ORIGINAL ARTICLE

Combined Effects of Plant-based Protein Supplementation with 8-week Resistance Training on Muscular Strength, Protein Catabolism, Immune Functions and Bone Metabolism Markers in Adult Males

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ABSTRACT

Introduction: This study investigated the effect of combined plant-based protein supplementation and resistance training on muscular strength, blood markers of protein catabolism, immune function, and bone metabolism in sedentary adult males. **Methods:** In this randomised, double-blinded study, 28 healthy males aged 19 – 29 years old were equally assigned into four groups: a combined plant-based protein with resistance training (PBPEX), plant-based protein alone (PBP), resistance training alone (EX) and control (C). Mode of resistance training was flat barbell press, machine shoulder press, wide grip lateral pull-down, seated cable row, barbell back squat, leg press and leg extension. The 8-week resistance training involved three sets of 60-70% of one-repetition maximum (1-RM) at 4-6 repetition/set/mode per session, three sessions/week. Participants in PBPEX and PBP groups consumed a plant-based protein supplement consisted of 9.8 g soy and pea protein for seven days/week. **Results:** PBPEX showed significant increases ($p<0.01$) in the knee and shoulder flexion peak torque compared to EX groups, respectively. PBP showed a significantly higher level ($p<0.05$) of serum urea, and blood urea nitrogen (BUN) compared to other groups. There were no changes in immune function and bone metabolism markers between pre- and post-exercise in all groups. **Conclusions:** These findings implied that a combination of plant-based protein supplementation and resistance training elicited greater beneficial effects on muscular strength than resistance training alone and plant-based protein supplementation alone. Therefore, combined plant-based protein with resistance training may be recommended in planning exercise and nutritional programme for sedentary male adults.

Keywords: Isokinetic peak torque, Soy isoflavone, Blood urea nitrogen, Creatinine, Immune function, Bone turnover

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INTRODUCTION

The depth of evidence for the most studied plant-based protein, soy protein, is well known (1). The bioactive components of the soy protein, isoflavones have been widely known to be beneficial to cardiovascular health but was less superior than animal-based protein in promoting gains in lean mass and physical performance (2, 3). As such, different components contained in various type of plant protein that yielded an essential role in muscle protein synthesis have received much attention. Recently, pea (Pisum sativum) protein isolate containing abundant essential branched-chain amino acids (BCAA) have shown to enhance muscle thickness, which is crucial to achieving gains in muscle strength (4). Although possessed an anabolic effect, the BCAA, if ingested in excess, undergoes rapid degradation to prevent toxicity caused by branched-chain α-keto acids, which is an intermediary product of catabolism (5). However, oxidation of BCAA in the skeletal muscle is greatly enhanced by exercise through activation of specific key enzymes (6). Therefore, to further enhance protein synthesis, the BCAA has been the preferred choice to elicit a greater effect.

Plant protein supplements in the form of soy protein isolate used to be the preferred choice for its higher protein quality per gram than other protein sources to promote exercise-induced muscle mass gains (7). Now, protein blends, from a mixture of various protein sources, have been offered with the rationale to extend or optimise protein delivery by increasing amino acid digestive rate (8, 9). Although the speed of protein delivery is important, the key to muscle gains has been the role of BCAA in reducing muscle damage (10). It was also reported that BCAA supplementation modifies the pattern of exercise-related cytokine production, by activating inflammatory macrophages, leukocytes and cytolytic responses leading to protection against

pathogens (11). In the malnourished and diseased, amino acids dietary intake could enhance the immune function through activation and proliferation of T and B lymphocytes, natural killer cells and macrophages, including antibodies, cytokines and other cytotoxic productions (12). However, protein blends have been less extensively studied in this respect.

Besides developing muscle strength, resistance exercise elicits a positive effect on general health and reduces chronic diseases (13, 14). Moderate intensity exercise enhances the immune response by providing a temporary boost in the production of macrophages (15). With regards to bone health, resistance exercise alone has been clinically shown to maintain bone strength by exerting mechanical load (16). Bone tissue continues to remodel itself by two opposing processes, which are the bone formation and bone resorption processes (17). An increase in bone formation and a suppression on bone resorption marker was observed among young male adults aged 23-31 years old following four months of resistance training (18). Dietary intake of protein increases the mediator of bone growth, the insulin-like growth factor-1, thereby indirectly increases bone mass (19). However, the effect of adaptation of soy and pea protein blend supplementation to resistance exercise has yet to be evaluated for its efficacy on bone metabolism.

