evidence has accrued sufficient evidence to allow the Academy of Nutrition and Dietetics (AND) to establish a position statement on the advantages of a vegetarian diet, including the ability for plant-based eating to lower cholesterol, risk of heart disease, hypertension, and Type 2 diabetes (T2DM) as well as reduction of BMI alongside higher intake of fibrous foods and certain micronutrients.[1] As a measure of health as it relates to chronic disease risk, vegans tend to have the lowest BMI (23.6kg/m^2) compared to mixed vegetarians (26.43kg/m^2) and omnivores (28.8kg/m^2) which suggests a protective effect against obesity, a known contributor to metabolic disease and chronic disease risk.[5] Additionally, when assessing overall occurrence of disease such as T2DM, omnivores had a 4.7% higher prevalence than vegans, and after adjustment for socioeconomic and lifestyle factors, vegans and lacto-ovo-vegetarians had one half the risk of development of T2DM in their lifetime.[5]

Adopting plant-based diets do not come without potential pitfalls, such as reduced status of certain micronutrients and decreased protein status. Omnivores have been known to consume three times the amount of B-12 compared to vegetarians and seven times the amount compared to vegans.[3] Additionally, vegan individuals consume roughly 400mg, on average, below the RDA for calcium daily compared to meat-eaters who met the RDA goals.[3] Cross-sectional data support the finding that vegetarians have lower intakes of calcium and iron as compared to omnivores.[10] The USDA goes even further, claiming that meeting the RDA for calcium may not be possible when consuming a vegetarian diet.[18] In addition to decreased micronutrient status, research suggests deficiencies in protein measured by lower levels of blood urea in vegetarians and vegans.[2] Research suggests when individuals consume upwards of 70% plant-based protein sources, the decreased bioavailability increases the risk of protein deficiency.[35]

Sustainability

The Environmental Protection Agency (EPA) defines sustainability as combining the efforts of social, environmental, and economic constructs in support of balanced resource utilization and environmental preservation for future generations.[28] Along these lines, it is possible a vegetarian lifestyle can support this approach in sustaining 'ecosystem services'. As such, food systems sustainability is an area that impacts food production and environmental health simultaneously.

Food production and consumption patterns are major contributors to the use of nonrenewable resources.[25] Upwards of 90% of phosphate demand comes from food production needs and phosphate emissions may be reducing biodiversity of the planet.[26] Overall, plant-based diets require less phosphorus, for example as used in fertilizer, compared to omnivorous diets.[26] Furthermore, producing animal-based foods compared to plant-based sources wastes an even larger amount of natural resources and produces more by-product waste; for example, beef production wastes 40 times the amount of energy compared to plant foods when assessing protein efficiency and an average of 11 times the fossil fuel energy is used to produce animal food sources compared to plant food sources.[25]

Plant-based diets may be the key to unlocking an efficient way of reducing the use of non-renewable resources and decreasing greenhouse grass emissions (GHGE). Livestock production may account for 18% of total GHGE due to emissions released during deforestation and conversion of land into pasture for animals as well as emissions from feed production, processing, manure, and transportation.[32] Some research suggests reductions as high as 20% in greenhouse gas emissions with individuals who consume a diet higher in grains, vegetables, fruits, and nuts.[33] Theoretically, if individuals were to adopt a vegetarian lifestyle in place of consuming animal foods, an average decrease of 29% of GHGE could be achieved, leading to a potential decrease in the rate of climate change.[34]

Protein Intake and Vegetarian Dietary Patterns

Protein is one of the three major macronutrients our body needs to function properly and its incorporation into our body cells, muscles, and tissues depend on the combination of twenty essential amino acids and the digestibility of protein food sources.[6] Proteins contribute a wide variety of functions in the body including cell growth, maintaining fluid and acid-base balance, boosting the immune system and even serving as a source of energy.[6] However, protein digestibility in animal-based foods can be as high as 90% whereas those found in grains and vegetables may only be 60% bioavailable.[6] Proteins may complement one another in plant-based sources to provide the twenty essential amino acids; however, bioavailability of these protein sources still may be lower in plant-based foods.[6] Some limiting factors, such as anti-nutrient factors, contribute to decreased bioavailability, including glucosinolates, trypsin inhibitors, tannins, and phytates.[29] To assess for protein digestibility and quality the Protein Digestibility Corrected Amino Acid Score (PDCAAS) was formulated and tested against fecal protein waste but did not account for the anti-nutrient factors listed above.[14] To improve the methodology, the Digestible Indispensable Amino Acid Score (DIAAS) was developed, which accounts for limiting anti-nutrient factors and ranks proteins such as whey on a higher scale than soy which would otherwise have been considered equal using the PDCAAS.[30]

The current RDA guidelines for protein intake do not consider the digestibility or quality of proteins. Furthermore, a 2018 meta-analysis reviewing nitrogen balance studies that assessed protein intakes for healthy adults revealed data collected was based on high quality animal-based protein to set the 0.8g/kg/day to meet 98% of the populations needs.[11] More recent research suggested protein intakes of 1.2-1.6g/kg/day to improve health standards.[31] Research by Kniskern and colleagues suggests governmental agencies, such as the Institute of Medicine (IOM), who regulate reports such as the Dietary Reference Intakes (DRI), have skewed perceptions of the types of protein plant-based eaters are ingesting. A 2010 study showed vegetarians consumed 21% of their protein from animal-derived sources whereas the IOM reported in their 2005 DRI report vegetarians were consuming 49% of their protein intake from animal-based sources.[8]

Concurrently, discrepancies between protein digestibility in study participants and the report showed a significant difference of 6%.[8] Those consuming low amounts of animal proteins, such as vegans, may have scores of digestibility range from 54-77%.[8]

Additionally, of the twenty essential amino acids, the branched-chain amino acids (BCAAs)-leucine, isoleucine, and valine-are important in stimulating the action of muscle protein synthesis (MPS).[38] These amino acids are found at considerably lower amounts in plant-based foods.[38] One of the most widely studied plant-based proteins, soy protein, with a digestibility score of 1.00, has diminished ability to stimulate MPS, possibly due to lower leucine content.[30,39] Dose-dependent protein increases stimulation of MPS, independent of exercise which exemplifies the need for correct protein recommendations to elicit lean body mass changes.[40] Furthermore, muscle protein breakdown is diminished as leucine content from high quality protein sources increases.[41] The rate at which MPS stimulates lean body changes utilizing animal-based sources compared to plant-based sources suggests the need for increased protein when consuming plant-based sources.

Vegetarian Diets and Lean Body Mass

The preservation of lean body mass is important throughout the lifespan to reduce the risk of sarcopenia, which is characterized by loss of muscle mass and strength impacting activities of daily living and increasing the risk of chronic disease.[36] Body composition measures in vegetarians compared to omnivores show an estimated 7kg difference in lean body mass.[37] Individuals who consume omnivorous diets have higher muscle mass compared to vegetarians consuming the same amount of total protein per day.[12] Few studies have examined lean body mass in vegetarians compared to omnivores without resistance training introduced. In resistance-based exercise training, vegetarians consuming plant-based proteins have smaller increases in lean body mass compared to those supplementing with the same amounts of animal-based proteins; yet, vegetarians may see similar lean body mass increases if plant-based protein intake is increased to more than 30g/meal.[14]

Research also focuses on type of dietary protein in conjunction with resistance training to elicit changes in lean body mass. Soy protein supplementation has a 1.8% lesser effect changes in lean body mass compared to milk-based proteins.[14] Similarly, examination of the effects of soy, whey, milk, and beef protein on muscle protein synthesis showed the lowest rates of lean body mass changes in soy protein supplementation compared to any of the animal-based sources, which may be attributed to decreased bioavailability, and found lower rates in soy compared to animal-based sources which was attributed to digestion and absorption bioavailability.[13] Plant-based protein intake may elicit small changes in lean body mass; however, bioavailability of protein source is important when considering recommendations to general populations for preservation of lean body mass.

Vegetarian Diets and Strength

Lean body mass relies on efficient protein utilization which may be affected by bioavailability and digestibility. Muscle mass and strength are highly correlated regardless of confounding variables such as age and gender.[42] Studies show increases in lean body mass and strength following various exercise regimens from walking to strength training.[14,43] To date, little research exists examining lean body mass and strength changes in sedentary individuals, specifically plant-based individuals without an omnivorous group for comparison.

A 2012 meta-analysis concluded that protein supplementation in comparison to placebo elicits changes in both lean body mass and strength when exercise is performed.[44] Research comparing omnivorous diet-as-usual to changing dietary patterns from omnivorous to lacto-ovo-vegetarian for thirteen weeks showed cross-sectional fiber type area of the muscle and one-rep max to increase in both groups.[17] However, individuals remaining on diet-as-usual showed greater changes in type II muscle area fibers.[17] Candow and colleagues assessed whey protein and soy protein supplementation in individuals consuming an omnivorous and found increases in both lean muscle mass and strength with no significant difference between whey and soy.[39] Research examining strength increases making small dietary changes or supplementing with varied proteins has generally shown no significant differences with regard to increases in strength and lean body mass.[39] However, individuals following a vegetarian or vegan diet may experience different changes in body composition and strength, possibly from lack of adequate protein due to decreased bioavailability or other issues. For example, one study compared vegetarians and omnivores for total body creatine, a substance known to enhance strength, and showed significant differences between the groups.[45] After separating vegetarians and omnivores into two further groups, creatine supplementation and placebo, individuals underwent resistance training to assess lean body mass change and strength within groups.[45] Vegetarians who supplemented and performed exercise had greater increases in type I and II muscle fiber area as well as greater increases in strength compared to vegetarians who only supplemented with creatine. While omnivores had the greatest increases in muscle area after exercise and supplementation, vegetarians who participated in both exercise and supplementation saw greater gains than omnivores not supplementing.[45] Similarly, Hartman and colleagues found consumption of fat-free fluid milk compared to fat-free soy protein elicited greater changes in lean body mass in young male weight-lifters, suggesting the need for further research comparing various protein sources for changes in lean body mass and strength.[14]

Taken together, the current body of literature fails to address a number of outstanding issues. In particular, the question of whether supplementation with plant-based protein among sedentary plant-based eaters could elicit changes in lean body mass and strength remains unanswered. The current study was therefore designed to explore this question.

CHAPTER 3

METHODS

Participants and Study Design:

The target population for the study included forty men and women who were healthy (disease free), sedentary vegetarians and vegans aged 18-50 years. Participants must have been consuming a vegetarian or vegan diet for at least one year prior to the study. If participants noted medication use, its use and dosage must have been stable for 3 months. Forty-five participants were stratified based on age, weight, BMI, gender, and years on diet and randomized into an experimental or control group. Power calculations (Table 1) were derived using similar studies of body composition in vegetarian men and women, resulting in a suggested sample size of fifty sedentary vegetarians and vegans. Participants were excluded if they were pregnant or planned to become pregnant, suffered from chronic disease, participated in any dieting (such as weight loss or gain attempts), supplemented with protein, had food allergies associated with the product, were not willing to travel to the downtown ASU campus or could not be tested for grip or leg strength. Sample size was determined by comparing other literature with similar outcomes in lean body mass and strength as seen below. The study has been approved by the Institutional Review Board (IRB) and after explanation, written consent was signed and provided by each participant. Subjects were randomly assigned to the control or experimental group before arriving at the Arizona Biomedical Collaborative Building (ABC) and were enrolled after consent was signed.

