

Article

Not peer-reviewed version

Effects of Four Weeks of Alternate-Day Fasting with or Without Protein Supplementation— A Randomized Controlled Trial

[Benedict Wei Jun Pang](#), [Yifan Yang](#)^{*}, [Nur Rashaqah](#), [Christopher Binggiang Huang](#), [Da Wei Sim](#)

Posted Date: 4 November 2025

doi: 10.20944/preprints202511.0245.v1

Keywords: weight loss; muscle preservation; intermittent fasting; alternate-day fasting; short-term; body composition; blood glucose; blood pressure



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Effects of Four Weeks of Alternate-Day Fasting with or Without Protein Supplementation – A Randomized Controlled Trial

Benedict Wei Jun Pang ^{1,2}, Yifan Yang ^{1,3,*}, Nur Rashiqa ¹, Christopher Bingqiang Huang ¹ and Da Wei Sim ¹

¹ Physical Education and Sports Science, National Institute of Education, Nanyang Technological University, Singapore

² Geriatric Education and Research Institute, Singapore

³ Science of Learning in Education Centre, National Institute of Education, Nanyang Technological University, Singapore

* Correspondence: yifan.yang@nie.edu.sg

Abstract

Background/Objectives: Long-term alternate-day fasting (ADF) effectively combats obesity, but short-term effects are less clear. Like other diets, ADF-induced weight loss often includes muscle loss, and whether protein supplementation mitigates this is uncertain. This study examined the effects of short-term ADF on body composition and health, and whether protein supplementation preserves muscle mass during weight loss in young Asian men with unhealthy BMI (≥ 23.0 kg/m²). **Methods:** Twenty participants were recruited for a single-arm trial to address the first objective, and 26 participants were randomly assigned to a control (C) or protein group (P) in a follow-up trial to address the second objective. Participants alternated between feeding (ad libitum) and fasting (400-600 kcal consumed between 12-2 PM) days for four weeks. P consumed 25 g whey protein as part of the fasting day meal. Pre-post body composition was assessed using bioelectrical impedance analysis. Anthropometry, fasting blood glucose (FG) and resting blood pressure (BP) were measured weekly. **Results:** Since interaction effects were absent, data from all three groups were combined for analyses. Four weeks ADF significantly ($p < .001$) reduced body (2.4 kg), fat (1.6 kg), and fat-free (0.8 kg) mass. BP and FG remained unchanged ($p = .753-.919$). No significant differences were detected between C and P in any measure. **Conclusions:** Short-term ADF effectively reduces body and fat mass, but also muscle mass which cannot be attenuated with protein supplementation during fasting days. Future studies should explore the effectiveness of protein or leucine supplementation, throughout the feeding and fasting days, in preserving muscle during weight loss.

Keywords: weight loss; muscle preservation; intermittent fasting; alternate-day fasting; short-term; body composition; blood glucose; blood pressure

1. Introduction

Globally, 43.5% of adults (age-standardized) are overweight (BMI 25.0-29.9 kg/m²) and 15.8% obese (≥ 30.0 kg/m²) [1], making obesity a global health epidemic. In Singapore, 40.7% and 13.9% are overweight and obese, respectively, with higher prevalence when guidelines for increased cardiovascular disease (CVD) risk in Asians are used instead (23.0-27.4 kg/m² and ≥ 27.5 kg/m²) [2,3]. Given that obesity is a risk factor for chronic diseases including metabolic syndrome, CVD, diabetes, cancer and overall mortality [4], its increasing prevalence constitutes a growing public health concern.

To combat obesity, various weight loss strategies have been promoted. Recently, the use of intermittent fasting (IF) has gained significant interest due to its purported benefits on lipid profile

[5], cardiovascular and metabolic health [6], on top of promoting weight loss [7]. Essentially, IF involves alternating between periods of food abstinence (fasting) and food consumption (feeding) [8]. Fasting periods can range from 16-20 hours/day, and up to 48 hours/week [7]. In particular, alternate day fasting (ADF), which involves alternating every 24 hours between feeding (ad libitum food intake) and fasting days (a small meal of ~400-600 kcal or 25% of daily caloric needs), has been one of the most well-researched and popularized IF protocols [9].

The health benefits associated with ADF are manifold. Current evidence suggests that ADF reduces BMI, body mass, fat mass, total and low-density lipoprotein cholesterol, triglycerides, and systolic and diastolic blood pressure [10,11]. Compared with very-low-calorie dieting (VLCD), 3-12 weeks ADF leads to greater reduction in fat mass and lower reduction in fat-free mass while decreasing hunger and improving satiety and dieting satisfaction, illustrating superior adherence rates and ease of compliance [12]. Of note, a longer-term study lasting six months showed non-superior adherence rates with ADF compared to a traditional calorie restriction diet [13]. Regardless, existing ADF studies typically range from 8-48 weeks, long durations that are hardly motivating to individuals looking to lose weight [10-12]. Moreover, short-term weight cut while maintaining muscle mass would be relevant to athletes, especially those participating in sports with weight categories or in which aesthetics plays a significant role. To date, only a handful of studies have investigated the effects of short-term (2-4 weeks) ADF, all of which conducted in predominantly Caucasian populations [14-20]. Collectively, these studies showed that two weeks ADF was insufficient to induce weight loss in overweight young men [16], and at least 3-4 weeks was required to reduce body mass and fat mass in non-obese and obese young to middle-age adults [14,15,17-20]. It remains unclear whether short-term ADF can improve body composition in a mixed normal weight to obese group, especially among Asian populations with increased CVD risks ($\text{BMI} \geq 23.0 \text{ kg/m}^2$).

Despite the purported health benefits associated with ADF, as with other weight loss diets, muscle loss is a major concern [21]. Importantly, studies comparing between ADF and no-diet controls consistently report significant muscle loss in the ADF group [11]. Additionally, while some studies suggest that high protein intake may preserve muscle during weight loss [22,23], others have reported no differences between high protein or control on muscle preservation [24,25]. At present, there is no evidence to suggest the preservatory effects of protein on muscle during ADF.

Considering these gaps, the objectives of the present study are to investigate: 1) whether short-term (4-week) ADF has beneficial effects on body composition, and 2) if increased protein intake through whey protein supplementation can preserve muscle during weight loss, among Asian young men with unhealthy BMI levels. To achieve these objectives, a single-arm pilot study was conducted with the main aim of addressing the first objective, and a follow-up randomized-controlled trial (RCT) was conducted to address the second objective. Additionally, the effects of 4-week ADF on other health markers, including fasting blood glucose and resting blood pressure, were explored. We hypothesized that 4-week ADF is sufficient to induce weight loss, and that protein supplementation during fasting days can reduce diet-induced muscle loss in normo-weight to obese young men with increased cardiovascular risks ($\text{BMI} \geq 23.0 \text{ kg/m}^2$).

2. Materials and Methods

2.1. Study 1 Design

In this single-arm study (S), 20 young men (21-35 years) with increased CVD risks ($\text{BMI} \geq 23.0 \text{ kg/m}^2$) were recruited to undergo four weeks (28 days) of ADF. Studies that found effective weight loss following ADF used a sample size of 8 or 16 [15,17,26] and hence, we targeted a sample size of 20 to buffer for dropouts. Body composition was assessed pre- and post-intervention, while anthropometry, fasting blood glucose and resting blood pressure were measured weekly. Participants completed weekly physical activity questionnaire and dietary records. Ethics approval was obtained from the Nanyang Technological University (NTU) Institutional Review Board (IRB-2016-09-055). There were no changes to methods and outcome measures after trial commencement.