To date, there are still limited studies to show whether plant-based soy and pea protein blend can support the skeletal muscle protein accretion, enhance immune function and bone metabolism in response to resistance training. Therefore, the present study was proposed to investigate the combined effects of plant-based protein supplement consisted of soy and pea and resistance training on muscular strength, blood markers of protein catabolism, immune function and bone metabolism in sedentary adult males.

MATERIALS AND METHODS

Participants

Twenty-eight healthy Malaysian sedentary male adults volunteered in this study . The study protocol was fully explained to the participants before obtaining their written consent. Participants who volunteered have met the inclusion criteria of the study: engaged in exercise for less than two times a week and obtained medical clearance to participate in physical activity . Exclusion criteria include intake of anabolic agents or supplements known to increase performance. The study was approved by the research ethics committee of Universiti Sains Malaysia (USM) (USM/JEPeM/17030173). The participants' physical characteristics and body composition are presented in Table I. The randomisation of participants into groups was done using Research Randomizer (20), i.e. 1) Control with placebo (C), 2) plant-based protein supplement alone (PBP), 3) resistance training with placebo (EX), and 4) resistance training with plant-based protein supplement (PBPEX) groups. Based on the primary strength measurement outcome of a previous study (21), sample size calculation (G*Power version 3.1.9.2) of 7 participants per group was needed to yield a statistical power of 0.8 with an alpha of 0.05 and a moderate effect size of 0.7 (22). The consolidated standards of reporting trials (CONSORT) is shown in Fig. 1.

Table I: Anthropometric and body composition data of all the participants

Parameters (N=28)	PBPEX	PBP	EX	C	*p* value
Age (years)	23.9 ± 3.3	23.2 ± 4.5	21.5 ± 2.3	22.2 ± 2.1	0.581
Body height (cm)	167.7 ± 4.4	171.2 ± 5.9	168.5 ± 9 .1	170.5 ± 2.5	0.702
Body weight (kg)	74.77 ± 12.7	73.05 ± 11.7	65.00 ± 9.2	68.90 ± 17.1	0.553
Body mass index (BMI)(kg/m^2)	26.5 ± 3 .5	30.1 ± 12.9	23.1 ± 3.6	23.7 ± 5.9	0.368
Fat percentage (%)	24.6 ± 7.7	24.1 ± 7.2	20.4 ± 6.5	23.3 ± 12.0	0.829
Fat mass (kg)	19.1 ± 8.5	18.2 ± 6.8	13.6 ± 5.6	17.5 ± 14.6	0.756
Fat-free mass (kg)	55.7 ± 5.6	54.8 ± 6.1	51.4 ± 5.4	50.6 ± 3.5	0.274

Data in mean ± SD. PBPEX = plant-based protein supplement with resistance exercise, PBP = plant-based protein supplement, EX = resistance exercise with placebo, and C = control with placebo

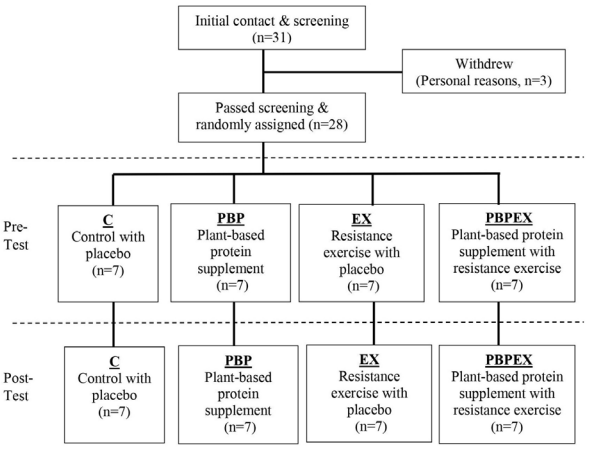

Figure 1: CONSORT (Consolidated Standards of Reporting Trials) diagram of the study

Muscular Strength Testing

All participants performed a familiarisation on a separate day based on previous recommendations (23). Two different angular velocities of 60°.s^{-1} and 180°.s^{1}, performed for 5 and 10 reps respectively with 60 s rests between angular velocities, were used to measure shoulder and knee flexion and extension muscular peak torque of the dominant (R) and non-dominant (L) limbs using an isokinetic dynamometer (Biodex System 3 Pro, New York, USA).

