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Effects Of Combined Strength And Endurance Training On Physical Performance And Biomarkers Of Healthy Young Women

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Short title: Training affects positively physical performance and health

Abstract

Cardiovascular fitness has decreased and obesity has increased in youth-adults world-wide during the last ten years. Therefore, there is an urgent need to find out optimal exercise training programs for improving physical performance and health outcomes, especially, among sedentary women. Subjects were 25-30-year-old females with very low physical activity, and 65 % of them were overweight (BMI >25). They performed endurance and strength training three times a week for nine weeks. Independent strength training and instructed endurance training by indoor cycling were prescribed. Measurements were performed before, in the middle and after the training period. No nutritional guidelines were given to the subjects. The 9-week training period led to 8.5 % increase in estimated maximal oxygen uptake. Maximal isometric strength of the leg and arm extensors as well as trunk flexors and extensors increased by 28.9 %, 7.8 %, 27.2% and 16.1%, respectively. Total cholesterol values lowered by 7.6 %, and high density lipoprotein increased by 8.8 %, while low density lipoprotein, haemoglobin, serum glucose and triglyceride remained unchanged. Serum cortisol increased by 22.7 % but no changes in plasma testosterone, estradiol or sex hormone binding globulin were observed. The skeletal muscle mass increased by 0.8 % without other changes in body composition. Our results indicated that only 27 combined endurance and strength training sessions in 9 weeks improved maximal endurance and strength capacity as well as some health outcomes. Thus, combined strength and endurance training itself can induce significant health benefits without the necessity of changes in dietary habits.

Key words: Sedentary women, physical training, body composition, physical fitness, hormone, blood lipids

INTRODUCTION

It is well established that incorporation of physical activity and exercise into persons' lifestyle improves their health and quality of life (22). In particular, regular exercise routines are advocated as a major element in body weight loss-management programs (21) as either a primary or adjunctive therapy. This is a critical consideration for many persons in light of the world-wide epidemic in obesity (23).

Recommendations by health agencies vary as to how much regular exercise and what type of exercise are necessary for optimal health. For example, the World Health Organization, Institute of Medicine, Center for Disease Control and Prevention are all strong proponents for the need for regular exercise, but the individual guidelines they have issued are not in total agreement. The variance in these guidelines has led to some confusion in the public as to what and how much to do in conducting an exercise regimen (5). Thus it is important to know how to develop training programs among people without preceding physical activity (especially women), and how quickly we can observe physiological and physical changes.

Modern life presents many advantages in time saving, but many young adults have a sense that their lives are extremely busy, too demanding, and full of stress. For example, a study commissioned by the United Kingdom health nutrition retailer Holland & Barrett revealed 68% of those questioned believe today's young adult generation are forced to endure more hardships than the previous generation at a comparable age (n > 4000; 20). In Canada, a national survey found that 90% of adult respondents felt high levels of daily "role overload," and that they were pushed daily to do too much and too many things at once (8). A survey by the American Psychological Association found 35% of adults polled since 2007 reported feeling more stress now compared to years earlier, and 53% said they received little or no support from their health care providers in coping with that heightened stress (2). These findings were especially applicable to young adults (2) and within that group more so young women, particularly if they were mothers (3). Regular exercise is recognized as a non-pharmacological means of dealing with the psychological and physiological consequence of life stress (7,12). Yet, in a busy lifestyle it can be difficult for adults to find the time to meet the complete exercise recommendations of health agencies.

In light of the above points and due to a lack of scientific studies of physical training of sedentary individuals, it is clearly relevant to understand effects of training in sedentary young adults, especially women, who seek to maintain or regain aspects of their overall health. The ultimate aim of the present study was to examine the influence of an exercise training program of combined strength and endurance exercise activities designed to be manageable to lifestyle demands (3 days per week, 9 weeks) on selected parameters of physical health and well-being in young adult women. This was chosen to do without nutritional guidance to our participants so as to focus on the impact of exercise training alone. It was hypothesized that physical fitness would improve and some health biomarkers would change positively while no changes were expected in body composition (due to training volume exposure).