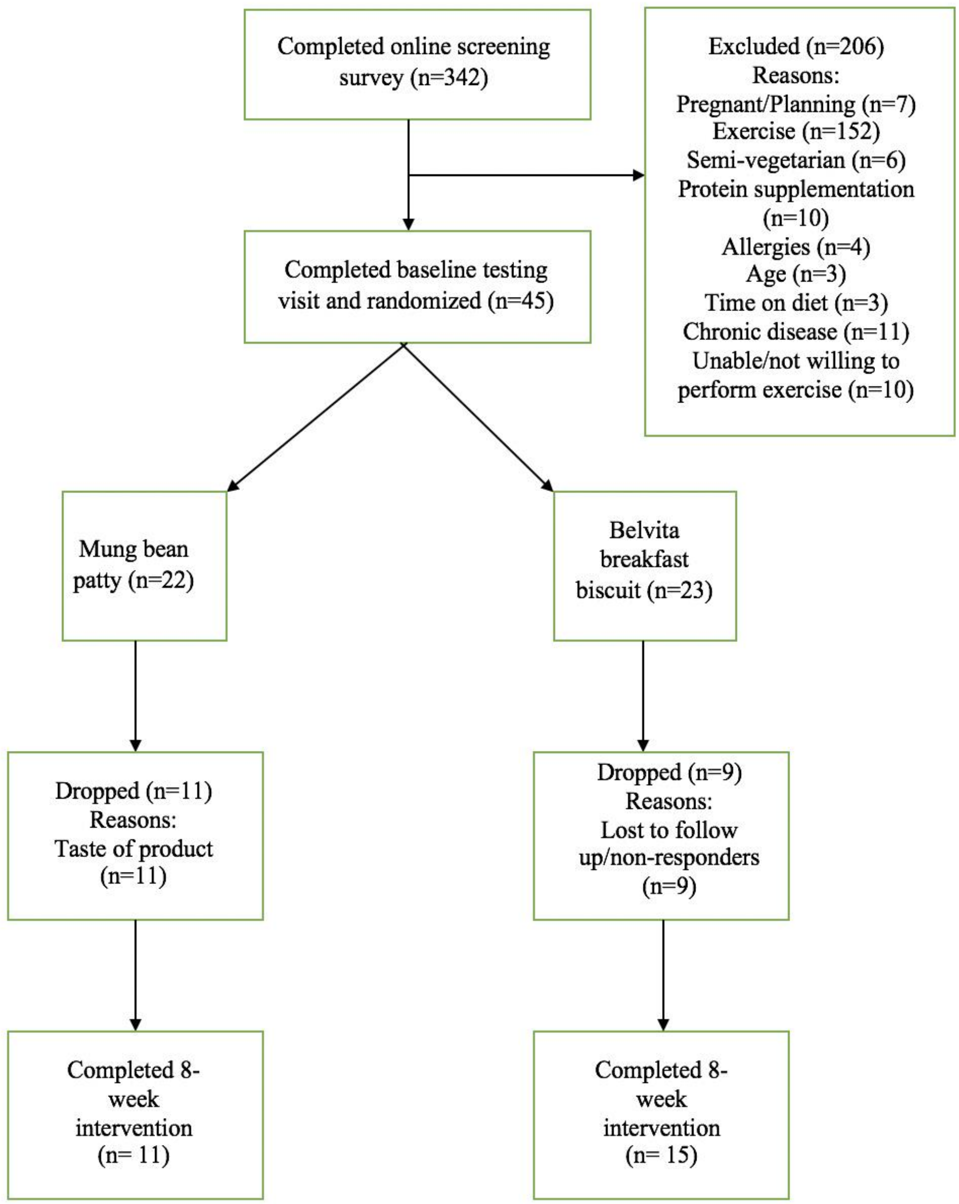
Recruitment was conducted around the Arizona State University (ASU) campus and Phoenix metropolitan area using flyers and Facebook posts to vegetarian and vegan organizations as well as word of mouth and list-serves to ASU students. The study used a parallel arm randomized controlled research design. The research team members who recorded measurements of the participants were blinded to groups; however, the primary investigator (PI) conducted the randomization and project coordinator supplied the food products to the participants. Participants were instructed to maintain a calendar over the eight-week trial period in which the product was to be consumed each day. Measurements were recorded pre-and-post supplementation at week 0 and week 8.

Table 1. Sample Size Determination: Studies used to calculate power for this study are listed along with their sample sizes, means, standard deviations (SD) or standard error of the mean (SEM), and study design.

Author	Article	Year	Mean ± SD/SEM	N per group	Calculated n per group	Subject state	Test
Lee et. al.	Body composition and nutrient intake of Buddhist vegetarians	2009	Vegetarians: 44.5 ± 3.75 Omnivores: 41.8 ± 5.1 Difference of means ± SD: 3 ± 4	85	58	Vegetarian Buddhist nuns Omnivorous Catholic nuns and college students	Parallel arm

V. Siani et. al.	Body composition analysis for healthy Italian vegetarians	2003	Vegetarians: 43.05 ± 8.39 Omnivores: 51.45 ± 8.77 Difference in means ± SD: 8.4 ± 8.5	30	36	Healthy Italian vegetarians, who had followed the diet their entire life and omnivores	Parallel arm
Aubertin-Leheudre et. al.	Relationship between animal protein intake and muscle mass index in healthy women	2009	Vegetarians: 18.2 ± 3.9 Omnivores: 22.6 ± 5 Difference in means ± SD: 4.4 ± 4.5	40	36	Healthy Caucasian women omnivores and vegetarians living in Helsinki	Parallel arm
				Average N=52	Average N=43		

Figure 1. Consort Flow Diagram



Independent Variable

The independent variable was the use of plant-based mung bean protein supplementation to elicit changes in lean body mass and strength. This was provided to experimental group participants in the form of two “egg replacement” patties per day.

This allotment, totaling an extra 21g/day, was given to the experimental group participants for daily use over an eight-week period. The product was supplied by JUST Foods corporation™. Participants in the experimental group were to consume the patties at breakfast as an iso-caloric substitute for their normal breakfast. Participants in the control group were supplied with an iso-caloric whole-grain, low protein (4g) Belvita™ breakfast bar to consume over the eight-week trial period. Participants were instructed to maintain their normal vegetarian or vegan diet, in addition to lifestyle patterns during the duration of the study.

Protocol procedures

The study protocol involved two visits to the downtown ASU campus that lasted approximately one hour each. During the first visit, participants were instructed to arrive fasted. They completed the informed consent document as well as a 24-hour dietary recall, healthy history and diet quality questionnaire. Anthropometric measurements were taken by trained laboratory professionals and include height, body weight, and waist circumference.

Height and weight were measured on a SECA™ stadiometer (Hamburg, Germany). Participants were instructed to remove excess clothing such as jackets and sweaters as well as shoes, stand straight with heels flat against the back of the stadiometer while the arm was moved flush with the top of the head and weight stabilized. Height was recorded in centimeters and weight in kilograms. Waist circumference was measured using a tension Creative Health Products™ tape measurer (Ann Arbor. MI) with the smallest part of the waist, slightly above the navel as the standard placement of the measuring tape. The measuring tape was pulled taut until equal tension was applied and the reading was recorded in inches.

A Tanita™ bioelectrical impedance test (Arlington Heights, IL) was performed to assess weight, fat mass, fat-free mass, body fat percentage, and BMI. The bioelectrical impedance analysis (BIA) measures body composition using a small electrical impulse which uses water as a medium to assess fat and fat free mass. Tissues and fluid such as blood with more water will conduct the current fasters, whereas fat tissue with less water will slow down the current. Participants were instructed once again to remove excess clothing such as jackets and sweaters, shoes, and socks. Age and height were entered into the BIA to obtain the reading. Age was reported as part of a standard health history questionnaire and the height measurement from the stadiometer was used. To assure equal measurements between subjects and testing visits, males and females were labeled as sedentary individuals and no weight was subtracted for clothing when completing the measurement.

Dual-energy x-ray absorptiometry (DXA) was also used to assess body composition. Due to minor radiation exposure, pre-menopausal female participants were then instructed to give a urine sample prior to the scan for a pregnancy test. The Ge Lunar iDXA™ (Chicago, IL) which is generally used to measure bone density by using radiation to produce a picture of the bones, was in this case used to assess lean body mass and fat mass. This procedure was conducted by a licensed radiology technician and involved the participant laying face up on a padded table for seven minutes while the entire body was scanned by the machine.

Additionally, a single-tube fasted venous blood draw was obtained for later assessment (data from which are not included in this thesis). After the blood draw, participants were instructed to rest for five minutes before obtaining resting blood pressure. Blood pressure was measured using the OMRON HEM-907XL™ device (Japan) with the participants sitting upright with feet flat on the floor and the arm extended on a flat surface.

Finally, strength tests were conducted using a Biodex™ multi-joint system dynamometer (Shirley, NY) and a grip-strength test. Written consent for participation in physical activity via a Physical Activity Readiness Questionnaire™ (PAR-Q) was completed before measurements of strength were taken. Lower body strength measurements included peak torque isokinetic flexion and extension at 150°, 120°, and 90° and peak torque isometric extension at 60°. The dynamometer uses isometric and isokinetic protocols to measure torque or force produced. Isometric exercise involves the muscle being contracted without movement of the joint. For this test, participants exerted force upon the machine’s arm, which was matched in a measurable way by the machine, which adjusts to press equal force back so as not to change the angle of the machine arm. For the isokinetic test, participants exerted force that results in motion of the arm but which is maintained at a constant speed throughout the range of motion regardless of the force applied. The Creative Health Products™ hand-grip strength dynamometer (Ann Arbor, MI) uses similar isometric measuring to assess hand-grip and forearm strength. This test was completed using three trial runs and averaging the three to compile a hand-grip strength measurement.

Following strength testing, the participants were introduced to the project coordinator where they received a calendar outlining the progression of the study, an allotment of patty supplements, and cooking instructions (if applicable). Participants were instructed not to begin any new medication(s) and to maintain their normal dietary patterns and exercise routine during the eight-week trial. Both groups were instructed to consume their supplement at breakfast as an iso-caloric replacement for another item. Participants randomized to the experimental group were instructed to consume two mung bean patties every day for eight weeks. Participants were instructed to mark on a calendar the days they consumed the food, were reminded they would not be penalized for not consuming the product, and should be forthcoming about the days they missed. Contact information was exchanged with the study coordinator for additional supplement pickup (if applicable) as well as to maintain contact for weekly check-ins throughout the study. Before departing, the participant scheduled their post-testing visit, eight weeks from day one and were instructed to return with their calendars.

Upon returning for post-testing, the participants were again asked to complete a 24-hour recall, diet quality questionnaire and a physical activity assessment that was measured at baseline in the health history questionnaire. The research team collected the participants completed calendars. Participants were then asked to complete an exit survey questionnaire assessing the taste and experience with their supplement. Contact information for payment of $120 was obtained for distribution of funds via check, cash, or Amazon gift card depending on participant preference. Post-testing measurements of height, weight, waist circumference and BIA followed the same procedure as baseline. Women were asked to give a urine sample before the DXA scan. Following anthropometric procedures, five-minute resting blood pressure was obtained before performing any strength testing. Post-testing measurements of hand-grip and lower body were the same as baseline. Following completion of strength testing, participants signed a release form for their results and copies were made of any available data the participant wished to receive.

Figure 2. Visit Flowchart

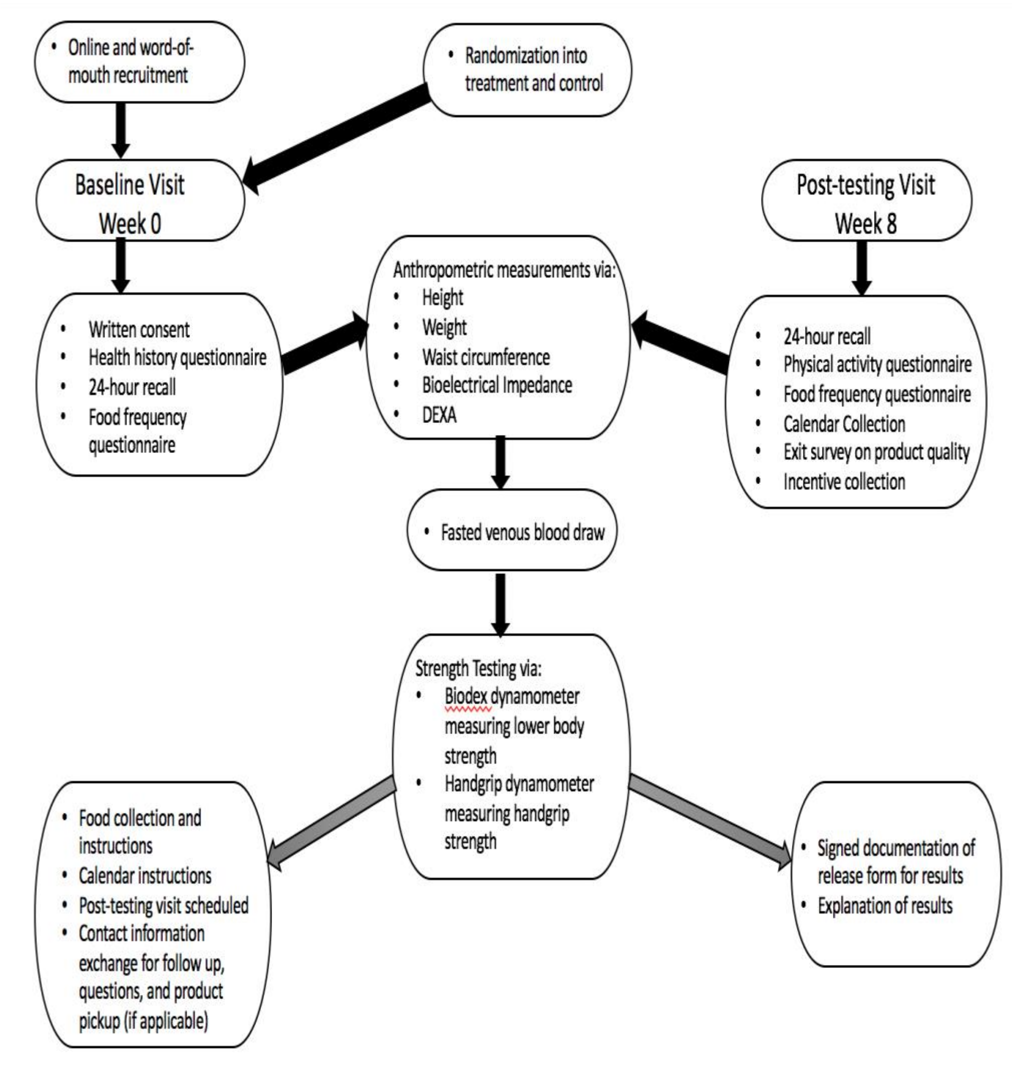


Table 2. Mung Bean Protein Isolate Amino Acid Composition[46]

Phenylalanine 90.3	Tyrosine 90.3	Leucine 74	Lysine 62.4	Valine 46.3	Histidine 27.9
Cysteine 13	Tryptophan 6.4	Glutamine 125.4	Asparagine 85.3	Arginine 64.4	Serine 38.5
Proline 30	Methionine 13	Glycine 32.2	Threonine 28.4	Alanine 36.6	Isoleucine 39.1

*Protein isolates given as a mg/g^{-1}

Laboratory Equipment

- Height and weight - SECA™ stadiometer (Hamburg, Germany).
- Body composition - Tanita™ bioelectrical impedance test (Arlington Heights, IL)
- Blood pressure - OMRON HEM-907XL™ device (Japan)
- DXA - Ge Lunar iDXA™ (Chicago, IL)
- Lower body strength - Biodex™ multi-joint system dynamometer (Shirley, NY).
- Hand-grip - Creative Health Products™ hand-grip strength dynamometer (Ann Arbor, MI)

Statistical Analysis

Statistical analysis of the sample was conducted using IBM SPSS Statistical Analysis software version 25 and all data are expressed as the mean ± the standard deviation. Data was assessed at baseline using an independent t-test to measure differences between the control and treatment group. Before the data was processed, normality testing was run and all non-normal data were attempted to be transformed but nonparametric testing was ultimately run. The data were then run through Mann-Whitney U tests, using a significance level of ($p<0.05$) to assess the differences between control and treatment over the eight-week trial period from baseline.