2.1.1. Participant Screening

Participants were screened according to the following inclusion criteria: 1) male, 2) 21-35 years old, 3) do not smoke or use tobacco products (including shisha), and 4) BMI \geq 23.0 kg/m². Additionally, participants who met any of the following exclusion criteria were excluded from the study: 1) unable to adhere to physical activity and diet requirements, 2) failed an exercise stress test, 3) health condition(s) that might be worsened with fasting, 4) taking long-term medication(s) for heart, blood, lung, liver, kidney or joint condition(s), and 5) taking other long-term alternative medication including traditional Chinese medicine that may affect the study's measurements. Eligible participants signed informed consent before their inclusion into the study.

2.1.2. ADF Protocol

The ADF protocol began at 12AM and alternated between fasting and feeding days in 24-hour cycles. On fasting days, no food and beverages were allowed except for plain water and/or zero-calorie beverages, and a small meal containing 400-600 kcal consumed [9] between 12-2PM to standardize the total duration of fasting and feeding. On feeding days, food and beverages were allowed to be consumed ad libitum. Participants maintained consistent physical activity levels throughout the study.

2.1.3. Laboratory Sessions

Participants visited the laboratory (Human Bioenergetics Laboratory, Physical Education and Sports Science, National Institute of Education, NTU, Singapore) on 5 occasions across 5 weeks. They abstained from intentional physical activity (except activities of daily living) for at least 48 hours, alcohol and/or caffeine for at least 24 hours, and fasted overnight for at least 10 hours before each laboratory session.

2.1.4. Body Composition

Body composition was assessed pre- and post-intervention (first and fifth laboratory sessions) through bioelectrical impedance analysis (BIA) using the InBody 720 (Biospace Co. Ltd., Seoul, Korea), where measurements of total body fat mass (FM), fat-free mass (FFM), skeletal muscle mass (SMM), appendicular fat-free mass (AFFM), percentage body fat (PBF), visceral fat area (VFA), and hydration status (intracellular water/extracellular water; ICW/ECW) were obtained. FM index (FMI), FFM index (FFMI), SMM index (SMMI) and AFFM index (AFFMI) were calculated using the respective measures in kilograms divided by square of height in meters (kg/m²). The use of multi-frequency BIA for the purpose of assessing body composition has been extensively validated to gold-standard methods [27–29] and used in other weight loss studies [17,26,30]. Participants started their 4-week ADF intervention within 1 week from the first session.

2.1.5. Anthropometry

Body height (m) and mass (kg) were measured weekly using the ID1Plus electronic weighing scale (Mettler Toledo, Singapore) and seca 242 stadiometer (seca GmbH & Co. KG, Hamburg, Germany), respectively. BMI was calculated using body mass in kilograms divided by the square of height in meters (kg/m²).

2.1.6. Health Assessments

Fasting blood glucose (FG) and resting blood pressure (BP) were also measured weekly. For FG (mmol/L), a tiny drop of blood was obtained through finger-pricking using the ACCU-CHEK Safe-T-Pro Plus lancet (Roche Diabetes Care GmbH, Mannheim, Germany), and analyzed using the OneTouch Ultra 2 blood glucose meter (LifeScan, Malvern, PA, USA). Resting systolic (SBP) and

diastolic (DBP) blood pressure (mmHg) were assessed using the OMRON HEM-907 blood pressure monitor (OMRON healthcare Co. Ltd., Kyoto, Japan).

2.1.7. Physical Activity Questionnaire and Dietary Record

The global physical activity questionnaire (GPAQ) [31] was administered weekly to estimate physical activity levels (MET-min). Participants completed a 2-day dietary record of any 2 fasting days each week, and total energy intake (kcal), protein (g), fat (g) and carbohydrate (g) content were analyzed for each recorded day.

2.2. Study 2 Design

In this two-arm RCT, 26 young men (21-35 years) with increased CVD risks ($\text{BMI} \geq 23.0 \text{ kg/m}^2$) were recruited to undergo four weeks (28 days) of ADF, and randomly assigned to one of two groups: control (C) or protein group (P) in blocks of 2 (CP or PC) to ensure equal sample size in each group. Participants in P additionally consumed protein supplements on the fasting day (Figure 1). Compromise power calculation using G*Power version 3.1.9.2 for a 2 (group) \times 2 (time) repeated measures ANOVA (rANOVA) interaction effect is 0.85 with 26 total participants (based on the final number of 13 participants in study 1), f effect size of 0.25, beta/alpha ratio of 1 and correlation of 0.5 among repeated measures. Ethics approval was obtained from the NTU IRB (IRB-2017-08-010). Inclusion/exclusion criteria were identical to Study 1, and eligible participants signed informed consent before their inclusion into the study. There were no changes to methods and outcome measures after trial commencement.

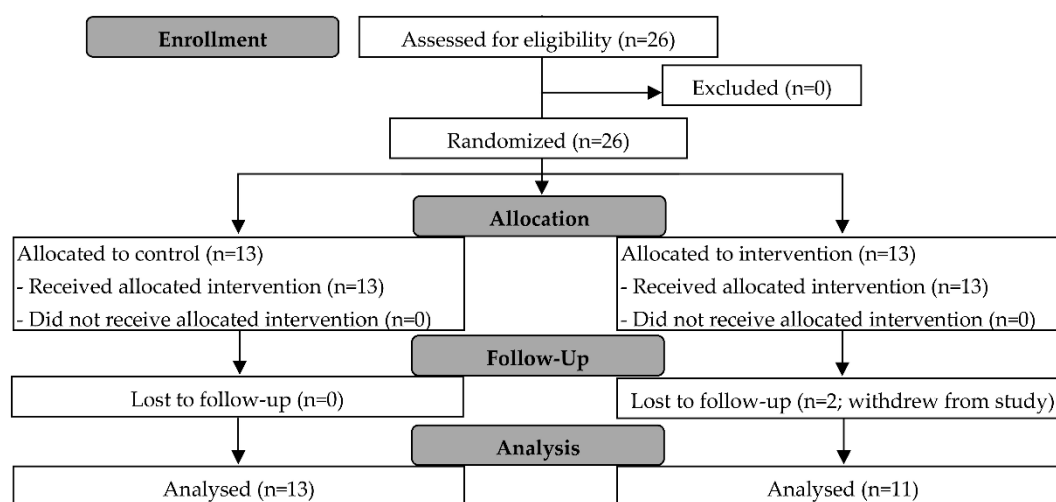


Figure 1. CONSORT Participant Flow Diagram for Study 2.

2.2.1. ADF Protocol

The ADF protocol was identical to Study 1, where a small, self-selected meal (400-600 kcal) was consumed between 12-2PM on fasting days. For group P, a 25 g whey protein supplement (125 kcal) was consumed as part of the fasting day meal, with the remaining 275-475 kcal coming from self-selected food items. On feeding days, food and beverages were allowed to be consumed ad libitum.

Apart from the distinction in fasting day meal for group P, the rest of the study design and protocols were identical to that of Study 1, including laboratory measurements, health assessments, and physical activity questionnaires and dietary records.

2.3. Statistical Analyses

SPSS Statistics version 27.0 (IBM, Chicago, IL, USA) and JASP version 0.16.3 (JASP Team) were used for data analyses. Differences in body composition pre- and post-intervention (and between groups for Study 2) were first analyzed separately for Study 1 (within-subjects analyses; group S) and Study 2 [time, group (C and P), and interaction effects]. When no interaction effects were present, data from all three groups were combined for within-subjects analyses. Significance level was set at $p \leq .05$.

