

Effect of Yeast Protein on Muscle Mass and Performance in an Adult Population – a Double Blind, Randomised Controlled Trial

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Abstract Protein supplementation is a practical and cost-efficient strategy to meet protein needs, essential for muscle maintenance and growth, especially during exercise. A critical factor in assessing protein sources is their impact on muscle protein synthesis. This study aimed to compare the effectiveness of yeast protein, whey protein, and a placebo when combined with resistance exercise, focusing on body composition, strength, and endurance outcomes. Seventy-nine participants consumed either 40 g of AnPro® yeast protein, whey protein, or a placebo (maltodextrin) twice daily for 8 weeks. Simultaneously, they engaged in thrice-weekly resistance training and underwent DEXA scans at baseline and week 8. Results showed increases in total mass, BMI, and strength for all groups compared to baseline, with both yeast and whey protein groups exhibiting gains in trunk and total lean mass. Additionally, the AnPro® group experienced reduced diastolic blood pressure and enhanced muscle endurance in bench press performance at 80% 1-RM, while the whey protein group showed improved muscle endurance in leg press performance. Furthermore, the whey protein group displayed a significant reduction in trunk fat mass compared to the placebo group. Subgroup analysis, focusing on individuals with low protein intake, revealed significant increases in lean mass and muscle strength, particularly in the 1-RM bench press, for both yeast and whey protein groups compared to placebo. These findings emphasize the effectiveness of yeast and whey protein in enhancing lean mass and strength compared to a placebo, particularly among individuals with low dietary protein intake.

Keywords: yeast protein, resistance exercise, lean mass, strength, protein supplementation

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1. Introduction

Protein provides the structural component of muscles, bones, and tissue [1], supports tissue metabolism [2], hormone and enzyme production [1], nervous system development [2], and acts as an energy source [1]. Dietary protein intake can be insufficient due to sources of protein typically being expensive [3]. The current recommendation for dietary protein intake for adults is 0.8-1.0 g/kg body weight per day [4,5,6]. The daily protein recommendation increases to a minimum of 1.2 g/kg body weight per day to increase muscle mass in combination with physical activity for optimal muscle maintenance [7]. Coupled with a rapidly expanding population, depleting resources and an increased demand for protein has made dietary protein supplementation from more sustainable, protein-rich food

ingredients a popular choice [3].

Protein supplements were first designed to enhance the physical performance of athletes and those constantly under exercise-induced stress [2]. Protein supplements are also becoming increasingly popular among the general population who recognize the health benefits [1,2]. The increased use of protein supplements comes from extensive research on the beneficial effects of protein supplementation on weight loss [8,10,11], muscle-enhancement [12,13,14,15,16], strength gains [10,11], [14,15,16] [17,18,19,20,21,22], tissue recovery [18,20] [23,24,25], and body composition [10,11,12] [19,21] [26,27,28].

Protein supplementation provides a practical, cost-effective manner to increase protein intake while minimising caloric consumption [3,29]. The primary outcome for the efficacy of a protein source is its effect on muscle protein synthesis (MPS) or muscle growth [1,15]

[30,31], accomplished through a combination of exercise and positive net protein balance [1] [32,33]. Protein supplementation can help maximise muscle gain from resistance exercise [32,33], facilitates faster recovery from stress-induced tissue damage [21,29,32], and enhances performance output [2,32]. The physiological benefits of protein intake subsequently favours a leaner body composition by increasing lean mass [1,2,9].

Protein sources derived from animal products are generally recognised as the highest quality protein. However, altering the amino acid composition of a protein source can modulate the protein quality [34]. Equalising the leucine content of plant-based proteins to the same level as animal proteins, such as whey protein, may enable both plant and animal derived protein to have equivalent efficacy for MPS rates, muscle thickness, force production, performance, strength, and body composition alterations [35,36].

There is currently a greater demand for supplements to be made from more sustainable sources where possible. AnPro® (Angel Yeast Company, China) is a novel yeast protein extracted from the species *Saccharomyces cerevisiae* [37] aimed to be equivalent in function to whey protein, but more sustainable. AnPro® contains more than 70% protein with a similar protein digestibility corrected amino acids score (PDCAAS) to whey protein concentrate (WPC) [37,38]. Zhixian and colleagues (2019) conducted an amino acid composition analysis of AnPro® yeast protein compared to soy protein isolate (SPI) and WPC. AnPro® was shown to have greater total branched chain amino acid (BCAA) content (23.3 g/100 g) than SPI (14.6 g/100 g) and WPC (20.9 g/100 g) and similar leucine content (10.2 g/100 g) to WPC (10.9 g/100 g) [38].

