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Correlation between physiological and biochemical variables during short term adequate protein intake combined with resistance exercise in sedentary adults

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This study aimed to clarify the pure synergistic effect of an adequate protein intake (1.5 g/kg body weight/day) and resistance exercise (RE) on muscle strength, body composition, and metabolic markers in sedentary adults, excluding confounders arising from excessive protein intake or impaired protein turnover. A double-blind randomized controlled trial was performed on apparently healthy sedentary adult participants ($n = 34$). RE was performed for 4 weeks in the placebo (PLA-EX, $n = 17$) and whey protein supplement groups (PRO-EX, $n = 17$). Body composition, isokinetic muscular function, resting metabolic rate, blood biochemical variables, and liver ultrasound findings were analyzed and compared before and after the intervention. Both the PLA-EX and PRO-EX groups experienced significantly reduced body weight (PLA-EX, $p < 0.001$; PRO-EX, $p < 0.01$), body mass index (PLA-EX, $p < 0.01$; PRO-EX, $p < 0.01$), and body fat percentage (PLA-EX, $p < 0.01$; PRO-EX, $p < 0.0001$) after the intervention. In addition, serum adiponectin (PLA-EX, $p < 0.0001$; PRO-EX, $p < 0.001$), leptin (PLA-EX, $p < 0.05$; PRO-EX, $p < 0.0001$), growth/differentiation factor 8 (PLA-EX, $p < 0.05$; PRO-EX, $p < 0.01$), albumin (PLA-EX, $p < 0.05$; PRO-EX, $p < 0.01$), total cholesterol (PLA-EX, $p < 0.001$; PRO-EX, $p < 0.0001$), triglycerides (PLA-EX, $p < 0.0001$; PRO-EX, $p < 0.0001$), and controlled attenuation parameters measured by liver ultrasound were significantly decreased (PLA-EX, $p < 0.05$; PRO-EX, $p < 0.0001$). The PRO-EX group showed no significant difference in circulating free fatty acid levels before and after the intervention, whereas PLA-EX levels decreased after the intervention ($p < 0.01$). Muscle mass ($p < 0.05$), muscle strength ($p < 0.001$), and insulin-like growth factor 1 ($p < 0.05$) were significantly increased by the intervention in the PRO-EX group but not in the PLA-EX group. Testosterone ($p < 0.01$), high-density lipoprotein cholesterol ($p < 0.01$), aspartate aminotransferase ($p < 0.0001$), and alanine aminotransferase ($p < 0.001$) were significantly reduced in the PRO-EX group but not in the PLA-EX group. The combination of RE and adequate protein intake (1.5 g/kg body weight/day) synergistically increased fat metabolism, induced an increase in IGF-1 in the blood, and increased muscle mass and strength in sedentary adults. Clinical Trial: The clinical trial described in this paper was registered at https://cris.nih.go.kr under the registration number KCT-0008696 (first trial registered 09/08/2023).

Keywords Protein intake, Protein supplements, Muscle strength, Resistance exercise

While there are differing opinions on the effectiveness of protein intake alone, the synergistic effect between resistance exercise (RE) and protein intake is clear in improving muscle mass and strength[1,2]. Current guidelines recommend a protein intake of 1.4–2.0 g/kg body weight [BW]/day for healthy adults engaging in RE[3]. However, recent studies have shown that consuming protein beyond 1.5 g/kg BW/day does not provide additional benefit for RE-induced strength gains[2]. Considering these findings and the potential side effects of excessive protein intake, such as kidney dysfunction and overstimulation of the mechanistic target of rapamycin (mTOR), which may accelerate cellular aging[4–11]. Thus, a protein intake of 1.5 g/kg BW/day represents a balanced

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approach, optimizing the efficacy of RE while minimizing potential health risks, positioning it as a prudent and scientifically supported threshold. Conflicting results have been reported on the adverse effects of protein intake on renal function. However, excessive consumption of animal proteins other than milk is associated with reduced renal function in individuals with mild renal impairment[12]. In the long term, the possibility of overstimulating the mTOR pathway, thus affecting premature aging, cannot be ignored[13,14]. It's important to note that not everyone will experience kidney problems if they consume more than 1.5 g/kg BW/day. The same applies to the stimulation of the mTOR signaling pathway. When combining protein intake and RE, limiting protein intake to 1.5 g/kg BW/day may be the optimal dose for increasing muscle mass and strength, while also limiting excessive protein intake.

To determine the net effect of protein intake on RE, randomized controlled trials (RCTs) should be conducted while controlling for various confounding variables. Excessive protein intake can have considerable effects on various physiological phenotypes. In addition, patients with diseases such as sarcopenia[15], frailty[16], arthritis[17], and obesity have potential factors that disrupt protein turnover[18,19] making it difficult to ascertain the net effects of RE and protein intake. Thus, to accurately determine the synergistic effect of RE and protein intake, protein turnover confounding factors and protein intake in habitual diets should be considered and this should be confirmed in RCTs. This is because more than 80% of studies on the relationship between protein intake and muscle strength improvement confirm that at least 1.2 g/kg BW/day of protein is consumed in a habitual diet[1]. This basic protein intake in habitual diets may be the cause of underestimating the synergistic effect of RE and protein intake.

It is surprising that no research, except for our previous study, has found a pure synergistic effect of resistance exercise (RE) and protein intake on muscle strength in healthy individuals without serious illnesses that could affect protein turnover[20,21]. Our previous study discovered that combining protein intake (1.5 g/kg BW/day) and RE increased muscle strength and decreased hepatic fat content[20,21]. However, we did not find any correlation between these changes and various blood biochemical variables. Therefore, no study has conducted multiple correlation between physiological variables and blood biochemical variables that change with the pure synergistic effect of protein intake and RE in a single RCT. Incorporating multiple factors within a single study provides critical insights beyond those obtained by focusing on a single variable. By comprehensively evaluating physiological parameteres alongside blood biochemical variables and hormonal profiles, researchers can more accurately identify the "pure synergistic effect" of RE and protein intake.

In this study, we employed a RCT design to systematically investigate the interplay between an optimal protein intake and RE. This multifactorial approach allows us to capture subtle causal relationships and potential confounders that may be overlooked in single-factor analyses, thus offering a more nuanced understanding of how protein intake and RE interact at the physiological level. Ultimately, our findings aim to inform the development of more targeted and effective exercise-nutrition intervention strategies, grounded in a comprehensive understanding of the mechanisms underlying RE-protein synergy. We believe it is important to correlate blood biochemical analysis with data on various physiological variables published in our previous studies. We proclaim that our analyzed datasets are large and present secondary research results that were not covered in previous studies, and are not subject to data salami slicing. The ultimate goal of our study was to confirm the pure synergistic effect of RE at appropriate protein intake levels and to confirm the correlation with blood biochemical variables related to the resulting physiological changes.