For Study 1 (S), within-subjects t-test were conducted for variables with parametric data: BM, FM, FFM, SMM, AFFM, BMI, PBF, VFA, and hydration status (ICW/ECW). Wilcoxon test was performed for non-parametric variables: FMI, FFMI, SMMI, and AFFMI.

For Study 2 (C and P), 2 (groups) \times 2 (time: pre-post) rANOVA was conducted for ICW/ECW. For the other variables (BM, FM, FFM, SMM, AFFM, FFMI, SMMI, AFFMI, BMI, PBF, FMI and VFA), due to non-normality of data, generalized estimating equations (GEE) were performed using robust estimator, normal distribution with log link function, and exchangeable correlation matrix. Model selections were done first based on smallest goodness of fit, and then smaller covariances of parameter estimate. There were no significant interactions and main effects were reported based on estimated marginal means to adjust for unequal sample size if any.

For combined analyses, within-subjects t-test were conducted for variables with parametric data: BM, FM, FFM, SMM, AFFM, BMI, PBF, FMI, VFA, and ICW/ECW. Wilcoxon test was performed for non-parametric variables: FFMI, SMMI, and AFFMI.

Differences in average calories and protein content of the fasting day meals between Groups P and C were analyzed using between-subjects t-test and Welch test, respectively. For all t-tests and Welch tests, effect sizes were calculated using Cohen's d (.2, small; .5, medium; .8, large). For Wilcoxon tests, effect sizes were calculated using the matched rank biserial correlation coefficient, r_{rb} (.1, small; .3, medium; .5, large). For rANOVA, partial eta squared, η^2p effect sizes were used (.01, small; .06, medium; .14, large).

As for weekly measurements (i.e., GPAQ, SBP, DBP, and FG), due to non-normality of data, GEE was performed for GPAQ, SBP, and DBP using robust estimator with normal distribution with log link function, and autoregressive correlation matrix, while an unstructured correlation matrix was used for FG. Similarly, model selections were done first based on smallest goodness of fit, and then smaller covariances of parameter estimate. There were no significant interactions for study 2 and any significant time effect was followed using simple contrast against baseline (pre-intervention) with Sidak correction for multiple comparisons. Results were based on estimated marginal means.

3. Results

3.1. Participants

Among the 20 participants recruited for Study 1 (between February and March 2017) and 26 participants for Study 2 (between January and March 2018), N=7 and N=2 of them dropped out, respectively (8 unable to commit; 1 uncontactable). Therefore, data from N=37 participants (N=13 and N=24, respectively) were used for analyses, with a total of N=13 in Group S, N=13 in Group C, and N=11 in Group P. Participant characteristics are displayed in Table 1.

Table 1. Participant characteristics for study 1 – single-arm group (S), study 2 – control (C) and protein (P) groups, and combined (All).

Characteristics		S (n=13)		C (n=13)		P (n=11)		All (n=37)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (y)	Pre	26	4	24	2	26	1	25	3
	Post	26	4	24	2	26	1	25	3
Height (m)	Pre	1.72	0.07	1.72	0.04	1.78	0.07	1.74	0.07
	Post	1.72	0.07	1.72	0.03	1.78	0.07	1.74	0.06

Body Mass (kg)	Pre	80.1	12.8	74.5	7.8	89.4	18.8	80.9	14.5
	Post	78.2	12.5	72.2	8.2	86.4	17.6	78.5	14.0
Fat Mass (kg)	Pre	19.8	7.6	16.4	5.9	23.4	12.2	19.7	9.0
	Post	18.8	7.6	14.6	6.2	21.3	12.0	18.1	8.9
FFM (kg)	Pre	60.3	7.2	58.1	4.8	66.0	8.7	61.2	7.5
	Post	59.4	6.7	57.6	5.2	65.1	8.0	60.5	7.2
SMM (kg)	Pre	34.2	4.2	32.8	2.9	37.4	5.1	34.6	4.4
	Post	33.6	4.0	32.6	3.2	36.8	4.7	34.2	4.3
AFFM (kg)	Pre	24.9	3.2	24.1	2.2	28.1	4.5	25.6	3.7
	Post	24.7	3.0	23.9	2.3	27.7	4.2	25.3	3.5
FFMI (kg/m ²)	Pre	20.4	1.8	19.6	1.3	20.7	1.9	20.2	1.7
	Post	20.0	1.5	19.4	1.5	20.5	1.7	20.0	1.6
SMMI (kg/m ²)	Pre	11.5	1.0	11.1	0.8	11.7	1.1	11.4	1.0
	Post	11.3	0.9	11.0	0.9	11.6	1.0	11.3	1.0
AFFMI (kg/m ²)	Pre	8.4	0.7	8.1	0.5	8.8	0.9	8.4	0.7
	Post	8.3	0.5	8.1	0.6	8.7	0.8	8.3	0.7
BMI (kg/m ²)	Pre	26.9	2.3	25.2	2.5	28.1	5.2	26.7	3.6
	Post	26.2	2.2	24.4	2.6	27.1	4.9	25.9	3.5
PBF (%)	Pre	24.2	5.8	21.7	6.0	25.1	7.7	23.6	6.4
	Post	23.4	5.8	19.8	6.6	23.6	8.0	22.2	6.8
FMI (kg/m ²)	Pre	6.6	2.0	5.5	2.0	7.4	3.7	6.4	2.6
	Post	6.2	2.0	4.9	2.1	6.7	3.6	5.9	2.6
VFA (cm ²)	Pre	81.2	33.3	67.7	25.4	96.8	44.3	81.1	35.6
	Post	76.1	33.2	59.1	26.7	88.3	44.2	73.8	35.9
ICW/ECW	Pre	1.7	0.0	1.7	0.0	1.7	0.0	1.7	0.0
	Post	1.7	0.0	1.7	0.0	1.7	0.0	1.7	0.0

Data presented in mean and standard deviation (SD) with minimum and maximum values. FM, fat mass; FFM, fat-free mass; SMM, skeletal muscle mass; AFFM, appendicular fat-free mass; FFMI, fat-free mass index; SMMI, skeletal muscle mass index; AFFMI, appendicular fat-free mass index; BMI, body mass index; PBF, percentage body fat; FMI, fat mass index; VFA, visceral fat area; ICW, intracellular water; ECW, extracellular water.

3.2. Fasting Day Meal

On fasting days, group S consumed on average 525 (SD 151) kcal for the fasting meal, of which 25 (7)%, 37 (15)%, and 38 (12)% came from protein, fat, and carbohydrates, respectively. Group P consumed 495 (50) kcal, of which 40 (8)%, 26 (6)%, and 34 (13)% came from protein, fat, and carbohydrates. Group C consumed 533 (38) kcal, of which 22 (4)%, 33 (5)%, and 46 (5)% came from protein, fat, and carbohydrates. Independent samples t-test revealed that, despite consuming fewer calories ($p=.045$, $d=-.9$), group P consumed significantly higher protein ($p<.001$, $d=3.0$) on the fasting day than group C (Figure 2), as intended.