Supplemental Intervention

A 9.8 g blend of isolated soy protein and pea protein was administered to PBP and PBPEX group, a dosage

recommended by the manufacturer to be safely administered to human (Summit Co., Malaysia). The C and EX groups consumed maltodextrin (DE10-12, China) as placebo to mimic the taste and colour of protein blends (24). This study was a double-blinded, placebo-controlled trial. A laboratory officer who was not participating in the data analysis of the study was assigned to prepare the supplementation. Each treatment supplement and placebo were prepared as dry powder in sealed sachets and with a number code only known to the laboratory officer to ensure the blinding of participants and researchers. Participants were asked to mix the powder with 150 ml of plain water and consumed every day after breakfast for 8 weeks. On resistance exercise days, PBPEX and EX groups consumed the supplement at 30 minutes after training. The participants recorded their daily intake of supplementation and was requested to maintain similar food intake throughout the duration of the study based on their baseline food diary, which they completed prior to the study period. The supplementation compliance was 95%. Participants maintained similar dietary intake and daily activities throughout the study period, and recorded their daily activities in the checklists provided by the researcher.

Resistance Training Intervention

The EX and PBPEX groups performed resistance training from 9.00-10.00 p.m. All participants attended the training sessions . One week before the commencement of training, both the one-repetition maximum (1-RM) bench press and back squat predictions were determined. Participants performed a warm-up, two sets of 10 repetitions at 20-30% of individual 10-RM with 3-min rest intervals between sets, before performing the highest number of repetitions until the resistance was intolerable to be sustained. The 1-RM was then calculated based on a previous method (25).

The resistance training was performed at seven stations with each station targeting training of major muscle groups: flat barbell press (pectoralis, anterior deltoid, triceps brachii), shoulder press (anterior and medial deltoids, triceps brachii), wide grip lateral pull-down (latissimus dorsi, biceps brachii), seated cable row (latissimus dorsi, rhomboid, biceps brachii), barbell back squat (quadriceps, gluteal, hamstrings), leg press (quadriceps, hamstrings, gluteal), and leg extension (quadriceps). Each prescribed resistance training mode began with 3 sets of 4-6 repetition per set at 60-70% of predicted 1-RM, with 2-5 mins of resting period between each set, based on a modified protocol recommended for beginners (13). The rate of repetitions was performed in a controlled manner, with concentric action and eccentric action of approximately 1 s and 2 s, respectively (26).

The participants completed three sessions per week to a total of 24 resistance training sessions, with at least 48 h of physical recovery between sessions. The intensity of training was progressively increased by 5% when participants could perform beyond the prescribed number of repetitions for each exercise mode.

Blood Sampling and Analyses

An 8 ml of venous fasting blood were drawn at 8.00-10.00 a.m. at pre and post 8 weeks. At post 8 weeks, blood was drawn from EX and PBPEX groups at 10-12 hours (8.00-10.00 a.m.) after training. Blood in the ethylenediamine tetra-acetic acid (EDTA) and plain tubes were centrifuged for 10 min at 3000 RPM in 4^{o}C (Hettich Zentrifuger-Rotina 46RS, Germany) to obtain supernatants. The blood supernatants were distributed equally by volume into sterile 1-ml polypropylene tubes before storing at -80^{o}C freezer (Thermo Forma, Model 705, USA) for later analysis. Then, serum creatinine, urea and blood urea nitrogen (BUN) were assayed spectrophotometrically as per the manufacturer's guideline (ARCHITECT c8000, Abbott Diagnostic, USA). The coefficient of repeatability for creatinine was 0.18 mg.dL^{-1}. For urea and BUN, the coefficient of repeatability was 2.2 mg.dL^{-1}.

White blood cell (WBC), lymphocyte, neutrophil, monocyte, eosinophil and basophil counts were determined using an automated haematology analyser (Sysmex XS-800i, Sysmex Corporation Kobe, Japan). For the immunophenotyping, a four-colour direct immunofluorescence reagent kit (BD MultitestTM IMK) and a flow cytometer (BD FACS Cantor ii, Becton Dickinson, USA) were used to analyse for T lymphocyte subsets (CD3+, CD4+, CD8+), B cell (CD19+) and Natural Killer (NK) cell (CD16+CD56+) absolute counts . Bone formation marker and serum alkaline phosphatase wereassayed using a reagent kit (Roche Diagnostics GmbH, Germany), whereas bone resorption marker, serum Cross-Linked C-telopeptide of type 1 Collagen (CTX1), was assayed using human CTX-1 kit (Elabscience Biotechnology Inc., USA), with both assays analysed via the calorimetric method (Hitachi Automatic Analyser 912, Bohringer Mannheim, Germany). All bone metabolism markers were assayed as per manufacturer's instructions.

Statistical Analysis

Statistical analyses were performed using SPSS version 22.0. Shapiro-Wilk test showed all data were normally distributed . Mixed factorial analysis of variance (ANOVA) was used to analyse mean differences in anthropometric and body composition parameters between groups. Repeated measures ANOVA with two fixed-effect factors design (treatment group and time trial) was used to analyse the differences in strength, catabolism markers, immune parameters and bone metabolism makers. A Bonferroni post hoc test was used to evaluate significant F-value. Degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity if the assumption of sphericity had been violated. P-value <0.05 was considered as statistically significant. All data

are presented as mean ± standard deviation (SD).