METHODS

Experimental Approach to the Problem

Among sedentary people, a beginning of training should be safe with low volume and intensity. In the gym, a supervisor should also teach correct techniques for each movement. In this way, it is possible to prevent injuries and/or troubles in the cardiorespiratory functions. However, there is a real lack of studies among truly sedentary individuals, concerning how much training and with what kind of progression is efficacious in improving physical fitness and body composition. These points were a key motivation in pursuing this study, where the sedentary women trained using combined strength and endurance training for 9 weeks without nutritional guidelines.

Subjects

Seventeen young women, without any training history, volunteered to participate in the present study. The criteria for their acceptance were that they had to be sedentary, 25-30 years old and without any hormonal contraceptive use. Their occupations involved them mainly working at offices. A final selection of the subjects was done after screening their physical activity, health and use of medicines according to a questionnaire they completed. All the subjects were informed of the benefits and risks of the investigation prior to signing an institutionally approved informed consent document to participate in the study. They also were given a permission to withdraw from the study at any point. The Ethical Committee of the University of Jyväskylä gave an approval statement for the present study.

The initial mean (±SD) age of the subjects was 27± 2 years (range 25-30 years), body height 168.2±5.3 cm (range 163.0-178.0 cm), body mass 72.3±12.9 kg (range 54.9-100.0 kg), and body mass index (BMI) 25.7±4.6 kg·m^{-2} (range 17.6 - 33.8 kg·m^{-2}). A total of 65 % of the subjects were overweight or obese (BMI>25.0), while 29% were in their normal weight (BMI=18.5-24.9 kg·m^{-2}) and 6 % underweight (BMI<18.49). The mean body fat was 32.8±8.6 % (range 17.4-45.7 %) at the beginning of the study.

Procedures

The subjects were initially tested twice, within an interval of one week, before the training intervention (PRE1 and PRE2). Thus the subjects served as their own controls. They were asked to maintain their physical activity and nutritional behaviour unchanged during the study period. After the two PRE measurements, they started their training programme of combined endurance and strength exercises for a 9-week period. All subjects were then tested after 4 (AFTER4) and 9 (AFTER9) weeks. All tests and measurements were performed at the same time in the morning, and the subjects were guided to have the same nutritional and hydrational status.

The participants trained 3 times a week during the 9 weeks. During the first 7 weeks, they performed two strength and one endurance training sessions while during the two last weeks, they performed two endurance and one strength training sessions. Because the aim of the present study was to focus only on the effects of exercise, they did not receive any nutritional guidelines. However, the participants kept food diaries during three days within each week including one weekend day. They were asked and instructed to write down all food they ate as accurately as possible.

Training

Endurance training consisted of spinning intervals with BodyBike indoor cycles (BodyBike International; Frederikshavn; Denmark). These researcher guided training sessions lasted from 30 min in the beginning of the training period to 55 min by the end of it. Mean intensities of cycling varied from 85 to 91 % of an individual maximal oxygen uptake. All training sessions were individually guided by monitoring heart rate (Firstbeat Memory Belt and Monitor software, Firstbeat Technologies, Jyväskylä, Finland). The participants also utilized a Fitness Trainer software (Firstbeat Technologies, Jyväskylä, Finland), where each participant had her own profile, and they also saved all their training and activity levels there.

Furthermore, the subjects were asked to maintain their normal lifestyle and not allowed to start any other new training programme or sport events.

Strength training was performed in a gym according to individual guidelines. The participants trained individually, mainly with TechnoGym equipment (TechnoGym, the Wellness Company; Cambettola; Italia) and free weights. At the beginning of each week, updated training programmes (movements, repetitions and weights) were given to each participant. The main training exercises were leg press, knee extension and flexion, toe rise, lateral pull down, bench press, biceps curl, triceps curl, back extension and abdominal curl. During the training period, intensity of loading increased gradually, and repetitions varied from 5 to 15 according to the load. During the first training week, for preventing injuries and learning correct exercise techniques, the participants performed more repetitions with lighter loads and a shorter recovery period as in the subsequent weeks (see table I).