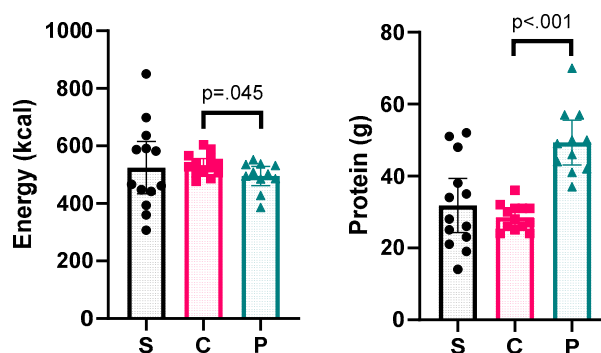


Figure 2. Fasting meal energy (kcal) and protein (g) intake for study 1 – single-arm group (S), and study 2 – control (C) and protein (P) groups. Bar chart presented as mean and 95% CI, with scatterplot of individual data points. Independent samples t-test was conducted only for Study 2 to compare the differences between groups C and P.

3.3. Hydration Status

ICW/ECW is an indicator of hydration status, which can affect BIA results. There was no significant difference in pre-post ICW/ECW for group S ($p=.827$, $d=-.06$), and no significant interaction ($p=.744$, $\eta^2p<0.01$) and main effect of time on ICW/ECW for groups P and C ($p=.978$, $\eta^2p<0.01$), suggesting that pre-post hydration status was consistent in both studies.

3.4. Body Composition

Post-intervention (Figure 3), group S lost significant amounts of BM ($p<.001$, $d=-1.79$), FM ($p=.007$, $d=-.91$), FFM ($p=.041$, $d=-.63$), SMM ($p=.032$, $d=-.67$), FFMI ($p=.027$, $d=-.69$), SMMI ($p=.021$, $d=-.71$), BMI ($p<.001$, $d=-1.62$), FMI ($p=.006$, $d=-.82$), and VFA ($p<.001$, $d=-1.36$). Changes in AFFM ($p=.235$, $d=-.35$), AFFMI ($p=.244$, $d=-.39$), and PBF ($p=.097$, $d=-.50$) were not significant. For groups P and C, there were no significant interaction effects of group and time on any measure ($p=.179-.807$). However, main effect of time was significant for all measures with reduction post-intervention: BM ($p<.001$, $d=-.21$), FM ($p<.001$, $d=-.23$), FFM ($p=.004$, $d=-.11$), SMM ($p=.004$, $d=-.10$), AFFM ($p=.003$, $d=-.08$), FFMI ($p=.002$, $d=-.14$), SMMI ($p=.002$, $d=-.13$), AFFMI ($p=.001$, $d=-.12$), BMI ($p<.001$, $d=-.23$), PBF ($p<.001$, $d=-.26$), FMI ($p<.001$, $d=-.24$), and VFA ($p<.001$, $d=-.27$).

Since no significant interaction effects were present between groups P and C, data from all three groups ($N = 37$) were combined for within-subjects analyses. The results showed significant losses in all measures of body composition post-intervention, which were BM, FM, FFM, SMM, AFFM, FFMI, SMMI, AFFMI, BMI, PBF, FMI, and VFA (Table 2). Corresponding changes in BM, FM, and FFM for each participant are depicted in Figure 4 to visualize individual responses. All but 2 participants lost BM (coefficient of variation, CV of change=.65) but the changes in FM (CV=.78) and FMM (CV=1.68) were more varied. While the change in BM is correlated to changes in FM (Spearman correlation coefficient, $\rho=.586$, $p<.001$) and FFM ($\rho=.537$, $p<.001$), respectively, there is no correlation between FM and FFM changes ($\rho=-.291$, $p<.081$; correlation data not shown).

3.5. Physical Activity and Blood Markers

There were no significant interaction effects between groups P and C (data not shown) in weekly physical activity level [GPAQ ($p=.426$)], SBP ($p=.653$), DBP ($p=.818$), and thus, data from studies 1 and 2 were combined (Table 3). Analysis of the combined data showed a significant difference in GPAQ levels across time ($p<.001$). Simple contrast against baseline revealed significant reductions in GPAQ levels between baseline and end of week 2 ($p=.002$), and between baseline and end of week 3 ($p=.004$).

Analyses of the combined data showed significant differences in DBP ($p=.021$) and FG ($p<.001$) across time but not for SBP ($p=.562$). Post-hoc analyses revealed significant reductions in DBP between baseline and end of week 3 (-2.8 mmHg, $p=.028$), and in FG between baseline and end of week 1 ($-.22$, $p=.026$). There were, however, no significant pre-post differences in DBP ($p=.809$), or FG ($p=.919$) from baseline to post-intervention.

