Serum Concentration of Myostatin, Insulin and Glucose Response to Continuous and Discontinuous Exercises in Overweight Menopausal and Menstrual Women

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Original Article

Abstract

Introduction: Myostatin regulates skeletal muscle mass and adipogenesis, but serum response to continuous and discontinuous exercises training it has not been studied in menopause and menstrual groups of women. The purpose of this study was to investigate the serum response of myostatin, insulin and glucose to use four different aerobic exercises training in menopause and menstrual groups of women.

Methods: Seventeen overweight women (BMI>30 and 40-50 years old) in Bandar abbas voluntarily participated in this study and randomly divided into two groups: menopausal (n=8) and menstrual (n=9) groups. Myostatin, insulin and glucose were measured before and after four different exercise sessions including continuous and discontinuous aerobic training in water and on treadmill. Data were analyzed by paired t-test and Analysis of covariance.

Results: Myostatin levels were significantly decreased after continuous and discontinuous training in water, and discontinuous training on treadmill in menopause group (pvalue <0.05). Meanwhile, myostatin level showed significant decrease in all four different conditions of training in menstrual group (pvalue <0.05). Insulin levels were significantly decreased after continuous and discontinuous training in water, and continuous training on treadmill in both menstrual and menopause groups (pvalue <0.05). Glucose levels were significantly decreased after continuous and discontinuous training in water, and continuous training on treadmill in menopause group (pvalue <0.05). In menstrual group, glucose levels were significantly decreased after continuous and discontinuous training on treadmill, and discontinuous training in water (pvalue <0.05).

Conclusion: The lowest myostatin response was seen in discontinuous training in water in both menopause and menstrual groups, while the highest myostatin response observed in menopause group who committed continuous training on treadmill. This study suggested that discontinuous than continuous exercise, and exercise in the water than on treadmill, Lead to better response in variables are listed.

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Introduction:

Aging is associated with a wide range of health deficiency. In this regard, menopause is a status in the aging process of women, which marks the cessation of menstrual cycle (1). Also, it is associated with dramatic reduction in circulating estrogen and progesterone concentrations in body which can result in reduced insulin sensitivity and consequently elevated risk of diabetes, and adipose tissue enhancement predominantly in the abdominal region and subsequently an increase in cardiovascular diseases, hyperglycemia and hyperinsulinemia (2-4). Available literatures support this notion that aerobic activities are useful for prevention or treatment of age-associated weight and fat gain, and therefore obesity-associated metabolic changes and diseases (5, 6).

Also, sarcopenia, reduced muscle mass by aging that may be induced by elevated expression of myostatin, can lead to the enhanced risk of cardiac, pulmonary, and metabolic diseases. Myostatin belongs to the transforming growth factor-β super family and is a growth factor protein that limits muscle tissue growth (7). The myostatin protein is produced primarily in skeletal muscle cells, circulates in the blood and lymph and acts on muscle tissue, apparently by slowing down the development of muscle stem cells. Recent evidence imply on myostatin reduction in response to muscle exercise e.g. acute and short-term swimming training; chronic wheel running; and muscle loading through treadmill running or isometric resistance training after atrophy induced by hindlimb unloading (8).

So, according to all above mentioned reasons, successful physical activities to reduce overweight and the dire consequences associated with aging are of utmost importance in both prevention and treatment of obesity and metabolic syndrome (9).

The main objective of this study was to examine myostatin, insulin and glucose responses to continuous and discontinuous aerobic training on treadmill and in water in overweight menopausal and menstrual women. It is hypothesized that following aerobic training in water and on treadmill, serum myostatin, insulin and glucose levels would be decreased.

Methods:

This is an interventional study. Nineteen overweight individuals, who had no previous history of routine exercise training, enrolled in our investigation. Two individuals did not complete the study because of either time constraints or medical issues unrelated to study participation. The 17 individuals (8 menopausal women and 9 menstrual women) who completed the study were more than 40 years old and had body mass index more than 30 kg/m^2. Informed consent was obtained from all participants, together with the manner in which informed consent was obtained.

