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CLINICAL TRIAL

Interactive effects of an isocaloric high-protein diet and resistance exercise on body composition, ghrelin, and metabolic and hormonal parameters in untrained young men: A randomized clinical trial

Hyun Ho Kim[1], Yun Jin Kim[1], Sang Yeoup Lee[2,3]*, Dong Wook Jeong[3], Jeong Gyu Lee[1], Yu Hyone Yi[1], Young Hye Cho[1,3], Eun Jung Choi[1,3], Hyun Jun Kim[4]*

[1]Department of Family Medicine, [2]Medical Education Unit and Medical Research Institute, Pusan National University School of Medicine, [3]Family Medicine Clinic and Research Institute of Convergence of Biomedical Science and Technology, Pusan National University Yangsan Hospital, Yangsan, and [4]Department of Physical Education, Kyungnam University College of Education, Masan, Korea

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***Correspondence**
Hyun Jun Kim Tel.: +82-10-9134-5959 Fax: +82-51-510-8125 E-mail: kimhj@kyungnam.ac.kr
Sang Yeoup Lee Tel.: +82-10-9134-5959 Fax: +82-51-510-8125
E-mail address: saylee@pnu.edu

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ABSTRACT

Aims/Introduction: The interactive effects of resistance training and dietary protein on hormonal responses in adults are not clear and remain controversial. We tested the effect of an isocaloric high-protein diet on body composition, ghrelin, and metabolic and hormonal parameters during a 12-week resistance training program in untrained healthy young men.

Material and Methods: We randomized 18 healthy young men to a standard diet (ST group) or an isocaloric high protein diet (HP group). Both groups participated in a 12-week resistance exercise program. We measured body composition, lipid profile, homeostatic model assessment of insulin resistance (HOMA-IR) indices, total ghrelin, and exercise-related hormones at baseline and 12 weeks.

Results: In the HP group, lean body mass (LBM), total ghrelin, growth hormone, testosterone and cortisol levels showed an increase, whereas body fat percentage and HOMA-IR showed a decrease at 12 weeks, compared with baseline ($P \leq 0.05$). In the ST group, no changes in these parameters were observed during the 12-week period. During the 12-week period, significant differences in the pattern of change of LBM ($P = 0.032$), total ghrelin ($P = 0.037$), HOMA-IR ($P = 0.040$) and high-density lipoprotein cholesterol ($P = 0.011$) over time were observed between the groups.

Conclusions: The findings of the present study suggest that an isocaloric high-protein diet can ameliorate body composition, metabolic profiles and energy metabolism during a 12-week scheduled resistance training program in untrained healthy young men. This trial was registered with ClinicalTrials.gov (no. NCT01714700).

INTRODUCTION

Resistance exercise is an essential part of preservation of lean body mass and weight maintenance after weight reduction[1]. When combined with resistance training, protein intake results in greater skeletal muscle mass through a higher stimulation of protein synthesis and a positive net protein balance[2–6]. Moderate-intensity resistance training exercise provides the greatest hormonal responses for optimal achievement of the benefits of muscular fitness[7]. The testosterone/cortisol ratio, which reflects the anabolic/catabolic properties, has been used for evaluation of exercise responses[7].

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Ghrelin induces release of growth hormone (GH) from the pituitary gland, increases appetite and stimulates weight gain[8]. Circulating ghrelin is sensitive to changes in bodyweight after diet, exercise or bariatric surgery[9–11]. Previous studies have reported an increase in ghrelin levels after ingestion of a protein rich test meal in both obese subjects and normal weight subjects[12,13]. However, little is known about the long-term effects of a high-protein diet on ghrelin release[14]. In the majority of studies, whey and soy supplement, not dietary protein, were the protein sources used most often with a resistance-training program. In addition, the interactive effects of resistance training and protein supplementation on hormonal responses are less clear, and remain controversial[3–7]; although, in those studies, these inconsistencies could be partially reconciled with factors such as nutrition, protein sources, overtraining, detraining and age. Therefore, we investigated the effect of an isocaloric high-protein diet on body composition, ghrelin, and metabolic and hormonal parameters during a 12-week resistance training program in previously untrained healthy young men.