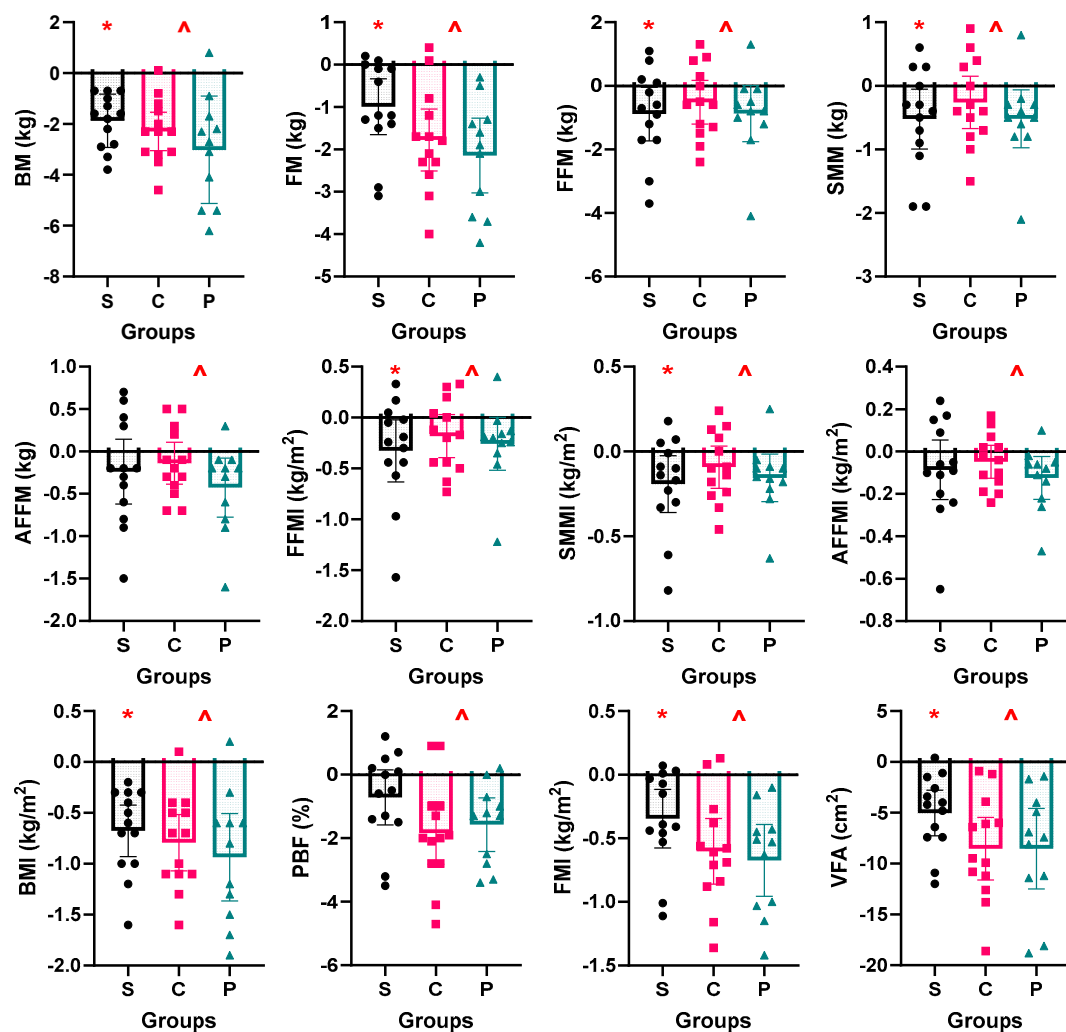


Figure 3. Pre-post changes in body composition for study 1 – single-arm group (S), and study 2 – control (C) and protein (P) groups. Bar chart presented as mean and 95% CI, with scatterplot of individual data points. BM, body mass; FM, fat mass; FFM, fat-free mass; SMM, skeletal muscle mass; AFFM, appendicular fat-free mass; FFMI, fat-free mass index; SMMI, skeletal muscle mass index; AFFMI, appendicular fat-free mass index; BMI, body mass index; PBF, percentage body fat; FMI, fat mass index; VFA, visceral fat area. Separate statistical analyses were conducted for studies 1 and 2. * indicates a significant pre-post change for study 1 ($p < .05$). ^ indicates a significant time effect for study 2 ($p < .05$). All interaction effects for study 2 were not significant ($p > .05$).

Table 2. Within-subjects Body Composition Analyses (Combined Data).

Characteristics	Test	Statistic	df	SE	p-value	Location Parameter ^a	95% CI		Effect Size ^b
							Difference	Lower	
Body mass (kg)	T-test	-9.378	36	0.252	<.001	-2.4	-2.9	-1.9	-1.5
Fat Mass (kg)	T-test	-7.752	36	0.208	<.001	-1.6	-2.0	-1.2	-1.3
FFM (kg)	T-test	-3.622	36	0.207	<.001	-0.8	-1.2	-0.3	-0.6
SMM (kg)	T-test	-3.691	36	0.116	<.001	-0.4	-0.7	-0.2	-0.6
AFFM (kg)	T-test	-2.842	36	0.088	0.007	-0.3	-0.4	-0.1	-0.5
FFMI (kg/m ²)	Wilcoxon	117	36	0.068	<.001	-0.2	-0.3	-0.1	-0.6
SMMI (kg/m ²)	Wilcoxon	114	36	0.038	<.001	-0.1	-0.2	-0.1	-0.7
AFFMI (kg/m ²)	Wilcoxon	173	36	0.029	0.006	-0.1	-0.1	0.0	-0.5

BMI (kg/m²)	T-test	-9.603	36	0.083	<.001	-0.8	-1.0	-0.6	-1.6
FMI (kg/m²)	T-test	-7.657	36	0.070	<.001	-0.5	-0.7	-0.4	-1.3
PBF (%)	T-test	-5.531	36	0.247	<.001	-1.4	-1.9	-0.9	-0.9
VFA (cm²)	T-test	-8.752	36	0.834	<.001	-7.3	-9.0	-5.6	-1.4

^a Mean difference for t-test, Hodges-Lehmann median difference for Wilcoxon. ^b Cohen's d for t-test, matched rank biserial correlation for Wilcoxon. FFM, fat-free mass; SMM, skeletal muscle mass; AFFM, appendicular fat-free mass; FFMI, fat-free mass index; SMMI, skeletal muscle mass index; AFFMI, appendicular fat-free mass index; BMI, body mass index; PBF, percentage body fat; FMI, fat mass index; VFA, visceral fat area.

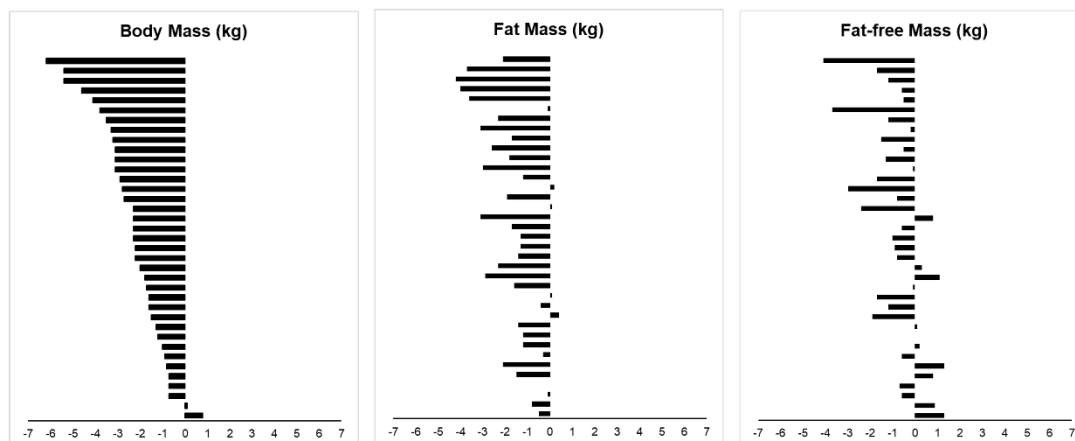


Figure 4. Corresponding changes in body mass, fat mass, and fat-free mass (ranked by change in body mass) for all 37 participants.

Table 3. Weekly Physical Activity and Health Indices (Combined Data).

	Week	N	Mean	SD
GPAQ (Met-min/week)	0	37	1443	1053
	1	37	1336	1051
	2	37	1089	823
	3	37	1139	800
	4	37	1189	1012
SBP (mmHg)	0	37	118	10
	1	37	118	9
	2	37	117	8
	3	37	116	8
	4	37	117	10
DBP (mgHg)	0	37	69	9
	1	37	68	8
	2	37	67	8
	3	37	67	9
	4	37	68	8
FG (mmol)	0	36	5.3	0.4
	1	37	5.1	0.3
	2	37	5.3	0.4
	3	36	5.1	0.4
	4	37	5.2	0.4

Data presented in mean and standard deviation (SD) with minimum and maximum values. GPAQ, global physical activity questionnaire; MET, metabolic equivalent of task; SBP, systolic blood pressure; DBP, diastolic blood pressure; FG, fasting blood glucose.

4. Discussion

To our knowledge, this is the first study to investigate the effects of short-term ADF on body composition, and the effects of whey protein supplementation on muscle preservation during short-term ADF, among normal weight to obese (Asian) young men with increased CVD risks.

4.1. Effectiveness of Short-Term ADF on Body Composition

Our results showed that four weeks ADF was sufficient to induce significant reductions (Figure 4) in body mass (35/37 participants) and fat mass (33/37). However, there was a concomitant decrease in muscle (fat-free mass) as well (28/37). Increased protein intake through whey protein supplementation during fasting days did not alleviate muscle loss.

There was a significant loss in body mass of 2.4 kg (2.9%) after four weeks of ADF, a rate that is clinically important and within safe recommendations of 0.45-0.9 kg/week [32]. This agrees with previous literature where 3-4 weeks [14,15,17-20], but not two weeks [16], of ADF was sufficient and effective for weight loss. Since weight loss is mainly determined by overall energy deficit [33], the energy deficit accumulated with < 3 weeks of ADF is likely insufficient to promote significant weight loss, and at least 3-4 weeks is required to accrue sufficient energy deficit to alter body mass among normal weight to obese individuals.

