Effects of Whey Protein Isolate Mixed with Vitamin D Emulsion Gels on Improving Muscle and Performance Recovery in Resistance-Trained Males

Jutawan Nuanchankong* Pattamaporn Jaroennon & Sakunta Manakla

Department of Nutrition and Dietetics, Faculty of Science and Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathumthani, 13180 Thailand

Abstract

Exercise performance, skeletal muscle repair, and physical recovery are influenced by protein and vitamin D. This study investigated the effects of whey protein isolate mixed with vitamin D emulsion gels (WPD-emulsion gels) on body composition and performance recovery in resistance-trained males. Various formulas of WPD-emulsion gels were developed with an optimal daily dose recommended protein content of 28 g from whey protein isolate, 1000 IU of vitamin D and three different amounts of rice bran oil and gelatin. A 9-point hedonic scale study was conducted to identify the highest rating formula on sensory acceptability and the effects on body composition and performance recovery. Eight resistance-trained males aged between 20-40 years old who spent at least 70% of total exercise time on resistance training for 3-5 days per week were chosen to participate. Participants were assigned WPD-emulsion gels consumed 30 min after resistance training (4 days/week) for 4 weeks. The study instruments were as follows: anthropometric and dietary assessments, the push-up and sit-to-stand test, muscle recovery times, perceived recovery status scale and rating of perceived exertion. Statistical analysis used one-way ANOVA and Paired t-tests. The results showed that the 5% rice bran with 14% gelatin formula received the highest sensory acceptability score. Skeletal muscle mass (at week 0: 59.9±3.4 kg, at week 4: 61.4±3.0 kg) and fat-free mass (at week 0: 63.1±3.6 kg, at week 4: 64.6±3.1 kg) were significantly increased from week 0 ($p<0.05$). In addition, participant's legs muscle mass and exercise recovery were also significantly improved. The study recommends that the consumption of WPD-emulsion gels is beneficial to muscles and could increase fat-free mass and improve exercise performance recovery.

Keywords: Whey Protein Isolate, Vitamin D, Muscle Mass, Fat-Free Mass, Performance Recovery

*** Corresponding Author**
e-mail: Jutawan@vru.ac.th

Introduction

Exercise enhances protein turnover, impacting both muscle function and the response to damage by increasing rates of muscle protein synthesis (MPS) and muscle protein breakdown. (Joanisse et al., 2021). Immediately following a workout for 1-4 hr, the focus shifts to rebuilding muscles. Athletes and individuals engaged in resistance training often utilize protein supplements to enhance skeletal muscle gains, improve exercise performance, and expedite muscle recovery. (Wilborn et al., 2013).

The whey fraction of milk is notably rich in highly bioavailable protein, specifically containing elevated levels of branched-chain amino acids (BCAAs) such as leucine, isoleucine and valine essential amino acids. Whey protein isolate is regarded as a pure and concentrated protein source, with protein concentrations exceeding 90% and it is rapidly absorbed by the body. Thus, whey protein is the optimal protein form as it reaches muscles faster, enhances muscle recovery and muscle mass gains and increases the total body protein synthesis (Sangwan, & Seth, 2021). The American College of Sports Medicine (2015) recommended a dietary reference protein intake of 1.2 -1.7 g/kg/day that is safe and effective for resistance-trained individuals attempting to maintain or increase lean muscle mass. The study by Williams et al. (2018), found that consuming whey protein isolate containing 28 g of protein in conjunction with engaging in high-intensity exercise for 8 weeks with non-adverse effects while increasing fat-free mass and muscle recovery compared with the control group, who consumed whey powder (Williams et al., 2018).

Vitamin D is a fat-soluble vitamin, it is important for normal skeletal muscle development and in optimizing muscle strength and performance. The recommended daily vitamin D intake is 1000 IU per day to maintain optimal serum 25(OH)D levels. Vitamin D deficiency is associated with oxidative stress in skeletal muscle, adverse mitochondrial function and skeletal muscle atrophy, which causes muscle pain and weakness (Gunton, & Girgis, 2018; Dzik, & Kaczor, 2019).

Emulsion gels as colloidal soft-solid materials have been widely applied to structure food products. The bioactive components protection of emulsion gels functions such as fat-soluble vitamin can be incorporated within the immobilized oil phase and protected by the oil-water interface and the gel network (Chen et al.,2020). Emulsion gels were prepared, liquid like protein and vitamin D were stabilized in the emulsion mixture by high-speed mixing (Dickinson, 2012).