Materials and methods

Participants

Thirty-four males residing in Busan, Korea were recruited and randomly assigned to either a placebo group (Placebo, PLA-EX, $n=17$) or a whey protein supplement intake group (Protein, PRO-EX, $n=17$) (Fig. 1). The participants were randomly assigned to either start with PLA-EX or PRO-EX to minimize any order effects using a block randomization procedure. Group allocation was based on skeletal muscle mass, body weight, body fat and age and completed using a computer-generated randomization. The number of participants was derived using the G*Power 3.1 software (Heinrich-Heine-Universität Düsseldorf, Germany). The analysis results showed that 30 participants were needed, and considering the dropout rate of 20%, it was decided to recruit 36 participants. For G*Power analysis, $\alpha=0.05$, effect size of 0.35, and power of 0.8 were considered. A physical activity readiness questionnaire (PAR-Q) was used to determine participants' medical history and readiness for physical activity. All participants were non-smokers and healthy sedentary individuals (no exercise within 3 months of the study). In addition, none of the participants were lactose-intolerant or had consumed alcohol within 3 months of the study. Outcome measures were assessed at baseline (August 2021) and following the 4-week intervention (September to October 2021). This study was approved by the Institutional Review Board of Pusan National University (No. 2020_136_HR, date of approval:31/05/2021). All participants provided written informed consent before participating in the study. The characteristics of the participants were as shown in Table 1.

Study design

This double-blind, randomized controlled trial was registered on (09/08/2023) at https://cris.nih.go.kr (KCT-0008696). Participants were randomly assigned to either the PLA-EX or PRO-EX group (initial recruitment, each $n=18$). Jung-Jun Park generated the random allocation sequence and assigned participants to interventions, and Jong-Hwa Won enrolled participants. All researchers checked the participants' group information after the study, except for Jung-Jun Park. Jung-Jun Park did not view the relevant information nor intervene in the intervention after the initial group assignment. Participants were unaware of their group information as they consumed products in plain packaging. Both groups underwent RE for 4 weeks. Body composition, respiratory gas analysis, and blood sampling were conducted in all participants before (pre-) and after (post-) intervention.

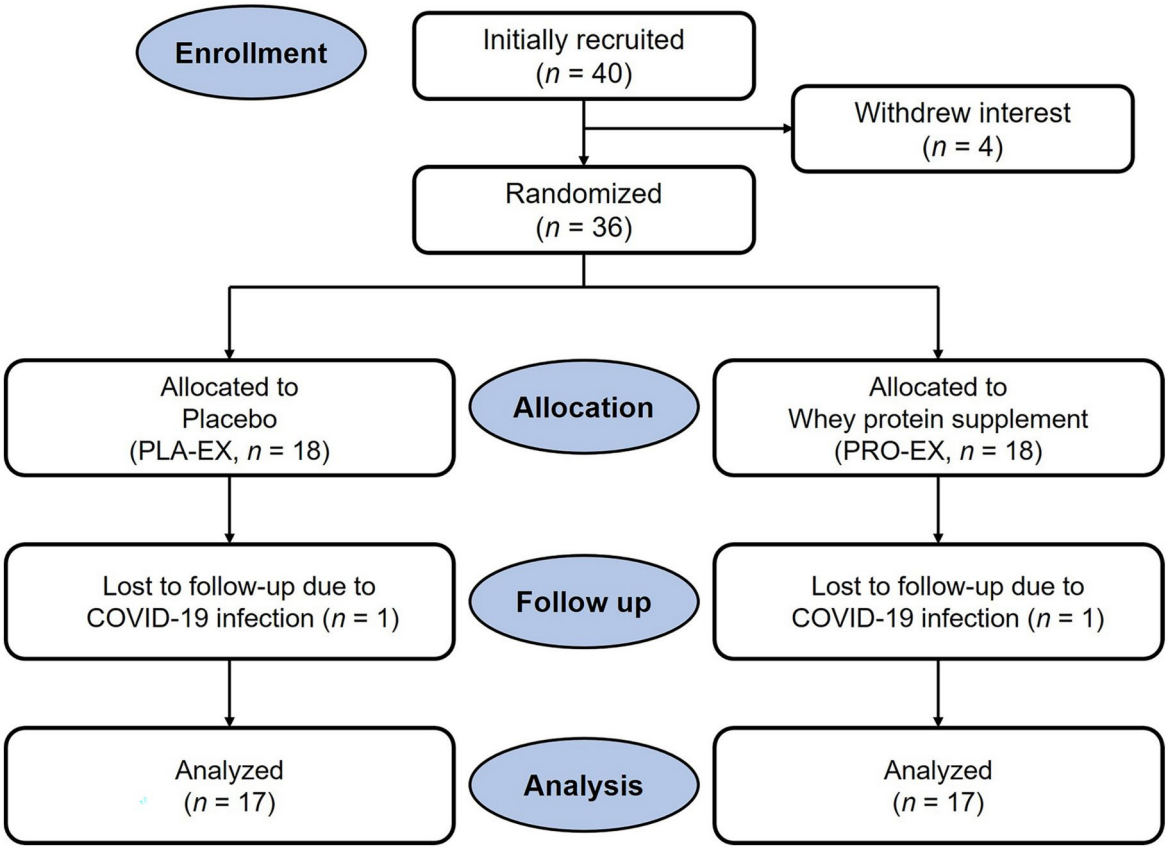

Fig. 1. Participant disposition.

Variable	Group		*p* value
	PLA-EX (*n* = 17)	PRO-EX (*n* = 17)	
Age (years)	24.00 ± 2.85	24.61 ± 3.11	0.55
Height (cm)	174.42 ± 3.05	173.77 ± 3.33	0.55
Body weight (kg)	72.78 ± 2.39	73.08 ± 4.19	0.80
Body mass index (kg/m^2)	23.96 ± 1.23	24.18 ± 1.75	0.66
Skeletal muscle mass (kg)	32.14 ± 2.17	32.01 ± 1.73	0.85
Body fat mass (kg)	15.99 ± 4.57	16.16 ± 3.90	0.91
%Body fat	21.86 ± 5.74	21.99 ± 4.47	0.94

Table 1. Participants characteristics.

Food, beverages (except water intake), alcohol, and caffeine were restricted 8 ± 1.5 h prior to all measurements. One individual from each group withdrew from the study during the four-week intervention period due to COVID-19 infection. Therefore, 17 participants from each group (PLA-EX, *n* = 17; PRO-EX, *n* = 17) completed the intervention. Since all participants provided complete data without any missing values for any measurement items, the data could be used for statistical analysis.

Dietary control

All participants were individually provided with 3 meals per day in a lunchbox based on the individual's total energy expenditure. Individual total energy expenditure was calculated using the Harris-Benedict formula[22]. Briefly, this was determined by multiplying the resting metabolic rate (RMR) by the physical activity index. Since the PAR-Q physical activity index of all participants was "sedentary," the physical activity index was 1.2. The food in the lunchbox diet were custom-made (Salady Inc., Seoul, Korea) and consisted of 10% protein, 30% fat, and 60% carbohydrates.

Whey protein supplement

The placebo (PLA-EX) and whey protein supplement (PRO-EX) had the same calorie content (99 kcal/pack) (Maeil Health Nutrition Co. Ltd., Pyeongtaek, Korea). The placebo contained 25 g of carbohydrate instead of protein. Each participant consumed either a whey protein supplement or placebo 3 times a day: morning, post-exercise, and evening. Thus, participants in the PLA-EX group consumed 0.8 g/kg BW/day, and those in the PRO-EX group consumed 1.5 g/kg BW/day of protein. Whey protein supplements and the placebo were designed such that there was no difference in taste, flavor, or texture and were provided in unlabeled packaging so that neither the participants nor researchers could distinguish between them. The protein intake of all participants in the study met the Korean Dietary Reference Intakes (KDRIs) set by the Korean Nutritional Society and Ministry of Health and Welfare of Korea[23]. Information on the amino acid profile of the whey protein supplement and its percentage relative to the KDRIs are presented in Table S1.