RESULTS

Combined plant-based protein with resistance training on muscle strength.

Fig. 2 shows the means of knee extension and flexion peak torque at 60°.s^{-1} and 180°.s^{-1} respectively. For knee flexion peak torque at 60°.s^{-1}, main effects of time, F(1, 21) = 26.1, $p < 0.001$, was found and this main effect was shown by an interaction between group and time, F(3,21) = 15.9, $p < 0.001$. The study showed significantly greater peak torque in the PBPEX for right (p<0.001) and left (p=0.001) knee flexion at 60°.s^{-1} in post-test compared to pre-test. From the test of between-group effect, the result showed significant greater value in PBPEX compared to EX (p<0.001), PBP (p<0.001) and C (p<0.001) groups respectively during post-test. For knee flexion peak torque at 180°.s^{-1}, main effect was shown by an interaction between group and time, F(3,21) = 24.9, $p < 0.001$. There was significantly greater peak torque in the PBPEX for right (p<0.001) and left (p<0.001) knee flexion at 180°.s^{-1} in post-test compared to pre-test. PBPEX showed significant greater peak torque when compared to EX, PBP and C groups in post-test.

Fig. 3 shows the means of shoulder extension and flexion peak torque at 60°.s^{-1} and 180°.s^{-1} respectively. For shoulder flexion peak torque at 60°.s^{-1}, main effects of time, F(1, 21) = 6.56, $p < 0.05$, was demonstrated but an interaction between group and time was not shown, F(3, 21) = 1.92, $p = 0.156$. Left shoulder flexion in PBPEX (p<0.001) showed significantly greater peak torque in post-test compared to pre-test, and to all the other groups during post-test. There were no significant differences in the right shoulder (p=0.019) and left shoulder (p=0.950) extension peak torque at 180°.s^{-1}of all the groups in post-test compared to pre-test. As for right shoulder flexion 180°.s^{-1}, main effects of time, F(1, 21) = 11.37, $p <$ 0.005, was found and this main effect was shown by an interaction between group and time, F(3,21) = 4.44, p

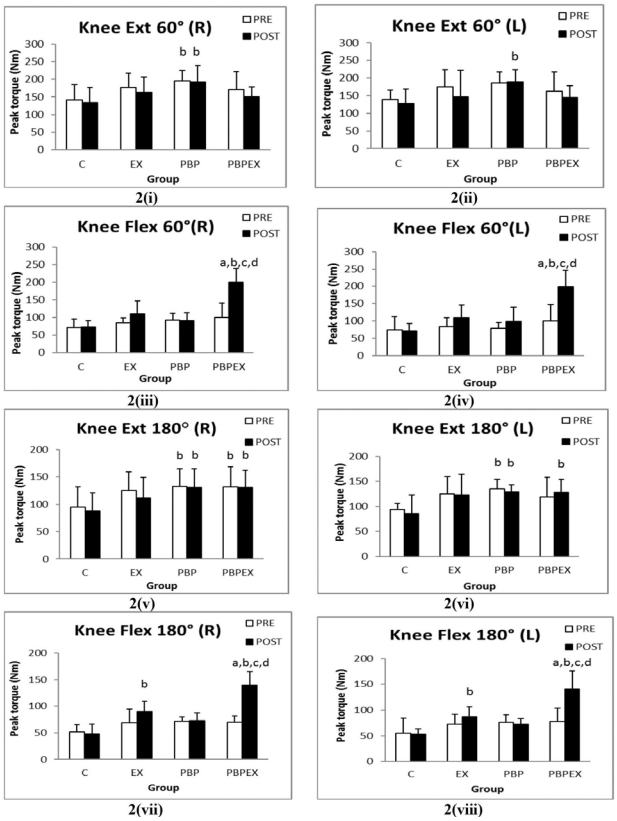

Figure 2: (i) mean dominant knee extension peak torque at 60°.s^{-1}, **(ii)** mean non-dominant knee extension peak torque at 60°.s^{-1}, **(iii)** mean dominant knee flexion peak torque at 60°.s^{-1}, **(iv)** mean non-dominant knee flexion peak torque at 60°.s^{-1}, **(v)** mean dominant knee extension average power at 180°.s^{-1}, **(vi)** mean non-dominant knee extension average power at 180°.s^{-1}, **(vii)** mean dominant knee flexion average power at 180°.s^{-1} and **(viii)** mean non-dominant knee flexion average power at 180°.s^{-1}.