Measurements

Maximal aerobic capacity (VO_2max) was indirectly determined using a graded cycle ergometer tests (Ergoline 800S, Bitz, Germany). A progressive protocol was used with an initial power output of 75 W and increased by 25 W every 2 min until exhaustion (i.e, until volitional fatigue or a decrease of pedalling cadence under 60 rpm/min). Heart rate was continuously recorded and monitored during the test using memory belts (Firstbeat Technologies; Jyväskylä; Finland). The heart rates were analysed with Firstbeat Sports software (Firstbeat Technologies; Jyväskylä; Finland) for estimating VO_2max.

Bilateral maximal isometric leg extension and arm extension forces were measured using dynamometers for leg press and bench press, respectively. Knee angle was set to 107° with a goniometer, and participants hands were placed on handle grips for the leg extension test (23). During the maximal bench press protocol, participants were in a supine position with their back flat on a bench and feet flat on the floor, with elbows and shoulders positioned at angles of 90°. Warm-up included at least two submaximal trials prior maximal trials. A total of three maximal trials were performed with 30 s recovery between trials. The best performance was included for further analysis. The participants were instructed on the testing protocol and correct technique for each test action prior to testing. They were also instructed to produce maximal strength as fast as possible and to maintain it for three seconds. Furthermore, they were encouraged by a research personnel during the maximal efforts.

In the maximal back and abdominal curls, the participants stood and they were tightly fixed with a belt around their hip and torso. A strain gage transducer was placed at the level of their chest. Then participants isometrically extended (back) or flexed (abdominal) their torso for 3 s. The best result from 3 trials was selected for further analysis.

Blood samples

Fasting (10-12 h) blood samples were drawn from the antecubital vein in the morning. Only water was allowed to be consumed ad libitum before the samples were drawn. They were collected into two vacuum tubes 5/2 ml K2E (B&D Vacutainer, Plymouth, UK), one 5/3 ml serum gel tube (B&D Vacutainer, Plymouth, UK) and one 5/2 ml FX-tube (B&D Vacutainer, Plymouth, UK). Haematological analyses were done immediately after blood draws with a Sysmex KX 21N-analyzer (Sysmex Co., Kobe, Japan). Serum gel tubes and FX-tubes were centrifuged at 3500 rpm for 10 min. Thereafter, total cholesterol (CHOL), high density lipoprotein (HDL) and triglycerides (TRIGLY) were analysed with Konelab 20 XTi-analyzer (Thermo Fisher Scientific Oy, Vantaa, Finland). Serum low density lipoprotein (LDL) was calculated according to the equation of Friedenwald et al. (10). The serum samples were stored in -80 °C until hormone analyses. Plasma glucose was also analysed with a Konelab 20 XTi analyser (Thermo Fisher

Scientific Oy, Vantaa, Finland). Serum hormones (testosterone, cortisol, estradiol) and SHBG were analysed with an Immulite 1000-analyzer (Siemens Healthcare Diagnostics LA, Ca, USA) (9).

Body composition

Body mass and composition were analysed with a bioelectrical impedance analysis (BIA) (Inbody 720, Seoul, South Korea) after an overnight fast of 10-12 h, to determine fat mass (FM), body fat percentage (BF%) and fat free mass (FFM). Body mass and height were measured to the closest 0.1 kg and 0.1 cm, respectively on a commercial scale. Body mass index (BMI) was calculated and waist circumference (WC) was measured at the level of iliac crest after exhaling by a standard anthropometric tape measure.

Statistical analyses

After verifying data normality and homogeneity of variances, standard statistical methods were used for the calculation of means, standard deviations (SD), standard errors (SE), Paired-Samples T-tests and Pearson bivariate correlation coefficients procedures. Paired-Samples T-test was used to compare PRE1 with PRE2 to verify stability on the measured variables before training and further PRE2 with AFTER4 and AFTER9 (also AFTER4 with AFTER9) to determine the effects of training program on measured parameters. The level of significance was set at $p \leq 0.05$. Analyses were carried out using statistical software PASW for Windows 18.0.1.

RESULTS

During the 9-week training period, VO_2max increased by 8.5% ($p<0.001$) by the end of training, with the largest development occurred during the first 4 first weeks by 6.9% ($p<0.001$) (Figure 1). At the same time, time to exhaustion was prolonged significantly from 10:35±2:19 min:s to 11:55±2:17 (after 4 weeks, $p<0.001$) and 12:39±2:33 min:s (at 9 weeks, $p<0.001$). Furthermore, maximal power output during the VO_2max test increased from 183±25 W to 199±26 W and 206±33 W ($p<0.001$), respectively. No changes between the PRE1 and PRE2 measurements were noticed for any of these outcomes.