The current study aimed to assess the effectiveness of AnPro® (yeast protein) for increasing muscle mass, strength and muscle endurance compared to whey protein and a placebo in otherwise healthy adult males aged over 40 years old. Yeast protein supplementation was hypothesised to result in equivalent gains when compared to whey protein and superior gains when compared to a placebo.

2. Methods

This study was conducted as a double blind, randomised, placebo controlled clinical study involving two active groups (yeast and whey protein) and one placebo group (maltodextrin). Participants were recruited from Brisbane, Australia between August 2021 and June 2022. Potential participants were provided with a participant information sheet, prior to screening and consent. Following screening, all participants that met the eligibility criteria provided written informed consent to participate in the study prior to completing any baseline measures and product allocation.

One hundred and sixteen male participants aged 40 years or older were recruited from databases and public media outlets. Participants were included in the study if they were able to provide informed consent, had a BMI between 20.0 and 34.9, were undertaking low impact cardiovascular exercise including, but not limited to, cycling, swimming, and walking no more than 5 times per

week, and agreed not to take other supplements (including protein or testosterone containing supplements) or medications (e.g., steroids) aimed at muscle mass growth for the duration of the trial. Exclusion criteria included those with unstable illnesses or impairments (e.g., diabetes, thyroid gland function, malignancy, lung conditions, chronic asthma, mood disorders or neurological disorders such as multiple sclerosis), serious illness or impairment (e.g., renal, hepatic, gastrointestinal, cardiovascular, neurological), acute sickness experienced within the previous two months, active smokers and/or nicotine or drug abuse, or chronic alcohol use (>14 alcoholic drinks per week). Participants were also excluded if they were currently undertaking resistance training exercise more than once a week, were allergic to any of the ingredients in the active or placebo formula (e.g., milk, whey, or yeast allergy), had participated in another exercise based clinical study during the previous three months, those who had treatment for cancer, HIV, or use of anabolic steroids in the previous year, a history of orthopaedic injuries or surgery in the previous six months, or any condition which in the opinion of the investigator made the participant unsuitable for inclusion.

Once enrolled, participants were randomly allocated to receive either AnPro® yeast protein, whey protein or a placebo. Randomisation was conducted using Random Allocation Software (sealedenvelope.com) by an individual not involved in the trial. Both participants and investigators were kept blinded to the allocation. Before starting on any trial product, all enrolled participants undertook baseline measures including: a full body scan for muscle mass [Dual Energy X-Ray Absorptiometry (DEXA)], a muscle strength and endurance test, blood test for safety markers, anthropometric measures (waist and hip circumference, height and weight), a Physical Activity Readiness Questionnaire (PAR-Q), an Ageing Males' Symptoms Questionnaire (AMS), and 24-hour diet recall. At baseline, an exercise familiarisation session was conducted to cover the prescribed exercises to be undertaken in the first 4 weeks, with a focus on teaching correct techniques. A similar session was held in week 4 to cover the prescribed exercises in the remaining 4 weeks of the study. Once all baseline measures were completed, including the familiarisation session, participants were provided with their trial product and required to take 40 g of their supplement daily (20 g in the morning and 20 g at lunch) for 8 weeks. The supplement was able to be taken as either a drink (e.g., mixed with water, milk or as a smoothie) or in food (e.g., added to cereal).

During the study, participants undertook an 8 week, at-home, training program using bodyweight for resistance. The training program consisted of exercises aimed to target the major muscle groups in the upper and lower body with variations to increase or decrease the intensity of each exercise. Participants completed 3 training sessions per week during weeks 1 to 3 and 5 to 7, and 2 sessions during the assessment weeks 4 and 8 (with the assessment counting as a 3rd session for the week). Each exercise session included an instructional video on how to complete each exercise. Exercise videos were available online via a secure link sent to the participants. In addition

to the videos, participants were guided through the first week's exercise session in person at the end of baseline testing. Additional webcam guided sessions were available for exercises undertaken in weeks 2 and 6 to help facilitate compliance and safety. Participants were also invited to attend the clinic at any stage throughout the trial to go through an exercise session should they feel they require additional support and guidance. At each completed exercise session, participants recorded all exercises completed in a diary provided.

During week 4, participants returned to the clinic to repeat the strength and endurance testing, anthropometric measures, and diet recall. During week 8, participants returned to the clinic for a repeat of all baseline measures. At both week 4 and week 8, participants were asked about any lifestyle changes (diet, exercise, and medication) in addition to subjective changes in exercise, performance (positive or negative), and any adverse events. Diet recall data was analysed using the online diet analysis software Foodworks (www.foodworks.online). Any participant that experienced an adverse event during the study was asked to contact a trial supervisor as soon as possible.