The training protocols were designed to provide 70-80% maximum heart rate, H.R.Max=208-(0.7× age), for 30 minutes. Subjects trained 1 time per week across 4-weeks. Training sessions included two types of discontinuous and continuous programs. Participants in discontinuous group performed 3 sets of 10 minutes repetitions with 5 minutes resting time at every session, while participants in continuous group performed a continuous exercise in a 30-minutes session.

Training protocols involved four different programs with one week interval between each one. Training sessions started with a continuous aerobic training in water in the first week, and followed by a discontinuous aerobic training on treadmill in the second week, a discontinuous aerobic training in water in the third week and a continuous aerobic training on treadmill in the fourth week.

Training program in water began with 5-10 minutes stretching and warm-up phase then followed by aerobic exercise in water and ends up with 5 minutes cool-down phase. Whilst, training program on treadmill started with 5-10 minutes slow walking on treadmill for warming, and then followed to reach approximately 70-80% maximum heart rate and ends up with 5 minutes cool-down.

All stages the exercise intervention was performed in a sports complex in the city of Bandar Abbas.

Blood samples were drawn before and after each training sessions. Blood samples were drawn from a superficial arm vein and were centrifuged at 1000 rpm for 15 min within 30 minutes of collection for obtaining serum. Serum samples were then aliquated into separate tubes and stored at -20 ° C till analysis.

Body composition Analysis ioi 353 was used for measurement of height, weight and Body Mass Index (BMI), which calculated by dividing body weight by square of height in meter, of participants.

Serum myostatin concentrations were assessed using a competitive enzyme linked immunosorbent assay (ELISA) method (Human Myostatin ELISA Kit, Cusabio Biotech, Chin). Initially, 50µl of standard or sample per well were added. Then, 50µl of HRP-conjugate was added to each well. Plate was incubated for 1 hour at 37° C. The liquid of each well was aspirated and washed for three times. After the last wash, any remaining liquid was removed by aspirating. Then, substrates were added to each well and incubated for 15 minutes at 37°C. Finally, stop solution was added and optical density was read using a microplate reader at 450 nm (10).

Insulin serum levels were measured by ELISA method (Mercodia insulin ELISA; Mercodia AB, Uppsala, Sweden). At first, 25 µl each of Calibrators and samples were poured into wells. Then, 100µl of enzyme conjugate was added to each well. Plate was incubated on a plate shaker for 1 hour at room temperature. After washing, 200 µl Substrate TMB was added into each well and incubated for 15 minutes at room temperature (18–25°C). After adding Stop Solution, optical density was read at 450 nm (11). For determination of glucose levels, 1 ml of reagent was added to all tubes. Then, 10 µl of standard or samples were added to tubes (one tube was filled with 10 µl of distilled water as blank) and incubated for 20 minutes at room temperature. Finally, absorbance against the blank was read at 546 nm (12, 13).

For comparison of myostatin, insulin and glucose response before and after four different training programs in two menopause and menstrual women groups, t-tests analysis was applied. Analysis of covariance was used to examine differences between groups after statistically removing the effect of selected covariates. Data were analyzed using SPSS software version 19.0. Data are presented as means SEM. Probability pvalue<0.05 is statistically significant.

Results:

Body composition is a reliable indicator to find possible health problems derived from the amount of body-composing constituents and imbalance between them. In this regard, the mean (±standard deviation) of body composition and age of menopause and menstrual women are indicated in Table 1.