Four weeks ADF reduced fat mass by an average of 1.6 kg (68.2% of total weight loss) and other obesity indices including BMI (-3.0%), FMI (-8.3%), PBF (-5.9%), and VFA (-9.0%), with large effect sizes for all indices. However, this was accompanied with significant muscle loss in terms of FFM by an average of 0.8 kg (31.8% of total weight loss), SMM (-1.2%), AFFM (-1.0%), FFMI (-1.3%), SMMI (-1.3%), and AFFMI (-1.0%), with moderate effect sizes for all indices (Table 2). This is supported by the findings of other weight loss studies, where regardless of dietary intervention, a significant loss of lean mass or FFM (25-42% of total weight loss) can be expected [12,34]. Due to the physiological demands of calorie restriction and weight loss, there is a natural decrease in basal and postprandial muscle protein synthesis (MPS) [35], which exerts deleterious effects on muscle regardless of the type of diet implemented [10].

4.2. Effectiveness of Protein Supplementation During Fasting Day

Interestingly, whey protein supplementation, despite increasing fasting day protein intake significantly by 1.7x in group P (49.4 g) compared to group C (28.5 g), was not effective in attenuating muscle loss. Corroboratively, several studies have reported that increased protein intake alone during energy restriction and weight loss does not improve or preserve muscle [24,25,36]. One possible reason for the absence of a significant effect could be that the difference in body mass-adjusted protein consumption between groups P (0.55-0.57 g/kg/day) and C (0.38-0.39 g/kg/day) was too small (0.16-0.19 g/kg/day) to detect any meaningful difference. Notably, fasting day protein intake for both groups was below the recommended dietary allowance of 0.8 g/kg (body mass)/day, an amount typically recommended to prevent protein deficiency [37]. Nonetheless, it is hardly feasible to enforce a fasting day protein intake of 0.8 g/kg/day, as that would represent an average protein intake of ~71.5 g (286kcal) for the P group, which equates to 47.7-71.5% of total fasting day calories (400-600 kcal) coming from protein alone. Protein intake on feeding days was also not measured or controlled in these studies, consistent with the nature of ADF and its evaluation for effectiveness. As such, a further explanation for group P not experiencing greater muscle preservation could be that the protein intake on feeding days was not sufficient to compensate for the lower protein consumed on fasting days in either group.

Indeed, a previous meta-analysis found that, during a weight loss diet, high protein intakes averaged at 1.25 g/kg/day, compared to standard protein intakes of 0.72 g/kg/day, resulted in greater losses in body mass and fat mass while preserving FFM [23]. Future ADF studies could explore increasing protein intake on both the feeding and fasting days in order to bring up the total protein intake, averaged across both feeding and fasting days, closer to the amount of 1.25 g/kg/day or higher.

Importantly, recent studies have demonstrated that protein quality (leucine amount) may be a better determinant of MPS and muscle anabolism than protein quantity [38,39]. While leucine intake was not monitored in the present study, there is merit in exploring the effectiveness of leucine interventions on muscle preservation during weight loss in future studies.

Nonetheless, increased protein consumption can improve satiety [40], which could explain the small (38 kcal) but significant ($p=.045$) lower caloric intake on fasting days in P group compared to C group. Although no significant differences were detected in terms of weight loss between groups, decreased energy intake and improved satiety through increased protein consumption could result in superior weight loss that is clinically relevant over longer periods of time.

4.3. Potential Influence of Physical Activity Type on Muscle Mass Preservation

Physical activity and resistance training habits could have further confounded the effects of protein supplementation on muscle preservation. While our GPAQ analyses revealed no significant changes in physical activity levels, and no interaction effect between groups P and C throughout the intervention, physical activity was quantified using energy expenditure (MET·min/week), and not differentiated by the type of activity. Given that resistance training provides potent anabolic stimulus for muscle hypertrophy through the stimulation of MPS with [41] or without [42] increased protein intake, more specific measures and analyses of resistance training and physical activity could elucidate our understanding of the effects of protein supplementation on muscle preservation during weight loss.

4.4. Effectiveness of Short-Term ADF on Blood Health Indices

The lack of a significant pre-post difference in blood pressure and fasting blood glucose suggests that short-term ADF does not benefit cardiovascular and diabetic health. Corroboratively, previous studies have demonstrated that three weeks ADF did not alter blood pressure [15], and two weeks ADF did not improve fasting blood glucose levels [16]. While it is possible that short-term ADF was simply insufficient in duration to improve cardiovascular and diabetic health, longer-term (8-48 weeks) ADF studies have also reported inconsistent findings in this regard [10,11]. The lack of significant changes in these indices may also be due to the fact that our participants were young, and that their average baseline SBP, DBP and FG were already within healthy ranges, limiting the potential for changes post-intervention. Nonetheless, other biomarkers of cardiovascular and diabetic health, including plasma triglycerides, adiponectin levels, and insulin sensitivity, were not assessed in this study. Future studies should explore these variables to further our understanding of the effects of ADF on cardiovascular and diabetic health.

4.5. Limitations

The main limitation of this study is that only young men were included, thus, findings may not be generalizable to women or the older population. Personal preferences in dietary habits could have confounded the effects of protein supplementation since food intake on feeding days were not controlled or monitored. Future studies could implement a controlled diet to ensure that total protein intake across the study duration is matched between groups.

5. Conclusions

Short-term ADF is effective in reducing body mass and fat mass in normal weight to obese young men. However, this is accompanied with significant muscle loss which cannot be attenuated with

protein supplementation during fasting days. Future studies should investigate the effectiveness of other interventions, such as protein or leucine supplementation throughout the ADF (i.e., on both feeding and fasting days), to preserve or improve muscular outcomes during weight loss.

Author Contributions: BWJP: Data analysis and statistical analysis; Wrote paper; Primary responsibility for final content. YY: Designed research; Conducted research; Provided essential materials; Data analysis and statistical analysis; Wrote paper; Primary responsibility for final content. NRBT: Designed research; Conducted research; Data analysis and statistical analysis. CBQH: Conducted research; Data analysis and statistical analysis. DS: Conducted research; Data analysis and statistical analysis. All authors have read and approved the final manuscript.

Funding: This research received no external funding

Institutional Review Board Statement: Both studies 1 and 2 were conducted in accordance with the Declaration of Helsinki, and approved by the NTU IRB (IRB-2016-09-055 approved on 8 December 2016 and IRB-2017-08-010 approved on 23 October 2017, respectively).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Dataset available from the corresponding author upon request.

Acknowledgments: The first author (BWJP) would like to thank the National Institute of Education (NIE), Nanyang Technological University (NTU), for providing him with the prestigious Nanyang President's Graduate Scholarship (NPGS) in support of his PhD journey. The authors graciously acknowledge all the participants for their time and effort in completing the 4-week ADF protocol.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ADF	Alternate-day fasting
AFFM	Appendicular fat-free mass
AFFMI	Appendicular fat-free mass index
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BP	Blood pressure
C	Control group
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
FFM	Fat-free mass
FFMI	Fat-free mass index
FG	Fasting blood glucose
FM	Fat mass
FMI	Fat mass index
GEE	Generalized estimating equation
GPAQ	Global physical activity questionnaire
ICW/ECW	Intracellular water/extracellular water
IF	Intermittent fasting
IRB	Institutional review board
MET	Metabolic Equivalent of Task
MPS	Muscle protein synthesis
NTU	Nanyang technological university
P	Protein group
PBF	Percentage body fat
rANOVA	repeated measures Analysis of Variance (ANOVA)
RCT	Randomized-controlled trial
S	Single-arm study

SBP	Systolic blood pressure
SMM	Skeletal muscle mass
SMMI	Skeletal muscle mass index
VFA	Visceral fat area
VLCD	Very low calorie dieting