Resistance exercise

All participants performed intensive REs (60 min/day) for 4 weeks (6 days/week). In this study, we implemented a 4-week RE program, drawing on previous findings indicating that even a 4-week period, increases in muscle cross-sectional area and isometric maximum voluntary contraction can be observed within an 8-week RE protocol[24]. In light of these prior studies and considering the limited study duration and participant adherence, we consequently selected a 4-week intervention. The RE program consisted of 5 min of warm-up, 50 min of workout, and 5 min of cool-down. The daily RE program included a combination of upper and lower body. The target body parts for the training were the chest, back, shoulders, arms, abdomen, and lower body. The exercise program consisted of 4 sets per exercise, with 8 to 12 repetitions per set, and a 1 to 2-min rest between sets. The exercises included chest exercises such as Smith bench press, incline bench press, and push-ups; back exercises including seated row, barbell row, and lat pulldown; lower body exercises such as squats, leg press, and lunges; shoulder exercises including Smith shoulder press, dumbbell press, and dumbbell side lateral raises; arm exercises like cable curls, cable pushdowns, and kickbacks; and abdominal exercises, primarily crunches. The one-repetition maximum (1-RM) was measured for each training exercise using Brzycki's 1-RM calculation formula[25]. The intensity of the RE program was gradually increased (1–2 weeks at 60% of 1-RM and 3–4 weeks at 70% of 1-RM).

Measurements

Body composition

The body composition of all participants was measured before and after the intervention using a bioelectrical impedance analyzer (BIA) (InBody 620, InBody Co. Ltd., Seoul, Korea). To reduce measurement error, participants were instructed to refrain from consuming food, beverages, alcohol, and caffeine for 8 h prior to the assessments. Each participant underwent three measurements on separate days. All assessments were conducted in the morning under similar conditions, and the mean values across the three measurements were utilized for statistical analysis.

Isokinetic muscular function

Muscle strength and endurance were measured using an isokinetic dynamometer (Cybex 770; Humac Norm, Stoughton, MA, USA). Muscle strength was evaluated using the peak torque at 60°/s with 5 repetitions. The peak torque is determined as the highest torque at any point within any repetition and range of motion and is expressed in Nm. Muscular endurance was evaluated by total work at 180°/s, with 15 repetitions. Total work is the sum of the work performed in each repetition or the total area under the torque curve and is expressed in Joules (J). Three practice repetitions were performed to familiarize the participants with the test. The range of motion of the knee was set to 0–90°. The isokinetic muscular functions of the dominant and non-dominant knee flexors and extensors were measured in all participants. These data were previously reported, but statistical analysis and graph plotting were re-conducted on paired samples to confirm the difference between pre and post of the same participants[21].

Resting metabolic rate

RMR analysis was performed using a respiratory gas analyzer (Quark RMR, Cosmed, Rome, Italy). Before measuring the RMR, participants were rested comfortably in a chair for 10–15 min. The participants were asked to lie down on a bed before a canopy was placed over their head and the breathing gas was measured for 15 min. Data from the first 5 min were excluded from the analysis, and the measurement data for the remaining 10 min were used to determine the RMR. All the participants remained awake during the measurement because the RMR is lower when asleep than at rest. These data were previously reported, but statistical analysis and graph plotting were re-conducted on paired samples to confirm the difference between pre and post of the same participants[21]. The oxidation rates of energy substrates were obtained from the Quark RMR system. The metabolic contribution of protein (Pro%) is calculated assuming 12 g of Ureic Nitrogen excretion in 24 h. This value is used to estimate the energy contribution from protein and is determined by the following formula (1). The oxidation rates of fat and carbohydrate are calculated after subtracting the protein contribution (Pro%) and are based on the respiratory exchange ratio (RER). The metabolic contributions of fat and carbohydrate are calculated using the following formulas (2) and (3).

$$\%Pro = (Ureic\,Nitrogen(g/day) \times 6.25 \times 4)/(Total\,Energy\,Expenditure(TEE, kcal/day)) \times 100 \quad (1)$$

$$\%Fat = (1 - \%Pro) \times (1 - RER) \quad (2)$$

$$\%Carb = 100 - \%Pro - \%Fat \quad (3)$$

Blood collection
Before and after the intervention, 10 mL of venous blood was collected from each participant into a serum-separating tube (#BD367985, BD Vacutainer® SST™, Becton Dickinson, NJ, USA) and was immediately centrifuged at 1762×*g* for 20 min (LABOGENE 1236MG, Gyrogen, Daejeon, Korea). The serum collected after centrifugation was immediately used for biochemical blood analysis. The remaining serum was stored at minimum – 70 °C and used for enzyme-linked immunosorbent assay (ELISA).

Blood biochemical analysis
Aspartate transaminase (AST), alanine aminotransferase (ALT), platelet (PLT), albumin, glucose, insulin, total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels were determined from the serum using an automatic biochemistry analyzer (7180 Clinical Analyzer, Hitachi, Tokyo, Japan).

Enzyme-linked immunosorbent assay
Adiponectin, leptin, free fatty acids (FFA), insulin-like growth factor 1 (IGF-1), GDF-8 (growth/differentiation factor 8, also known as myostatin), and testosterone levels were analyzed using a commercial ELISA kit. All analyzed variables were simultaneously assayed in a 96-well plate, with serum collected before and after the intervention. Excluding the 24 wells for establishing the standard curve, the remaining 72 wells were used to analyze pre- and post-collection serum samples for all participants (total of 34 samples × 2). Therefore, it was not possible to perform the analysis in triplicate on one microplate and eliminating intra- and inter-experimental errors with 3 experiments for each variable. The absorbance was measured using a microplate reader (2030 Multilabel Radar, VICTOR X5™; Perkin Elmer, Norwalk, CT, USA) and the data were analyzed using GainData (Arigo Biolaboratories, https://www.arigobio.com/elisa-analysis). All procedures were performed according to the manufacturer's instructions for each commercial ELISA kit (Adiponectin, Cat #. DHWAD0, R&D Systems, Minneapolis, MN, USA; leptin, Cat #. DLP00; R&D Systems, Minneapolis, MN, USA; FFA, cat. MBS041165, MyBioSource, San Diego, CA, USA); IGF-1, Cat #. EI1001-1, AssayPro, St. Charles, MO, USA; GDF-8, Cat #. DGDF80, R&D Systems, USA; testosterone, Cat #. EK-311-15, Phoenix Pharmaceuticals, Burlingame, CA, USA).

Hepatic fat content
A portable ultrasound device (FibroScan® Mini + 430; Echosens, Paris, France) was used to analyze the hepatic fat content of the participants. The controlled attenuation parameter (CAP) identified by the ultrasound device was used to represent hepatic fat content. All measurements were performed with a 3.5 MHz standard probe in the right hepatic lobe through the intercostal space with the participant in the supine position[26]. The criteria for determining measurement validity were: (1) at least 10 valid shots; (2) a success rate of at least 60%; and (3) the interquartile range was less the 30% of the median CAP values. The final CAP was recorded as the median value of all the measurements and expressed in dB/m[27]. These data were previously reported, but statistical analysis and graph plotting were re-conducted on paired samples to confirm the difference between pre and post of the same participants[20].