[a], significantly different from pre-test (p<0.05); [b], significantly different from respective control group (p<0.05); [c], significantly different from respective EX group (p<0.05); [d], significantly different from respective PBP group (p<0.05)

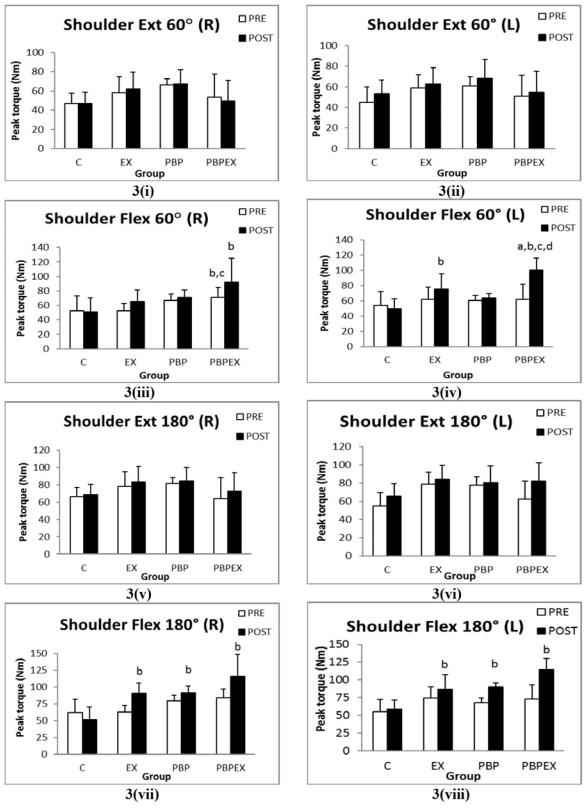

Figure 3: (i) mean dominant shoulder extension peak torque at 60°.s^{-1}, **(ii)** mean non-dominant shoulder extension peak torque at 60°.s^{-1}, **(iii)** mean dominant shoulder flexion peak torque at 60°.s^{-1}, **(iv)** mean non-dominant shoulder flexion peak torque at 60°.s^{-1}, **(v)** mean dominant shoulder extension average power at 180°.s^{-1}, **(vi)** mean non-dominant shoulder extension average power at 180°.s^{-1}, **(vii)** mean dominant shoulder flexion average power at 180°.s^{-1} and **(viii)** mean non-dominant shoulder flexion average power at 180°.s^{-1}.

[a], significantly different from pre-test (p<0.05); [b], significantly different from respective control group (p<0.05); [c], significantly different from respective EX group (p<0.05); [d], significantly different from respective PBP group (p<0.05)

< 0.05.

There was significantly greater peak torque in the PBPEX (p<0.001), PBP (p=0.004) and EX (p=0.005) compared to C group respectively in post-test. For left shoulder flexion 180°.s^{-1}, there was significantly greater peak torque in the PBPEX (p<0.001), PBP (p=0.019) and EX (p=0.034) compared to C group respectively in post-test.

Combined plant-based protein with resistance training on protein catabolism and bone metabolism

Mean serum creatinine, urea, BUN, ALP, and CTX-1 are presented in Fig. 4. For BUN, main effects of time was not significant, F(1, 20) = 0.614, p = 0.443, but there was an interaction between group and time, F(3, 20) = 5.39, p < 0.01. For serum urea, main effects of time, F(1, 21) = 0.773, p < 0.005, was found and there was an interaction between group and time, F(3, 21) = 6.98, p < 0.005. There was significantly higher serum urea (p=0.002) and BUN (p=0.007) found in the PBP group compared to C, EX and PBPEX groups, respectively at post-test. Serum creatinine in PBP (p=0.006) was significantly higher than the C group in post-test. Serum ALP and serum CTX-1 were not significantly different at post-test compared to pre-test in PBP, EX and PBPEX groups. For serum ALP, main effects of time was not significant, F(1, 21) = 2.78, p =0.110, and there was no interaction between group and time, F(3, 21) = 1.258, p = 0.314, whereas for serum CTX-1, main effects of time was not significant, F(1, 21) = 1.91, p =0.181, and there was no interaction between group and time, F(3, 21) = 1.015, p = 0.406.