The primary outcome measure for this study, was change in lean muscle mass as measured by DEXA, and included whole body, trunk, and limbs. Secondary measures included muscle strength, as measured by 1-RM leg press and 1-RM bench press, muscle endurance, as measured by 80% 1-RM leg press repetitions to fatigue and 80% 1-RM bench press repetitions to fatigue, and additional body composition (fat mass, % body fat, body weight, waist circumference, hip circumference).

To achieve statistical power, 23 participants were required per group for power to detect a 50% difference in the change from baseline for fat free mass compared to the placebo group (e.g., 900 g vs 600 g; Effect size: 1, Alpha error probability: 0.05, Power: 0.95). Analysis was conducted using SPSS 22 and Microsoft Excel. To allow

for dropouts, up to 40 participants were recruited to each group. All results were first tested for normality before any other test was conducted. Differences between groups was assessed using independent t-tests and covariates were accounted for with an ANCOVA. A significant difference between groups was considered at a level of $p < 0.05$. Any participant that presented a result that was considered an outlier ($\pm 2SD$ away from the mean) was excluded from analysis. Subgroup analysis was conducted on any participant that reported low protein intake for individuals undertaking physical activity. Participants were considered to have low protein intake if their average reported protein intake over the 8-weeks was less than 1.2 g/kg body weight [7].

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by Bellberry Limited, application number 2020101023. This trial was registered with the Australia and New Zealand Clinical Trial Registry (ACTRN12621000212853p).

3. Results

One hundred and sixteen participants were enrolled, with 79 completing the full study requirements. Of those who did not complete the study, 10 withdrew after providing consent but prior to receiving product (i.e., did not receive product or complete baseline measures), 14 withdrew, 2 were lost to follow up, and 11 withdrew due to an adverse event (Figure 1). Of those who withdrew due to an adverse event, 4 were in the whey protein group (nasal congestion, $n = 1$; gastrointestinal upset, $n = 1$; nausea, $n = 1$; head injury, $n = 1$), 5 in the yeast protein group (shoulder injury, $n = 1$; asthma, $n = 1$; fainting, $n = 1$; nausea, $n = 1$; gastrointestinal upset, $n = 1$), and 2 in the placebo group (pain during exercise, $n = 1$; back injury, $n = 1$).

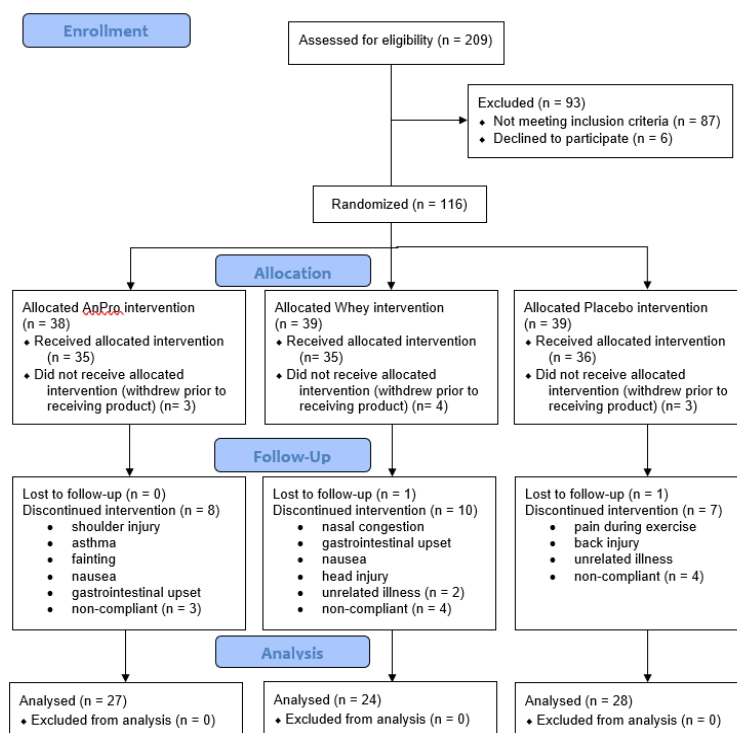


Figure 1. CONSORT participant flow diagram

Diet recall analysis over the 8 weeks showed groups were equally matched for total energy intake, carbohydrates, protein, and fat at each time point (baseline, week 4 and week 8; Table 1). Comparing change over the 8-weeks showed no significant changes from baseline for any group. No participant reported consuming protein supplements during the study (Table 1).

At baseline, the groups were similar in most measures. However, the AnPro® group had a significantly lower android to gynoid ratio compared to the placebo group and a significantly lower heart rate compared to the whey protein group. The placebo group had a significantly lower maximum bench press compared to both the AnPro® and whey protein groups at baseline, which was

consistent at week 4 and week 8.

At the completion of the study (week 8), all three groups had a significant increase from baseline in weight, BMI, total gynoid mass and maximum bench press and leg press.