CHAPTER 4

RESULTS

Baseline demographics are presented below. Normality tested showed non-normal distribution; therefore; non-parametric Mann-Whitney tests show no group differences for any baseline characteristics.

Table 3. Characteristics of groups at baseline

	Control	Experimental	P value
Male/Female	1/14	1/10	0.822
Age (yr)	34.1 ± 10.6	34.1 ± 6.4	0.856
Weight (kg)	66.9 ± 16.4	68.0 ± 17.9	0.775
Height (cm)	166.8 ± 7.8	162.2 ± 7.6	0.102
BMI (kg/m^2)	23.9 ± 4.7	25.7 ± 6.1	0.678
Waist Circumference (cm)	30.7 ± 5.1	31.8 ± 5.1	0.516
METS (1kcal/kg/hr)	32.7 ± 19.5	27.0 ±16.7	0.275

*Data is presented as mean ± SD. P value represents Mann-Whitney.

Of the forty-five participants who were randomized and stratified, twenty-two were placed in the mung bean patty group and twenty-three in the Belvita™ group. Eleven participants dropped from the experimental group and nine from the control group before post-testing was completed (Figure 1.) Mann-Whitney tests show no significant differences between experimental and control groups after completing the intervention in lean body mass (Table 2), lower body strength expressed in both absolute (Table 3) and relative force (Table 4) or grip strength (Table 5).

Significant difference existed between baseline METS (32.7 ± 19.5 EXP, 27.0 ±16.7 CON) for the current study and guidelines from the Office of Disease Prevention and Health Promotion (ODPHP) (500-1,000 MET minutes) for the average age of participants (p=0.000).

Table 4. Body Composition as measured by fat mass, lean mass, and visceral fat*

		Control	Experimental	P value	Effect size
Fat mass (kg)	Pre	23.71 ± 8.86	25.57 ± 10.89		
	Post	23.57 ± 8.88	26.15 ± 9.43		
	Change	-0.14 ± 1.15	0.56 ± 1.76	0.471	0.023
Lean mass (kg)	Pre	40.39 ± 9.43	39.71 ± 8.28		
	Post	40.39 ± 9.27	40.77 ± 8.13		
	Change	0.007 ± 0.69	0.78 ± 0.86	0.437	0.002

Visceral mass (kg)	Pre	0.47 ± 0.49	0.57 ± 0.42		
	Post	0.46 ±0.51	0.59 ±0.39		
	Change	-0.08 ± 0.96	0.22 ± 0.82	0.405	0.06

*Values are reported as mean ± SD. P value indicates Mann-Whitney testing.

Table 5. Absolute lower body strength*

Peak Torque (N-M)		Control	Experimental	P value	Effect Size
90° flexion	Pre	77.8 ± 27.7	72.4 ± 19.9		
	Post	70.8 ± 23.7	73.9 ± 18.4		
	Change	-5.9 ± 18.9	1.5 ± 6.4	0.218	0.064
90° extension	Pre	105.7 ± 43.3	64.7 ± 19.8		
	Post	90.1 ± 36.3	98.0 ± 33.7		
	Change	-11.9 ± 22.3	1.2 ± 14.1	0.90	0.110
120° flexion	Pre	64.7 ± 19.8	71.8 ± 29.5		
	Post	64.1 ± 22.1	67.3 ± 18.7		
	Change	0.1 ± 5.5	-4.5 ± 16.1	0.681	0.042

120° extension	Pre	91.7 ± 36.5	90.6 ± 27.8		
	Post	83.4 ± 35.2	91.1 ± 30.5		
	Change	-4.3 ± 7.5	0.5 ± 11.3	0.250	0.068
150° flexion	Pre	53.9 ± 17.1	57.2 ± 17.9		
	Post	57.1 ± 21.1	61.7 ± 17.0		
	Change	4.2 ± 7.9	4.5 ± 5.7	0.827	0.001
150° extension	Pre	75.3 ± 29.5	82.3 ±31.9		
	Post	73.3 ± 32.4	83.3 ± 24.7		
	Change	0.6 ± 11.2	1.0 ± 11.9	0.848	0.000
Isometric	Pre	136.9 ± 60.7	141.5 ± 43.2		
	Post	128.1 ± 59.0	142.8 ± 52.9		
	Change	-5.2 ± 31.5	1.3 ± 17.5	0.494	0.16

*Values are reported as mean ± SD. P value indicates Mann-Whitney testing.

Table 6. Relative lower body strength*

(Peak Torque/BW)		Control	Experimental	P value	Effect Size
90° flexion	Pre	110.8 ± 22.9	107.8 ± 20.5		
	Post	109.6 ± 22.9	111.3 ± 19.2		
	Change	-2.5 ± 10.2	3.4 ± 11.1	0.112	0.077

90° extension	Pre	149.7 ± 38.4	142.5 ± 26.9		
	Post	139.2 ± 38.1	144.7 ± 34.0		
	Change	-9.7 ± 19.4	2.2 ± 18.0	0.125	0.097
120° flexion	Pre	97.5 ± 19.4	106.7 ± 36.3		
	Post	99.3 ± 22.3	100.5 ± 18.5		
	Change	0.3 ± 8.5	-6.2 ± 23.6	0.547	0.038
120° extension	Pre	136.6 ± 35.1	133.0 ± 21.3		
	Post	128.6 ± 37.0	135.3 ± 30.7		
	Change	-7.1 ± 11.4	2.3 ± 13.1	0.139	0.137
150° flexion	Pre	81.5 ± 17.8	85.1 ± 20.1		
	Post	88.3 ± 20.9	92.2 ± 17.4		
	Change	5.8 ± 10.5	7.1 ± 8.1	0.827	0.005
150° extension	Pre	112.8 ± 29.0	120.6 ± 32.3		
	Post	113.1 ± 34.9	124.1 ± 20.8		
	Change	0.4 ± 15.6	3.5 ± 19.1	0.827	0.009
Isometric	Pre	192.6 ± 52.1	197.7 ± 40.5		
	Post	188.2 ± 58.5	204.4 ± 53.8		
	Change	-5.5 ± 35.4	6.6 ± 22.8	0.687	0.041

*Values are reported as mean ± SD. P value indicates Mann-Whitney testing.

Table 7. Grip Strength*

		Control	Experimental	P value	Effect Size
Hand Grip (kg)	Pre	26.51 ± 9.17	24.19 ± 5.46		
	Post	24.42. ± 11.33	24.69 ± 4.49		
	Change	-2.09 ± 7.75	0.50 ± 2.12	0.203	0.046

*Values are reported as mean ± SD. P value indicates Mann-Whitney testing.

Individual change in lean body mass is shown for both the control (Figure 2) and experimental (Figure 3) below.

Figure 3. Individual change characteristics in lean body mass in control group

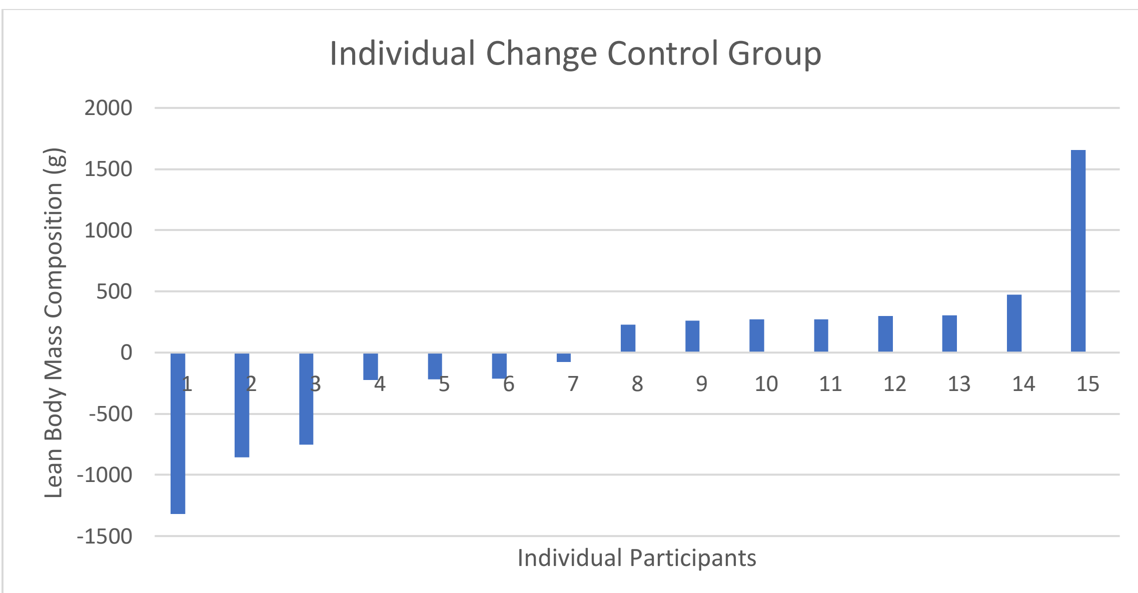
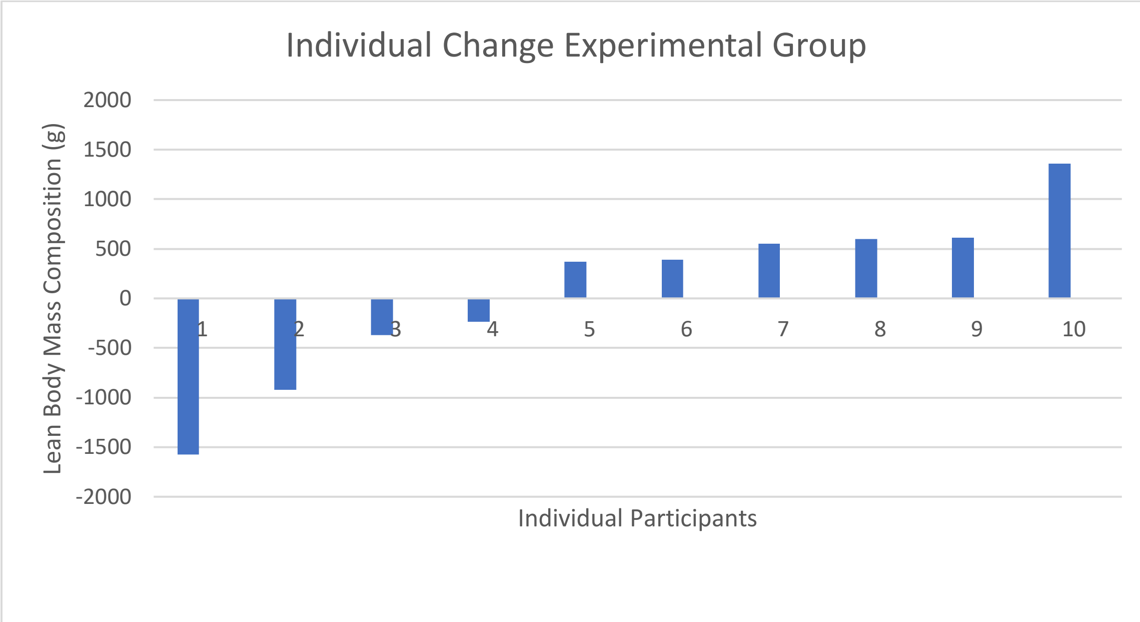
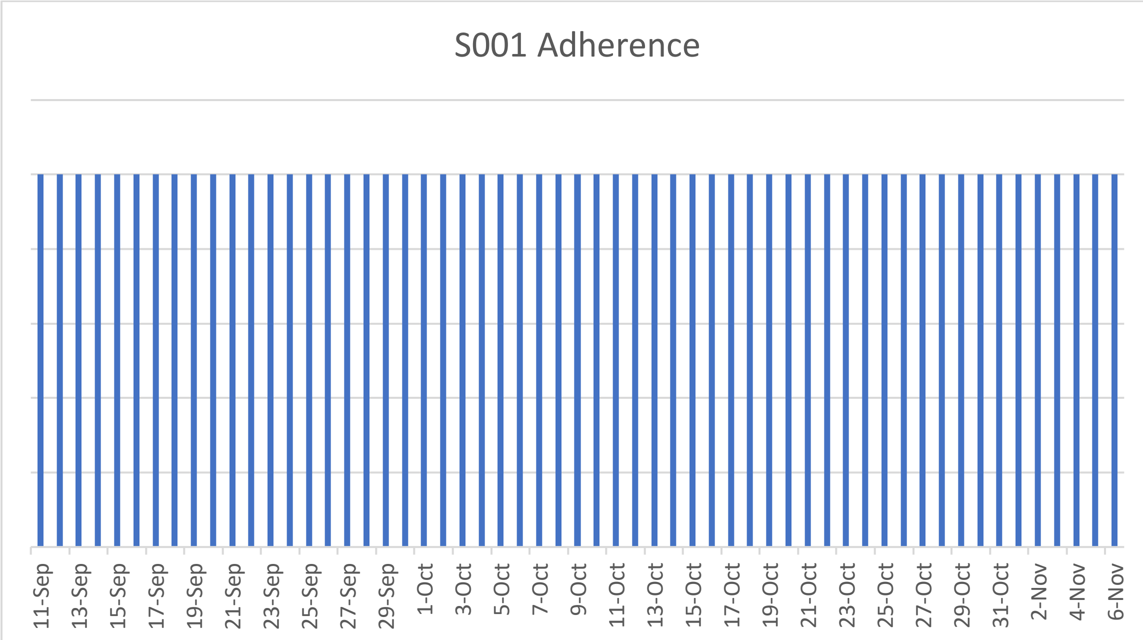