Maximal isometric bilateral force of the leg extensor muscles increased by 28.9% from 1911±182 to 2464±240 N ($p<0.001$) by the end of the training period, and by 11.2% ($p=0.053$) at the first four weeks (Figure 2 A). In dynamic leg extension, one repetition maximum (1 RM) increased from 97.6±5.7 to 129.4±6.1 kg ($p<0.001$) and 10 repetitions maximum from 77.1±4.5 to 113.1±5.4 kg ($p<0.001$) by the end of training. During the 9-week training period, maximal isometric bench press force improved by 7.8% from 325±24 N to 350±29 N ($p=0.025$) but was not affected by the first 4-week period (Figure 2 B). The rate of force development was unchanged throughout the study period. Maximal abdominal curl force increased by 25.0% ($p<0.001$) and 27.2% (377±52 vs. 505±38 N, $p=0.011$) during the first 4-week and entire training periods, respectively. Maximal back curl force was also increased by 16.1% from 488±43 to 566±43 N after 4 weeks ($p=0.058$) but not further after 9 weeks. No changes between the PRE1 and PRE2 measurements were found in any of the present physical tests measured except in maximal force of the leg extensor muscles, which was 10.8 % higher in PRE2 than in PRE1 ($p=0.006$).

Serum triglyceride concentrations did not change throughout the study, but total cholesterol values decreased. This reduction was already apparent during the first 4 weeks of training (Table 2) and reached 7.6% at week 9 ($p=0.046$). HDL increased by 8.8% ($p=0.012$) at week 9, and its changes were positively correlated with the changes in VO2max (Figure 3) and the changes in skeletal muscle mass ($r=0.46$, $p<0.05$). LDL did not change ($p>0.05$), and fasting blood glucose also remained unchanged as well as all these parameters when comparing PRE1 and PRE2 measurements (Table 2).

Serum testosterone, estradiol, and SHBG did not change during the training period (Table 3). However, cortisol concentration increased from PRE2 to the end of the training period (i.e. at week 9; p=0.014). No differences were observed in testosterone-SHBG-ration and testosterone-cortisol-ratio as well as between PRE1 and PRE2 values for any of these hormonal measures.

Total energy intake did not change from PRE to the 4- or to the 9-week training period assessment (2043±629 vs. 2049±595 vs. 1842±568 kcal/day, p=0.223), respectively. Neither any change was found in consumed macro-nutrients: as carbohydrate relative contribution varied from 42.6±4.6 to 47.86.9%, fat from 35.4±4.4 to 38.5±3.9%, and proteins from 15.1±3.6 to 17.8±4.8%, respectively.

In body composition, there was a slight and significant increase in skeletal muscle mass (26.4±9.8 vs. 26.6±3.4 kg, p=0.008) during the 9-week training period, while body fat percent decreased (32.8±8.6 vs. 32.0±8.5%, p=0.03) during the first 4-week of training but not after that. Total body mass did not change throughout the entire training period (PRE = 72.3±12.9, 4-week = 72.3±13.3, 9-week = 72.0±13.1 kg).

DISCUSSION

Public health researchers have made many recommendations as to how much exercise should be done; but what is an exact optimal amount that can match the time constraints of modern people, and hence is manageable, is an issue of much debate (5). This study attempted to provide some insight on this issue as we examined the influence of an exercise training program of combined strength and endurance exercise activities, designed to be manageable to life-style demands, on measures of physical health and well-being in young adult sedentary women. Indeed, we showed that both cardiovascular and muscular functions were improved as were blood lipid profiles, and small positive but significant changes also in body composition were observed in response to the training.