Statistical analysis
GraphPad Prism 9 software (GraphPad Software Inc., San Diego, CA, USA) was used for all statistical analysis and graphing. Statistical analyses were performed after pairing all individual measurement variables and also pairing pre- and post-values. Two-way repeated measures ANOVA and Sidak's post hoc test were conducted to confirm changes and interactions according to the group and time of each variable. To identify the correlation between all independent variables (Δ values before and after intervention), Pearson's correlation coefficient was analyzed, and the correlation matrix was plotted. The statistical significance level was set at $p < 0.05$.

Results
Dietary consumption
During the experimental period, the caloric intake of the study participants was 2249.55 ± 122.77 kcal in the PRO-EX group and 2320.76 ± 158.86 kcal in the PLA-EX group.

Body composition
BW, body mass index (BMI), and body fat percentage were significantly reduced by the intervention in both the PLA-EX (BW, $p < 0.001$; BMI, $p < 0.01$; %body fat, $p < 0.01$) and PRO-EX (BW, $p < 0.01$; BMI, $p < 0.01$; %body fat, $p < 0.0001$) groups (Fig. 2A–C). Muscle mass increased in the PRO-EX group ($p < 0.05$) but there was no significant difference in the PLA-EX group (Fig. 2D).

Isokinetic muscular function
In the PRO-EX group, the torque at 60° flexion ($p < 0.001$;. Cohen's $d = 1.022$) and the total work of 180° extension ($p < 0.01$, Cohen's $d = 0.753$) of the dominant knee were increased (Fig. S1A and D). In the non-dominant knee, the torque at 60° flexion ($p < 0.01$; Cohen's $d = 0.765$) and total work of 180° extension ($p < 0.05$; Cohen's $d = 0.636$) in the PRO-EX group were also increased (Fig. S1E and H). However, the PLA-EX group showed a significant increase in total work in only the 180° extension of the non-dominant knee among the isokinetic muscular function test items ($p < 0.01$) (Fig. S1H). In both the dominant and non-dominant knees, no significant change

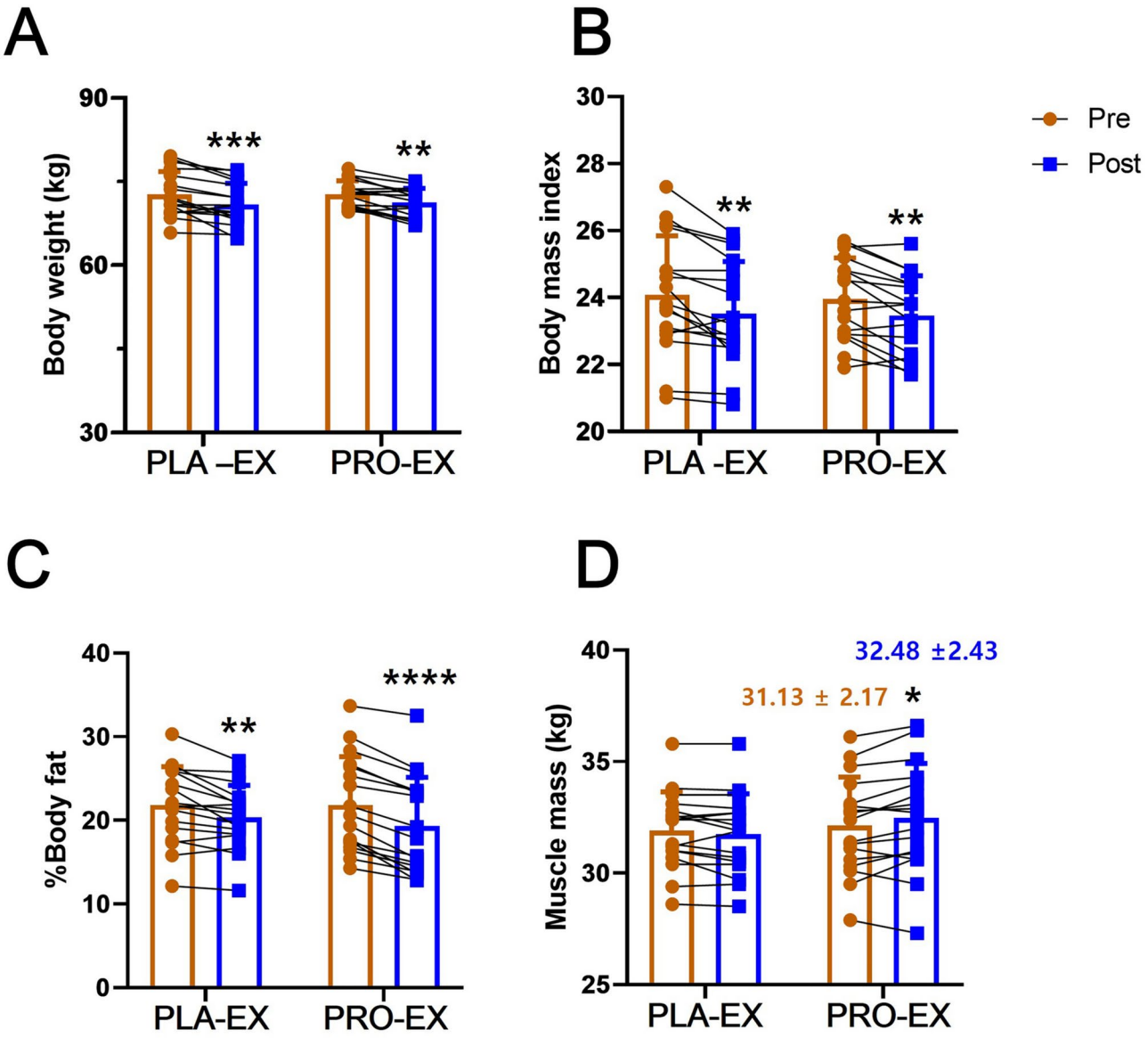

Fig. 2. Changes in body composition resulting from the combination of placebo or protein intake with resistance exercise (RE). (**A**) Body weight. (**B**) Body mass index. (**C**) %Body fat. (**D**) Muscle mass. All data presented as the mean (M) ± standard deviation (S.D.). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$, vs. Pre.

due to the intervention in torque or total work was confirmed at either 60° extension or 180° flexion (Fig. S1B,C,F,G).

Metabolic rate

While there was no significant difference in RMR between pre- and post-intervention in the PLA-EX, RMR significantly increased in the PRO-EX ($p < 0.05$) (Fig. 3A). In the PLA-EX group, fat metabolism was significantly increased ($p < 0.05$) and carbohydrate metabolism was significantly decreased ($p < 0.05$) (Fig. 3B). In the PRO-EX group, fat metabolism was increased, but carbohydrate and protein metabolism were decreased (both $p < 0.05$).