Both the AnPro® and whey protein groups increased lean trunk mass and total lean mass from baseline. The whey protein group was the only group to increase their 80% bilateral leg press score and fat mass in the right leg. The AnPro® group was the only group to decrease diastolic blood pressure and increase their 80% bench press score, total android mass, android to gynoid ratio, fat mass in the left arm, and lean mass in both the left and right leg (Table 2 Table 3 Table 4).

Table 1. Anthropometric measures for all completed participants (n = 79)

	Baseline			Week 4			Week 8		
	Placebo (n = 28)	AnPro® yeast (n = 27)	Whey protein (n = 24)	Placebo (n = 28)	AnPro® yeast (n = 27)	Whey protein (n = 24)	Placebo (n = 28)	AnPro® yeast (n = 27)	Whey protein (n = 24)
Energy Intake (kJ)	9,688.4 (4,693.0)	9,327.6 (3,911.2)	8,608.6 (1,987.2)	8,613.0 (3,423.0)	7,867.8 (2,642.4)	9,321.1 (2,667.1)	9,519.4 (4,317.7)	8,518.7 (3,234.6)	10,243.0 (3,292.1)
Carbohydrates (g)	212.8 (93.7)	221.5 (136.4)	209.9 (63.8)	207.4 (95.4)	180.4 (91.7)	219.3 (91.9)	254.9 (218.3)	217.3 (90.3)	253.3 (108.9)
Protein (g)	107.3 (65.4)	96.5 (42.4)	86.2 (27.2)	95.0 (40.3)	86.1 (35.6)	112.8 (35.4)	94.5 (44.3)	91.2 (34.4)	111.9 (39.8)
Fat (g)	104.1 (68.3)	92.2 (40.6)	82.9 (29.0)	82.0 (39.7)	78.6 (34.9)	88.1 (32.2)	85.8 (40.3)	73.9 (37.2)	100.9 (57.5)

Values are mean (SD); kJ = kilojoules; g = grams.

Table 2. Anthropometric measures for all completed participants (n = 79)

	Baseline			Week 4			Week 8		
	Placebo (n = 28)	AnPro® yeast (n = 27)	Whey protein (n = 24)	Placebo (n = 28)	AnPro® yeast (n = 27)	Whey protein (n = 24)	Placebo (n = 28)	AnPro® yeast (n = 27)	Whey protein (n = 24)
Waist circumference (cm)	97.5 (10.4)	94.5 (9.8)	99.1 (9.8)	97.7 (10.5)	94.9 (9.6)	99.3 (10.0)	97.9 (10.1)	95.3 (8.7)	99.0 (10.2)
Hip circumference (cm)	103.1 (6.7)	102.5 (5.6)	103.9 (5.9)	103.4 (6.7)	102.7 (5.4)	103.7 (5.9)	103.3 (6.5)	102.5 (5.4)	104.2 (5.8)
BP Systolic (mmHg)	131.1 (12.4)	128.0 (9.0)	129.7 (11.7)	130.3 (11.1)	129.9 (9.5)	130.8 (14.3)	127.3 (9.5)	126.0 (8.3)	129.9 (10.5)
BP Diastolic (mmHg)	83.4 (8.8)	83.7 (7.4)	85.0 (9.3)	83.5 (7.7)	82.8 (8.4)	84.1 (9.9)	83.1 (6.4)	81.4 (7.5)	85.2 (7.7)
Heart rate (bpm)	68.9 (12.7)	65.0 (10.0)	71.5 (11.2)	69.0 (12.8)	68.3 (10.9)	72.3 (11.1)	70.3 (12.0)	66.6 (8.7)	71.6 (10.6)
Height (m)*	1.8 (0.1)	1.8 (0.1)	1.8 (0.1)	-	-	-	-	-	-
Weight (kg)	87.1 (13.2)	85.1 (10.7)	88.7 (12.3)	87.9 (12.9)	86.2 (10.5)	89.6 (12.6)	88.0 (13.0)	86.1 (10.5)	89.4 (12.6)
BMI (kg/m²)	26.8 (3.3)	26.2 (2.9)	27.3 (3.6)	25.1 (7.7)	26.5 (2.9)	25.3 (8.5)	27.1 (3.3)	26.5 (2.9)	27.6 (3.7)
Max bench press (kg)	54.3 (13.0)	56.7 (14.2)	62.9 (17.0)a	58.5 (14.7)	60.8 (16.6)	65.4 (17.7)	58.7 (15.0)	62.5 (15.4)	68.5 (17.8)
80% bench press reps (n)	8.2 (2.6)	9.0 (2.3)	9.2 (2.8)	9.0 (2.7)	9.3 (2.4)	9.6 (2.6)	9.5 (2.5)	10.2 (2.3)	10.1 (2.8)
Max leg press (kg)	208.4 (37.6)	255.0 (67.8)	255.0 (73.2)	225.7 (47.7)	279.2 (70.0)	272.8 (85.9)	237.2 (52.5)	287.4 (69.8)	286.1 (76.3)
80% leg press reps (n)	16.3 (6.8)	17.1 (4.7)	16.8 (9.0)	18.8 (9.8)	16.9 (5.3)	23.0 (18.1)	21.0 (18.6)	18.5 (4.9)	21.3 (13.2)

Values are mean (SD); BP = blood pressure; BMI = body mass index; a significantly different to placebo $p < 0.05$; *Height was only measured at baseline.