Table 1- The mean and standard deviation of participants' characteristics are indicated

Groups	Age	Height (cm)	Weight (kg)	BMI
Menopause	51.37±6.04	158.77±4.72	82.72±11.79	33.16±3.30
Menstrual	47.37±2.91	159.33±5	88.95±10.93	34.98±3.46

In menopause group, myostatin levels in continuous/water, discontinuous/treadmill, discontinuous/water and continuous treadmill groups were decreased -1.36±0.63 (pvalue=0.000) from 9.17±4.38 to 7.81±4.21, -1.65± .92 (pvalue=0.000) from 9.11±4.43 to 7.46±4.32, -2.075 ±1.64 (pvalue=0.000) from 9.69±4.60 to 7.61±3.72, and -1.21±2.48 (pvalue= 0.210) from 9.07±4.14 to 7.86±3.53 respectively. In menstrual group, myostatin levels in continuous/water, discontinuous treadmill, discontinuous/water and continuous treadmill groups were decreased -1.49±1.45 (pvalue=0.017) from 7.50±2.29 to 6.01±2.88, -1.44±0.52 (pvalue=0.000) from 7.73±3.05 to 6.28±3.42, -2.18±0.75 (pvalue=0.000) from 8.58±3.87 to 6.40±3.48, and –1.63±1.13 (pvalue=0.003) from 8.32±3.35 to 6.69±3.81 respectively (Table 2).

Table 2- Measured myostatin levels (ng/ml) in two studied groups in four different exercises. Asterisk shows statistically significant decrease (pvalue<0.05)

Group / serum collection		Activity			
Group	**Training/Session**	**Continuous/Water**	**Discontinuous/Treadmill**	**Discontinuous/Water**	**Continuous/Treadmill**
Menopause	**Pre**	9.17±4.38	9.11±4.43	9.69±4.60	9.07±4.14
	Post	7.81±4.21	7.46±4.32	7.61±3.72	7.86±3.53
	t-test	t_9= -6.769 pvalue=0.000*	t_9= -5.660 pvalue=0.000*	t_7= -3.565 pvalue=0.000*	t_7= -1.381 pvalue=0.000*
	Covarance	7.32±0.398	7.03±0.398	6.66±0.447	7.47±0.44
Menstrual	**Pre**	7.50±2.29	7.73±3.05	8.58±3.87	8.32±3.35
	Post	6.01±2.88	6.28±3.42	6.40±3.48	6.69±3.81
	t-test	t_8= -3.078 pvalue= 0.017*	t_8= -8.374 pvalue= 0.000*	t_8= -8.754 pvalue= 0.000*	t_8= -4.331 pvalue= 0.003*
	Covarance	7.05±0.442	7.12±0.421	6.48±0.419	6.98±0.419

Table 3- Measured insulin levels (mU/l) in two studied groups in four different activities. Asterisk shows statistically significant decrease (pvalue<0.05)

Group / serum collection		Activity			
Group	TrainingSession	Continuous/Water	Discontinuous/Treadmill	Discontinuous/Water	Continuous/Treadmill
Menopause	Pre	24.65±18.73	27.54±13.95	26.22±13.91	33.98±13.63
	Post	15.81±11.43	19.89±9.26	15.69±8.51	21.70±11.75
	t-test	t_9= -2.671	t_9= -1.861	t_7= -2.721	t_7= -4.326
		pvalue= 0.026*	pvalue= 0.096	pvalue= 0.030*	pvalue= 0.003*
	Covarance	17.25±2.69	19.99± 2.68	16.39±3.00	18.79±3.02
Menstrual	Pre	21.48±14.52	24.18±23.22	30.95±18.91	33.82±19.11
	Post	10.37±6.54	14.22±8.49	16.47±16.72	19.98±16.35
	t-test	t_8= -3.718	t_8= -1.743	t_8= -2.931	t_8= -3.021
		pvalue= 0.006*	pvalue= 0.120	pvalue= 0.019*	pvalue= 0.017*
	Covarance	13.28±2.85	15.87±2.83	14.98±2.83	17.15±2.85

Table 4- Measured glucose levels (mg/dl) in two studied groups in four different exercises. Asterisk shows statistically significant decrease (pvalue <0.05)