MATERIALS AND METHODS

Participants

The present study was approved by the institutional review board at the Medical Research Institute, Pusan National University, and was carried out in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrolment between August 2006 and January 2007. The study was registered with ClincialTrials.gov, number NCT01714700. A total of 18 healthy young men participated in the present study. They had not participated in any weight-management program during the previous 6 months. An open-label design was used. At enrolment, participants were randomly assigned to one of two groups: a control group with a standard diet (total calories 2,400 kcal/day, 60% carbohydrate, 15% protein and 25% fat; ST group) and an interventional group with an isocaloric high protein diet (total calories 2,400 kcal/day, 55% carbohydrate, 30% protein and 15% fat; HP group). Participants in both groups took part in a scheduled resistance exercise training program. Participants did not take part in any other resistance training program or competitive sport over the study duration. At the start of the study, participants were instructed to maintain their assigned diet throughout the study period. This was assessed using a food frequency questionnaire (FFQ) with a 24-h recall after 12 weeks. Participants were presented with two-dimensional food pictures of the actual size of portions on a computer by a single trained dietitian to minimize interviewer bias. Medium was defined as the portion sizes shown, small was approximately half the medium portion size, and large was one-and-a-half times the medium portion size[15]. None of the participants withdrew from the study until 12-week follow-up measures.

Exercise Protocol

All participants were under supervision during participation in the progressive supervised resistance exercise-training program. They participated in an onsite supervised protocol for 12 weeks (6 days per week).

Exercise duration was increased gradually, from 50 min at week 1 to 80 min at week 12, including warm-up for 20 min and cool-down periods for 10 min by carrying out gentle stretching exercises of the upper and lower limbs. A one repetition maximum (1-RM) is the maximum weight that can be lifted for one complete repetition of the movement. For each participant, the RM was calculated for a particular muscle group. Each movement was carried out at 60–80% of his 1-RM. Resistance was increased slowly by three steps; step 1 (60–70% of his 1-RM for week 1–4), step 2 (65–75% of his 1-RM for week 5–8) and step 3 (70–80% of his 1-RM for week 9–12). For the purpose of this analysis, compliance at each visit was set at 100%.

Baseline and Follow-up Measurements

Height and bodyweight were measured using a digital scale with the examinee wearing a light gown, but no shoes. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). The percentage body fat and total fat mass were measured by bioelectric impedance analysis (Inbody 3.0; Biospace Co., Ltd., Seoul, Korea), a sensitive test for quantification of changes in lean and fat mass *in vivo*[16] and for assessment of regional fat distribution[17]. A mercury sphygmomanometer was used for measurement of blood pressure (BP) of each participant, in the sitting position, after a 10-min resting period. Two readings each for the systolic and diastolic BPs were recorded at 3-min intervals, and the average of each measurement was included in our analysis.

Blood samples were obtained preprandially at 0.800 hours from each participant's antecubital vein after a 12-h fast, and were used for determination of plasma glucose, insulin, lipid profile, total ghrelin, GH, insulin-like growth factor-1 (IGF-1), testosterone and cortisol. Blood samples were obtained at baseline (prerandomization) and 12 weeks after randomization. Plasma glucose was measured immediately after sampling into tubes containing NaF. Other blood samples were drawn into chilled tubes containing Na_2 ethylenediaminetetraacetic acid (1 mg/mL) and aprotinin (500 U/mL). Plasma was immediately separated by centrifugation at 4°C and stored at −70°C until assayed. Plasma for ghrelin analysis was immediately acidified with 1 N HCl (1:10 relative to the plasma volume) in order to prevent ghrelin degradation before freezing. Glucose was determined by a glucose oxidase method using the Synchron LX 20 (Beckman Coulter, Fullerton, CA, USA). Insulin was determined by an electrochemiluminescence method (Roche Diagnostics GmbH, Mannheim, Germany) with a detection limit of 0.20 μIU/mL, and inter- and intra-assay coefficients of variation (CVs) of 1.0 and 2.6%, respectively. Homeostatic model assessment (HOMA) index was calculated using the formula for estimation of insulin sensitivity: fasting insulin (μIU/mL) × fasting glucose (mg/dL)/405[18]. Lipid profile was measured by an enzymatic colorimetric method using with an autoanalyzer (Hitachi

747; Hitachi Corp., Tokyo, Japan). Total ghrelin was determined by a radioimmunoassay (RIA) kit (BioSource Europe S.A., Nivelles, Belgium) with a detection limit of 0.35 ng/L, and inter- and intra-assay CVs of 7.0 and 5.2%, respectively. GH was determined by two-site immunoradiometric assay (Daiichi Radioisotope Laboratories, Ltd., Tokyo, Japan) with a detection limit of 0.1 ng/mL, and inter- and intra-assay CVs of 1.6 and 1.4%, respectively. IGF-1 was determined by an enzyme-labeled chemiluminescent immunometric assay (DPC Diagnostic Products Inc., Los Angeles, CA, USA) with a detection limit of 20 ng/mL, and inter- and intra-assay CVs of 2.3 and 3.9%, respectively. Cortisol was determined by a solid phase RIA (DPC Diagnostic Products Inc.) with a detection limit of 0.2 μg/dL, and inter- and intra-assay CVs of 3.5 and 4.9%, respectively. Testosterone was determined by a RIA (Spectria Testosterone, Orion Diagnostica, Espoo, Finland) with a detection limit of 0.14 ng/mL and inter- and intra-assay CVs of 8.0 and 6.0%, respectively. All procedures were carried out at 4°C.