Table 3. DEXA measurements for all participants (n = 79)

	Baseline			Week 8		
	Placebo (n = 28)	AnPro® yeast (n = 27)	Whey protein (n = 24)	Placebo (n = 28)	AnPro® yeast (n = 27)	Whey protein (n = 24)
Total Android Mass (g)	7080.4 (1559.1)	6713.9 (1336.7)	7287.2 (1506.4)	7178.3 (1614.2)	6793.0 (1322.6)	7309.2 (1535.6)
Total Gynoid Mass (g)	13269.0 (2051.8)	13355.0 (1853.4)	13780.3 (1912.4)	13505.8 (1967.2)	13498.0 (1770.7)	13916.4 (2019.8)
Android/Gynoid ratio	1.29 (0.25)	1.14 (0.22)a	1.26 (0.26)	1.30 (0.30)	1.19 (0.23)	1.24 (0.25)
VAT Mass (g)	601.3 (274.7)	505.8 (236.0)	569.3 (223.3)	607.6 (290.4)	505.6 (220.2)	577.5 (231.2)
Fat Mass L Arm (g)	971.6 (357.0)	913.6 (360.5)	1028.5 (405.2)	986.2 (360.9)	949.5 (380.7)	1029.7 (388.4)
Fat Mass R. Arm (g)	990.8 (371.5)	948.8 (365.9)	1059.5 (441.4)	1006.2 (371.1)	962.7 (368.9)	1083.3 (451.8)
Fat Mass Trunk (g)	10275.9 (4775.5)	9220.5 (4329.4)	10812.5 (4562.1)	10607.5 (4906.1)	9350.5 (4153.1)	10542.1 (4481.4)
Fat Mass L. Leg (g)	3271.1 (1355.7)	3182.7 (1206.4)	3286.4 (1149.3)	3319.6 (1430.6)	3185.6 (1159.2)	3376.0 (1248.8)
Fat Mass R. Leg (g)	3348.6 (1395.6)	3257.0 (1290.5)	3348.1 (1286.4)	3360.1 (1379.8)	3242.8 (1221.7)	3457.8 (1350.3)
Fat Mass Total (g)	19849.7 (7958.4)	18520.1 (7183.3)	20545.5 (7539.1)	20268.5 (8127.4)	18691.3 (6957.6)	20498.5 (7546.0)
Lean Mass L Arm (g)	3797.9 (484.6)	3681.4 (511.4)	3802.6 (579.4)	3804.6 (462.3)	3740.1 (552.2)	3814.5 (588.4)
Lean Mass R. Arm (g)	3931.6 (407.4)	3922.8 (571.3)	4113.6 (590.1)	3997.7 (423.1)	3955.8 (521.9)	4163.3 (612.7)
Lean Mass Trunk (g)	31605.8 (3417.3)	31172.9 (3153.0)	32068.8 (3832.4)	31741.5 (3335.9)	31510.0 (3285.1)	32513.2 (3811.9)
Lean Mass L. Leg (g)	10643.3 (1346.5)	10515.6 (1290.0)	10730.6 (1121.9)	10771.5 (1323.4)	10684.5 (1202.9)	10869.7 (1297.8)
Lean Mass R. Leg (g)	11011.4 (1300.9)	10915.1 (1350.0)	11082.1 (1325.4)	11133.9 (1274.7)	11140.4 (1283.0)	11180.3 (1409.3)
Lean Mass Total (g)	64573.5 (6480.0)	63815.0 (6240.3)	65450.7 (7083.0)	65021.4 (6262.5)	64642.6 (6297.4)	66192.1 (7290.5)

Values are mean (SD); a significantly different to placebo $p < 0.05$; VAT = Visceral Adipose Tissue; L = left; R = right

Table 4. Change from baseline for all participants (n = 79)