Group / serum collection		Activity			
Group	TrainingSession	Continuous/Water	Discontinuous/Treadmill	Discontinuous/Water	Continuous/Treadmill
Menopause	Pre	80.70±17.29	85.70±16.99	85.87±23.22	84.63±13.14
	Post	66.80±9.34	77.50±16.31	73.00±7.75	72.75±8.08
	t-test	t_9= -3.985	t_7= -1.799	t_9= -2.408	t_7= -4.395
		pvalue= 0.003*	pvalue= 0.115	pvalue= 0.039*	pvalue= 0.003*
	Covarance	67.34±2.18	76.05±2.19	71.48±2.45	71.72±2.44
Menstrual	Pre	76.55±13.81	77.11±9.70	84.44±16.08	81.88±15.79
	Post	69.88±6.09	66.55±4.36	71.22±9.41	71.66±7.21
	t-test	t_8= -1.751	t_8= -3.101	t_8= -4.726	t_8= -2.534
		pvalue= 0.118	pvalue= 0.015*	pvalue= 0.001*	pvalue= 0.035*
	Covarance	72.08±2.32	68.53±2.31	70.27±2.30	71.73±2.30

In menopause group, insulin levels in continuous/water, discontinuous/treadmill, discontinuous/water and continuous/treadmill groups were decreased -8.84±10.47 (pvalue=0.026) from 24.65±18.73 to 15.81±11.43, -7.65±13.00 (pvalue=0.096) from 27.54±13.95 to 19.89±9.26, -10.53±10.95 (pvalue=0.030) from 26.22±13.91 to 15.69±8.51, and -12.28±8.04 (pvalue=0.003) from 33.98±13.63 to 21.70±11.75 respectively.

In menstrual group, insulin levels in continuous/water, discontinuous/ treadmill, discontinuous/ water and continuous/ treadmill groups were reduced by -11.11±8.96 (pvalue=0.006) from 21.48±14.52 to 10.37±6.54, -9.96±17.16 (pvalue=0.120) from 24.18±23.22 to 14.22±8.49, -14.47±14.81 (pvalue=0.019) from 30.95±18.91 to 16.47±16.72, and -13.84±13.74 (pvalue=0.017) from 33.82±19.11 to 19.98±16.35 respectively (Table 3).

In menopause group, glucose levels in continuous/water, discontinuous/treadmill, discontinuous/water and continuous/treadmill groups were decreased -13.90±11.03 (pvalue=0.003) from 80.70±17.29 to 66.80±9.34, -8.20±10.76 (pvalue=0.115) from 85.70±16.99 to 77.50±16.31, -12.87±20.23 (pvalue=0.039) from 85.87±23.22 to 73.00±7.75, and -11.87±7.64 (pvalue=0.003) from 84.63±13.14 to 72.75±8.08 respectively.

In menstrual group, glucose levels in continuous/water, discontinuous/treadmill, discontinuous/water and continuous/treadmill groups were decreased -6.66±11.42 (pvalue=0.118) from 76.55±13.81 to 69.88±6.09, -10.55±10.21 (pvalue=0.015) from 77.11±9.70 to 66.55±4.36, -13.22±8.39 (pvalue=0.001) from 84.44±16.08 to 71.22±9.41, and -10.22±12.10 (pvalue=0.035) from 81.88±15.79 to 71.66±7.21 respectively (Table 4).

Conclusion:

Obesity is a common phenomenon among menopause women, especially central obesity. Obesity is associated with an increased risk of insulin resistance and type 2 diabetes by increasing concentrations of insulin and glucose (14). Also, myostatin, a negative regulator of muscular growth, is increased in older age and should be taken into account as a potential causative reason for health problems in older men and women. Although the effects of exercise training on myostatin expression in skeletal muscle have been discussed by several studies, the results remain disputable. For example, both increasing and decreasing levels of myostatin mRNA has been seen after different types of exercise training in healthy persons (15). Thus, we decided to design the present study to elucidate the response of myostatin, insulin and glucose to discontinuous and continuous training on treadmill and in water in two groups of middle aged menopause and menstrual women. Given t-test analysis, the main findings from this investigation were that all myostatin, insulin and glucose reduced after different aerobic training in water and on treadmill.