The combination of whey protein and vitamin D supplements resulted in beneficial increases in muscle mass in males undergoing resistance training (Chen et al., 2022). However, many trainers have used a high dose of whey protein and vitamin D over the recommended dose for building muscle, which may not be pure and safe. In this study, we prepared an emulsion gel containing the recommended dose of whey protein isolate and vitamin D that can be used to safely improve muscle mass, fat-free mass and performance recovery.

This study aimed to investigate the effects of whey protein isolate mixed with vitamin D emulsion gels on body composition and performance recovery in resistance-trained males.

Materials and methods

1. Ethical approval

Ethical approval was obtained from Human Research Committee of Valaya Alongkorn Rajabhat University under the Royal Patronage, Pathumthani, Thailand. (Rec.N0 41/2563) The participants received an information form about the study and signed an informed consent form to show that they agreed to participate in the study.

2. Preparation of whey protein isolate mixed with vitamin D emulsion gels (WPD-emulsion gels)

The composition of WPD-emulsion gels was whey protein isolate (Matell Intertrade Co., Ltd., Khonkaen, Thailand), vitamin D (Now Corporate Offices, United States of America), orange juice (Thainamthip Co., Ltd., Bangkok, Thailand), sugar (Mitr Phol Sugar Co., Ltd., Bangkok, Thailand), gelatin (McGarrett, JR F & B Co., Ltd., Bangkok, Thailand), orange flavor (Best Odour Co., Ltd., Bangkok, Thailand) and rice bran oil (Thai Edible Oil Co., Ltd., Bangkok, Thailand). The 3×3 full factorial design was varied with 3 levels of rice bran oil at 5%, 10%, 15% and gelatin at 12%, 13%, 14% while the other ingredients were fixed. The amounts of whey protein isolate in each gel was 31 g (28 g of protein) and 28 mcg (1000 IU of vitamin D3: Cholecalciferol) of vitamin D based on previous studies which showed the effective daily recommended dose for improving lean body mass and exercise performance (Williams et al., 2018; Gunton, & Girgis, 2018; Dzik, & Kaczor, 2019).

Emulsion gels were prepared as described in Dickinson (2012). Briefly, the oil phase was prepared by mixing vitamin D and rice bran oil and orange juice was used as the water phase. A high-speed blender (Em-Smart-4, Sharp, Japan) was used to homogenize the water and oil phases at 17200 rpm for 5 min to obtain the emulsion forms. Whey protein isolate was subsequently added to the emulsion mix by a high-speed blender again. The gelling property of the emulsion was induced by heat (50°c) while adding gelatin, sugar and orange flavor to homogenization.

3. Sensory acceptability

The WPD-emulsion gel samples were evaluated on their sensory acceptability by 50 untrained participants (20-40 years old), who were fitness members of the Valaya Alongkorn Rajabhat University under the Royal Patronage. This study used a 9-point hedonic scale, rating scales of 1–9 (1 = dislike extremely, 9 =like extremely) for appearance, taste, color, odor, texture and overall acceptance (Society of Sensory Professionals, 2023). The highest-rated formula was selected to investigate the effects of whey protein isolate mixed with vitamin D emulsion gels on body composition and performance recovery.

4. Participants and recruitment for clinical study

4.1 Inclusion criteria

Resistance-trained males aged between 20-40 years old who are members fitness of the Valaya Alongkorn Rajabhat University under the Royal Patronage were recruited for the study. Participants spent at least 70% of total exercise time on resistance training for 3-5 days per week. Participants had a normal range of body fat percentage (8-20 %) and muscle mass percentage (33-39%) (Omron Healthcare, 2020). All participants did not use protein products, supplements and medication or any hormones that may affect muscle mass gain e.g., testosterone, creatine and dehydroepiandrosterone (DHEA) etc.

4.2 Exclusion criteria

Participants who had allergies to whey protein, metabolic disorders and gastrointestinal dysfunctions were not allowed to enroll in the study. Participants also did not have a history or present serious clinical problems such as liver disease, kidney disease, thyroid disease, or infectious diseases. Only eight male participants met the inclusion criteria and therefore were selected by purposive sampling based on the previous study (Sangwanna et al., 2019).

5. Clinical study protocol

This investigation was a one group pretest-posttest control design, participants were a self-control group. We determined the effects of WPD-emulsion gels on the body composition and performance recovery in resistance-trained males for 4 weeks. Week 0 was baseline before intervention, participants stabilized lifestyle at the initial phase. During weeks 1-4, participants were provided with WPD-emulsion gel 1 bag (100 g) to consume 30 min after resistance training for four days per week. Participants were instructed to maintain their current dietary intake, exercise activity and lifestyle activity for the duration of the study.