Figure 4. Individual change characteristics in lean body mass in experimental group



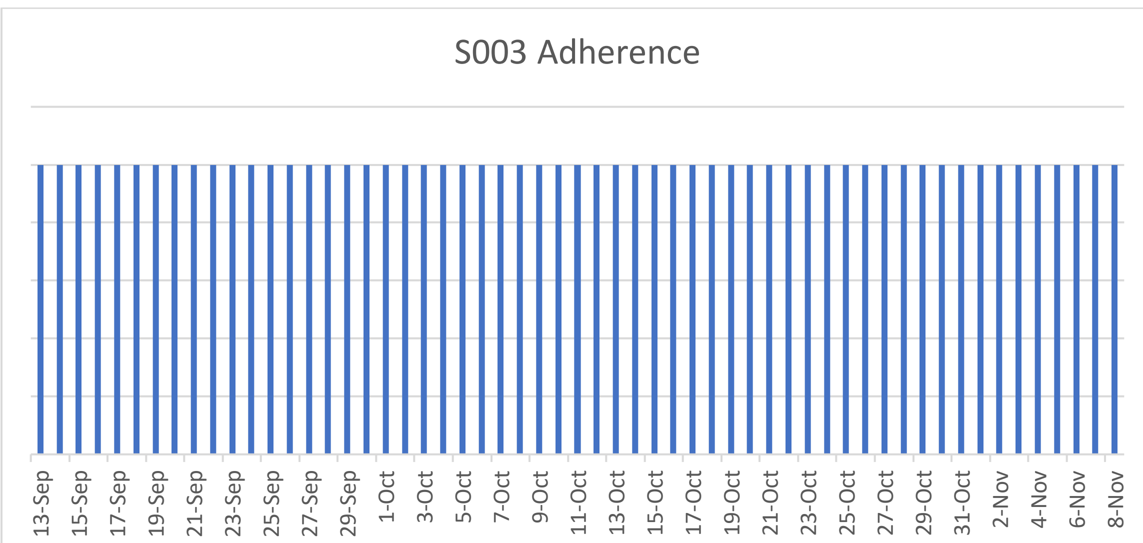
Additionally, participant adherence rates between groups varied as low as 28% and as high as 100%. Adherence for the control group was 82% whereas the experimental group averaged 84%. One participant from the experimental group failed to return a calendar. Individual participant adherence is shown below (Figures 4-28). Each line represents one day of adherence, individual adherence is reported within the graphs.

Figure 5. S001 Participant Adherence*



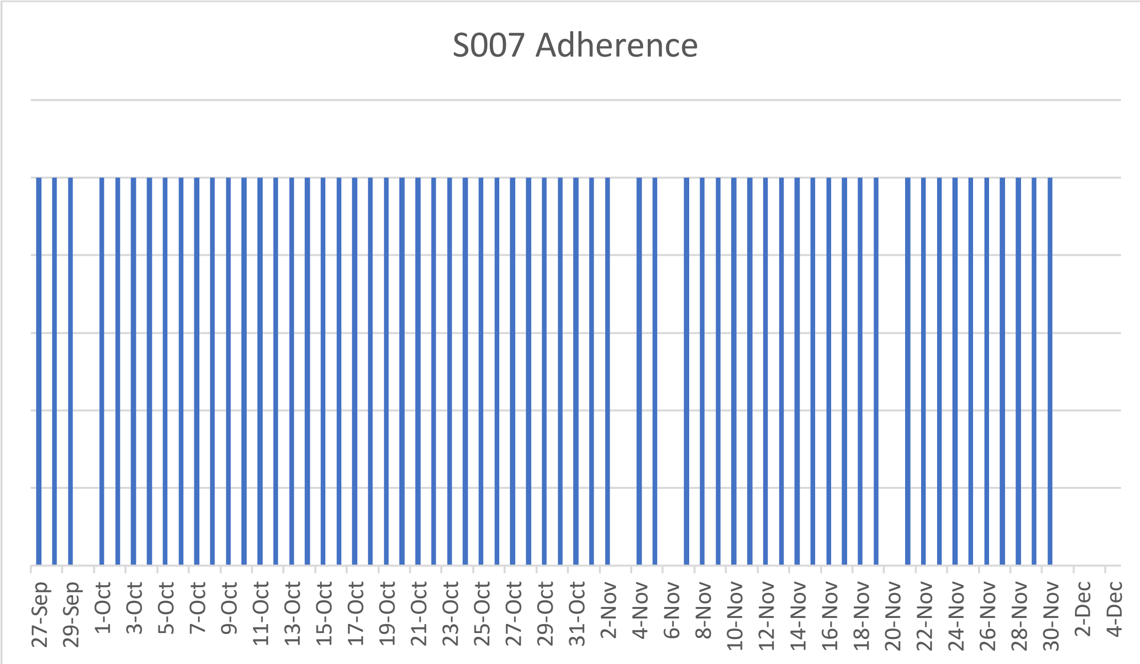
*100% adherence (exp)

Figure 6. S003 Participant Adherence*



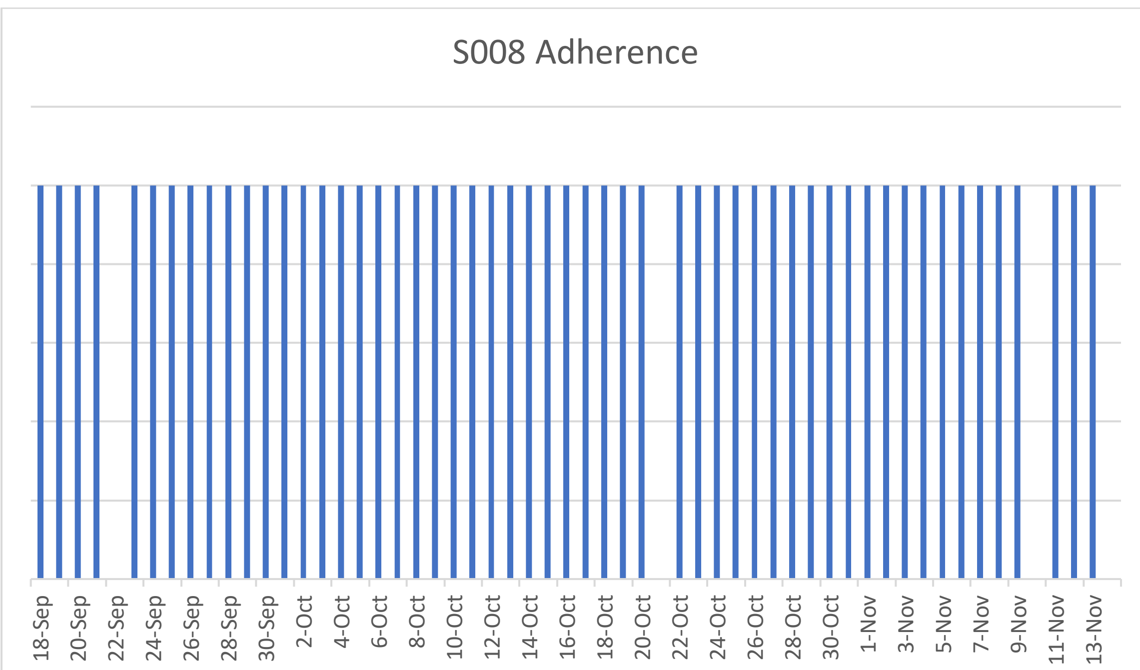
*100% adherence (con)

Figure 7. S007 Adherence*



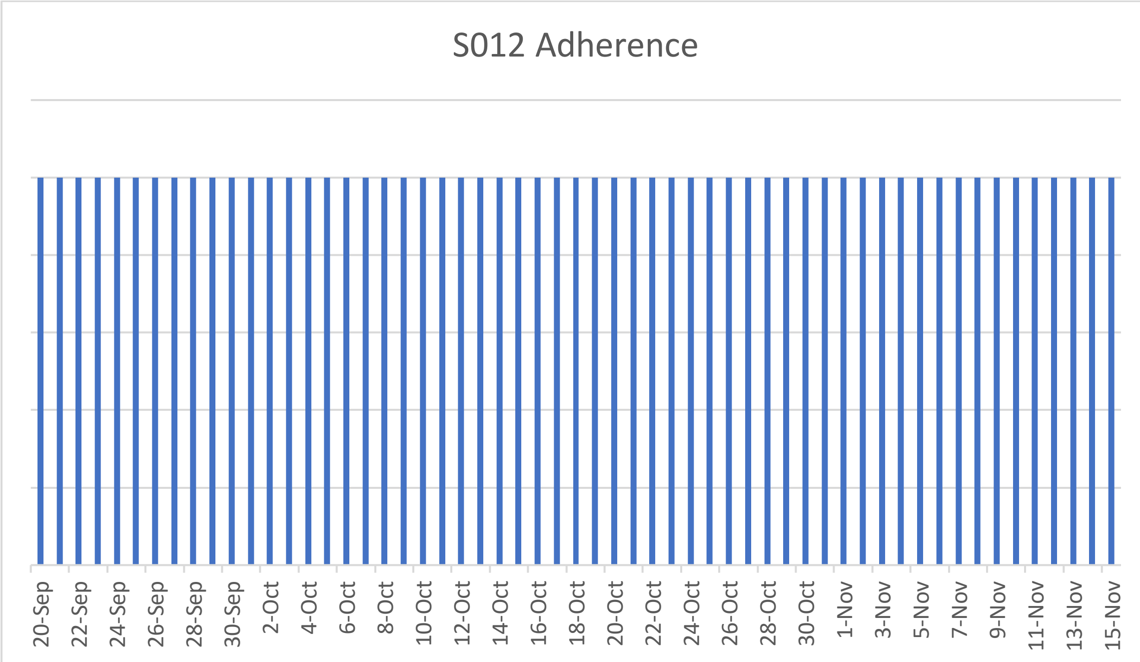
*88% adherence (con)

Figure 8. S008 Adherence*



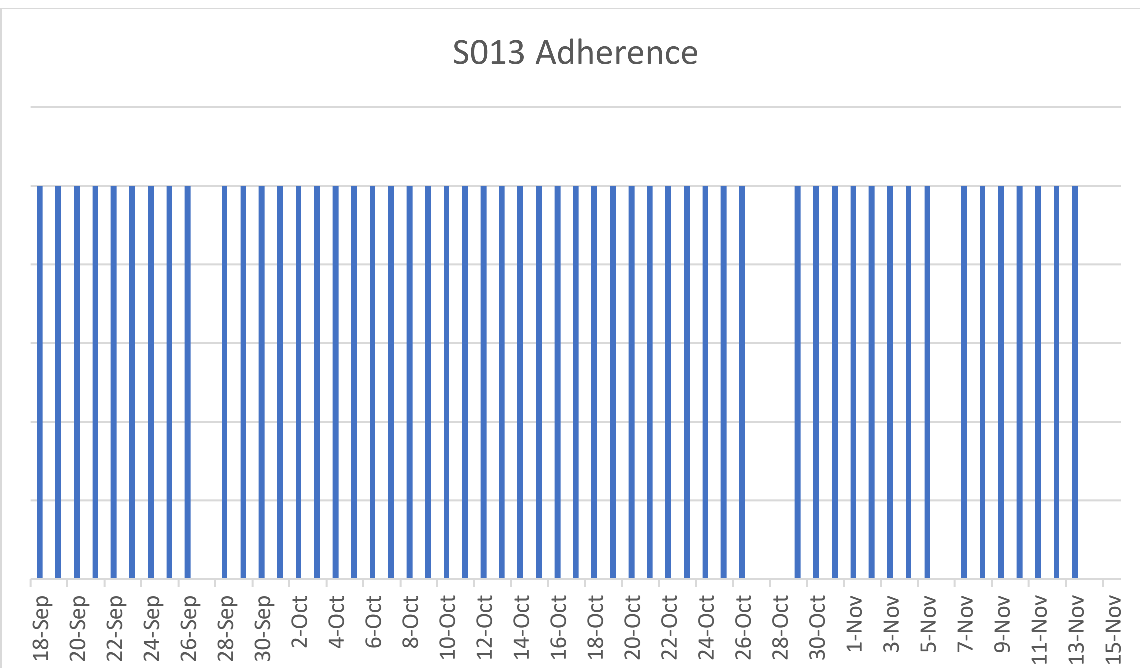
*93% adherence (con)