Blood biochemical variables

Albumin, TC, and TG levels were significantly decreased by the intervention in both PLA-EX (albumin, $p < 05$; TC, $p < 0.001$; TG, $p < 0.0001$) and PRO-EX (albumin, $p < 0.01$; TC, $p < 0.0001$; TG, $p < 0.0001$) (Fig. S2B,E,G). PLT, fasting glucose, and fasting insulin levels were not significantly altered by the intervention (Fig. S2A,C,D). There was a significant decrease in HDL-C levels after the intervention in the PRO-EX group ($p < 0.01$), but no change was observed in the PLA-EX group (Fig. S2F). However, in both the PLA-EX ($p < 0.01$) and PRO-EX ($p < 0.01$) groups, the ratio of TC to HDL-C was significantly lower after the intervention than before the intervention (Fig. S2H). Adiponectin, leptin, and GDF-8 (myostatin) levels were significantly reduced by the intervention in

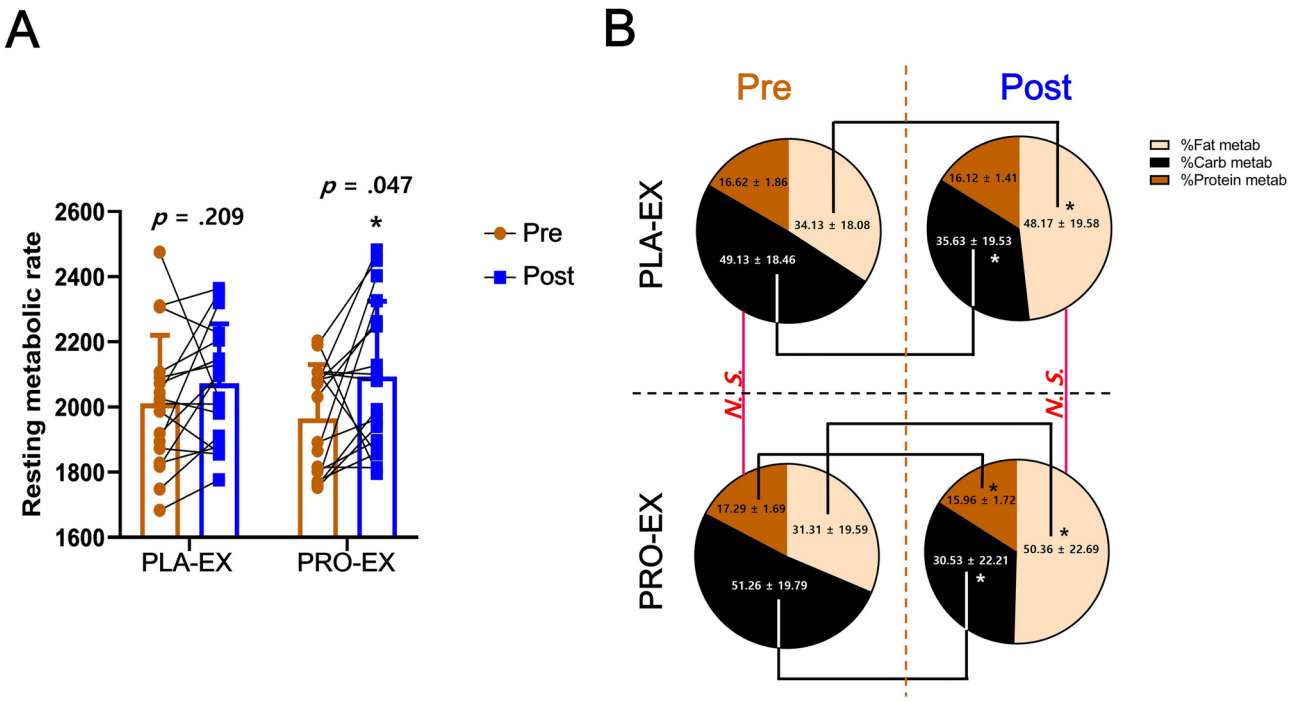

Fig. 3. Metabolic changes induced by combinations of placebo or protein intake with RE. (**A**) Resting metabolic rate. (**B**) Changes in energy substrate contribution. All data presented as the M ± S.D. *$p < 0.05$, vs. Pre.

both the PLA-EX (adiponectin, $p < 0.0001$; leptin, $p < 0.05$; GDF-8, $p < 0.05$) and PRO-EX (adiponectin, $p < 0.001$; leptin, $p < 0.0001$; GDF-8, $p < 0.01$) (Fig. 4A,B,E) groups. A significant difference in FFA was confirmed only in the PLA-EX group ($p < 0.01$) (Fig. 4C), and a significant difference in IGF-1 and testosterone was confirmed only in the PRO-EX group (IGF-1 increased, $p < 0.05$; testosterone decreased, $p < 0.01$) (Fig. 4D,F).

Hepatic fat content and related blood chemical variables

CAP was significantly reduced by the intervention in both the PLA-EX ($p < 0.05$) and PRO-EX ($p < 0.0001$) groups (Fig. 5A). The AST and ALT levels were significantly decreased in the PRO-EX group only (AST, $p < 0.0001$; ALT, $p < 0.001$) (Fig. 5B,C).

Correlation analysis

A Venn diagram was made to clarify the differences according to the intervention (PLA-EX vs. PRO-EX) for each independent variable identified in this study. In the PLA-EX group, there was a significant decrease in FFA only, whereas in the PRO-EX group, IGF-1 and muscle mass were significantly increased, and testosterone, HDL-C, AST, and ALT were significantly decreased (Fig. 6). As a result of the correlation analysis, 35 interactions were identified in the PLA-EX group and 20 interactions were identified in the PRO-EX group ($p < 0.05$–$p < 0.0001$) (Fig. S3, Table S2). Cases in which an interaction between variables inevitably appeared (e.g., the interaction between BW and BMI) were ignored. Correlation analysis confirmed that FFA, which was reduced in the PLA-EX group only, was negatively correlated with IGF-1 ($r = -0.612$, $p < 0.01$), and positively correlated with testosterone ($r = 0.506$, $p < 0.05$), insulin ($r = 0.515$, $p < 0.05$) and CAP ($r = 0.495$, $p < 0.05$) (Fig. S3, Table S2). IGF-1, testosterone, muscle mass, HDL-C, AST, and ALT levels decreased in the PRO-EX group only and were not correlated with other variables (Fig. S3, Table S2).

Discussion

It is generally assumed that a higher protein intake will increase the effectiveness of RE. Therefore, many people attempt to increase their protein intake based on the level of RE. A recent meta-analysis found that increasing protein intake had a synergistic effect when performing RE, but the effect plateaued when protein intake exceeded 1.5 g/BW/day[2]. Therefore, it is necessary to consider the known side effects of excessive protein intake during RE and daily life[28–31]. Thus, in this study, protein intake was limited to 1.5 g/kg BW/day to minimize confounding variables due to excessive protein intake. In this study, a 4-week RE program was implemented, drawing on evidence from an 8-week RE study suggesting that the interaction between protein intake and exercise can be observed even within a relatively short period (i.e., 4 weeks)[24]. Considering these previous findings, along with the limited study duration and participant adherence, we therefore opted for a four-week intervention.