6. Measurements of clinical study

Participants were measured on the following: dietary intakes, body composition, exercise performance and exercise recovery at baseline (week 0) and the end of study (week 4). The instruments used in the study were:

6.1 Dietary intakes

Dietary Assessment used the 24-hr dietary recall method (Salvador et al., 2015). Participants recorded dietary intakes with interview. Ensure compliance and digestive irregularities were assessed by the dietary record review when participants returned to receive WPD-emulsion gels on day 4 of every week. Total energy and macronutrient intakes were assessed by INMUCAL- Nutrients version 4 software developed by the Institute of Nutrition, Mahidol University (2018).

6.2 Body composition

The body composition including weight, body fat, skeletal muscle, fat-free mass and segmental muscle mass were evaluated using the bioelectrical impedance analysis: BIA (Tanita BC-601 Body Composition Analyzer, Tanita Co. Ltd., Japan). The BIA measured the electric resistance of electric current passing at different speeds through the body. Due to the electrical conductivity among various bodily tissues (body fat, muscle, fat-free mass), the instrument was able to measure the impedance of the estimated body composition (Marra et al., 2019). Muscle circumferences such as mid-arm circumference and mid-thigh circumference were measured with an inelastic tape according to Lee et al., (2017).

6.3 Exercise performance

The push-up test was assessed for the upper body dynamic strength and endurance according to the American College of Sports Medicine (2014) guidelines for exercise testing and prescription. The procedure of the push-up test started from the lowest face-down position and the hands are kept at the width of the shoulders. Phase one was arm extension keeping the body straight. In the second phase, the body was lowered to the down position with an elbow angle of 90°. The number of push-ups performed consecutively without rest in 60 sec was counted as the score. The number of male push-ups was classified into 4 categories: excellent (25-36), very good (17-35), good (13-28) and fair (10-21).

The sit-to-stand test was assessed for lower body strength and endurance which was modified from the procedures used by the Department of Physical Education, Ministry of Tourism and Sports, Thailand (2019). Participants were instructed to stand as quickly as possible with their full legs extended on the chair while keeping their arms folded across their chests. The sit-to-stand test was counted, and the maximal number of chair stands completed in 60 sec was categorized into excellent (48-54), good (41-53), moderated (33-45) and fair (25-38).

6.4 Exercise recovery

The exercise recovery was evaluated as muscle recovery times, a perceived recovery status scale and a rating of perceived exertion. Muscle recovery time was counted as hr from the time of resistance training day. The perceived recovery status was performed following the method of Laurent et al. (2011), which used a rating of 0 (very poor recovered) to 10 (very well recovered). The rating of perceived exertion was used to measure the intensity of the exercise. The scale runs from 0 (None at all) to 10 (Extremely strong) (Williams, 2017).

7. Statistical analysis

Statistical analysis using one-way ANOVA for different changes in sensory acceptability outcomes and Paired t-tests were used to analyze changes in dietary intakes, body composition, exercise performance and exercise recovery in the study.

Results and discussion

1. Sensory acceptability

The sensory acceptability of the WPD-emulsion gels formulas is shown in Table 1. The effects of different amounts of rice bran oil and gelatin on the appearance and color scores were not statistically significantly different. Increasing the amount of rice bran oil at 10% and 15% showed rice bran oil formulas at a statistically significantly decrease regarding the odor scores below 5% of rice bran oil formula (P-value < 0.05) while increasing the amount of gelatin at 13% and 14% formulas showed a statistically significantly increase in the texture scores compared to the 12% of gelatin formula (P-value < 0.05). An increase in the amount of rice bran oil in the formulas resulted in a lower acceptability of odor scores. This is due to the organoleptic properties of rice bran, in which unreactive volatile compounds are changed into hydrocarbons, aldehydes and ketones, leading to the unpleasant odor of rice bran (Gao et al., 2021). As a result, the WPD-emulsion gel formulas, that were low in rice bran and high in gelatin were scored higher by participants. Thus, we selected the 5% rice bran with 14% gelatin formula as the highest overall acceptance and taste scores for the WPD-emulsion gels study on body composition and performance recovery.

2. The nutritional information of the WPD-emulsion gels

The highest sensory acceptability score of 5% rice bran with 14% gelatin formula of WPD-emulsion gels was used for the dietary intervention study. A serving size of WPD-emulsion gels was 1 bag (100 g). The nutritional information of the WPD-emulsion gels is shown in Table 2.