Combined plant-based protein with resistance training on immune function

Fig. 5 shows the means of white blood cell, lymphocyte, monocyte, neutrophil, eosinophil and basophil count. Main interaction effects for white blood cell [F(3, 21) = 0.218, p = 0.883], lymphocyte [F(3, 21) = 0.369, p = 0.776], monocyte [F(3, 21) = 0.471, p = 0.706], neutrophil [F(3, 21) = 0.451, p = 0.719], eosinophil [F(3, 21) = 0.531, p = 0.666] and basophil [F(3, 21) = 1.079, p = 0.379] were not significantly different in all the groups. Fig. 6 shows the means total T-lymphocyte, T-helper, T-cytotoxic, B and NK cells. No significant differences in main interaction effects were found in the immunophenotyping of B-cells [F(3, 21) = 0.287, p = 0.834] and NK cells [F(3, 21) = 0.360, p = 0.783], as well as T-lymphocyte [F(3, 21) = 0.522, p = 0.672], T-helper [F(2, 16) = 0.055, p = 0.946] and T-cytotoxic [F(3, 21) = 1.77, p = 0.202] cells in PBP, EX and PBPEX groups.

DISCUSSION

This study observed the effect of combined plant-based protein with resistance training on muscle strength. Combined plant-based protein with resistance training demonstrated a significant increase in knee and shoulder flexion peak torque compared to resistance training alone. Additionally, plant-based protein alone has no significant effect on both knee and shoulder

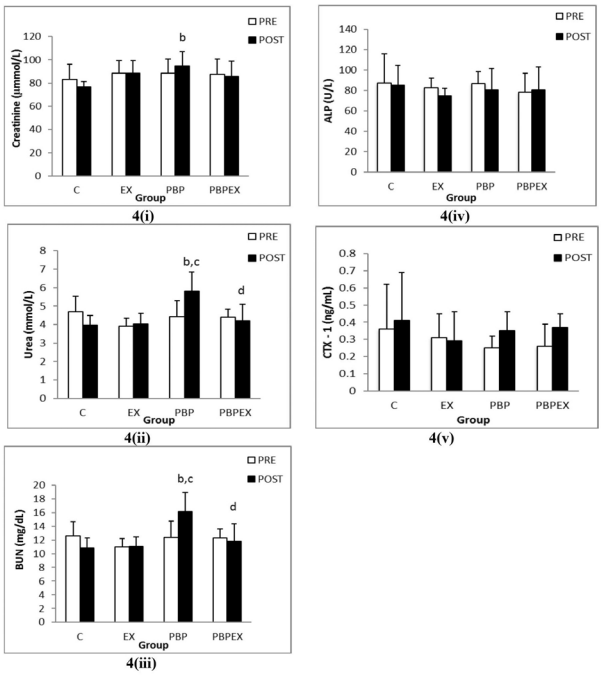

Figure 4: (i) mean serum creatinine, **(ii)** mean serum urea, **(iii)** mean serum blood urea nitrogen (BUN), **(iv)** mean serum alkaline phosphatase (ALP), and **(v)** mean serum cross linked C- telopeptide of type 1 procollagen (CTX-1)..

[a], significantly different from pre-test (p<0.05); [b], significantly different from respective control group (p<0.05); [c], significantly different from respective EX group (p<0.05); [d], significantly different from respective PBP group (p<0.05)

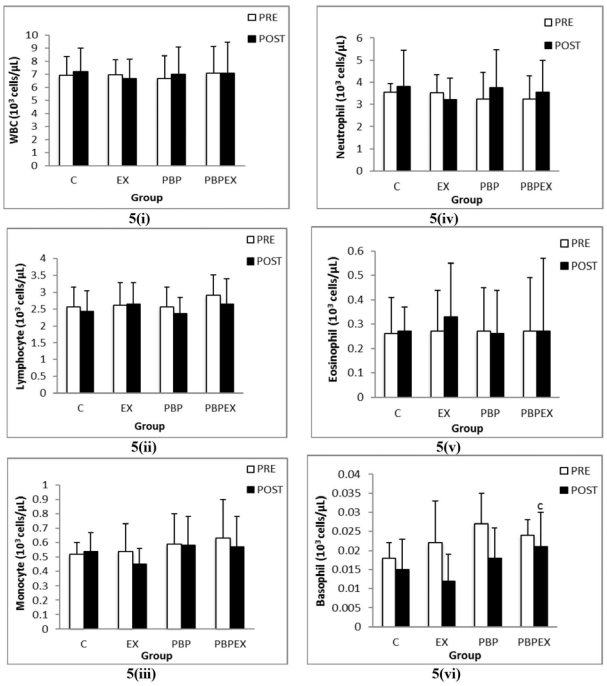

Figure 5: (i) mean white blood cell (WBC), **(ii)** mean lymphocyte, **(iii)** mean monocyte, **(iv)** mean neutrophil, **(v)** mean eosinophil, and **(vi)** basophil counts.