Regardless of the health organization, all agree that improvement of cardiovascular function is a key element to enhancement of the overall health and quality of life in an individual (4). Our training program succeeded in improving cardiovascular function as we observed a considerable and significant increase in relative VO_2max. These changes seemed to be due to true systemic and cellular adaptations, and not just the function of total weight (mass) loss as no changes occurred in the latter measure and aerobic performance was enhanced (i.e., time to exhaustion and peak power output). The magnitude of change detected agrees with previous findings using similar subject populations and elements of combined training. For example, Ho et al. reported a 13.3% increase in VO_2max while Blake et al. (2000) found closer to 20% increases (15). However, the study of Ho et al. involved a 12-week training period with 5 days per week training, while the study of Blake et al. used 14 weeks – 3 days per week regimes (6,15). Hence, those studies had slightly greater training dosages compared to our program. Nonetheless, our findings track well with the magnitude of change within those studies, but clearly demonstrate that significant alterations can occur in less time and with few training sessions per week in the exercise program.

Muscle function is another key element to improved overall health and quality of life in individuals (22). We found substantial changes in several key muscular parameters; e.g. maximal isometric and dynamic strength in the lower limbs, upper body as well as trunk extension and flexion. The muscle groups displaying these changes and the magnitude of the change corresponds with previous work from our research group (16,19), but these earlier works focused primarily on males. The current work in women supports that the positive muscular benefits of combined training are applicable across the genders.

When endurance-cardiovascular and strength-resistance exercise training are used in combination there is always the question of adaptive “interference”. That is, in the landmark study by Hickson, he demonstrated that when combined endurance and strength training was conducted concurrently maximal strength performance is compromised (14). The inference concept is an issue of much debate right now in the exercise science research community facilitating a large number of current studies on the topic. A series of such studies from our research group have shown that during more realistic training programs (i.e. less intensive than that used by Hickson, (14)) for physically active but systematically untrained populations, consisting of a much lower training frequency (i.e., 2-3 sessions endurance and strength, respectively), adaptations in strength and power development as well as muscle growth are not compromised (13,19). Furthermore, a most recent study from our group has shown that the increases in endurance performance were larger in magnitude when strength and endurance were performed on different days in comparison with that produced by same-session training (9). This latter study has also shown that body fat mass decreased only following combined strength and endurance training performed on the different days. Thus, “separating strength and endurance training into more frequent sessions performed on the different days seems to be a valid option for healthy adults who wish to simultaneously optimize body composition and improve physical fitness” (9). This work by Eklund et al. is well in line with and support of the present findings (9).

Our prescribed exercise training program worked well in improvement in both cardiovascular and muscular performance. The magnitude of improvements did tend to be larger in muscle performance (~8 to 29% increases) than cardiovascular performances (~8.5 to 12.5%). Whether this is due to the strength programme (Table 1) being more optimal in design for our subjects or perhaps their initial very low level of muscular fitness (which would allow greater improvement) is unclear and cannot be verified from our data (1). Nonetheless, we have no reason to suspect that other women with the characteristics of our study population would not experience similar positive improvements in cardiovascular-muscular functionality.

Assessment of blood parameter responses is a critical diagnostic means for healthcare providers to quantify the medical implications of an exercise training program. To this end we found that total cholesterol was significantly decreased and HDL-cholesterol was increased. These are two critical parameters indicative of positive cardiovascular adaptation and these findings are in agreement with numerous previous findings and are extremely positive health outcome (see review by Leon and Sanchez, 17). There were no changes in LDL-cholesterol, triglycerides, or glucose. However, at the end of the study (9 week) serum cortisol concentration was significantly elevated. We speculate that this hormonal response was also a positive training adaption reflecting in our participants becoming better at lipid metabolism, a common training adaptation (1). We venture that this was mediated in part by the higher cortisol observed evoking a greater level of fat oxidation as this hormone is a key lipolysis regulator (11,12).

As mentioned, the body weight of our participants did not change over 9 weeks of training. This finding was disappointing as body weight is usually a critical, and simple, indicator to sedentary individuals that their training program is being effective (6). Nevertheless, there were significant changes within body composition: a small increase in the muscle component (lean body mass) and decrease in the percentage of body fat. Additional compositional changes (e.g., fat mass) and larger changes in muscle with training programs of similar or slightly longer duration have been reported in earlier studies (15,18,21). These latter studies have typically integrated a nutrition element to the program where caloric energy and macro-nutrient intake have been modified. The combination of an exercise program and nutritional modification are well established as efficacious means of substantially altering body weight and composition (1). Our goal though was to focus only on the effect of an exercise training program in our research subjects. The rationale for this approach was based upon the idea that inducing too many changes in lifestyle and behaviour within the participants would be too demanding and likely compromise compliance and completion of the training on their part.