3. The characteristics of the participants

Eight participants successfully completed all aspects of the study, with an average age of 27.6±1.4 (21-34) years old. The average weekly training frequency was 4±0.4 (3-6) days and the repetition and set program of trainings were 13.6±1.1 (10-20) and 3.1±0.1 (3.0-4.0), respectively. Their exercise activity and lifestyle activity were stabled throughout the study. Resistance training volume between 6-12 reps for 3-6 sets will help the muscle hypertrophy (Schoenfeld & Grgic, 2017). The characteristics of the participants at week 0 is shown in Table 3.

Table 1 The sensory acceptability of the WPD-emulsion gels formulas

Rice bran oil (%)	Gelatin (%)	Appearance^{ns}	Color^{ns}	Odor	Taste	Texture	Overall acceptance
	12	5.62±1.68	6.34±1.34	6.94±1.58^{a}	5.32±1 .71^{c}	4.91±1.88^{c}	6.11±1.88^{a}
5	13	5.81±1.62	6.26±1.28	6.26±1.48^{b}	5.79±1.84^{ac}	5.85±1.71^{ab}	6.02±2.01^{a}
	14	5.96±1.67	6.34±1.42	6.19±1.49^{b}	6.30±1.82^{a}	6.15±2.00^{a}	6.38±1.79^{b}
	12	5.88±1.81	6.31±1.32	5.58±1.49^{c}	5.10±2.49^{c}	5.38±2.17^{bc}	5.48±2.08^{c}
10	13	6.09±1.65	6.19±1.60	5.43±1.65^{c}	5.36±2.28^{c}	5.83±1.82^{ab}	5.74±2.09^{c}
	14	5.85±1.71	6.11±1.56	5.57±1.49^{c}	5.49±1.96^{bc}	5.68±1.85^{ab}	5.87±1.69^{c}
	12	6.23±1.65	6.34±1.37	6.11±1.65^{b}	6.15±1.63^{ab}	5.96±1.93^{ab}	6.23±1.63^{b}
15	13	6.26±1.62	6.21±1.43	5.77±1.55^{c}	6.17±1.71^{ab}	5.98±1.66^{ab}	6.28±1.58^{ab}
	14	6.30±1.91	6.26±1.51	5.49±1.50^{c}	5.47±1.94^{bc}	6.11±1.89^{a}	6.13±1.84^{ab}

Remark: Means ± SD in each column followed by the different letters are significantly different at $P < 0.05$, ns: non-significant

Table 2 Nutritional information of WPD-emulsion gels 1 bag (100 g)

Nutritional Information	Amounts
Energy, kcal	187
Carbohydrate, g	7.5
Sugar, g	0.5
Protein, g	28
Fat, g	5
Sodium, g	65
BCAA, g	6
Vitamin D, IU	1000

Table 3 The characteristics of the participants at week 0

Parameters	Min-Max	Mean±SE
Age, yr.	21-34	27.6±1.4
Weight, kg	60.3-96.2	77.7±4.4
Height, cm	168.0-185.0	177.4±2.2
Weekly training frequency, day	3-6	4±0.4
Resistance training time, min	50-120	108.8±12.6
Repetition	10.0-20.0	13.6±1.1
Set	3.0-4.0	3.1±0.1

4. Dietary intakes

The daily energy and macronutrient intakes throughout the study were reported by participants based on the self-recorded 24-hr dietary recall method (Table 4). During weeks 1-4, participants received 1 bag (100 g) of WPD-emulsion gels taken 30 min after resistance training for 4 days/weeks. Taking the WPD-emulsion gels did not cause digestive discomfort. The average WPD-emulsion gels consumption was 84% of the intended amount (approximately 23 g protein and 840 IU vitamin D intake amounts from WPD-emulsion gels). The consumption of WPD-emulsion gels may not be affected by the total energy and macronutrient intakes. Participants had stable dietary intake from regular diet throughout the study. No significant change was observed in the vitamin D intake from a regular diet (data not shown).