References

1. WHO. Global Health Observatory (GHO) data – Overweight and obesity 2022. Available online: https://www.who.int/gho/ncd/risk_factors/overweight_obesity/obesity_adults/en/ (accessed on 30 Aug 2024).
2. WHO. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* **2004**, *363*, 157–163. [https://doi.org/10.1016/s0140-6736\(03\)15268-3](https://doi.org/10.1016/s0140-6736(03)15268-3).
3. Chen, K.K.; Wee, S.-L.; Pang, B.; Lau, L.K.; Jabbar, K.A.; Seah, W.T.; Ng, T.P. Relationship between BMI with percentage body fat and obesity in Singaporean adults – The Yishun Study. *BMC Public Health* **2021**, *10.21203/rs.3.rs-53522/v2*. <https://doi.org/10.21203/rs.3.rs-53522/v2>.
4. Wang, Y.C.; McPherson, K.; Marsh, T.; Gortmaker, S.L.; Brown, M. Health and economic burden of the projected obesity trends in the USA and the UK. *Lancet* **2011**, *378*, 815–825. [https://doi.org/10.1016/s0140-6736\(11\)60814-3](https://doi.org/10.1016/s0140-6736(11)60814-3).
5. Meng, H.; Zhu, L.; Kord-Varkaneh, H.; H, O.S.; Tinsley, G.M.; Fu, P. Effects of intermittent fasting and energy-restricted diets on lipid profile: A systematic review and meta-analysis. *Nutr.* **2020**, *77*, 110801. <https://doi.org/10.1016/j.nut.2020.110801>.
6. Cho, Y.; Hong, N.; Kim, K.W.; Cho, S.J.; Lee, M.; Lee, Y.H.; Lee, Y.H.; Kang, E.S.; Cha, B.S.; Lee, B.W. The Effectiveness of Intermittent Fasting to Reduce Body Mass Index and Glucose Metabolism: A Systematic Review and Meta-Analysis. *J. Clin. Med.* **2019**, *8*. <https://doi.org/10.3390/jcm8101645>.
7. Welton, S.; Minty, R.; O'Driscoll, T.; Willms, H.; Poirier, D.; Madden, S.; Kelly, L. Intermittent fasting and weight loss: Systematic review. *Can. Fam. Physician* **2020**, *66*, 117–125.
8. Patterson, R.E.; Sears, D.D. Metabolic Effects of Intermittent Fasting. *Annu. Rev. Nutr.* **2017**, *37*, 371–393. <https://doi.org/10.1146/annurev-nutr-071816-064634>.
9. Varady, K.A.; Bhutani, S.; Klempel, M.C.; Kroeger, C.M.; Trepanowski, J.F.; Haus, J.M.; Hoddy, K.K.; Calvo, Y. Alternate day fasting for weight loss in normal weight and overweight subjects: a randomized controlled trial. *Nutr. J.* **2013**, *12*, 146. <https://doi.org/10.1186/1475-2891-12-146>.
10. Park, J.; Seo, Y.G.; Paek, Y.J.; Song, H.J.; Park, K.H.; Noh, H.M. Effect of alternate-day fasting on obesity and cardiometabolic risk: A systematic review and meta-analysis. *Metab.* **2020**, *111*, 154336. <https://doi.org/10.1016/j.metabol.2020.154336>.
11. Cui, Y.; Cai, T.; Zhou, Z.; Mu, Y.; Lu, Y.; Gao, Z.; Wu, J.; Zhang, Y. Health Effects of Alternate-Day Fasting in Adults: A Systematic Review and Meta-Analysis. *Front. Nutr.* **2020**, *7*, 586036. <https://doi.org/10.3389/fnut.2020.586036>.
12. Alhamdan, B.A.; Garcia-Alvarez, A.; Alzahrnai, A.H.; Karanxha, J.; Stretchberry, D.R.; Contrera, K.J.; Utria, A.F.; Cheskin, L.J. Alternate-day versus daily energy restriction diets: which is more effective for weight loss? A systematic review and meta-analysis. *Obes. Sci. Pract.* **2016**, *2*, 293–302. <https://doi.org/10.1002/osp4.52>.
13. Trepanowski, J.F.; Kroeger, C.M.; Barnosky, A.; Klempel, M.C.; Bhutani, S.; Hoddy, K.K.; Gabel, K.; Freels, S.; Rigdon, J.; Rood, J., et al. Effect of Alternate-Day Fasting on Weight Loss, Weight Maintenance, and Cardioprotection Among Metabolically Healthy Obese Adults: A Randomized Clinical Trial. *JAMA Intern. Med.* **2017**, *177*, 930–938. <https://doi.org/10.1001/jamainternmed.2017.0936>.
14. Stekovic, S.; Hofer, S.J.; Tripolt, N.; Aon, M.A.; Royer, P.; Pein, L.; Stadler, J.T.; Pendl, T.; Prietl, B.; Url, J., et al. Alternate Day Fasting Improves Physiological and Molecular Markers of Aging in Healthy, Non-obese Humans. *Cell. Metab.* **2019**, *30*, 462–476.e466. <https://doi.org/10.1016/j.cmet.2019.07.016>.
15. Heilbronn, L.K.; Smith, S.R.; Martin, C.K.; Anton, S.D.; Ravussin, E. Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. *Am. J. Clin. Nutr.* **2005**, *81*, 69–73. <https://doi.org/10.1093/ajcn/81.1.69>.