Figure 9. S012 Adherence*



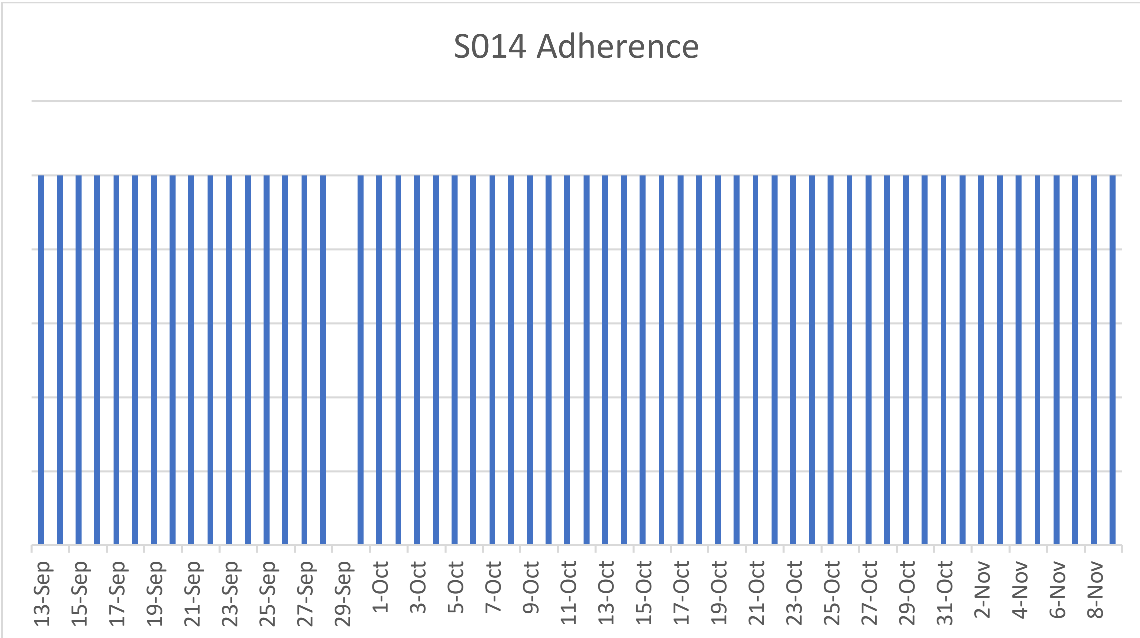
*100% adherence (con)

Figure 10. S013 Adherence*



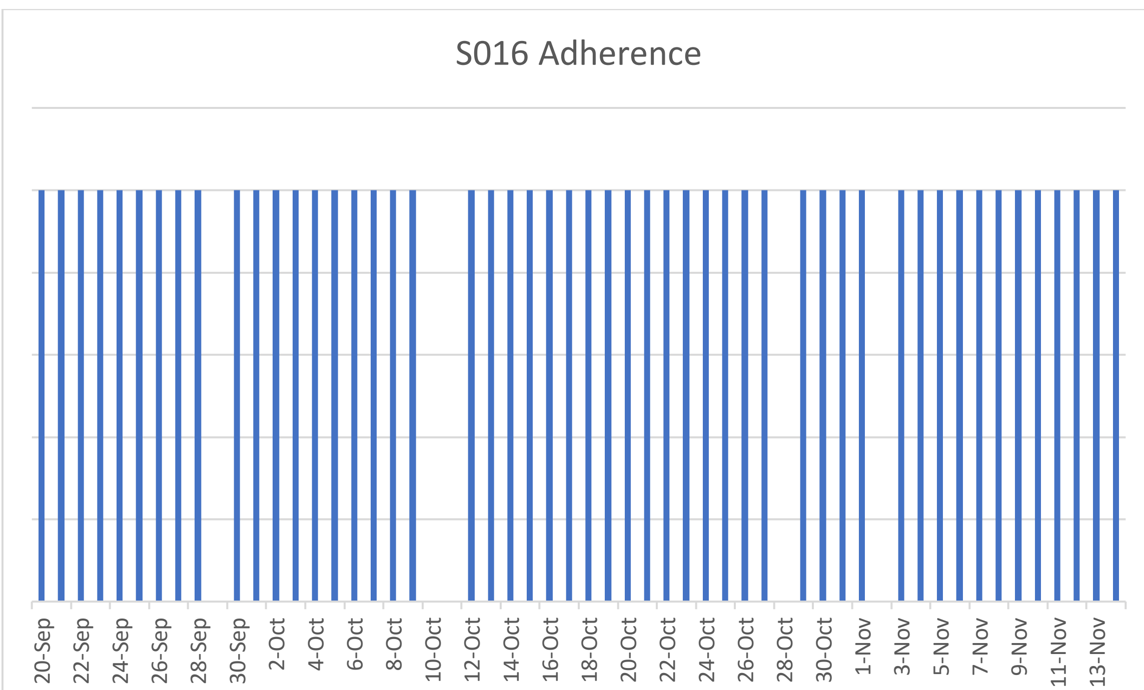
*90% adherence (exp)

Figure 11. S014 Adherence*



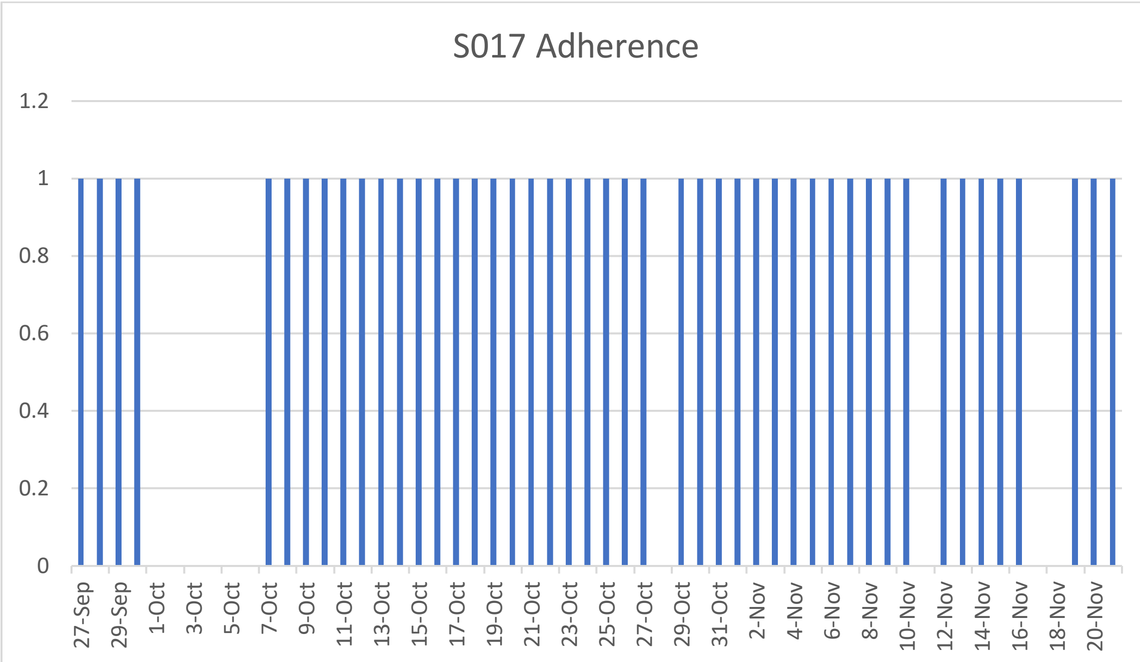
*98% adherence (con)

Figure 12. S016 Adherence*



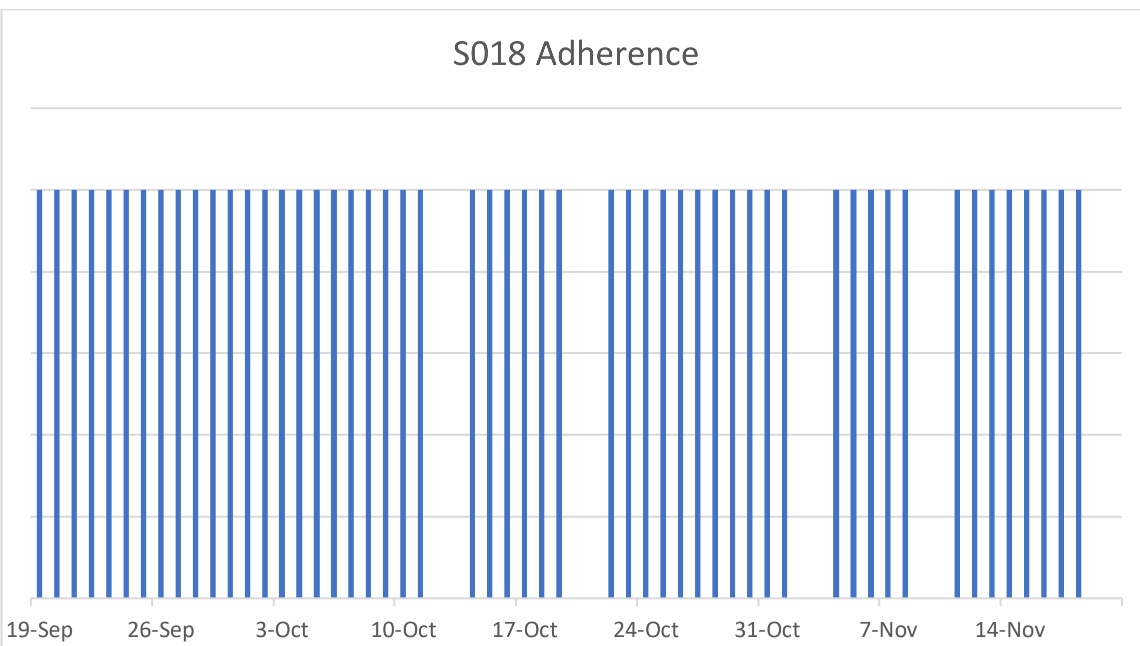
*91% adherence (con)

Figure 13. S017 Adherence*



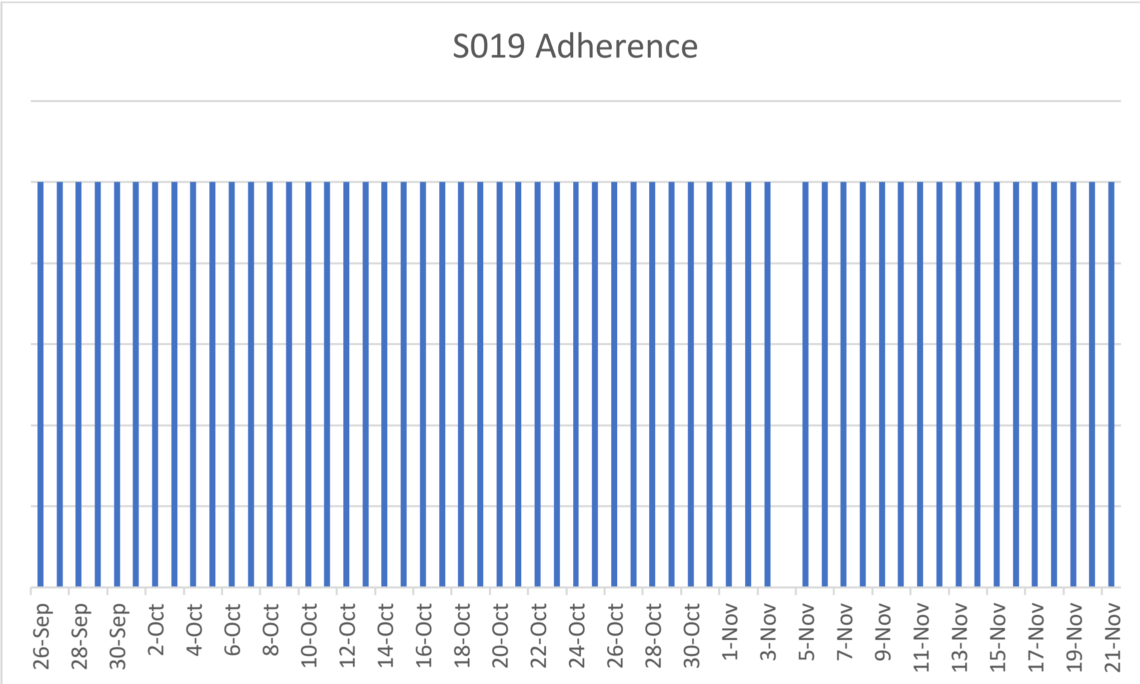
*82% adherence (exp)