5. Effects of WPD-emulsion gels on body composition

The body compositions after 4 weeks of WPD-emulsion gels consumption resulted in a significantly increased skeletal muscle mass and fat-free mass from week 0. (59.9±3.4 kg, 63.1±3.6 kg at week 0 and 61.4±3.0 kg, 64.6±3.1 kg at week 4, P-value < 0.04 and P-value < 0.05, respectively). Both right and left leg muscles also had significant gain from 11.0±1.0 kg, 10.9±1.0 kg at week 0 to 11.7±0.8 kg, 11.8±0.9 kg at week 4, respectively (P-value < 0.05). The result may be due to the augmenting protein intakes. The trend of total protein intake of 113.9±8.5 g (1.5±0.1 g/kg/d) at week 4 was higher than 83.4±16.4 g (1.1±0.2 g/kg/d) at week 0, with amounts of protein intake increasing approximately 23.4 ± 3.1 g derived from WPD-emulsion gels. Consistency with no significant differences in protein intake amounts from regular diet occurred throughout the study (83.4±16.4 at week 0, 90.1± 8.05 at week 4, P-value = 0.75). Approximately 20 g of protein isolate is appropriate for muscle protein synthesis rates during the first few hours of post-resistance training (Trommelen et al., 2019). The optimal amount of total protein intake of 1.5 g/kg/d is sufficient for maintaining and increasing muscle strength while engaging in resistance training (Tagawa et al, 2022). Ingestion of approximately 20 g protein is adequate to maximize post-exercise muscle protein synthesis rates (Van Loon, 2013). Protein intakes ranging from 1.2-1.7 g/kg/d are adequate for nitrogen balance and optimization of fat-free mass response to resistance exercise training (McAdam et al., 2018). Whey protein contains branch chain amino acids (BCAA), which is called fast protein which means quick digestion and absorption. It was the rapid transport of amino acids to skeletal muscle used in the stimulation of muscle protein synthesis and reduction of protein breakdown, presumably optimizing the tissue repair, or assisting in the resynthesis of glycogen stores when enough carbohydrate is not readily available (Vliet et al., 2018; Jäger et al., 2017). In a short time, whey protein supplementation with resistance training for 3 weeks contributed to an increase in muscle mass and strength in athletes (Sobral et al., 2020). Interestingly, the participant's carbohydrate and protein intakes were 202.4 g and 113.9 g respectively at week 4. Consistency with a ratio of carbohydrate to protein intakes of 2:1. This affects glycogen synthesis, it has been attributed to amino acids on insulin releasing which resulted in higher circulating insulin concentrations thereby increasing muscle glucose uptake refueling, rebuilding and recovery of muscles after a strength training workout (Margolis et al., 2021). In this study, only lower segmental muscle mass increased as leg muscles had more muscle fibers than arms and therefore the results were more obvious. The other body composition parameters are shown in Table 5.

6. Effects of WPD-emulsion gels on performance recovery

Effects of WPD-emulsion gels on push-ups significantly increased from week 0 (31.5±4.5 at week 0 and 38.6±3.3 at week 4, P-value < 0.05). In addition, the exercise recovery was significantly improved in recovery time, perceived recovery status scale and rating of perceived exertion (P-value < 0.05). The performance recovery is shown in Table 6. The dose of vitamin D intake during weeks 1-4 was 840 IU from WPD-emulsion gels, lower than vitamin D 1000 IU recommendation (Gunton, & Girgis, 2018). However, that made a difference in the upper body performance as push-ups and recovery status were significantly improved from the beginning of this study. Gunton, & Girgis (2018) reported the effect of a daily vitamin D intake of 800 to 1000 IU in the elderly and found improvements in balance and muscle function. Muscle tissue damage caused by exercise results in muscle degeneration by degradation of structural proteins, including myofibrils and cytoskeletal proteins. Whey protein supplements consumed after eccentric exercise for at least 7 days resulted in increased fibroblast proliferation and faster muscle strength recovery after exercise (Kim et al., 2017). Vitamin D receptor (VDR) is present in muscle, as is CYP27B1, the enzyme that hydroxylates 25(OH)D to its active form. Vitamin D increases VDR gene expression, which mitigates reactive oxygen species (ROS) production, augments antioxidant capacity and prevents oxidative stress, a common antagonist in muscle damage, improves mitochondrial function and inhibits muscle atrophy (Dzik, & Kaczor, 2019; Latham et al., 2021).

Interestingly, at the end of the study, muscle circumferences showed an upward trend in this study. Moreover, the rating of perceived exertion of participants increased from 4.9±0.6 (strong) to 6.9±0.4 (very strong).