	Placebo (n = 28)	AnPro® yeast protein (n = 27)	Whey protein (n = 24)
Waist circumference (cm)	0.41 (2.69)	0.77 (2.22)	-0.12 (2.86)
Hip circumference (cm)	0.20 (1.57)	0.04 (1.68)	0.28 (1.82)
BP Systolic (mmHg)	-3.79 (12.38)	-2.04 (8.43)	0.21 (8.39)
BP Diastolic (mmHg)	-0.36 (8.60)	-2.30 (4.87)*	0.21 (6.32)
Heart rate (bpm)	1.39 (7.94)	1.59 (7.10)b	0.13 (10.29)
BMI (kg/m²)	0.30 (0.49)*	0.31 (0.42)*	0.28 (0.57)*
Max bench press (kg)	4.63 (4.26)*	5.83 (4.07)*	5.58 (4.87)*
80% bench press reps (n)	1.26 (3.68)	1.19 (2.72)*	0.70 (2.12)
Max leg press (kg)	27.66 (24.22)*	32.98 (19.50)*	29.81 (23.42)*
80% leg press (n)	4.50 (15.22)	1.16 (5.68)b	4.61 (5.29)*
Total Mass(g)	875.8 (1,633.7)*	1,019.2 (1,081.2)*	685.6 (1,127.2)*
Total Android (g)	97.9 (280.7)	79.1 (183.2)*	22.0 (242.3)
Total Gynoid (g)	236.8 (353.6)*	142.9 (263.9)*	136.2 (267.0)*
Android/Gynoid ratio	0.01 (0.09)	0.05 (0.07)*,b	-0.02 (0.10)
VAT mass(g)	6.32 (69.25)	-0.15 (43.59)	8.25 (54.71)
Fat L Arm (g)	14.64 (93.98)	35.94 (81.00)*	1.28 (79.97)
Fat R. Arm (g)	15.38 (77.11)	13.92 (88.18)	23.86 (96.02)
Fat Trunk (g)	331.7 (876.9)	130.0 (795.7)	-270.4 (927.9)a
Fat L. Leg (g)	48.5 (250.5)	3.0 (190.2)	89.6 (220.0)
Fat R. Leg (g)	11.5 (233.3)	-14.2 (232.8)	109.8 (257.3)*
Fat Total (g)	418.8 (1,220.75)	171.3 (1,102.3)	-46.9 (1,271.2)
Lean L Arm (g)	6.7 (203.3)	58.8 (179.1)	11.9 (154.7)
Lean R. Arm (g)	66.2 (175.0)	32.9 (175.0)	49.8 (201.7)
Lean Trunk (g)	135.7 (1,392.3)	337.1 (827.7)*	444.4 (918.3)*
Lean L. Leg (g)	128.2 (330.3)	168.9 (329.8)*	139.1 (334.6)
Lean R. Leg (g)	122.5 (329.7)	225.3 (270.3)*	98.2 (347.6)
Lean Total (g)	447.8 (1,781.6)	827.6 (875.6)*	741.4 (1,158.6)*

Values are mean (SD); * Significant change from baseline ($p < 0.05$); a Significant difference to placebo ($p < 0.05$); b Significant difference to Whey protein ($p < 0.05$).

When the change at week 8 was compared between groups, the whey protein group had a significant reduction in android to gynoid ratio and increase in 80% leg press

reps compared to the AnPro® group. The whey protein group also had a significant reduction in total trunk fat mass compared to the placebo group (Table 4).

Table 5. Change from baseline – subgroup analysis (low protein intake) (n = 48)