As with any study there are limitations to our work. The addition of a control group would have enhanced the interpretation of our findings. To counter this point we chose to have two pre-training testing sessions to allow for a stable baseline assessment for the majority of our measurements. We feel this allowed us to identify with a greater sensitivity the changes in our participants even without an actual control group. Certainly the introduction of a nutrition intervention as a part of the study design could have led to larger positive changes in many of our health biomarkers (e.g., blood lipids, body composition). As noted earlier though, this was not a focus of our study and we tried to identify the effects of exercise training alone.

In conclusion, the results of this study support the concept that women engaged in an exercise training program of combined strength and endurance exercise activities designed to be manageable to lifestyle demands can have positive health changes in a multiple of performance and health biomarkers. These findings point to it being feasible that an efficacious exercise program can be conducted by individuals living in a busy and demanding societal setting.

PRACTICAL APPLICATIONS

The present study has demonstrated that a limited exposure to combined strength and endurance training can induce significant health benefits in sedentary women without the necessity of intervening to change dietary habits. These benefits were achieved within the constraints of the busy life-style that our subjects maintained. This is a critical finding as many people, especial women, find work-life time limitation as a barrier to participation in a successful exercise program (6,22). Thus, for achieving good health benefits, it can be recommended in sedentary women a combined strength and endurance training three times a week with a high intensity (80-90% from the maximum) during the first 2-3 months of training.

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FIGURE LEGENDS

Figure 1. Mean (±SE) estimated maximal oxygen uptake (VO_2max) measured twice before (PRE1 and PRE2) the training intervention, after 4 weeks, and after 9 weeks, i.e. at the end of the entire training period. ***p<0.001.

Figure 2. Mean (±SE) maximal force of the leg extensor muscles (A) and arm extensor muscles (B) measured twice before (PRE1 and PRE2) the training intervention, after the training period of 4 and 9 weeks. ***p<0.001; *p<0.05.

Figure 3. Relationship between changes in maximal oxygen uptake (VO2max) and serum HDL concentration during the 9-week training period.

Table I. An individual example of the progressively increased strength training sessions. Recovery between the sets varied 45-60 s in the first week and 120-150 s in the last one.

	Weeks 1	Week 8
Leg press	3 x 15 x 80 kg	3 x 5-8 x 130 kg
Lateral pull down	1 x 15 x 15 kg	3 x 12 x 22.5 kg
Bench press	3 x 15 x 15 kg	3 x 5-8 x 30 kg
Biceps curl	1 x 15 x 5 kg	2 x 12 x 7 kg
Vertical rowing	1 x 15 x 4 kg	2 x 12 x 6 kg
Triceps curl	3 x 15 x 7.5 kg	3 x 5-8 x 20 kg
Back extension	1 x 15 x 20 kg	3 x 12 x 30 kg
Abdominal curl	1 x 15 x 20 kg	3 x 12 x 30 kg

Table 2. Mean (±SD) serum triglyceride, cholesterol; high-density lipoprotein (HDL), low-density lipoprotein (LDL) and glucose values in each measuring point after fasting. *p<0.05; **p<0.01

	PRE1	PRE2	AFTER4	AFTER9
Triglyceride (mmol·l^{-1})	1.17±0.34	1.04±0.35	0.95±0.24	1.01±0.29
Cholesterol$_{tot}$ (mmol·l^{-1})	5.0±0.5	4.8±0.3	4.6±0.3**	4.4±0.7*
HDL (mmol·l^{-1})	1.37±0.29	1.37±0.26	1.40±0.25	1.51±0.27*
LDL (mmol·l^{-1})	2.6±0.4	2.6±0.5	2.5±0.4	2.5±0.5
Glucose (mmol·l^{-1})	5.3±0.4	5.1±0.3	-	5.3±0.4

Table 3. Mean (±SD) serum testosterone, cortisol, estradiol, and SHBG values in each measuring point after fasting. **p=0.014

	PRE1	PRE2	AFTER4	AFTER9
Testosterone (nmol·l^{-1})	2.25±1.06	2.25±1.06	2.27±1.11	2.57±1.87
Cortisol (nmol·l^{-1})	524±129	439±86	439±96	563±134**
Estradiol (pmol·l^{-1})	453±371	461±455	461±271	505±355
SHBG (nmol·l^{-1})	56.2±16.4	58.2±14.6	58.2±15.3	55.6±22.5

FIGURE 1.