Table 4 Daily energy and macronutrient intakes throughout the study

Dietary intakes	Week0		Week4		*P*-value
	Min-Max	Mean±SE	Min-Max	Mean±SE	
Energy, kcal	1162.0-2422.3	1596.9±175.0	1504.9-2447.2	1933.4±136.3	0.17
Carbohydrate, g	105.0-286.4	174.6±19.2	148.8-331.8	202.4±20.0	0.10
Total Protein, g	42.9-181.2	83.4±16.4	85.1-149.6	113.9±8.5	0.17
Source of protein					
Regular diet	42.9-181.2	83.4±16.4	67.1-126.6	90.1± 8.05	0.75
WPD-emulsion gels	-	-	17.5 – 28.0	23.4 ± 3.1	
Protein, g/kg/d	0.60-2.50	1.1±0.2	1.14-1.92	1.5±0.1	0.18
Animal protein, g	24.0-165.8	62.6±16.8	65.7-155.5	98.6±11.7	0.10
Vegetable protein, g	2.4-22.2	12.2±32.3	11.8-20.4	15.2±1.1	0.29
Fat, g	36.0-114.8	62.8±13.9	40.1-118.8	78.7±8.4	0.19

Remark: Means ± SE in each row followed by significantly different at $P<0.05$

Table 5 Differences in Body composition throughout the study

Parameters	Week 0		Week 4		*P*-value
	Min-Max	Mean±SE	Min-Max	Mean±SE	
Weight, kg	60.3-96.2	77.7±4.4	61.3-95.6	78.5±4.2	0.11
Body fat, % of weight	13.3-23.6	18.6±1.2	12.7-24.9	17.5±1.8	0.40
Body fat, kg	9.7-19.9	14.5±1.3	8.0-20.3	14.0±1.8	0.60
Skeletal muscle mass, kg	46.3-74.9	59.9±3.4	50.5-75.1	61.4±3.0	<0.04*
Fat-free mass, kg	48.8-79.0	63.1±3.6	53.3-79.0	64.6±3.1	<0.05*
Segmental muscle mass					
Right arm, kg	2.3-4.1	3.1±0.2	2.4-4.1	3.1±0.2	0.84
Left arm, kg	2.2-4.1	3.0±0.2	2.3-4.1	3.0±0.2	0.88
Right leg, kg	7.8-16.4	11.0±1.0	9.3-16.3	11.7±0.8	<0.05*
Left leg, kg	7.7-15.6	10.9±1.0	9.1-16.7	11.8±0.9	<0.01*
Trunk, kg	26.3-37.8	31.8±1.1	26.6-37.9	31.6±1.1	0.44
Muscle circumference					

Right mid-arm, cm	31.0-40.0	34.4±1.1	31.0-40.0	34.9±1.0	0.09
Left mid-arm, cm	31.0-39.0	33.6±0.9	30.3-40.0	34.2±1.1	0.39
Right mid-thigh, cm	49.7-65.5	55.9±1.8	52.5-65.5	56.5±1.6	0.12
Left mid-thigh, cm	49.6-63.0	56.0±1.5	50.0-63.0	56.3±1.4	0.26

Remark: Means ± SE in each row followed by significantly different at $P<0.05$

Table 6 Differences in performance recovery throughout the study

Parameters	**Week0**		**Week4**		***P*-value**
	Min-Max	**Mean±SE**	**Min-Max**	**Mean±SE**	
Exercise performance					
Sit-to-stand test	27.0-50.0	37.8±3.1	31.0-56.0	43.6±3.6	0.16
Push-up test	12.0-51.0	31.5±4.5	29.0-55.0	38.6±3.3	<0.05*
Exercise recovery					
Recovery time, hr./day	1.0-2.5	2.0±0.2	1.0-2.5	1.4±0.2	<0.02*
Perceived recovery status scale	3.0-8.0	6.9±0.4	6.0-9.0	7.3±0.4	<0.003*
Rating of perceived exertion	3.0-8.0	4.9±0.6	5.0-9.0	6.9±0.4	<0.01*

Remark: Means ± SE in each row followed by significantly different at $P<0.05$

Remarkably, we found that the minimum intervention period was 4 weeks to promote muscle hypertrophy outcomes and performance recovery. The limitation of this study was a small sample size. The outcome of this study suggested that further research should focus on larger sample size. In addition, an assessment of nitrogen balance, serum creatinine kinase and serum 25(OH)D related muscle synthesis should be conducted.

Conclusion

WPD-emulsion gels, which contains the optimal daily recommended dose of whey protein isolate and vitamin D consumption taken 30 min after resistance training (4 days/week) for 4 weeks can be used to safely improve muscle mass, fat-free mass and performance recovery. The study recommends the consumption of WPD-emulsion gels to promote muscle and fat-free mass and potentially improve exercise performance recovery.

Acknowledgement

The research was supported by the Fitness Club of the Valaya Alongkorn Rajabhat University under the Royal Patronage. The authors would like to express their appreciation to the participants who contributed to the present study.