Statistical Analysis

Normally distributed data were expressed as means ± standard deviation, and skewed distribution data are expressed as median (interquartile ranges). The percentage of change in variables was calculated as the difference between the values at baseline and 12 weeks divided by the baseline value and multiplied by 100. The D'Agostino–Pearson normality test was used for testing of normal distribution. Between-group comparisons were carried out by two-sample *t*-test or Mann–Whitney *U*-test, as appropriate; and within group comparisons by paired *t*-test or Wilcoxon signed rank test, as appropriate. Comparisons of the change during 12 weeks between the two different diet groups were made by two-way ANOVA for repeated measures with age and BMI as covariates. SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. A *P*-value of <0.05 was deemed statistically significant. All statistical tests were two-tailed.

RESULTS

Basal Characteristics of the Study Participants

There were no significant differences in age (23.6 ± 2.8 vs 25.0 ± 2.4 years, $P = 0.256$), baseline BMI, and body fat percent between the ST and HP groups. In addition, no statistically significant differences in BP, HOMA-IR, lipids, GH, IGF-1, testosterone, cortisol and ghrelin were observed at baseline between the groups (Table 1).

Within-Group Analyses

After the intervention, BMI remained unchanged in both groups during the experimental period. However, after

Table 1 | Changes in characteristics and metabolic parameters of study participants at baseline and 12 weeks after intervention

Variables	Resistance exercise + high-protein diet group ($n = 9$)				Resistance exercise + standard diet group ($n = 9$)				*P*-value§
	Baseline†	After 12 weeks	Δ	*P*-value‡	Baseline†	After 12 weeks	Δ	*P*-value‡	
BMI (kg/m^2)	23.6 ± 2.8	23.3 ± 2.1	−1.0 ± 3.6	0.331	24.5 ± 2.8	24.1 ± 2.3	−1.6 ± 2.7	0.115	0.713
BF (%)	22.4 ± 5.8	20.0 ± 4.9	−10.3 ± 6.5	0.004	19.2 ± 7.9	18.3 ± 7.3	−4.7 ± 14.8	0.320	0.312
LBM (kg)	51.5 ± 4.6	52.7 ± 4.1	2.4 ± 2.0	0.006	57.5 ± 6.4	57.2 ± 5.6	−0.4 ± 2.8	0.596	0.032
SBP (mmHg)	135.4 ± 18.7	131.8 ± 13.8	−2.2 ± 6.9	0.283	134.6 ± 9.0	131.7 ± 11.9	−1.8 ± 10.0	0.550	0.935
DBP (mmHg)	79.6 ± 11.5	78.8 ± 9.1	−0.0 ± 12.9	0.823	78.1 ± 10.2	78.4 ± 7.2	1.9 ± 16.4	0.938	0.785
HOMA-IR	3.7 (2.1–6.2)	1.6 (1.4–2.0)	−55.3 [(−65.0) −(−32.3)]	0.015	2.5 (2.0–3.0)	2.5 (1.8–3.9)	24.0 [(−35.0) −49.2]	0.767	0.040
TC (mg/dL)	182.2 ± 33.3	181.1 ± 18.3	1.7 ± 16.3	0.916	180.1 ± 16.1	184.7 ± 24.8	2.8 ± 12.9	0.573	0.871
HDL-C (mg/dL)	50.7 ± 7.5	55.7 ± 13.2	9.1 ± 13.2	0.080	60.8 ± 12.41	56.1 ± 12.8	−7.9 ± 11.7	0.060	0.011
LDL-C (mg/dL)	120.7 ± 32.0	119.8 ± 18.2	3.0 ± 18.4	0.919	112.2 ± 15.5	120.0 ± 21.3	8.1 ± 20.0	0.321	0.583
TG (mg/dL)	102.6 ± 47.0	67.4 ± 19.8	−23.5 ± 30.8	0.068	82.1 ± 30.6	96.4 ± 44.7	27.8 ± 66.8	0.363	0.053
GH (ng/mL)	0.6 (0.2–4.2)	9.8 (3.7–24.6)	828.5 (315.4 −2445.4)	0.028	0.3 (0.2–3.7)	0.7 (0.3–6.6)	14.8[(−49.8) −319.5]	0.484	0.063
IGF-1 (ng/mL)	252.1 ± 79.4	262.3 ± 72.83	6.8 ± 20.1	0.593	233.4 ± 77.4	260.0 ± 58.5	16.1 ± 23.7	0.091	0.384
TT (ng/mL)	4.4 (4.3–4.8)	5.5 (4.5–5.8)	15.7 (4.0–40.1)	0.050	4.8 (4.3–5.7)	4.6 (4.3–5.2)	0.7 [(−23.6) −34.4)]	0.953	0.222
Cortisol (ug/dL)	9.7 ± 3.9	12.9 ± 3.9	52.8 ± 73.4	0.026	11.5 ± 4.0	11.2 ± 3.0	8.2 ± 44.1	0.782	0.138
Ghrelin (pg/mL)	658.2 ± 73.8	817.2 ± 87.0	25.0 ± 14.3	0.001	707.3 ± 133.0	760.5 ± 91.2	9.3 ± 15.0	0.192	0.037
TT/cortisol ratio	0.6 ± 0.4	0.5 ± 0.2	2.7 ± 58.9	0.740	0.5 ± 0.3	0.5 ± 0.2	11.6 ± 69.6	0.964	0.773