A) Effects of aerobic exercise on myostatin concentration. With respect to t-test analysis, myostatin levels were significantly decreased after continuous and discontinuous training in water, and discontinuous training on treadmill in menopause group (pvalue<0.05). In contrast, myostatin did not decrease significantly after continuous training on treadmill in menopause group (pvalue>0.05). Meanwhile, myostatin level showed significant decrease in all four different conditions of training in menstrual group (pvalue<0.05).

Given covariance analysis results for myostatin levels in menstrual and menopause groups, the lowest myostatin response was found in discontinuous training in water in both menopause and menstrual groups, while the highest myostatin response belonged to menopause group who conducted continuous training on treadmill. These results were statistically meaningless (pvalue>0.05).

These results are in accordance with previous study which imply on significant reduction of myostatin mRNA contents by training in water for 4 weeks (8). Also, our finding that aerobic exercise is associated with a reduction in serum myostatin levels in women with age of older than 40 years old is consistent with Konopka et al. (9), who recently reported that aerobic exercise training performed for 12 weeks can reduce myostatin mRNA expression in older women (pvalue <0.05).

Although reduction of myostatin levels after different aerobic training protocols varies in menstrual and menopause groups, data from our laboratory suggest that aerobic exercise in general can play a critical role in myostatin reduction and consequently decline the risk of sarcopenia and its associated diseases in older women (15).

B) Effects of aerobic exercise on insulin concentration. Secretion of insulin is mainly controlled by plasma glucose concentration, and the hormone has a number of important metabolic actions. Its main function is to control the uptake and utilization of glucose in peripheral tissues by the glucose transporter. Insulin concentrations are severely reduced in insulin-dependent diabetes mellitus (IDDM), while its levels are raised in non-insulin-dependent diabetes mellitus (NIDDM) and obesity (16). Serum insulin levels have been used as an estimate of insulin sensitivity (14).

With regard to t-test analysis, our findings in this study present that insulin levels were considerably decline after continuous and discontinuous training in water, and continuous training on treadmill in both menstrual and menopause groups (pvalue<0.05), while no substantial decrease was observed in insulin levels after discontinuous training on treadmill, in spite of a medium decrease (pvalue>0.05).

Considering covariance analysis results for insulin levels in menstrual and menopause groups, the lowest insulin response was detected in continuous and discontinuous training in water in menstrual group, while the highest insulin response belonged to menopause group who performed discontinuous training on treadmill. These results were statistically meaningless (pvalue>0.05).

Overall, increasing oxidative enzymes and glucose transporters in the muscle by exercise training can be considered as an explanation for insulin reduction after aerobic training in both menstrual and menopause overweight women. Our findings are in concordance with Donnelly et al. reported significant decreases in insulin concentrations in both discontinuous and continuous exercise (17).

C) Effects of aerobic exercise on glucose concentration. Glucose is one of the most important carbohydrates in body and considered as the primary source of energy for the body's cells. Glucose levels are tightly regulated in the human body. The determination of glucose levels in blood is critical in the control of diabetes (18).

With respect to t-test analysis, glucose levels were significantly decreased after continuous and discontinuous training in water, and continuous training on treadmill in menopause group (pvalue<0.05). Moreover, glucose levels were declined after discontinuous training on treadmill in menopause group, although this did not reach significance (pvalue>0.05). In menstrual group, glucose levels were significantly decreased after continuous and discontinuous training on treadmill, and discontinuous training in water (pvalue<0.05). On the contrary, no noticeable drop was detectable after continuous training in water (pvalue>0.05).

Regarding covariance analysis results for glucose levels in menstrual and menopause groups, the lowest glucose response was seen in continuous training in water in menopause group, while the highest glucose response belonged to menopause group who committed discontinuous training on treadmill. These results were statistically meaningless (pvalue>0.05).

These results suggest that aerobic exercise (in water or on treadmill) can reduce the risk of metabolic disease associated with obesity, such as high insulin and glucose concentrations (19).