	Placebo (n = 16)	AnPro® yeast protein (n = 20)	Whey protein (n = 12)
Waist circumference (cm)	1.18 (1.86)	0.53 (2.40)	0.50 (2.57)
Hip circumference (cm)	0.29 (1.53)	-0.11 (1.75)	0.12 (2.1)
BP Systolic (mmHg)	-3.50 (11.74)	-2.80 (7.74)	2.25 (6.76)
BP Diastolic (mmHg)	0.25 (9.23)	-2.15 (4.93)	0.58 (6.92)
Heart rate (bpm)	2.25 (8.68)	1.45 (7.77)	-0.67 (12.62)
BMI (kg/m²)	0.26 (0.37)	0.26 (0.47)	0.29 (0.64)
Max bench press (kg)	2.43 (3.52)	5.47 (3.84) ^a	5.83 (6.14)
80% bench press reps (n)	2.07 (4.33)	0.60 (2.37)	1.18 (2.75)
Max leg press (kg)	28.32 (25.65)	31.92 (19.17)	32.01 (26.54)
80% leg press (n)	2.21 (7.54)	1.16 (5.39) ^b	6.18 (6.76)
Total Mass(g)	587.7 (1,312.1)	950.9 (1,244.2)	716.5 (1,274.7)
Total Android (g)	123.6 (220.13)	74.6 (193.5)	7.0 (264.5)
Total Gynoid (g)	148.5 (288.7)	120.15 (285.0)	234.7 (294.0)
Android/Gynoid ratio	0.00 (0.08)	0.06 (0.06) ^{a,b}	-0.04 (0.12)
VAT mass(g)	6.69 (75.92)	-4.15 (46.63)	18.08 (61.29)
Fat L Arm (g)	8.33 (82.94)	35.64 (86.30)	8.67 (87.86)
Fat R. Arm (g)	19.93 (79.41)	15.69 (94.35)	64.38 (88.23)
Fat Trunk (g)	520.25 (684.51)	72.57 (806.12)	-132.51 (1,001.15) ^a
Fat L. Leg (g)	74.04 (268.39)	-16.73 (210.24) ^b	159.59 (240.76)
Fat R. Leg (g)	56.60 (182.67)	-48.09 (245.53) ^b	192.96 (247.85)
Fat Total (g)	665.6 (920.1)	63.7 (1,124.1)	286.4 (1,280.7)
Lean L Arm (g)	20.22 (197.9)	46.75 (198.60)	-15.86 (132.86)
Lean R. Arm (g)	57.79 (188.64)	30.00 (177.04)	25.35 (234.34)
Lean Trunk (g)	-227.4 (1,157.8)	387.5 (845.6)	125.7 (558.8)
Lean L. Leg (g)	75.95 (392.3)	126.27 (337.06)	207.36 (403.55)
Lean R. Leg (g)	20.28 (311.65)	263.08 (298.58) ^a	109.52 (316.96)
Lean Total (g)	-99.1 (1,778.6)	867.14 (844.7) ^a	439.7 (680.8)

Values are mean (SD); ^a Significant difference to placebo ($p < 0.05$); ^b Significant difference to Whey protein ($p < 0.05$).

No significant differences were seen for exercise compliance, with both groups reporting an exercise session completion rate of $> 96\%$. No significant differences were seen in the AMS questionnaire either within or between groups throughout the study.

When subjects were analysed for protein intake, 48 participants were identified as having a daily protein intake below the recommendation (Table 5). Subgroup analysis showed at baseline the AnPro® group had a significantly lower android to gynoid ratio, as seen in the full group analysis. Comparing groups for change at week 8, the AnPro® group had a significantly greater change in

android to gynoid ratio compared to both the placebo and whey protein group. The whey protein group had a significantly greater trunk fat mass reduction compared to the placebo group. Fat mass in both legs was significantly reduced in the AnPro® group compared to the whey protein group. Total lean mass and right leg lean mass was increased significantly more in the AnPro® group compared to the placebo (Table 5).

4. Discussion

The aim of this study was to assess the effectiveness of yeast protein on increasing muscle mass and strength over 8 weeks compared to whey protein or placebo supplementation in otherwise healthy adult males aged 40 years and older. Analysis of diet recall data revealed that all groups exhibited similar nutrient intake profiles, including protein. Notably, one participant in the whey protein group disclosed consuming a protein supplement during screening. However, this individual ceased supplement intake two weeks prior to study commencement. Consequently, it is anticipated that dietary intake exerted minimal influence on the overall study outcomes, with any observed differences between groups likely attributable to the assigned study product.

At baseline, most outcome measures were similar across all three groups. The primary difference observed was that the placebo group exhibited a significantly lower maximum bench press compared to the whey protein group. Additionally, differences included the AnPro® group displaying a lower android to gynoid ratio in contrast to the placebo group, and a lower heart rate compared to the whey group. The higher starting bench press in the whey protein group might have potentially posed challenges for improvements within this group. Nevertheless, it is improbable that these baseline disparities significantly influenced the study outcomes, as changes observed throughout the study appeared to be relative to each group's respective starting points (see Table 4).

At the completion of the study, all three groups exhibited increases in weight, BMI, total gynoid mass, as well as improvements in maximum bench press and leg press from baseline. These outcomes suggest that all groups experienced strength gains and adhered to the prescribed exercise regimen. The primary focus of this study was to assess changes in lean muscle mass. Notably, only the AnPro® and whey protein groups demonstrated increases in lean trunk mass and total lean mass compared to baseline. These results show that both whey protein and AnPro® supplementation can significantly augment lean mass compared to a placebo, with AnPro® showing comparative efficacy to whey protein.

The whey protein group demonstrated an increase in their 80% leg press score, whereas the AnPro® group exhibited an increase in their 80% bench press score from baseline. These findings suggest that both whey protein and AnPro® supplementation can enhance strength endurance. The reason for the differential improvements between the two groups, with one showing progress in leg press score and the other in bench press score, remains unclear but may stem from various factors. One possibility

is that participants in each group may have favoured upper or lower body exercises, potentially leading to greater investment of effort and endurance in the respective muscle groups.