References

Burd, N.A., West, D.W., Moore, D.R., Atherton, P.J., Staples, A.W., Prior, T., … Tang, J.E. (2011). Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. *J Nutr.*, *141*(4),568–573.

Chen, H., Mao, L., Hou, Z., Yuan, F., & Gao, Y. (2020). Roles of additional emulsifiers in the structures of emulsion gels and stability of vitamin E. *Food Hydrocoll*, *99*, 105372.

Chen, Y., Liang, Y., Guo, H., Meng, K., Qiu, J., & Benardot, D. (2022). Muscle-Related Effect of Whey Protein and Vitamin D_3Supplementation Provided before or after Bedtime in Males Undergoing Resistance Training. *Nutrients*, *14*(11), 1-18.

Dickinson, E. (2012). Emulsion gels: The structuring of soft solids with protein-stabilized oil droplets. *Food hydrocolloids*, *28*(1), 224-241.

Dzik, K. P., & Kaczor, J. J. (2019). Mechanisms of vitamin D on skeletal muscle function: oxidative stress, energy metabolism and anabolic state. *Eur J Appl Physiol*, *119*(4), 825–839.

Gao, C., Li, Y., Pan, Q., Fan, M., Wang, L., & Qian, H. (2021). Analysis of the key aroma volatile compounds in rice bran during storage and processing via HS-SPME GC/MS. *J. Cereal Sci, 99*, 103178.

Gunton, J. E., & Girgis, C. M. (2018). Vitamin D and muscle. *Bone Rep*,*8*, 163–167.

Institute of Nutrition, Mahidol university. (2018). *Nutrient calculation computer software INMUCAL-Nutrients V4 database NB1*. Nakornpathom

Jäger, R., Kerksick, C. M., Campbell, B. I., Cribb, P. J., Wells, S. D., Skwiat, T. M., … Purpura, M. (2017). International Society of Sports Nutrition Position Stand: protein and exercise. *J Int Soc Sports Nutr*, *14*(20), 1-25.

Joanisse, S., McKendry, J., Lim, C., Nunes, E.A., Stokes, T., Mcleod, J.C., & Phillips, S.M. (2021). Understanding the effects of nutrition and post-exercise nutrition on skeletal muscle protein turnover: Insights from stable isotope studies. *Clin. Nutr. Open Sci.*,*36*, 56-77.

Kim, J., Lee, C., & Lee, J. (2017). Effect of timing of whey protein supplement on muscle damage markers after eccentric exercise. *J Exerc Rehabil, 13*(4), 436-440.

Latham, C. M., Brightwell, C. R., Keeble, A. R., Munson, B. D., Thomas, N. T., Zagzoog, A. M., … Fry, C. S. (2021). Vitamin D Promotes Skeletal Muscle Regeneration and Mitochondrial Health. *Front Physiol*, *12*, 660498.

Laurent, C. M., Green, J. M., Bishop, P. A., Sjökvist, J., Schumacker, R. E., Richardson, M. T., & Curtner-Smith, M. (2011). A practical approach to monitoring recovery: development of a perceived recovery status scale. *J Strength Cond Res*, *25*(3), 620–628.

Lee, Y. S., Kim, S. D., Kang, H. J., Kim, S. W., Shin, I. S., Yoon, J. S., & Kim, J. M. (2017). Associations of Upper Arm and Thigh Circumferences with Dementia and Depression in Korean Elders. *Psychiatry Investig*, *14*(2), 150–157.

Margolis, L. M., Allen, J. T., Hatch-McChesney, A., & Pasiakos, S. M. (2021). Coingestion of Carbohydrate and Protein on Muscle Glycogen Synthesis after Exercise: A Meta-analysis. *Med Sci Sports Exerc*, *53*(2), 384–393.

Marra, M., Sammarco, R., De Lorenzo, A., Iellamo, F., Siervo, M., Pietrobelli, A., … Donini, L. M (2019). Assessment of Body Composition in Health and Disease Using Bioelectrical Impedance Analysis (BIA) and Dual Energy X-Ray Absorptiometry (DXA): A Critical Overview. *Contrast Media Mol Imaging*, *2019*, 3548284.

McAdam, J. S., McGinnis, K. D., Beck, D. T., Haun, C. T., Romero, M. A., Mumford, P. W., …. Roberson, P. A., (2018). Effect of Whey Protein Supplementation on Physical Performance and Body Composition in Army Initial Entry Training Soldiers. *Nutrients*, *10*(9), 1248.