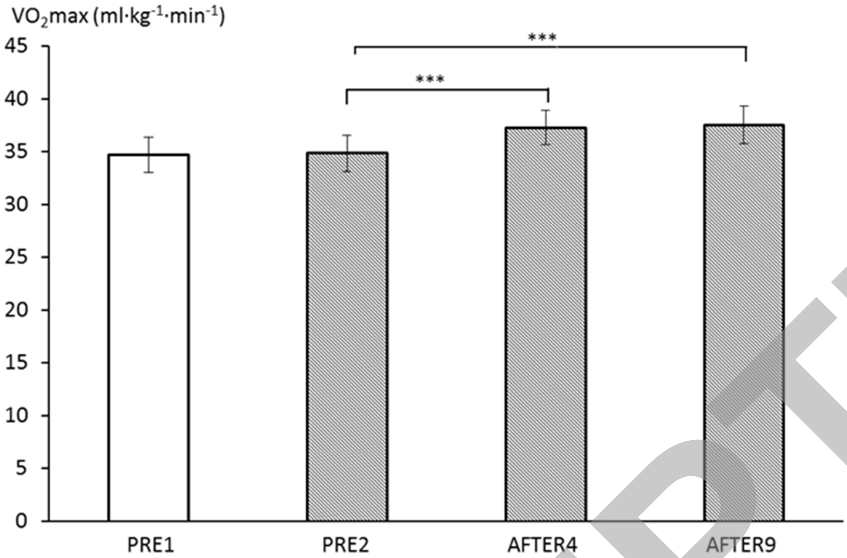
VO2max (ml·kg-1·min-1)
45
40
35
30
25
20
15
10
5
0
***
***
PRE1
PRE2
AFTER4
AFTER9

Figure 2.

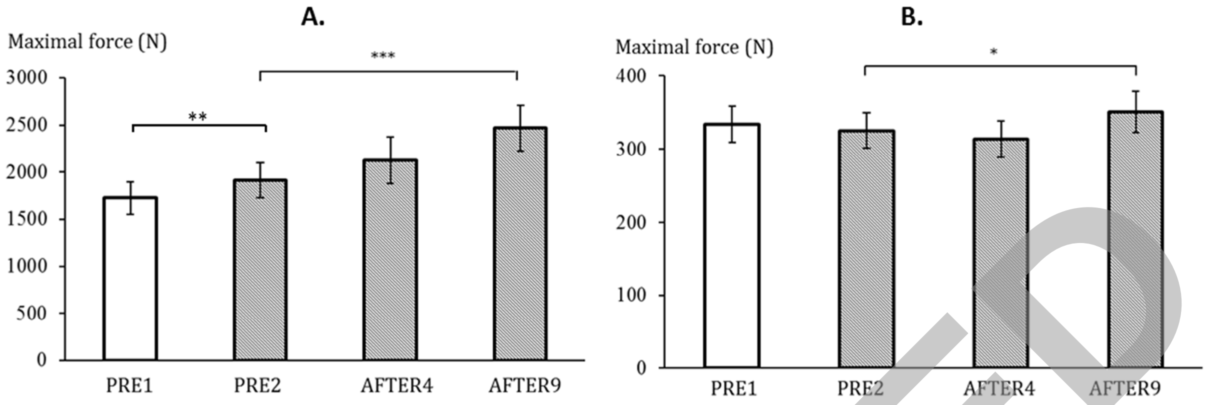
A.
Maximal force (N)
3000
2500
2000
1500
1000
500
0
**
***
PRE1
PRE2
AFTER4
AFTER9
B.
Maximal force (N)
400
300
200
100
0
*
PRE1
PRE2
AFTER4
AFTER9

Figure 3.