Data are expressed as means ± standard deviation or median (interquartile ranges). Δ defined as end of study minus baseline divided by baseline and multiplied by 100. BMI, body mass index; BF, body fat; DBP, diastolic blood pressure; GH, growth hormone; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance index; IGF, insulin growth factor; LBM, lean body mass; LDL-C, low density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceridemia; TT, total testosterone. †$P > 0.05$ by two sample *t*-test or Mann–Whitney *U*-test between two groups at baseline. ‡Paired *t*-test or Wilcoxon signed ranks test for within group. §Two-way repeated measures ANOVA over time for between groups.

12 weeks, body fat percent showed a significant decrease ($P = 0.004$ by paired t-test), whereas lean body mass showed a significant increase ($P = 0.006$ by paired t-test) in the HP group. No significant difference in BP, lipid profiles and IGF-1 was observed in either group during the experimental period. Among hormonal parameters, median HOMA-IR showed a significant decrease ($P = 0.015$), whereas median GH showed a significant increase ($P = 0.028$) only in the HP group. Median testosterone showed a marginal increase in the HP group ($P = 0.05$). Cortisol and ghrelin levels also showed a significant increase only in the HP group (Table 1).

Between-Group Analyses

Table 1 also shows the differences in the change of metabolic characteristics between the groups during the experimental period. The results of two-way ANOVA for repeated measures showed no significant effect of a high-protein diet on BP and low-density lipoprotein cholesterol over time.

During the 12-week resistance-exercise program, a significantly greater lean body mass was observed with a high-protein diet than with a standard diet ($P = 0.032$). Results of repeated-measures ANOVA showed a significant decrease in HOMA-IR over time in the HP group ($P = 0.040$). The increase in high-density lipoprotein cholesterol (HDL-C) and ghrelin was greater in the HP group than in the ST group. No significant effect of a high protein diet over time on any other metabolic characteristics was observed between the groups at week 12. There was no significant difference between the ST and HP groups with respect to energy at week 12 (2382.5 ± 364.8 kcal/day vs 2425.6 ± 367.7 kcal/day, $P = 0.806$). By design, protein intake was greater ($30.2 \pm 0.9\%$ of total intake) in the HP group compared with the ST group ($15.1 \pm 0.9\%$ of total intake, $P < 0.001$), whereas fat intake was lower in the HP group ($15.5 \pm 6.2\%$ of total intake) compared with the ST group ($25.0 \pm 2.5\%$ of total intake, $P < 0.001$) at week 12.

DISCUSSION

The present study was designed to investigate the effect of an isocaloric high-protein diet on body composition, ghrelin, and metabolic and hormonal parameters during a 12-week scheduled resistance-training program in untrained healthy young men. This design is critical to providing an understanding of the interactive effects of protein intake and resistance exercise on those responses.

