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EFFECT OF TEN WEEKS OF COMBINED EXERCISE ON GROWTH HORMONE, INSULIN-LIKE GROWTH FACTOR-2 AND MYOSTATIN LEVELS IN ELDERLY KOREAN WOMEN

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ABSTRACT

The purpose of the present study was to examine the effect of 10 weeks of combined exercise on growth hormone (GH), insulin-like growth factor-2 (IGF-2) and myostatin levels in elderly Korean women. Subjects were randomly assigned to the combined exercise group (CEG; n=17) or the control group (CG; n=17). The CEG performed 80 minutes of exercise, 5 days per week for 10 weeks. Each exercise session included four phases: a 10-minute warm-up; a 30-minute resistance exercises (10 to 15 repetitions maximum); a 30-minute aerobics exercise (60 to 80% of heart rate reserve); and a 20-minute cool-down. The interaction effect (time × group) on the levels of GH ($F=6.934$, $p=0.013$) and IGF-2 ($F=8.592$, $p=0.006$), increased significantly more in the CEG than in the CG, whereas the interaction effect for the myostatin levels ($F=13.544$, $p<0.001$) decreased significantly more in the CEG than in the CG. The 10 weeks of supervised combined exercise was effective for increasing GH and IGF-2 levels and decreasing myostatin levels in elderly Korean women.

Key words: Combined exercise; Elderly women; Growth hormone; Insulin-like growth factor-2; Myostatin.

INTRODUCTION

According to a statistics report on the Republic of Korea, 12.7% of the population (6.4 million people) in 2014 were aged (65 years and older), and this population has almost tripled since 1990 (Statistics Korea, 2015). The increasing senior population is becoming a serious social and public health problem. This increase in the aged population tightly correlates with drastic increases in falls, frailty and medical costs (Kim *et al*., 2013; Gelbard *et al*., 2014; Buckinx *et al*., 2015).

Rosenberg (1989) first reported that sarcopenia (muscle loss) is associated with aging. Sarcopenia is directly linked to a decrease in muscle strength and function due to physical inactivity and increased dependency, which is associated with cardiovascular diseases (Kim & Choi, 2015; Wannamethee & Atkins, 2015). To prevent or treat sarcopenia, the most effective approach is to improve muscle mass via regular exercise (Landi *et al*., 2014). In particular, combined exercise (consisting of aerobic exercises and resistance training) has a major positive effect on muscle mass, function and strength (Landi *et al*., 2014).

Previous studies, however, have only focused on muscles and not on other related factors influencing muscle growth, such as the growth hormone (GH). In particular, GH insulin-like growth factor-2 (IGF-2) and myostatin levels are strongly associated with an increase in gene expression, morphology and function of muscles and tendons (Boesen *et al*., 2014; Livingstone & Borai, 2014; Kalinkovich & Livshits, 2015).

PURPOSE OF RESEARCH

The purpose of this study was to examine the effect of 10 weeks of combined exercise on GH, IGF-2 and myostatin levels in systemic blood from the antecubital vein in elderly Korean women.

METHODS

Ethical compliance

All study procedures were approved by the A-si Senior Welfare Centre, and all subjects provided written informed consent.

Subjects

Subjects were randomly assigned to the Combined Exercise Group (CEG; n=20) and the Control Group (CG; n=20), which included subjects older than 65 years from the A-si Senior Welfare Centre in Gyeongsangbuk-Do, Republic of Korea. These subjects did not exercise regularly and had no previous diagnosis of abnormal glucose metabolism or other health problems. Subjects were instructed to maintain their normal diet and activity pattern throughout the study, and compliance with this instruction was assessed via physical activity and food-frequency questionnaires administered at the start and end of the study.

Using repeated analysis of variance (2×2 design), an anticipated statistical power of 0.80 and a α-error probability of 0.05 with an effect size of 0.25, a sample size of 34 subjects was required for the analysis (G-power programme 3.1.3, Kiel, Germany). Thus, 40 subjects were included in the analysis after applying the exclusion criteria. In both groups, 3 subjects dropped out of the study because they did not complete the exercise programme and/or the final test. All assessments were thus completed with only 17 subjects in the CEG and 17 in the CG.

Anthropometrical measurements

The following parameters were measured 2 days before the start of the study: anthropometrical measures; including height, weight, body mass index (BMI); waist circumference; and hip circumference.