[a], significantly different from pre-test (p<0.05); [b], significantly different from respective control group (p<0.05); [c], significantly different from respective EX group (p<0.05); [d], significantly different from respective PBP group (p<0.05)

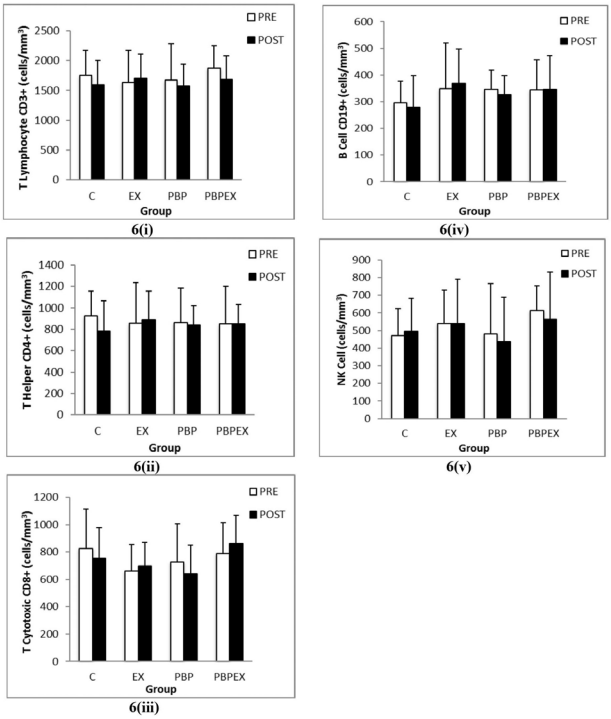

Figure 6: (i) mean total T lymphocyte ($CD3^+$), **(ii)** mean T helper lymphocyte ($CD4^+$), **(iii)** mean T cytotoxic lymphocyte ($CD8^+$), **(iv)** mean B cell ($CD19^+$) and **(v)** mean Natural Killer (NK) cell counts.

[a], significantly different from pre-test ($p<0.05$); [b], significantly different from respective control group ($p<0.05$); [c], significantly different from respective EX group ($p<0.05$); [d], significantly different from respective PBP group ($p<0.05$)

strengths. These observations supported our hypothesis that combined dietary protein and resistance training are needed to facilitate improvement in skeletal muscle strength.

We found significant increases in peak torque of knee flexion at $60^o.s^{-1}$ and $180^o.s^{-1}$ with plant-based protein and resistance training combination. Conversely, knee extension peak torque had shown a decrement in both angular velocities in the combined plant-based protein and resistance training as well as resistance training alone participants, which may have resulted from the prescribed resistance training protocol that focuses on concentric action rather than eccentric action. Our finding was similar to a previous study, in which after 8 weeks of concentric muscle training resulted in significantly accelerated strength gains in untrained men (27).

Significant increases in peak torque of left shoulder flexion at $60^o.s^{-1}$ and $180^o.s^{-1}$ were observed in the non-dominant shoulder after 8 weeks of combined plant-based protein and resistance training. This positive effect on the non-dominant shoulder may be associated with motor unit recruitment and firing behaviour in skeletal muscles. In a previous study of the motor unit recruitment and firing behaviour of the first dorsal interosseous (FDI) muscles for both hands, the variability of abduction force was significantly higher in the non-dominant hand (28). This phenomenon had been explained in the previous study among elderly who had a 30% reduction in their force variability during submaximal FDI contractions after strength training (29).

After 8 weeks of the study period, there were significantly higher levels of protein catabolism for serum urea and BUN with plant-based protein supplementation alone group compared to control, resistance training alone and combined supplementation and resistance training groups. This finding reflects that protein supplementation alone can cause a rise in BUN with increased daily protein intake by 5% (9.76 g) above the recommended daily allowance for a sedentary man (30). The increased BUN in our study was similar to most high-protein intake effect regardless of its source, as the protein ingested in excess of those needed for biosynthesis cannot be stored without prior transformation. Thus, increasing protein intake without physical activity in the long-term could lead to an increase of urea production as protein cannot be stored in the muscle tissue. The reduction of adenosine triphosphate during exercise will reduce protein synthesis within the cell. Increased physical exercise, particularly strength exercise can lead to a reduction in skeletal muscle protein synthesis (11). The combination of plant-based protein supplement with resistance training prescribed in our study seems to have potential in attenuating the increased of protein catabolism induced by plant-based protein supplementation alone.