Figure 14. S018 Adherence*



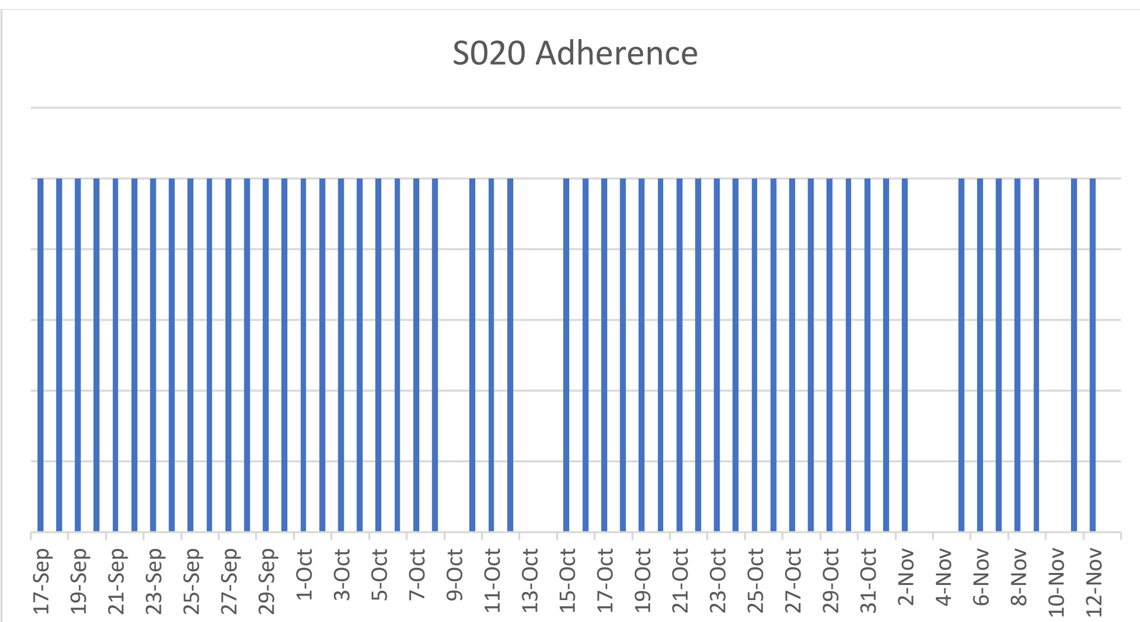
*84% adherence (exp)

Figure 15. S019 Adherence*



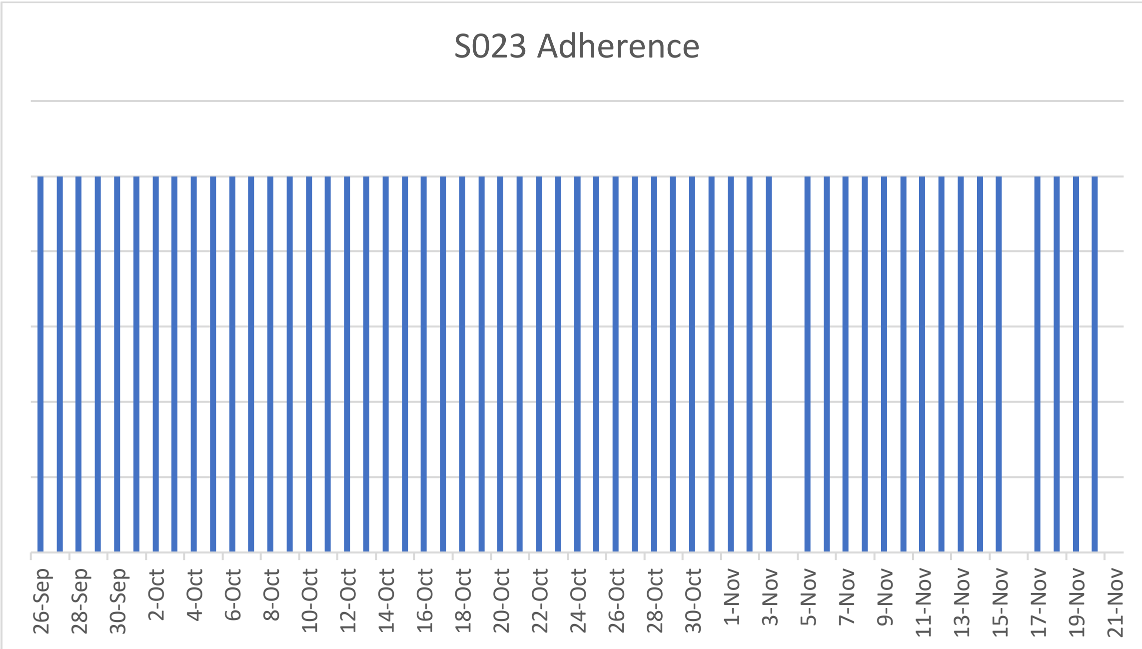
*98%adherence (con)

Figure 16. S020 Adherence*



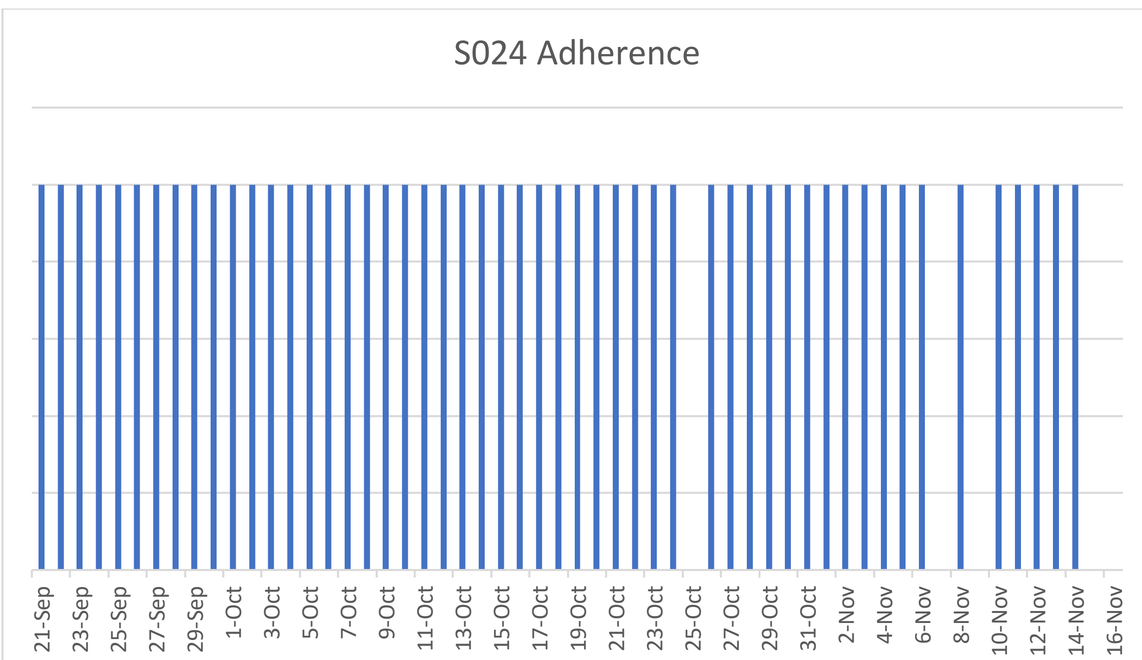
*88% adherence (con)

Figure 17. S023 Adherence*



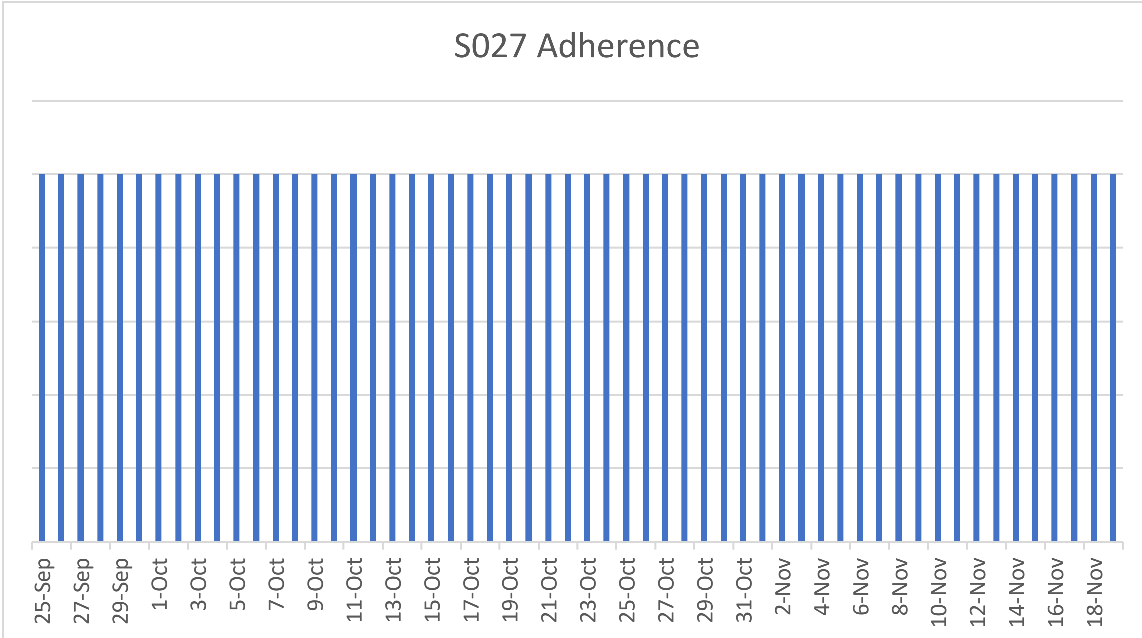
*95% adherence (con)

Figure 18. S024 Adherence*



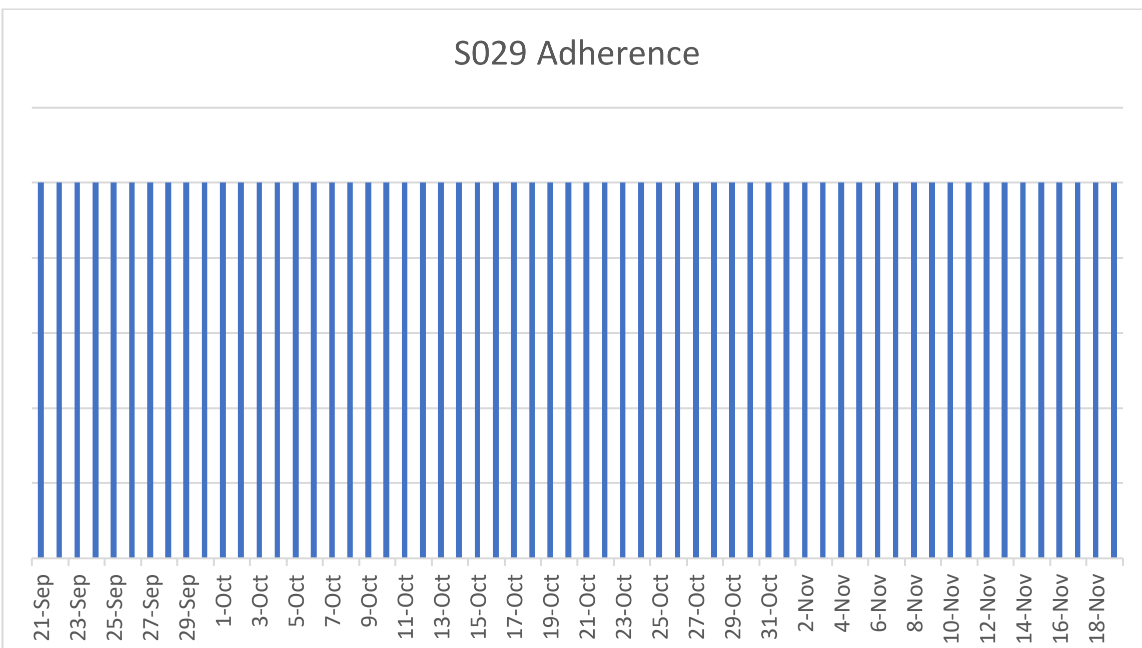
91% adherence (exp)

Figure 19. S027 Adherence*



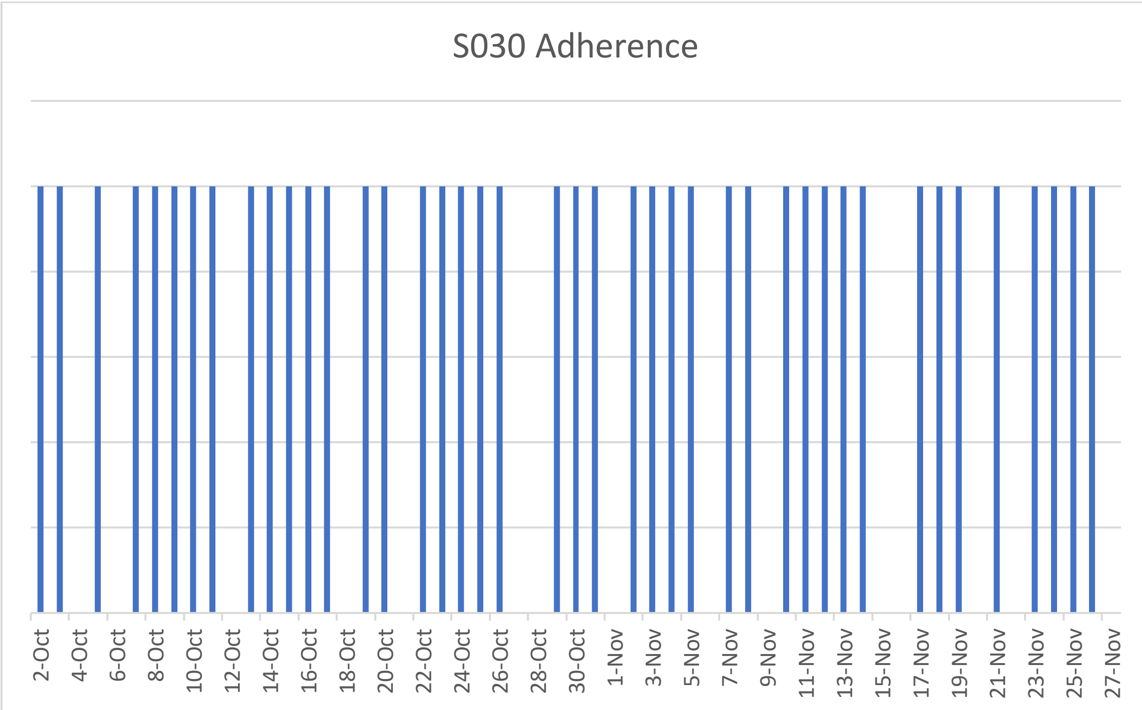
*100% adherence (exp)