We confirmed that there was an increase in muscle mass and strength in the PRO-EX group when RE was performed with a protein intake of 1.5 g/kg BW/day (Fig. 2D, Fig. S1A and B, and Fig. 6). These results mean that protein intake of only 1.5 g/kg BW/day is sufficient to induce increases in muscle mass and muscle strength

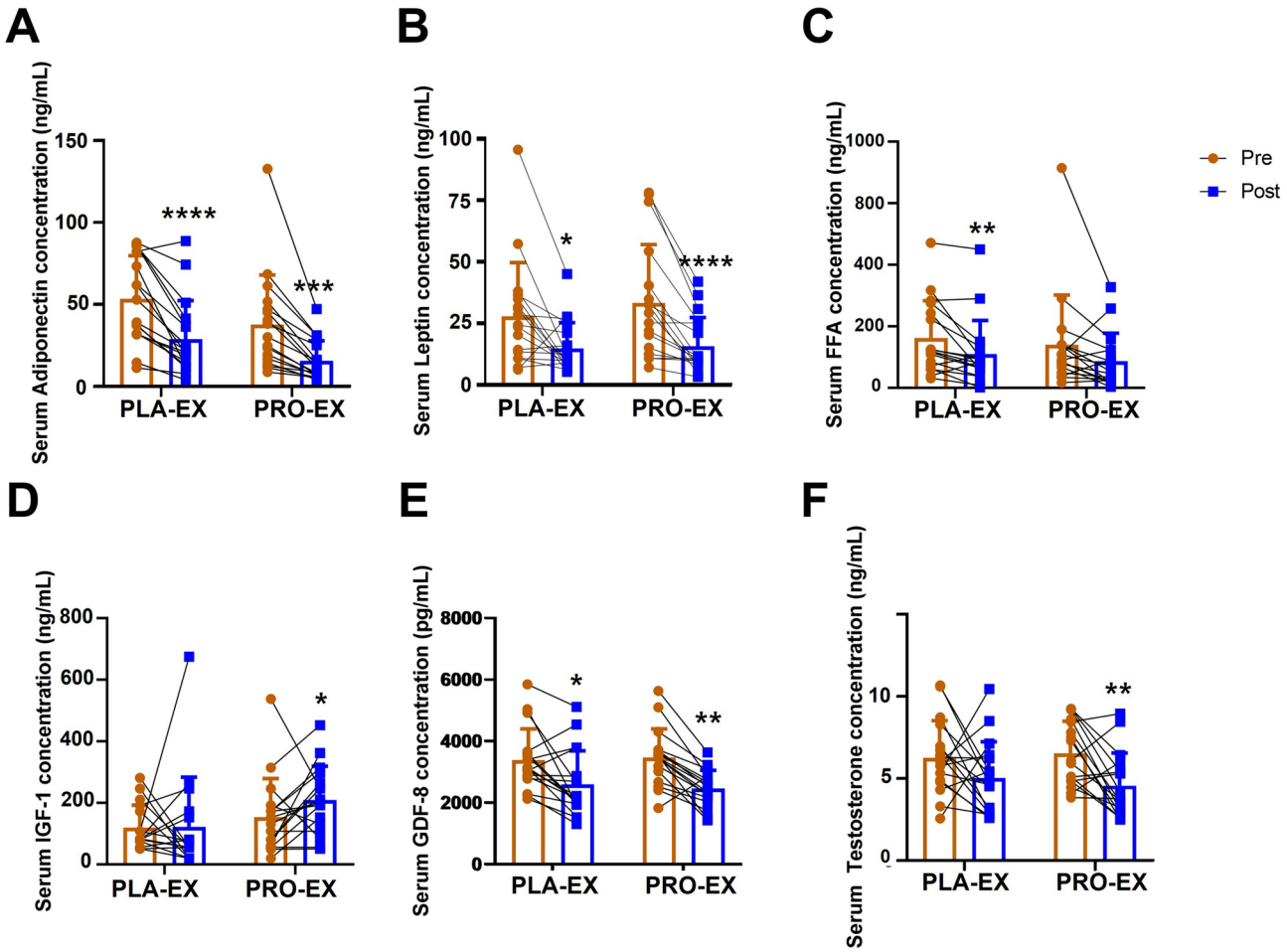

Fig. 4. Changes in serum circulating hormone levels by combinations of placebo or protein intake with RE. (**A**) Adiponectin. (**B**) Leptin. (**C**) Free fatty acids. (**D**) Insulin-like growth factor 1. (**E**) Growth/differentiation factor 8. (**F**) Testosterone. All data presented as the M ± S.D. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$, vs. Pre.

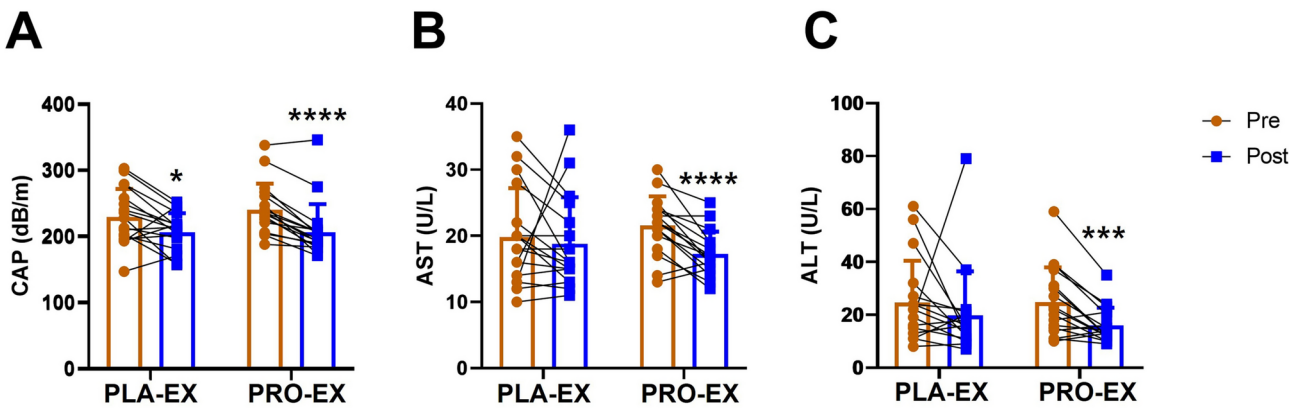

Fig. 5. Changes in liver health indices by combinations of placebo or protein intake with RE. (**A**) Controlled attenuation parameter. (**B**) Aspartate transaminase. (**C**) Alanine aminotransferase. All data presented as the M ± S.D. *$p < 0.05$, ***$p < 0.001$, and ****$p < 0.0001$, vs. Pre.

Fig. 6. Venn diagram categorizing the variables changed by combinations of placebo or protein intake with RE into union, intersection, and subset.

through a synergistic effect with RE. Additionally, protein intake above 1.5 g/kg BW/day suggests that RE may be unnecessary for those performing RE, considering the side effects of excessive protein intake. Other physiological and biochemical variables presented in this study are believed to be the result of a combination of RE and protein intake, excluding the influence of excess protein intake. Since excessive protein intake induces various side effects, the results of this study provide an important basis for determining the optimal protein intake for RE. Nevertheless, it is important to note that our study did not directly compare multiple levels of protein intake (e.g., 1.2, 1.8, or 2.0 g/kg BW/day) and thus cannot conclusively determine 1.5 g/kg BW/day as the absolute optimal dose. Rather, our findings provide preliminary evidence indicating that a moderate intake set at 1.5 g/kg BW/day can yield significant synergistic benefits in conjunction with resistance exercise. This conclusion also aligns with several meta-analyses suggesting that protein intakes above 1.6 g/kg BW/day do not offer additional advantages for muscle strength gains. However, further research involving direct comparisons among different intake levels is warranted to refine and validate these recommendations.