Evans et.al reported that endurance exercise training was associated with statistically significant improvement in glucose tolerance and reduction in insulin resistance in older adults (20). In comparison, our data show that aerobic training can also reduce insulin and glucose levels in serum. Another study by Ryan et.al describing a significant decrease in plasma glucose and insulin after aerobic and resistive training combined with weight loss in overweight postmenopausal women (21). These data are in support of our findings that demonstrated a drop in insulin and glucose after aerobic training.

It can be concluded that aerobic training provided considerable response in measured factors in studied women groups, in spite of some variations. The lowest myostatin response was seen in discontinuous training in water in both menopause and menstrual groups, while the highest myostatin response observed in menopause group who committed continuous training on treadmill. In summary, this study suggested that discontinuous than continuous exercise, and exercise in the water than on treadmill, Lead to better response in variables are listed.

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پاسخ سرمی میواستاتین، انسولین و گلوکز به فعالیت ورزشی تداومی و تناوبی در زنان یائسه و غیریائسه دارای اضافه وزن

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چکیده

مقدمه: میواستاتین به تنظم توده عضله اسکلتی و آدیپوژنر منجر می‌شود، اما پاسخ سرمی آن به فعالیت ورزشی تداومی و تناوبی در زنان یائسه و غیریائسه بررسی نشده است. هدف از پژوهش حاضر، بررسی پاسخ سرمی میواستاتین، انسولین و گلوکز به چهار مدل متفاوت از فعالیت ورزشی هوازی در زنان یائسه و غیریائسه بود.

روش‌کار: بدین منظور، ۱۷ نفر از زنان دارای اضافه وزن (BMI>۳۰ و ٤۰ تا ۵۰ سال) شهر بندرعباس داوطلبانه در این پژوهش شرکت کردند و در دو گروه یائسه و غیریائسه قرار گرفتند. میواستاتین، انسولین و گلوکز قبل و بعد از چهار جلسه متفاوت فعالیت ورزشی شامل فعالیت ورزشی هوازی تداومی و تناوبی در آب و روی تردمیل اندازه‌گیری شدند.

نتایج: مقادیر میواستاتین پس از فعالیت ورزشی تداومی و تناوبی در آب و تناوبی روی ترمیل در زنان یائسه به طور معنی داری کاهش یافت (pvalue<۰/۰۵). همچنین، مقادیر میواستاتین کاهش معنی‌داری را پس از هر چهار مدل فعالیت ورزشی در زنان غیریائسه نشان داد (pvalue<۰/۰۵). مقادیر انسولین پس از فعالیت ورزشی تداومی و تناوبی در آب و تداومی روی ترمیل در هر دوی زنان یائسه و غیریائسه به طور معنی‌داری کاهش یافت (pvalue<۰/۰۵). مقادیر گلوکز پس از فعالیت ورزشی تداومی و تناوبی در آب و تداومی روی ترمیل در زنان یائسه کاهش معنی‌داری داشت (pvalue<۰/۰۵). در زنان غیریائسه، مقادیر گوکز پس از فعالیت ورزشی تداومی و تناوبی روی ترمیل و تناوبی در آب به طور معنی‌داری کاهش یافت (pvalue<۰/۰۵).

نتیجه‌گیری: کمترین پاسخ میواستاتین پس از فعالیت ورزشی تناوبی در آب در هردو گروه یائسه و غیریائسه مشاهده شد، در حالی که بیشترین پاسخ میواستاتین در گروه یائسه پس از فعالیت ورزشی تداومی روی تردمیل مشاهده گردید. این مطالعه نشان داد فعالیت ورزشی تناوبی در مقایسه با تداومی و فعالیت ورزشی در آب در مقایسه با روی تردمیل، به پاسخ بهتری در متغیرهای ذکر شده منجر می‌شود.

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کلیدواژه‌ها: فعالیت ورزشی، اضافه وزن، یائسه، میواستاتین.

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