Figure 20. S029 Adherence*



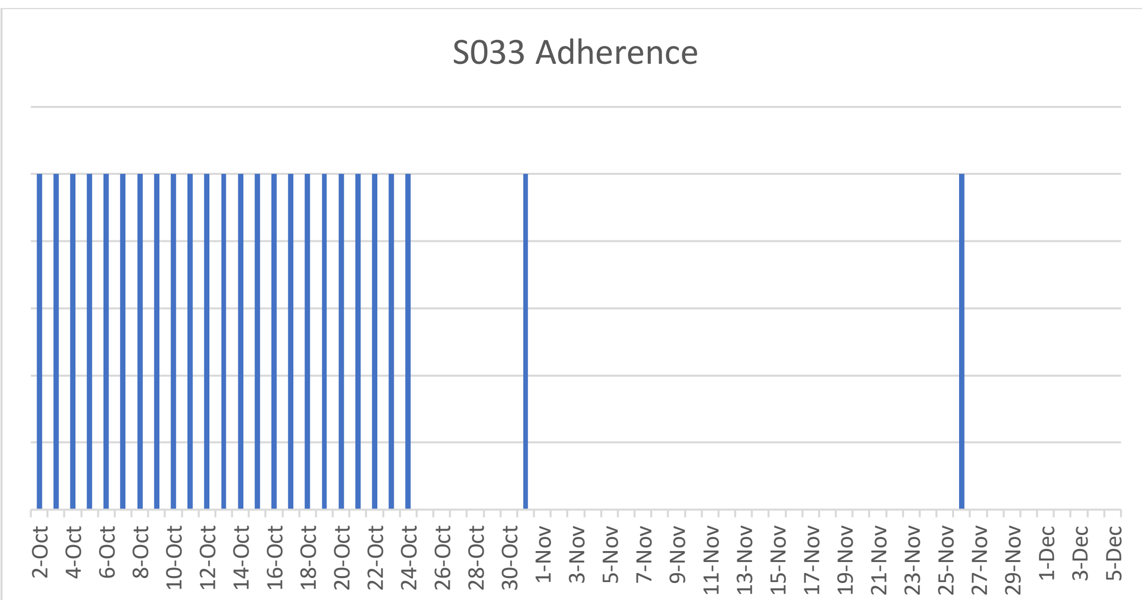
*100% adherence (exp)

Figure 21. S030 Adherence*



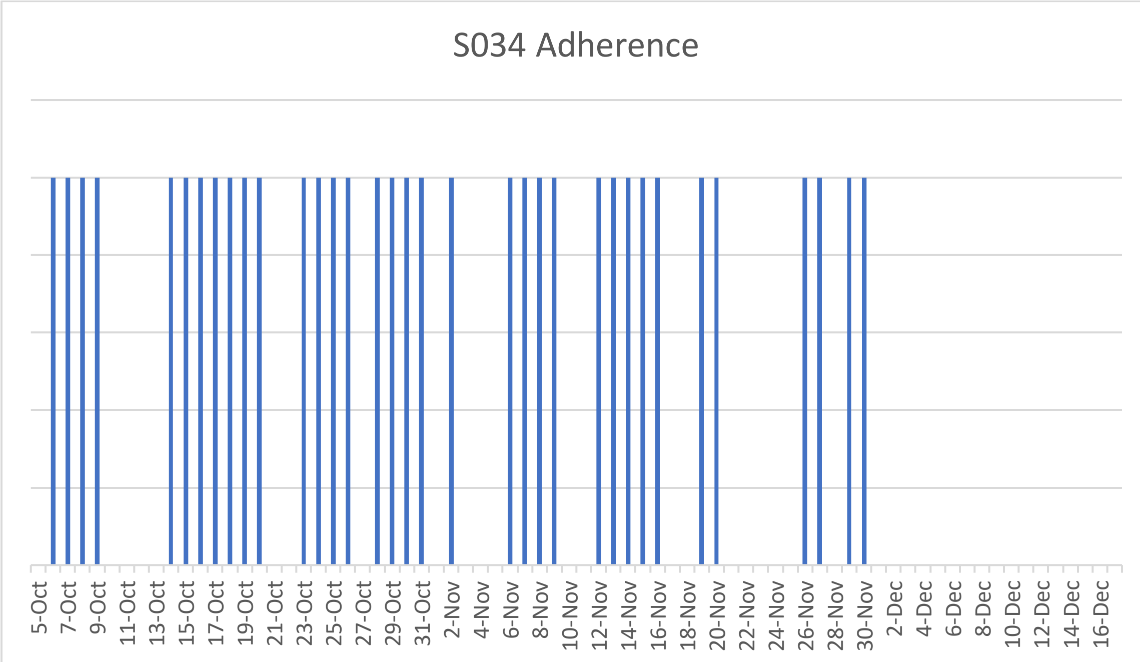
*74% adherence (con)

Figure 22. S033 Adherence*



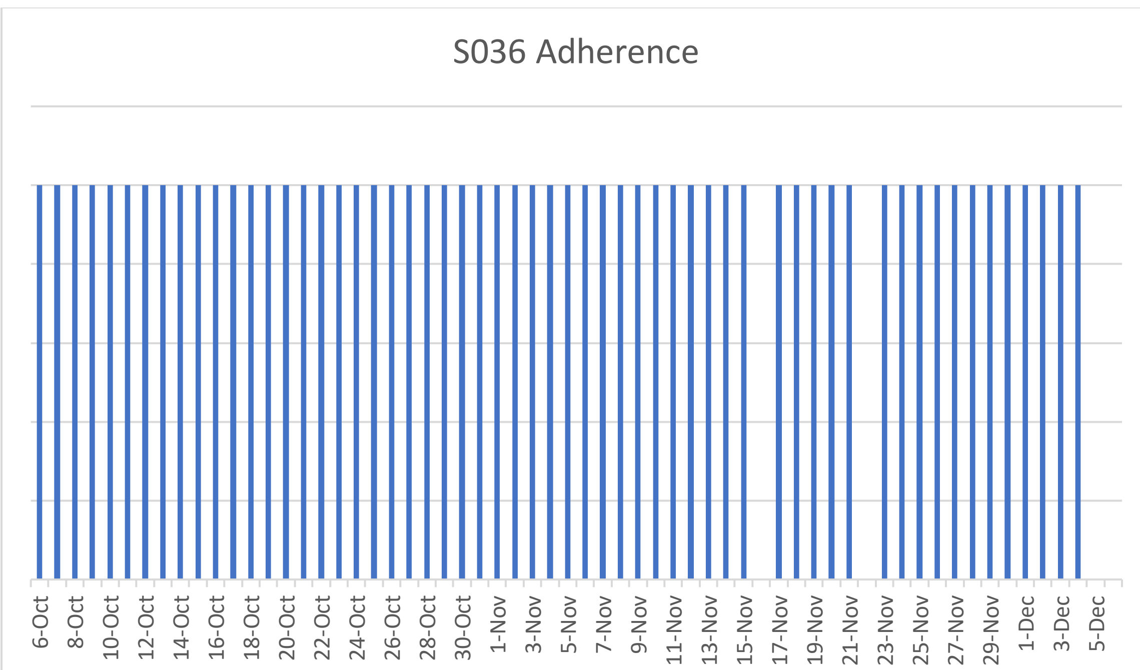
*38% adherence (exp)

Figure 23. S034 Adherence



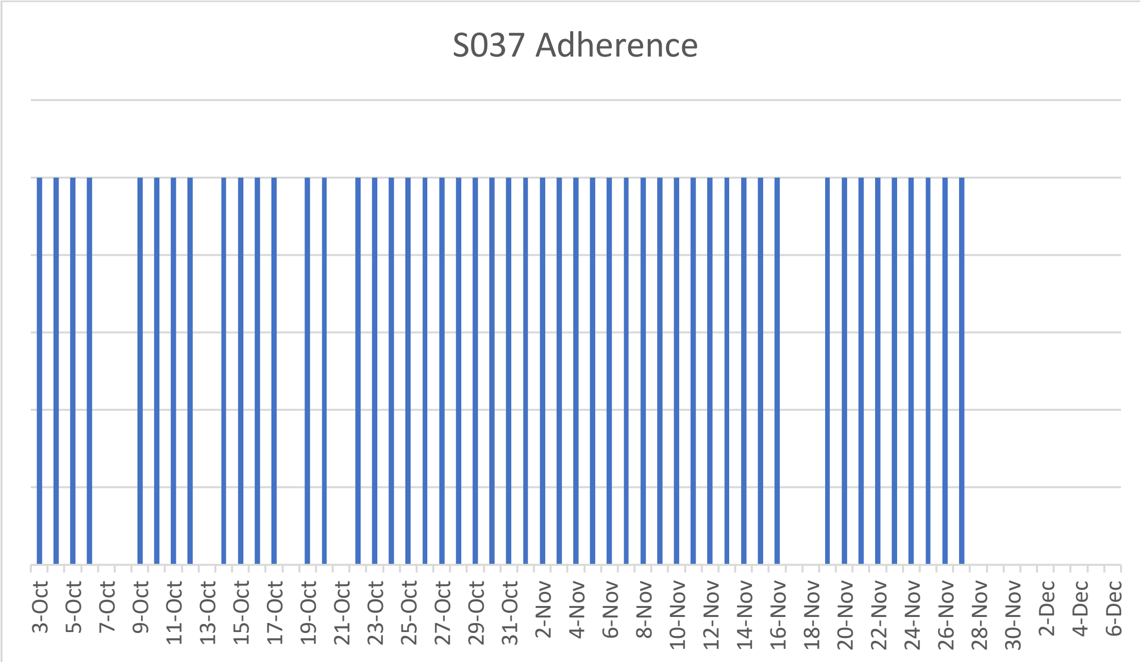
*47% adherence (con)

Figure 24. S036 Adherence



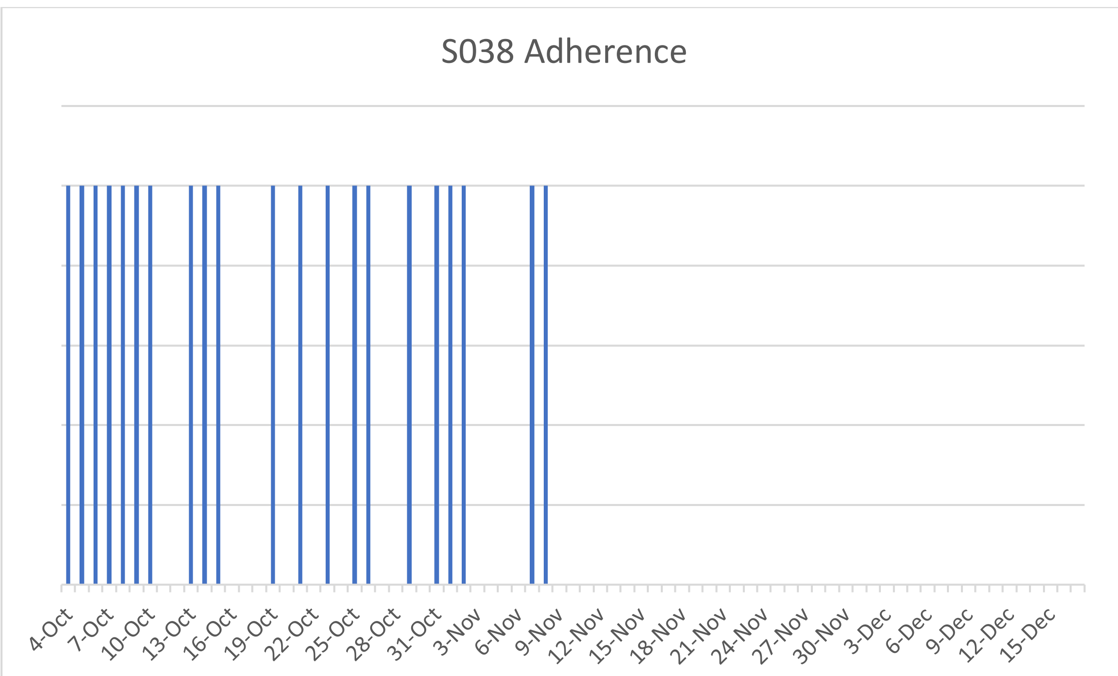
*94% adherence (con)