The BMI (kg/m^2) of each subject was calculated as weight divided by the square of height. Waist circumference was measured at the part of the trunk midway between the lower costal margin (the bottom of the lower rib), and the iliac crest (the top of the pelvic bone) with the subject standing with her feet about 25 to 30cm apart. Hip circumference was measured at the part of the hip located midway. The measurement was taken by fitting the tape snugly around

the hips, without compressing any underlying soft tissues. The circumference was measured to the nearest 0.5cm at the end of a normal expiration.

Experimental procedures

The CEG participated in a 10-week supervised combined aerobic and resistance exercise programme. An exercise session consisted of a: warm-up phase (10 minutes); a resistance exercise phase (10 to15 repetitions maximum, 30 minutes); an aerobic exercise phase (60 to 80% of heart rate reserve, 30 minutes); and a cool-down phase (10 minutes). It was performed for 80 minutes, 5 times per week. The CG was asked to maintain a normal sedentary lifestyle.

Blood sampling and analyses

All subjects were prohibited from consuming anything for 8 to 10 hours prior to blood sampling in the morning after an overnight fast. Blood samples collected from the antecubital vein were centrifuged at 3,000rpm for 20 minutes, and the serum was extracted for analysis and frozen at -80°C to analyse GH, IGF-2 and myostatin levels. The following parameters were measured 2 days before the start of the study and 2 days after the study, hormone levels, including GH, IGF-2 and myostatin.

GH analysis

GH concentrations were determined using GH Quantikine ELISA Kits (R&D Systems, Inc., Minneapolis, MN, USA). One hundred µL of Assay Diluent RD1-57 and 50µL of serum were added to each well and incubated for 2 hours at room temperature. Each well was washed with the aspirate 4 times to prevent contamination. To determine the optical density of each well, the samples were red within 30 minutes of adding 50µL of the stop solution. The colour in the well changed from blue to yellow. When the colour change was not uniform, the plate was gently tapped to ensure that it was mixed sufficiently. The optimal density of each well was determined within 30 minutes at 450nm and 540nm using a micro plate reader (Molecular Devices, Orleans, CA, USA).

IGF-2 analysis

IGF-2 concentrations were determined using IGF-2 Quantikine ELISA Kits (R&D Systems, Inc.). Serum samples were pre-treated to release IGF-2 from the binding proteins and diluted 100-fold with a pre-treatment constituent prior to the assay. One hundred and fifty (150)µL of Assay Diluent RD1-53 and a 50µL serum sample were added to each well and incubated for 2 hours at room temperature. The same methods previously described for GH analysis were used to prevent contamination and determine optical density for IGF-2 analysis.

Myostatin analysis

Myostatin concentrations were determined using Myostatin Quantikine ELISA Kits (R&D Systems, Inc.). Fifty (50)µL of Assay Diluent RD1-17 and serum samples was added to each well and incubated for 2 hours at room temperature on a horizontal orbital micro-plate shaker. The same methods previously described for GH analysis were used to prevent contamination and determine optical density for the myostatin analysis.

Exercise programme

All subjects in the CEG performed a whole-body stretch before (10-minute warm-up) and after

(10-minute cool-down) each training session. The exercise group performed the main exercise programme for 60 minutes, which consisted of 30 minutes of resistance exercise training (3 sets of 10 to 15 repetitions maximum of the following exercises: leg press; leg curl; chest press; lat-pull-down; shoulder press; biceps curl; triceps extension; and sit-ups). This was followed by 30 minutes of aerobic exercise at an intensity of 60 to 80% of their heart rate reserve.

Statistical analysis

All descriptive data are presented as mean ± standard deviation. Independent t-tests were used to examine differences between groups at baseline. Repeated analysis of variance was used to evaluate significant changes in the dependent variables before and after combined exercise in the CEG compared to those in the CG. All analyses were performed using SPSS, version 18.0 (SPSS, Chicago, IL, USA). Statistical significance was set at $p<0.05$.

RESULTS

Characteristics of the subjects are shown in Table 1.