16. Halberg, N.; Henriksen, M.; Soderhamn, N.; Stallknecht, B.; Ploug, T.; Schjerling, P.; Dela, F. Effect of intermittent fasting and refeeding on insulin action in healthy men. *Journal of applied physiology (Bethesda, Md. : 1985)* **2005**, *99*, 2128–2136. <https://doi.org/10.1152/jappphysiol.00683.2005>.
17. Klempel, M.C.; Bhutani, S.; Fitzgibbon, M.; Freels, S.; Varady, K.A. Dietary and physical activity adaptations to alternate day modified fasting: implications for optimal weight loss. *Nutr. J.* **2010**, *9*, 35–35. <https://doi.org/10.1186/1475-2891-9-35>.
18. Templeman, I.; Smith, H.A.; Chowdhury, E.; Chen, Y.C.; Carroll, H.; Johnson-Bonson, D.; Hengist, A.; Smith, R.; Creighton, J.; Clayton, D., et al. A randomized controlled trial to isolate the effects of fasting and energy restriction on weight loss and metabolic health in lean adults. *Sci. Transl. Med.* **2021**, *13*. <https://doi.org/10.1126/scitranslmed.abd8034>.
19. Varady, K.A.; Bhutani, S.; Church, E.C.; Klempel, M.C. Short-term modified alternate-day fasting: a novel dietary strategy for weight loss and cardioprotection in obese adults. *The American Journal of Clinical Nutrition* **2009**, *90*, 1138–1143. <https://doi.org/10.3945/ajcn.2009.28380>.
20. Derron, N.; Güntner, A.T.; Weber, I.C.; Braun, J.; Koska, I.Ö.; Othman, A.; Mönch, L.; von Eckardstein, A.; Puhán, M.A.; Beuschlein, F., et al. Alternate-day fasting elicits larger changes in fat mass than time-restricted eating in adults without obesity – A randomized clinical trial. *Clinical Nutrition* **2025**, *53*, 212–221. <https://doi.org/10.1016/j.clnu.2025.08.033>.
21. Aragon, A.A.; Schoenfeld, B.J.; Wildman, R.; Kleiner, S.; VanDusseldorp, T.; Taylor, L.; Earnest, C.P.; Arciero, P.J.; Wilborn, C.; Kalman, D.S., et al. International society of sports nutrition position stand: diets and body composition. *J. Int. Soc. Sports Nutr.* **2017**, *14*, 16. <https://doi.org/10.1186/s12970-017-0174-y>.
22. Westerterp-Plantenga, M.S.; Lemmens, S.G.; Westerterp, K.R. Dietary protein - its role in satiety, energetics, weight loss and health. *Br. J. Nutr.* **2012**, *108 Suppl 2*, S105–112. <https://doi.org/10.1017/s0007114512002589>.
23. Wycherley, T.P.; Moran, L.J.; Clifton, P.M.; Noakes, M.; Brinkworth, G.D. Effects of energy-restricted high-protein, low-fat compared with standard-protein, low-fat diets: a meta-analysis of randomized controlled trials. *The American Journal of Clinical Nutrition* **2012**, *96*, 1281–1298. <https://doi.org/10.3945/ajcn.112.044321>.
24. Soenen, S.; Bonomi, A.G.; Lemmens, S.G.; Scholte, J.; Thijssen, M.A.; van Berkum, F.; Westerterp-Plantenga, M.S. Relatively high-protein or 'low-carb' energy-restricted diets for body weight loss and body weight maintenance? *Physiol. Behav.* **2012**, *107*, 374–380. <https://doi.org/10.1016/j.physbeh.2012.08.004>.
25. Backx, E.M.P.; Tieland, M.; Borgonjen-van den Berg, K.J.; Claessen, P.R.; van Loon, L.J.C.; de Groot, L.C.P.G.M. Protein intake and lean body mass preservation during energy intake restriction in overweight older adults. *Int. J. Obes.* **2016**, *40*, 299–304. <https://doi.org/10.1038/ijo.2015.182>.
26. Bhutani, S.; Klempel, M.C.; Kroeger, C.M.; Trepanowski, J.F.; Varady, K.A. Alternate day fasting and endurance exercise combine to reduce body weight and favorably alter plasma lipids in obese humans. *Obesity (Silver Spring, Md.)* **2013**, *21*, 1370–1379. <https://doi.org/10.1002/oby.20353>.
27. Faria, S.L.; Faria, O.P.; Cardeal, M.D.; Ito, M.K. Validation study of multi-frequency bioelectrical impedance with dual-energy X-ray absorptiometry among obese patients. *Obes. Surg.* **2014**, *24*, 1476–1480. <https://doi.org/10.1007/s11695-014-1190-5>.
28. Ramírez-Vélez, R.; Tordecilla-Sanders, A.; Correa-Bautista, J.E.; González-Ruíz, K.; González-Jiménez, E.; Triana-Reina, H.R.; García-Hermoso, A.; Schmidt-RioValle, J. Validation of multi-frequency bioelectrical impedance analysis versus dual-energy X-ray absorptiometry to measure body fat percentage in overweight/obese Colombian adults. *Am. J. Hum. Biol.* **2018**, *30*. <https://doi.org/10.1002/ajhb.23071>.
29. Bosy-Westphal, A.; Later, W.; Hitze, B.; Sato, T.; Kossel, E.; Gluer, C.C.; Heller, M.; Müller, M.J. Accuracy of bioelectrical impedance consumer devices for measurement of body composition in comparison to whole body magnetic resonance imaging and dual X-ray absorptiometry. *Obes. Facts* **2008**, *1*, 319–324. <https://doi.org/10.1159/000176061>.
30. Bhutani, S.; Klempel, M.C.; Berger, R.A.; Varady, K.A. Improvements in coronary heart disease risk indicators by alternate-day fasting involve adipose tissue modulations. *Obesity (Silver Spring, Md.)* **2010**, *18*, 2152–2159. <https://doi.org/10.1038/oby.2010.54>.
31. Armstrong, T.; Bull, F. Development of the World Health Organization Global Physical Activity Questionnaire (GPAQ). *J. Public Health* **2006**, *14*, 66–70. <https://doi.org/10.1007/s10389-006-0024-x>.

32. NHLBI Obesity Education Initiative Expert Panel on the Identification, E., and Treatment of Obesity in Adults (US). Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults: The Evidence Report. Available online: <https://www.ncbi.nlm.nih.gov/books/NBK2003/> (accessed on 2 Feb 2024).
33. Volek, J.S.; Vanheest, J.L.; Forsythe, C.E. Diet and exercise for weight loss: a review of current issues. *Sports Med.* **2005**, *35*, 1–9. <https://doi.org/10.2165/00007256-200535010-00001>.
34. Davis, C.S.; Clarke, R.E.; Coulter, S.N.; Rounsefell, K.N.; Walker, R.E.; Rauch, C.E.; Huggins, C.E.; Ryan, L. Intermittent energy restriction and weight loss: a systematic review. *Eur. J. Clin. Nutr.* **2016**, *70*, 292–299. <https://doi.org/10.1038/ejcn.2015.195>.
35. Cava, E.; Yeat, N.C.; Mittendorfer, B. Preserving Healthy Muscle during Weight Loss. *Adv. Nutr.* **2017**, *8*, 511–519. <https://doi.org/10.3945/an.116.014506>.
36. Larsen, A.E.; Bibby, B.M.; Hansen, M. Effect of a Whey Protein Supplement on Preservation of Fat Free Mass in Overweight and Obese Individuals on an Energy Restricted Very Low Caloric Diet. *Nutrients* **2018**, *10*. <https://doi.org/10.3390/nu10121918>.
37. Wolfe, R.R.; Miller, S.L. The Recommended Dietary Allowance of Protein: A Misunderstood Concept. *JAMA* **2008**, *299*, 2891–2893. <https://doi.org/10.1001/jama.299.24.2891>.
38. Churchward-Venne, T.A.; Breen, L.; Di Donato, D.M.; Hector, A.J.; Mitchell, C.J.; Moore, D.R.; Stellingwerff, T.; Breuille, D.; Offord, E.A.; Baker, S.K., et al. Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men: a double-blind, randomized trial. *Am. J. Clin. Nutr.* **2014**, *99*, 276–286. <https://doi.org/10.3945/ajcn.113.068775>.
39. Devries, M.C.; McGlory, C.; Bolster, D.R.; Kamil, A.; Rahn, M.; Harkness, L.; Baker, S.K.; Phillips, S.M. Leucine, Not Total Protein, Content of a Supplement Is the Primary Determinant of Muscle Protein Anabolic Responses in Healthy Older Women. *The Journal of nutrition* **2018**, *148*, 1088–1095. <https://doi.org/10.1093/jn/nxy091>.
40. Weigle, D.S.; Breen, P.A.; Matthys, C.C.; Callahan, H.S.; Meeuws, K.E.; Burden, V.R.; Purnell, J.Q. A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations. *The American Journal of Clinical Nutrition* **2005**, *82*, 41–48. <https://doi.org/10.1093/ajcn/82.1.41>.
41. Yang, Y.; Breen, L.; Burd, N.A.; Hector, A.J.; Churchward-Venne, T.A.; Josse, A.R.; Tarnopolsky, M.A.; Phillips, S.M. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *Br. J. Nutr.* **2012**, *108*, 1780–1788. <https://doi.org/10.1017/s0007114511007422>.
42. Frimel, T.N.; Sinacore, D.R.; Villareal, D.T. Exercise attenuates the weight-loss-induced reduction in muscle mass in frail obese older adults. *Med. Sci. Sports Exerc.* **2008**, *40*, 1213–1219. <https://doi.org/10.1249/MSS.0b013e31816a85ce>.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.