Figure 25. S037 Adherence



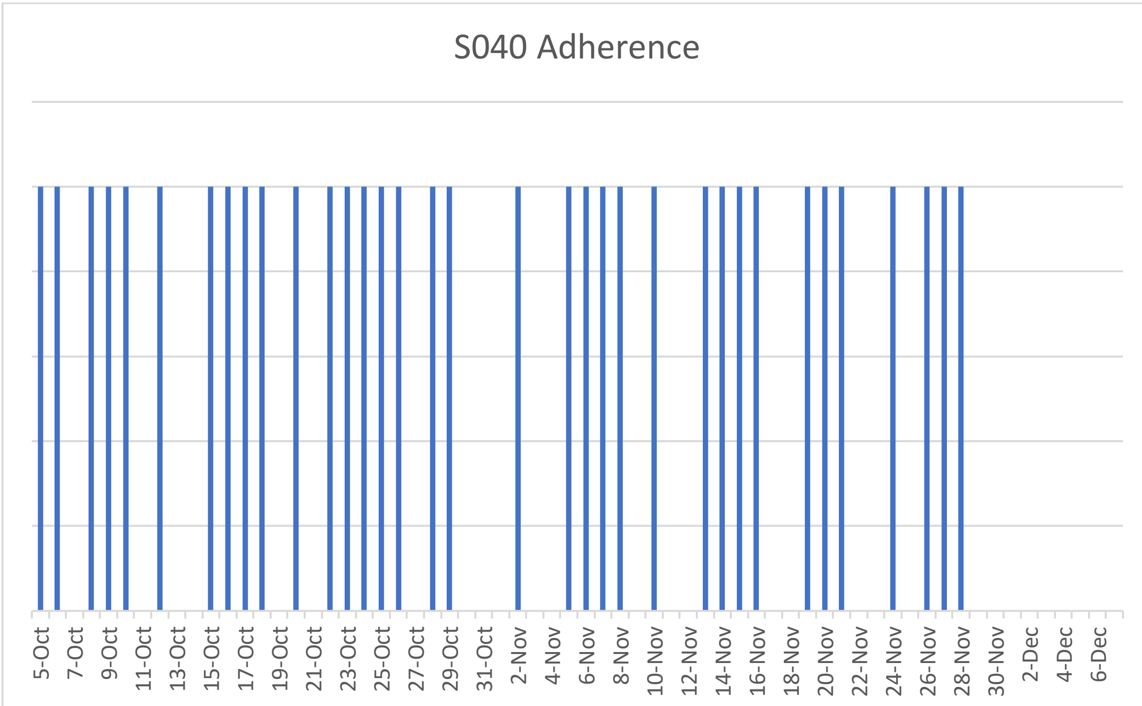
*75% adherence (exp)

Figure 26. S038 Adherence*



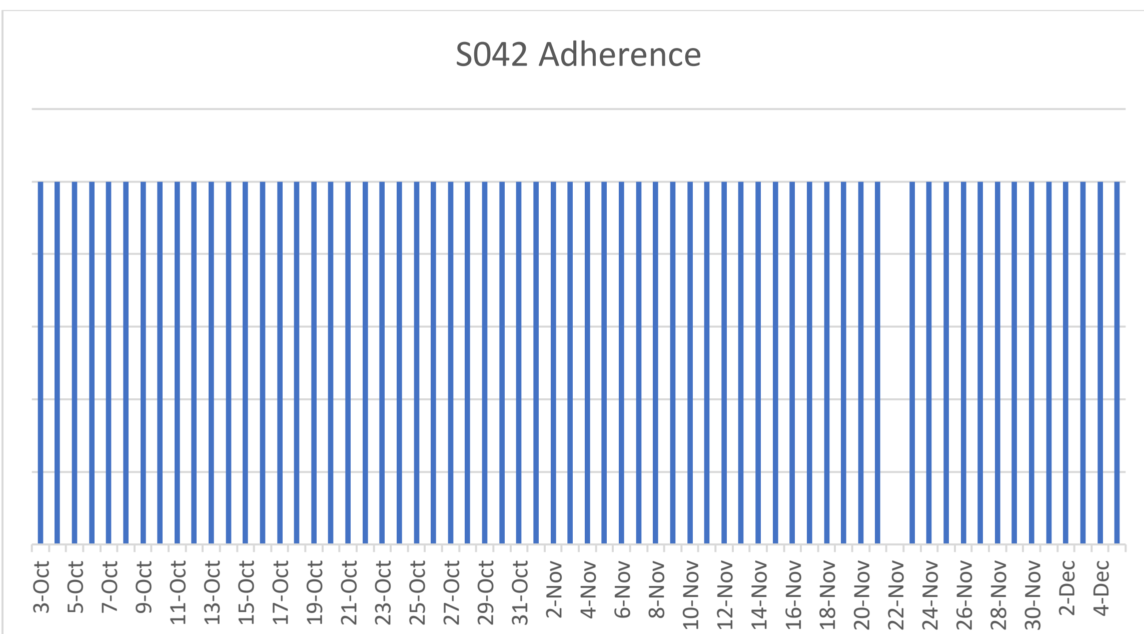
*28% adherence (con)

Figure 27. S040 Adherence*



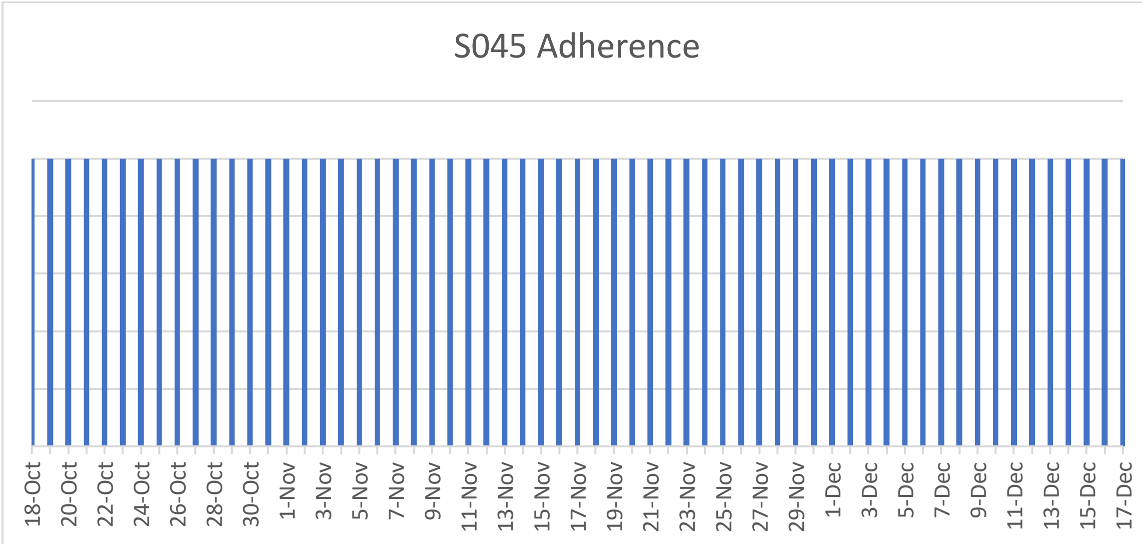
*55% adherence (exp)

Figure 28. S042 Adherence*



98% adherence (con)

Figure 29. S045 Adherence*



*100% adherence (con)

After conducting a post-hoc power analysis using G power software, with 0.05 alpha level, an effect size of 0.5, and total sample of 26, results indicated an observed power of 0.22.

CHAPTER 5

DISCUSSION

To date, much of the literature focused on protein intake as it relates to lean body mass or strength in vegetarians and omnivores compares plant-based protein sources to animal-based sources or relates the two using resistance exercise interventions.[1-3] It is known that increased protein intake in conjunction with resistance training can lead to increases in lean body mass and strength; and animal-based protein sources may induce higher increases in both areas compared to plant-based sources. However, little to no research exists examining the effects of increased plant-based protein in vegetarian and vegans compared to those not exercising regularly.

The current literature that shows the importance of protein intake in those not exercising focuses on individuals in energy deficits, the obese, or elderly populations.[4-6] Ingestion of high-quality protein intake spread throughout the day at 20-30g per meal showed decreases in loss of muscle tissue during times of energy deficit.[4] Similarly, obese individuals undergoing bariatric surgery saw significant preservation of lean body mass after surgery when supplementing with 30g of additional protein during a six month period ($p=0.05$) compared to those not supplementing.[5] Sarcopenia, the loss of lean body mass associated with the aging process, may be reduced with increased protein intake.[6] Elderly men and women supplementing with 210g/day of ricotta cheese spread through the day, providing 15.7g of protein, for two weeks showed twice as much lean body mass gains as those maintaining the habitual diets.[6] These studies examined the effects of high-quality animal-based protein ingestion as it related to preservation and increases in lean body mass. However, vegetarian and vegan individuals may benefit from research utilizing plant-based sources to elicit similar responses due to their decreased ability to maintain muscle mass.[7] Most studies use whey protein to examine lean body mass changes due to its high leucine content, a possible key regulator in muscle protein synthesis pathways, suggesting vegetarian and vegan populations should increase plant-based food sources to match leucine content in whey when attempting to elicit similar changes in lean body mass.[7]

The present study not only targets the issue of plant-based protein as it may relate to changes in lean body mass but also considers changes in lean body mass and strength in those not resistance training. Additionally, to the knowledge of the authors, this study may be one of the first examining an incomplete, non-soy protein as an alternative source to plant-based dietary protein for vegetarians and vegans. While there were no significant differences between control and experimental groups, small gains in lean body mass were observed in the experimental group as well as minimal fat losses. Additionally, various measures of strength in absolute and relative lower body measures as well as hand grip showed small losses in the control group and gains in the experimental group. These small changes may indicate the need for further research in this area of study.

Limitations

This study is no different than others in that many important limitations existing preventing significant findings. The research team emphasized for the participants to maintain their usual diet and exercise regimen and abstain from any new medications or supplements. Verification of adherence to diet and exercise was reliant on two 24-hour recalls and physical activity questionnaires at baseline and post-testing. Abstention of supplements and medications was also reliant on self-report. The inability to objectively measure compliance to consumption of the products was due to the use of a calendar in which participants marked the number of days they consumed their product.

Perhaps the largest two potential limitations of the study were the lack of qualifiers and lack of completers due to taste and acceptability of the product. Many people know the benefits of engaging in regular physical activity which disqualified almost half of the responders. Additionally, people consuming a vegan diet are educated on the risks of nutrient deficiency and regularly consume protein supplements also adding to reduced number of qualifiers. The estimated sample size to see an effect was fifty and only forty-five were enrolled; of that twenty-six completed with only eleven receiving the treatment, decreasing the effect size.

Finally, other questions remained regarding plant-based protein source and amount needed to elicit changes without exercise as well as duration of the intervention. Notably, Vliet et. al. believes that if plant-based protein intake is ingested at >30g/meal in conjunction with resistance changes, similar responses in lean body mass and strength can be seen in vegetarians and vegans compared to omnivores ingesting animal-based sources undergoing training.[13] Therefore, this idea can be expanded on without the addition of exercise training.

Generalizability

The present study did include both males and females, but only two of the twenty-six participants were male. Therefore, the results are more generalizable to females. The study population included healthy vegetarian and vegan adults and may not be generalizable to older adults following a plant-based diet. The study was also performed on sedentary individuals and may not be generalizable to those currently engaging in exercise, specifically resistance trained individuals.

CHAPTER 6

CONCLUSIONS AND APPLICATIONS

There are numerous areas of research that support adoption of a plant-based diet for health benefits and reduction in environmental impact.[5,7,10,23-25]The health benefits relating to plant-based diets range from reduced BMI to increased micronutrient status, fiber intake, reduced intake of saturated fat and cholesterol as well as overall reduced risk of chronic disease such as hypertension, Type 2 diabetes, and heart disease.[5,7,10,23,24] Additionally, there are large advantages in environmental impacts of adopting a plant-based diet. Consuming plant-based food sources utilizes less fossil fuel and natural resources such as nonrenewable phosphate rock, and improves sustainability of food systems.[25,26] Although benefits exist in adopting a plant-based diet there are additional areas of concern including decrease in certain micronutrients and inadequate protein intake.[3,10] This discrepancy in the literature provides the framework for future research to investigate the specific needs of individuals following plant-based diets.

The importance of protein in the body cannot be overstated in terms of functions need for proper body performance. While absorption of the other two macronutrients, carbohydrates and fats, are not a problem between omnivores and vegetarians, protein's digestibility ranges in different sources.[6] The digestibility and absorption of protein from plant-based sources can be as low as 54%, whereas animal-based sources can be upwards of 90%.[8,9] Protein contributes to the production of lean body mass, an important factor in preventing sarcopenia, loss of lean body mass in the aging process.[6]

The present study examined whether moderate dietary plant-based protein increases, 21g/day for eight weeks, may elicit changes in body composition and in turn strength, in vegetarians and vegans. No significant changes were observed in lean body mass or strength in the study population but this pilot study provides the framework for future research. While the current study had low adherence mainly due to taste of products, future work ought to increase dietary protein and spread it throughout the day to examine changes in body composition. Previous work suggests higher plant-based protein intake spread throughout meals may have similar effects on body composition as animal-based sources at lower intakes.[14]

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