The lack of effect on bone formation marker of alkaline phosphatase (ALP) and bone resorption marker of cross-linked C-telopeptide of type 1 collagen (CTX-1) in all groups of our study was similar to a previous study, in which high-intensity resistance training did not promote the osteogenic effect in bone-specific ALP (31). Although serum ALP was unchanged in all groups, PBPEX group showed a trend of increase in this bone formation marker, whereas this trend of the increase was not observed in other groups. This indicates that resistance training combined with a plant-based protein supplement may have the potential in enhancing bone formation. EX group showed a decreased trend in CTX-1 marker, implying that resistance training alone may have the potential to reduce bone resorption . In an animal model, 8 weeks of jumping exercise for 40 times per days reduced bone resorption (32). However, no significant changes were observed in our study, but the increased trends in bone resorption marker of CTX-1 in PBP and PBPEX groups implied one. Nevertheless, a study of longer period is necessary to confirm this speculation.

Two major mechanisms, the neuroendocrine factor and muscle damage, trigger the modulation of the immune response in resistance training. These mechanisms are believed to be linked to an increased volume and intensity of exercise characterised as a temporary

perturbation on the immune system (15). Generally, a moderate exercise regime is beneficial for immune function. The increase of acute immune response in the phagocytosis and degranulation of blood granulocytes and monocytes were observed in moderate exercise intensity (33). Also, previous studies that explained the relationship between volume and intensity of exercise with upper respiratory tract infection (URTI) frequency indicated that those who are engaged in moderate-intensity exercise regime had less URTI incidence than the sedentary individual (33, 34). Leukocyte response to the level of exercise intensity. However, our study corroborates with another study, showing unchanged circulating lymphocyte subsets, monocytes or neutrophils with the chronic resistance training of 2-3 times per week (35). Our study found no differences in the total WBC, monocyte, neutrophil, eosinophil and basophil counts following 8 weeks of combined plant-protein supplementation and resistance training.

Microtrauma to muscle fibres from resistance training may lead to activation of neutrophils with approximately 60% of leukocytes act as the first-line defence to eliminate infectious agent involved in muscle inflammation (36). These innate immune cells respond primarily by mobilising plasma proteins to the injury sites (37). Nevertheless, this response was not seen in our study. We assumed that the circulating leukocyte cells might have returned to baseline during recovery and the injury was insignificant (35). The blood was sampled at 10-12 hours post-exercise as opposed to immediate sampling in a previous study (33). As mentioned by Nieman et al. (38), following high-intensity exercise, total leukocytes could increase immediately post-exercise from 50% to 100% above pre-exercise values and could drop 30% to 50% below pre-exercise levels within 30 min of recovery, remaining low for 2 to 6 hours. Hence, we postulate that the timing of blood sampling might have produced unchanged effects compared to other previous studies.

There were no significant differences in T ($CD3^+$), T-helper ($CD4^+$), T cytotoxic ($CD8^+$), B (CD19+) and Natural Killer (NK) ($CD16^+56^+$) cells among all the groups in our study. However, we found that the significantly higher level of basophil counts in PBPEX compared to the EX group at post-exercise indicates possible increased activation of IL-3 (39). After IL-3 activation, high level of basophil may have inhibited the proliferation of T cell, specifically $CD4^+$ (40); however, it was not the case in our study. The basophil counts in PBPEX group were within normal values (0.02-0.1x109/l) (41).

We noted that circulating lymphocytes and other leukocytes subsets counts may have decreased slightly and returned to baseline 24 hours after exercise. It was reported that lymphocytes and other subsets counts returned to baseline within 3 hours following exercise (42). Resistance exercise involves the recruitment of large muscle mass and multi-joint movement, which caused NK cell to be substantially reduced immediately after exercise and up to 2 hours post-exercise. Moderate intensity exercise will not suppress immune function (15, 33, 34). Our study observed no significant decreases of immune parameters in PBPEX, EX and PBP. These findings implied that the prescribed intensity and frequency of the resistance training might be appropriate to maintain, but not suppress the immune functions of the sedentary adult males.

Our study was limited by participants' lifestyles and calorie intake, which were not strictly controlled. Further studies with a longer duration are warranted to evaluate long term effects from combining soy-pea protein ingestion and resistance exercise.

CONCLUSION

The current study provided evidence of the efficacy of 9.8 g plant-based protein supplementation with a combination of 3 times per week resistance training for 8 weeks duration that can be potentially used to assist untrained athletes or sedentary individuals in improving muscle strength and to maintain the immune system. Ingestion of plant-based protein alone may increase serum urea and blood urea nitrogen whereas combination of plant-based protein and resistance training has the potential in reducing the increase of protein catabolism markers. The combination of plant-based protein and resistance training did not elicit significant effect on both bone formation and resorption markers. Additionally, this combination may be proposed for formulating guidelines in planning exercise training programme and nutritional promotion in sedentary adult males' population.

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