An increased protein intake during RE caused a decisive difference in energy metabolism (Fig. 3A,B). Although no significant change in the RMR by intervention was confirmed in either group, the PRO-EX group showed a more significant change than the PLA-EX group (PLA-EX, $p < 0.209$; PRO-EX, $p < 0.047$) (Fig. 3A). In particular, in the PRO-EX group, protein and carbohydrate metabolism significantly decreased, and fat metabolism increased at rest (Fig. 3B). Although ureic nitrogen was not measured in this study, the protein metabolism ratio was assumed to be based on the default value of 12 g, as protein metabolism remains relatively stable at rest. The significant decrease in the protein metabolism ratio observed in the PRO-EX group of this study is interpreted as a relative reduction due to the increase in RMR (Fig. 3A), with the actual percentage decrease being around 1–2% (Fig. 3B). In addition, proper protein intake combined with RE may be slightly more effective in increasing fat metabolism. This is in line with a study that concluded a protein intake of 1.6 g/kg BW/day along with an adequate carbohydrate amount promotes FA oxidation in skeletal muscle[32]. Although there is a difference in the extent of change in different intervention methods, it should not be overlooked that RE itself induces a change in metabolism. It should be noted that RE should precede protein intake for health management such as weight control or body fat reduction.

Differences in the HDL-C levels were observed (Fig. S2F). HDL-C is well known as a good cholesterol that cleans blood vessels, but the fact that it was significantly reduced in the PRO-EX group may seem puzzling. However, it can be inferred that the decrease in TC caused a decrease in HDL-C because the change in TC was more significant in the PRO-EX group than in the PLA-EX group. Crucially, significant reductions in TC occurred in both the PLA-EX and PRO-EX groups; therefore, reductions in both TC and HDL-C levels are independent of RE. In other words, the results show that protein intake improves fat metabolism slightly more through its synergistic effect with RE. In addition, in a study conducted in overweight adults, when a high-protein diet was followed and exercise was performed, TC and HDL-C levels decreased, and when a high-protein diet was followed without exercise, TC levels decreased but HDL-C levels increased[33]. This suggests the possibility that excessive protein intake can reduce HDL-C. Furthermore, considering that the TC/HDL-C ratio in both the PLA-EX and PRO-EX groups was lower after the intervention than before, the decrease in HDL-C observed in this study may be interpreted as a temporary reduction attributable to the decrease in TC. Indeed,

previous meta-analyses have reported that short-term weight loss can reduce HDL-C, which further supports the notion that the findings of this study are influenced by short-term weight loss[34].

Adiponectin and leptin are hormones produced and released by adipose tissue. However, to date, the relationship between these two hormones and RE combined with protein intake is not fully understood. However, based on our data (Fig. 4A,B), it seems that increased fat metabolism due to exercise causes a decrease in body fat, which in turn causes a decrease in the appetite-suppressing hormones adiponectin and leptin. It is natural to reduce appetite suppressing hormones due to BW loss through exercise and diet management, or to replenish the energy consumption due to exercise. A study showed that changes in adiponectin due to acute exercise in healthy adults are insignificant[35]. Therefore, changes in appetite-related hormones are likely due to secondary effects, such as reduction of body fat by a combination of chronic dietary control and exercise, rather than the direct effects of exercise. FFA was a variable in which a significant change after intervention was identified only in the PLA-EX group (Fig. 4C and Fig. S3). This result shows that PLA-EX can be more effective in reducing FFAs in the blood using RE. However, the decrease in FFA showed that there was no significant difference in the PRO-EX group, but it decreased on average ($p = 0.0657$) (Fig. 4C). This result shows that the improved metabolism and increased glucose uptake by RE can be effective in regulating FFAs[36,37].

The circulating level of IGF-1 significantly increased only in the PRO-EX group after the intervention. These results imply that the synergistic effect of RE and protein intake increases IGF-1 concentration at rest, leading to increased muscle mass and strength. Morton et al. reported no relationship between circulating IGF-1 levels and muscle strength gain in young men who had undergone 12 weeks of RE[38]. These results may seem contradictory to the results of this study, but instead highlight the importance of these results. In the study by Morton et al., protein supplementation was not administered to the participants, and the increase in IGF-1 in this study was obviously due to protein intake together with RE[38]. IGF-1 seems to be an important factor that helps muscle recovery and growth during the recovery period, but at this time, the effects of GDF-8 and testosterone seem to be insignificant. Testosterone is a factor that is increased by RE. However, there is a report that there is no change or rather a decrease at rest after long-term RE[39]. It is well known that RE promotes muscle growth and strength gain; however, the direct effects of GDF-8, a factor that inhibits muscle growth and strength gain, and exercise have not been well established. Since most studies have focused on the genetic and molecular regulation of GDF-8 rather than on its response to RE, its indirect effect had to be inferred from our study. In our study, GDF-8 tended to show a slightly negative correlation with IGF-1 (PLA-EX, $r = -0.22$; PRO-EX, $r = -0.09$) inducing muscle growth, which probably has some antagonism with IGF-1. However, it is important to consider GDF-8 and IGF-1 as independent factors. In addition, the correlation of circulating GDF-8 with protein intake and RE is still unclear. In this study, we analyzed multiple blood-based biochemical parameters associated with muscle physiology. However, not all factors involved in muscle synthesis were addressed. This limitation leaves open the possibility that more comprehensive investigations—such as metabolomic or proteomic analyses—could provide a broader understanding of changes in blood biochemical profiles. Furthermore, on the basis of the known correlations between the representative muscle-related factors examined here and other biochemical variables, one could infer additional alterations in unmeasured parameters. For example, cortisol, a glucocorticoid, has been reported to exhibit a positive correlation with circulating GDF-8 levels[40], suggesting that an increase in GDF-8 may be accompanied by a concurrent rise in cortisol. Although our investigation primarily centered on the synergistic effects of protein intake and RE, it is important to acknowledge that various micronutrients (such as vitamin D, calcium, magnesium, iron, zinc, and B vitamins) play significant roles in muscle protein synthesis and metabolic regulation[41–43]. For instance, vitamin D status may influence both muscle strength and endocrine factors, while minerals like magnesium and iron are essential for adenosine triphosphate production and oxygen transport, respectively[44,45]. If baseline micronutrient levels varied considerably among participants, such discrepancies could have modulated outcomes like IGF-1 elevation and muscle adaptation. Although we did not measure these micronutrient levels, the standardized meals likely minimized inter-individual nutrient variability. Future studies could benefit from incorporating detailed micronutrient assessments in order to further elucidate how they interact with protein intake and RE training to optimize muscle mass and strength gains.

There was a decrease in CAP due to RE in both the PLA-EX and PRO-EX groups. It is noteworthy that the level of CAP, which indicates the degree of liver fibrosis, decreased even though the participants in this study were young, healthy people. This indicated that liver health further improved within the normal range. This is because, in general, a CAP value of 238 dB/m or lower is considered normal. AST and ALT levels were significantly decreased only in the PRO-EX group, and the reduction of the two variables related to liver function showed that the combination of RE and protein intake can improve liver function. Many studies have shown that RE improves nonalcoholic fatty liver disease[46]. However, to the best of our knowledge, our report is the first to show that liver health is improved when RE and adequate protein intake are combined in healthy adults.