Table 1. **CHARACTERISTICS OF SUBJECTS INCLUDED IN THE STUDY**

Variables	Control group (n=17)	Exercise group (n=17)	t Value	p Value
Age (years)	78.41±4.84	78.82±5.02	-0.244	0.809
Height (cm)	149.31±5.90	146.72±5.19	1.361	0.183
Weight (kg)	52.38±7.14	51.62±5.97	0.333	0.741
Body mass index (kg/m^2)	23.58±2.79	24.19±2.55	-0.667	0.510
Waist circumference (cm)	86.97±9.06	89.32±8.07	-0.800	0.430
Hip circumference (cm)	92.41±6.28	93.32±5.27	-0.461	0.648

Table 2. **CHANGES IN GH, IGF-2 AND MYOSTATIN LEVELS BEFORE AND AFTER 10-WEEK INTERVENTION**

Parameters	Group	Before	After	Interaction	
				F Value	p Value
GH (ng/mL)	Control	1469.6±861.6	1138.2±767.5	6.934	0.013*
	Combined	1284.9±624.1	1678.7±768.8		
IGF-2 (ng/mL)	Control	289.8±215.5	288.6±178.0	8.592	0.006**
	Combined	298.0±212.6	357.0±238.0		
Myostatin (ng/mL)	Control	1404.4±483.5	1489.5±556.3	13.544	<0.001***
	Combined	1548.5±752.7	987.0±620.5		

GH=Growth hormone; IGF-2=Insulin-like growth factor-2; Interaction=(Group × Time)
* $p<0.05$ ** $p<0.01$ *** $p<0.001$ Analyses=Repeated analysis of variance

Changes in GH, IGF-2, and myostatin levels before and after the 10-week intervention in the CEG and CG are shown in Table 2. The interaction effect (time×group) on the levels of GH ($F=6.934$, $p=0.013$) and IGF-2 ($F=8.592$, $p=0.006$), increased significantly more in the CEG than in the CG, whereas the interaction effect for the myostatin levels ($F=13.544$, $p<0.001$) significantly decreased more in the CEG than in the CG.

DISCUSSION

The current study investigated the effectiveness of a 10-week training programme on potential changes in the levels of GH, IGF-2 and myostatin. Significant improvements were observed in all growth-related hormones in elderly women who performed the combined exercise.

Aging is associated with a decrease in muscle mass, strength, power and maximal exercise capacity. To prevent these aging-related changes, Landi *et al*. (2014) suggested that resistance exercise training is more effective for increasing muscle mass and strength, whereas endurance exercise training is superior for maintaining and improving the maximum aerobic capacity. It is, therefore, recommended that the elderly should perform a balance of both endurance and strength exercises for at least three days per week.

Both aerobic and resistance exercises improve muscle growth. Acute exercise rapidly increases growth-related hormone levels with a physiological stimulus, which induces tissue remodelling. Furthermore, small incremental changes with repeated regular exercise eventually cause hypertrophy (Wideman *et al*., 2002; Kraemer & Ratamess, 2005). GH is the major IGF carrier in the plasma of muscle and it induces hepatic synthesis. Exercise stimulates the secretion of GH within 10 to 20 minutes, causing hypertrophy (Roth *et al*., 1963). IGFs have an anabolic effect on skeletal muscles via paracrine/autocrine secretion (Velloso, 2008; Frystyk, 2010), which simultaneously cause an increase in GH levels.

Myostatin, a potent regulator of muscle development and size, is a member of the transforming growth factor β superfamily (Guo *et al*., 2009). Myostatin inhibits the proliferation and differentiation of myoblasts and is involved in the Akt/m TOR pathway that regulates protein synthesis (Hittel *et al*., 2009). Previous studies have reported that aerobic and resistance exercises reduce circulating myostatin levels in humans whether there is acute or chronic myostatin inhibition (Roth *et al*., 2003; Louis *et al*., 2007; Hulmi *et al*., 2009).

The current study showed an increase in the levels of GH and IGF-2 and a decrease in the levels of myostatin after 10 weeks of combined exercise training. These results strongly support those of previous studies that reported positive effects on hypertrophy and muscle function in the elderly, which ultimately prevent sarcopenia.

This study had limitations. The subjects were recruited from the A-si Senior Welfare Centre in Gyeongsangbuk-Do, Republic of Korea, thus the subjects do not necessarily represent the entire elderly Korean population. Furthermore, only a small sample of elderly women was included (N=34). However, this study had a strength in that it focused on elderly women who underwent a combined exercise training programme, which positively affected relevant myokine and anabolic hormone levels.

CONCLUSIONS

The 10-week supervised combined exercise programme presented in this study has the potential to be effective in increasing GH and IGF-2 levels and decreasing myostatin levels in elderly Korean women. Combined aerobic and resistance exercise benefited the anabolic hormonal state of these elderly individuals, suggesting that it does play an integral role in attenuating age-associated sarcopenia.

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