The absence of change in leg press score in the AnPro® group contrasts with their observed increase in leg lean mass. Notably, the AnPro® group demonstrated augmented lean mass in both the left and right legs, indicative of increased muscle development. While the increase in lean mass is typically associated with enhanced maximum strength, it would also be anticipated to result in improved endurance.

Potential explanations for the lack of observed changes in endurance could stem from factors such as the duration of the study period and the intensity of the prescribed exercises. Although an 8-week duration should theoretically induce neurological adaptations and muscle growth, it might have been insufficient to fully optimize muscle adaptations. Additionally, the effectiveness of the observed effects may have been influenced by the quantity and intensity of the prescribed exercise regimen. While the exercises provided opportunities to increase intensity, being predominantly body weight-based, participants might not have challenged themselves as rigorously as those engaged in weight-based resistance training.

Future studies might consider extending the duration of the intervention and incorporating more gym-based exercises to potentially enhance outcomes. However, implementing gym-based exercises in a research setting poses logistical challenges and typically necessitates in-clinic monitored gym sessions. Despite these potential limitations, the observed effects still lend support to AnPro® and whey protein supplementation being superior to a placebo, with AnPro® comparable to whey protein.

The outcomes of this study align with findings from other research focusing on protein supplementation. For instance, a study conducted by Griffen and colleagues (2022) involving older men showed whey protein supplementation, when combined with resistance exercise, led to increased leg press performance, augmented lean mass, and decreased fat mass compared to protein supplementation alone [39]. Similarly, research by Bell and colleagues (2017) among older men demonstrated that 6 weeks of whey protein supplementation without exercise resulted in participants gaining strength and lean mass, with an additional 6 weeks of exercise yielding further increases in upper body strength [40]. A study focusing on older men found ingestion of 40 g of whey protein stimulated a more pronounced response of MPS post-exercise [41].

These studies collectively underscore the importance of both protein intake and exercise in optimizing gains in strength and lean mass. Notably, the findings from these studies mirror the results of the current study, where supplementation with both whey and yeast protein led to enhancements in lean mass and muscle strength and endurance. Such improvements in muscle mass and strength are likely attributed to the optimal stimulation of muscles induced by resistance training, complemented by the availability of protein to enhance myofibrillar protein synthesis [40].

Adequate dietary protein plays a crucial role in

maintaining various physiological functions within the body. Inadequate protein intake can lead to conditions such as anaemia, physical weakness, and compromised immunity [42]. that the majority of exercising individuals can benefit from a daily protein intake ranging between 1.2 to 2.0 grams per kilogram of body weight to effectively build and sustain muscle mass [6,7] [43]. Subgroup analyses targeting individuals with low dietary protein intake, as per the recommendations of the American College of Sports Medicine (ACSM), revealed significant enhancements in lean mass and strength following supplementation with either yeast or whey protein.

Research conducted on younger men identified that a daily dose of at least 20 g of whey protein stimulated muscle protein synthesis, with similar effects observed at higher doses [44]. This finding aligns with the dosage employed in the present study. However, it's important to note that the optimal amount of protein required for muscle gain may vary depending on the intensity and volume of exercise performed. Engaging in high workload activities may necessitate a higher protein intake to support muscle recovery and growth.

Muscle protein synthesis (MPS) is closely intertwined with both nutrition and exercise, as both factors have been demonstrated to trigger this process. Nutrition and exercise act synergistically to promote protein anabolism within skeletal muscle, with sustained increases in protein synthesis providing the stimulus necessary for preserving and augmenting skeletal muscle strength and size [45]. The postprandial availability of essential amino acids also plays a crucial role in MPS, with the quantity required to meet these demands contingent upon the protein quality [46].

Protein quality is often assessed using the PDCAAS, with supplements boasting higher PDCAAS values typically exhibiting greater efficacy regulating skeletal muscle mass. An examination of the composition of AnPro® yeast protein revealed comparable PDCAAS values to whey protein [37,38], which has been assigned the highest PDCAAS value of 1.00 [46]. This evidence suggests that the observed outcomes among individuals supplemented with yeast protein, in comparison to whey protein, are likely attributable to its ability to effectively stimulate MPS.

In summary, this study demonstrated that supplementation with either AnPro® or whey protein, particularly in individuals with low dietary protein intake, improves lean mass and muscle strength among healthy men aged over 40 when combined with resistance training. AnPro® is a viable and sustainable protein source, exhibiting an effectiveness comparable to that of whey protein.

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Conflict of Interest

None

Authorship

The authors confirm contribution to the paper as follows: formulating the research question: DB, HZ, ZC and AR; designing the study: DB and AR; carrying out the study: DB, RS and AR; analysing the data: AR; interpreting the findings: DB and AR; manuscript preparation: DB, HZ, ZC and RS.

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