Omron Healthcare (2020). Full Body Sensor Body Composition Monitor and Scale Model HBF-510. Retrieved January 20, 2023, from https://omronhealthcare.com/wp-content/uploads/hbf-510w-instruction-manual.pdf

Sangwan, S., & Seth, R. (2021). Whey Protein Supplement: An Exclusive Food or Need of the Hour: Review. *Annu Res Rev Biol*, *36*(4), 110-119.

Sangwanna, S., Jaroennon, P., Nuanchankong, J., Suchaiya, W., & Manakla, S. (2019). Body composition among fitness club members of Valaya Alongkorn Rajabhat University under the Royal Patronage Pathum thani Thailand: a cross-sectional study. *The Proceeding of the 10th napa 2019*, 15-20.

Salvador Castell, G., Serra-Majem, L., & Ribas-Barba, L. (2015). What and how much do we eat? 24-hour dietary recall method. *Nutr Hosp*, *31*(3), 46–48.

Schoenfeld, B.J., & Grgic, J. (2017). Evidence-Based Guidelines for Resistance Training Volume to Maximize Muscle Hypertrophy. *Strength Cond J.*, *40(4),*107-12.

Sobral,C., Gomes, D., Silva, M., Martins, P., & Baltazar, A. (2020). Whey protein supplementation in muscle hypertrophy. *Eur. J. Public Health*, *30*(2).

Society of Sensory Professionals (2023). The 9-point Hedonic Scale. Retrieved January 15, 2023, from https://www.sensorysociety.org/knowledge/sspwiki/Pages/The%209point%20Hedonic%20Scale.aspx

Tagawa, R., Watanabe, D., Ito, K., Otsuyama, T., Nakayama, K., Sanbongi, C., … Miyachi, M. (2022). Synergistic Effect of Increased Total Protein Intake and Strength Training on Muscle Strength: A Dose-Response Meta-analysis of Randomized Controlled Trials. *Sports Med*, *8*(1),1-12.

The American College of Sports Medicine (2014). *ACSM's Guidelines for Exercise Testing and Prescription*. (9th Ed). Philadelphia : Lippincott Williams & Wilkins.

The American College of Sports Medicine. (2015). Protein Intake for Optimal Muscle Maintenance. Retrieved January 22, 2023, from https://www.acsm.org/docs/default-source/files-for-resource-library/protein-intake-for-optimal-muscle-maintenance.pdf

The Department of Physical Education. Ministry of Tourism and Sports. (2019). Criteria of Physical fitness standard test for aged 19 - 59 years old. Retrieved January,10,2023 from https://www.dpe.go.th/manual-files-411291791796

Trommelen, J., Betz, M. W., & van Loon, L. J. C. (2019). The Muscle Protein Synthetic Response to Meal Ingestion Following Resistance-Type Exercise. *Sports Med*, *49*(2), 185–197.

Wilborn, C. D., Taylor, L. W., Outlaw, J., Williams, L., Campbell, B., Foster, C. A., Smith-Ryan, A., Urbina, S., & Hayward, S. (2013). The Effects of Pre- and Post-Exercise Whey vs. Casein Protein Consumption on Body Composition and Performance Measures in Collegiate Female Athletes. *J Sci Med Sport*, *12*(1), 74–79.

Williams, J., Vieira, K., & Williams, S. (2018).The Effects of Supplementing Ingredient Optimized Whey Protein Isolate (ioProtein) Versus Whey Protein Comparator Following High Intensity Exercise for 8-Weeks. *J Food Process Technol, 9*(2),1-5.

Williams, N. (2017). The Borg Rating of Perceived Exertion (RPE) scale. *J Occup Med, 67*(5):404–405.

Van Loon L. J. (2013). Role of dietary protein in post-exercise muscle reconditioning. *Nestle Nutr Inst Workshop Ser*, *75*, 73–83.

Vliet, S. V., Beals, J. W., Martinez, I. G., Skinner, S. K., & Burd, N. A. (2018). Achieving Optimal Post-Exercise Muscle Protein Remodeling in Physically Active Adults through Whole Food Consumption. *Nutrients*, *10*(2),1-14.

Volek, J. S., Volk, B. M., Gómez, A. L., Kunces, L. J., Kupchak, B. R., Freidenreich, D. J., … Aristizabal, J. C. (2013). Whey protein supplementation during resistance training augments lean body mass. *J Am Coll Nutr*, *32* (2), 122–135.

Article info
Nuanchankong et al.

Received: 5 October 2023
Revised: 18 December 2023
Accepted: 20 December 2023