Previous studies have reported that ghrelin levels decrease after acute high-protein intake, whereas ghrelin levels increase or remain unchanged after chronic high-protein intake, despite sustained reductions in appetite[19–24]. Long-term aerobic or combined exercise usually results in an increase in circulating levels of total ghrelin through weight loss[25]. In previous studies, acute resistance exercise without weight change resulted in a decrease or no significant change in ghrelin levels[26–28]. However, little is known about plasma ghrelin levels, especially during long-term resistance exercise only. In the present study, participants in both groups carried out only resistance exercise, but not aerobic exercise, over a 12-week period. As a result, we observed no significant effect of resistance exercise on ghrelin concentrations in the ST group. However, when combined with resistance exercise, a high-protein diet increased ghrelin levels at 12 weeks during the experimental period. Furthermore, at week 12 after resistance training, the plasma total ghrelin levels showed a much greater increase in the HP group than in the ST group. The present results are consistent with those of previous studies[23,26,27]. Acylated ghrelin is known to enhance appetite and increase food intake in humans, whereas des-acylated ghrelin has been reported to inhibit food intake and delay gastric emptying[11,28]. In this regard, we have previously shown that a compensatory increase in total ghrelin levels by long-term combined exercise-induced weight loss was attributed not to increased levels of acylated ghrelin, but to increased levels of des-acylated ghrelin[11].

In the present study, a 12-week resistance exercise program with high-protein intake (HP group) resulted in decreased body fat percentage and HOMA-IR, but increased lean body mass, GH and cortisol. No changes in these parameters were observed in the ST group during the 12-week period. Participants in the ST group had higher fat intake and lower protein intake than those in the HP group. This could explain, in part, why individuals in the ST group failed to lose body fat with long-term resistance exercise. In addition, at week 12, HOMA-IR showed a decrease, whereas lean body mass and HDL-C showed a much greater increase in the HP group than in the ST group. The present findings regarding change in lean body mass are consistent with those of a previous study reporting that whey and soy protein supplements induced gains in lean body mass during a 12-week period of resistance exercise. There is still a great deal that remains to be understood regarding the effects of a protein diet on the physiological and hormonal response to resistance exercise.

Protein supplementation or intake is believed to stimulate anabolic hormone-induced muscle protein synthesis, and counteract the deleterious effects of muscle degradation observed after bouts of resistance exercise[19]. Several hormones play a critical role in exercise in general and strength training. Testosterone, GH, and IGF-1 provide strength and muscle growth stimulus, whereas cortisol increases muscle protein breakdown, inhibits protein synthesis and is secreted during exercise in order to enhance the use of proteins for fuel. The testosterone/cortisol ratio is suggested as a marker of the anabolic/catabolic status of an individual and changes in relation to the training stress, such as intensity and duration of physical exercise[4]. These hormones work together to maximize muscle and strength. According to the results of the present study, resistance exercise in long-term untrained young men resulted in increased cortisol and marginally increased testosterone, when a high-protein diet was combined with resistance exercise, although previous clinical studies have yielded inconsistent results[2–4,6,30–32]. However, the testosterone-to-cortisol ratio

remained unchanged throughout the 12 weeks in both groups. In the present study, no change in levels of IGF-1 was observed from baseline throughout the study in both groups. In addition, no change was observed in IGF-1, despite a higher GH during 12 weeks of resistance-exercise training in the HP group. Previous studies of the acute or chronic effect of exercise on circulating IGF-I have produced inconsistent results, with levels being reported to decline, to increase, or to remain unchanged after the onset of exercise[33,34]. Finally, data from the present study appear to support the importance of a fine balance between anabolism and catabolism during chronic moderate-high intensity resistance training with proper diet. Our study had some limitations, including its relatively small number of participants and the variability in resistance exercise-induced hormone levels. However, the prospective design of the present study compensates, in part, for these limitations. The other limitation was that our methods did not include assessment of energy expenditure, the composition of dietary fat or protein intake, such as nitrogen urinary nitrogen excretion, although we instructed all participants to maintain their assigned diet and lifestyle unchanged throughout the study period and we used a food frequency questionnaire with 24-h recall for assessment. However, previous studies have reported that there was no difference in mean nitrogen level estimated from urinary nitrogen output and well-designed FFQ[35,36]. Unfortunately, we did not also measure acylated ghrelin, des-acylated ghrelin, leptin or serum creatinine concentration. However, for evaluation of general safety, a vital check-up, taking detailed history and physical examination were carried out on all participants at all visits by a single physician. All participants were followed-up until 3 months after completion of this study, and did not show any adverse effects. We have previously reported that the plasma leptin concentration showed a gradual decrease from baseline over the course of a study on the effects of exercise-induced weight loss on ghrelin and leptin in overweight children[11]. This change in leptin levels appears to reflect exercise induced fat loss.

In conclusion, these findings suggest that there are hormonal interactions to ameliorate body composition, metabolic profiles, and energy metabolism after a long-term higher protein diet and resistance exercise. However, replication studies with various types of resistance-exercise programs and high-protein diet are required in order to confirm the results of the present study for current practice in the field.

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