As previously mentioned, several findings in our study require additional clarification. First, the decrease in resting testosterone observed in the PRO-EX group may reflect an adaptive response rather than a straightforward suppression of androgenic activity. Chronic RE training, particularly under calorie-restricted conditions, can lead to lower baseline testosterone levels despite acute exercise-induced elevations[47,48]. Second, although HDL-C is often considered "good cholesterol," its reduction in the PRO-EX group likely coincided with a proportional decrease in TC. Short-term weight loss has been shown to induce concurrent declines in both TC and HDL-C, not only in middle-aged individuals across multiple trials[49] but also in overweight women[50], despite differences in participant age and baseline health status compared with our cohort. Notably, the TC-to-HDL-C ratio improved in both of our groups, suggesting that, overall, lipid profiles trended favorably. Lastly, the selective decrease in AST and ALT in the PRO-EX group is consistent with enhanced hepatic lipid metabolism and reduced liver inflammation. Studies indicate that regular RE can help lower hepatic steatosis and circulating liver enzymes[51], while higher-protein diets, particularly in conjunction with exercise, appear

to improve body composition and metabolic parameters associated with liver health[52]. Although our findings point to a potentially beneficial synergy, future research incorporating advanced imaging or hepatic biomarker panels could further elucidate the precise mechanisms behind these improvements. There are some limitations to our study that we would like to mention. We cannot rule out the possibility that the PRO-EX difference was widened because the protein intake of PLA-EX did not reach the recommended daily amount. However, our method may be useful in showing the difference in protein intake more clearly. In future research, we recommend adding a research group that meets the recommended daily intake of protein to fill in the gaps in our research. Additionally, since the participants of our study performed resistance exercise while consuming a regular diet in accordance with the recommended daily intake, their calorie intake was lower than their usual calorie intake. This regular diet may have resulted in the downregulation of factors related to anabolism such as muscle synthesis. Despite being on a calorie-restricted diet, the group that consumed protein supplements showed significant improvements in muscle quantity and quality, along with an increase in IGF-1 levels. It is important to note that our study was conducted on untrained sedentary individuals, and the findings cannot be generalized to trained individuals. Thus, the limitations of this study that were mentioned earlier could actually be a factor that further emphasizes the positive effect of protein intake. A further limitation lies in our conservative sample-size calculation. Although a prior meta-analysis suggested a moderate effect size ($d = 0.58$) for protein supplementation and RE[53], we adopted a smaller effect size ($d = 0.35$) to mitigate the risk of inflating intervention effects and to capture subtle yet clinically meaningful changes[54]. Accordingly, we determined that at least 30 participants would be required and ultimately recruited 34 to accommodate attrition. Nevertheless, subdividing this cohort for exploratory analyses may have reduced statistical power, and the relatively short (4-week) intervention window further constrains the generalizability of our findings. Future studies with larger cohorts and longer durations are warranted to validate and extend these observations Despite carefully controlling for total energy and macronutrient composition, we did not quantify or monitor participants' intake or serum levels of key micronutrients (e.g., vitamin D, calcium, iron). These nutrients are known to impact muscle hypertrophy, metabolic homeostasis, and hormonal responses[43,44]. Consequently, we cannot exclude the possibility that unmeasured micronutrient deficiencies or surpluses influenced our findings, such as the observed differences in IGF-1 and testosterone concentrations. A more comprehensive assessment of micronutrient status in future trials would likely provide deeper insights into how these factors integrate with adequate protein intake and RE to further optimize muscle adaptation and overall metabolic health. Additionally, our study assessed body composition using BIA. While BIA is practical, cost-effective, and non-invasive (making it well-suited for repeated measures in a relatively short-term intervention) it is also sensitive to factors such as hydration status and meal timing, potentially affecting measurement accuracy. Moreover, BIA is generally considered less precise than dual-energy X-ray absorptiometry or computed tomography for quantifying lean mass and fat distribution. Nevertheless, given our sample size and the need for multiple assessments, BIA was a pragmatic choice that minimized participant burden. Future research with smaller cohorts or more extensive resources might benefit from advanced imaging modalities to yield a more comprehensive and precise understanding of body composition changes.

In summary, we confirmed that differences in various physiological variables, including blood biochemical variables in the human body, appeared depending on the presence or absence of protein supplementation during RE. Protein supplementation during RE appears to be more effective than carbohydrate intake in improving fat metabolism and liver function. In addition, supplementation with protein during RE induces an increase in the concentration of IGF-1 in the blood during the resting period, which increases muscle mass and strength. In particular, the role of IGF-1 in increasing muscle mass and strength appears to be independent of resting testosterone and is negatively correlated with GDF-8. These results were clearly due to the synergistic effects of additional protein intake and RE. This is because this study was a double-blind RCT in which protein confounders and the effects of protein intake and excessive protein intake by a habitual diet were excluded. We suggest that a protein intake of 1.5 g/kg BW/day is sufficient to achieve a synergistic effect with RE. Although our findings indicate that 1.5 g/kg BW/day may be sufficient for realizing a synergistic effect with resistance exercise, caution is advised when generalizing these results. Future randomized controlled trials comparing multiple protein intake levels (e.g., 1.2, 1.5, and 2.0 g/kg BW/day) in parallel are necessary to establish a clearer dose–response relationship and to offer more nuanced guidelines for individuals aiming to optimize muscle mass and strength gains. Many studies have reported cases of ingesting 1.2 g/kg BW/day in habitual diets. Therefore, for most young adults, adding 0.3 g/kg BW/day of protein to the existing diet when performing RE can enhance the effects of RE, while avoiding the side effects of excessive protein intake. In other words, if an individual consumes sufficient protein in their habitual diet, protein supplementation is not necessary. However, if a person maintains a diet based on basal metabolic rate, it would be good to increase protein intake by about 0.5–0.8 g/kg BW/day.

Conclusions

RE clearly increased fat metabolism, muscle mass, and strength. Increases in IGF-1, when combined with RE and adequate protein intake, appear to be associated with increases in muscle mass and strength, but not testosterone and GDF-8. In addition, the protein intake during RE should consider the protein intake in the habitual diet, and in general, protein intake of + 0.3 g/kg BW/day is considered sufficient for the habitual diet.

Data availability

The data that support the findings of this study are available from the corresponding author, [Kyung-Wan Baek], upon reasonable request. Most of the data supporting the research results were disclosed in the Supplementary Material of this manuscript.

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Author contributions

KWB and JJP contributed to conception/design of the research; KWB, JHW and CBK, contributed to acquisition, analysis, or interpretation of the data; KWB drafted the manuscript; JJP critically revised the manuscript; and JJP agree to be fully accountable for ensuring the integrity and accuracy of the work. All authors read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

This study was approved by the Institutional Review Board of Pusan National University (No. 2020_136_HR, date of approval:31/05/2021). All participants provided written informed consent before participating in the study. The data were collected at Pusan National University in Republic of Korea. This study was conducted in accordance with the Declaration of Helsinki.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-89925-x.

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