Int J Diabetes & Metab (2011) 19:63-68

Twelve-week resistance training decreases myostatin level and improves insulin sensitivity in overweight-obese women

Abbas Saremi and Mohammad Parastesh
Department of Sport Sciences, Arak University, Arak, Iran

Abstract
Myostatin is a secreted protein that acts as a potent inhibitor of skeletal muscle growth. Inactivation of myostatin increases muscle mass and decreases insulin resistance. The aim of this study was to determine if the improvement in insulin resistance after resistance training is associated with a decline in serum levels of myostatin. A sample of 19 obese-overweight women (age= 23.2±3.4 yr, body mass index= 29.18±1.72 kg/m^2) were assigned to resistance training (n= 10) and control (n= 9) groups. Resistance training was performed 3 times weekly for 12 weeks. Body composition, metabolic parameters, and myostatin were measured prior to and after the intervention. Insulin resistance improved significantly after 12wk of resistance training ($p<0.05$), but without changes in adiposity parameter such as body weight, body mass index, fat percent and waist circumference ($p>0.05$). Also, following the resistance training, muscle strength and lean body mass were significantly increased ($p<0.05$). Concurrently, myostatin concentration significantly decreased in response to resistance training ($p<0.05$). In conclusion, 12-wk resistance training program increases insulin sensitivity in obese-overweight women, and this improvement was associated with decreased myostatin levels but is independent of measurable changes in body fat. These data suggest that resistance training-induced alterations in myostatin levels may have a role in resistance training-induced insulin sensitivity improvement.

Keywords: *Insulin sensitivity, Myostatin, Resistance training, Obesity*

Introduction

Despite remarkable progress in diagnosis, prevention and treatment, cardiovascular diseases are the main cause of mortality and morbidity throughout the world. This, to a great extent, is related to the development of obesity.[1] It has been widely demonstrated that obesity is a major risk factor for the development of insulin resistance, diabetes, dyslipidemia and hypertension.[2] While the majority of previous research have associated obesity, insulin resistance and increased risk of cardiovascular disease with abnormalities in the endocrinal function of fat tissue (including secretion of adipokins such as leptin, resistin, adiponectin and inflammatory factors such as tumor necrosis factor-alpha [TNF-α]),[2,3] recent research indicate that skeletal muscle also play an important role in insulin resistance through the secretion of myostatin.[4] Myostatin is a member of the transforming growth factor–β (TGF-β) superfamily and acts on skeletal muscle as a growth inhibitor.[5] Myostatin is highly synthesized in the skeletal muscle and then released into the circulation. Myostatin impairs muscle growth by inhibiting satellite cell proliferation and differentiation.[6,7] Targeted disruption of the myostatin signaling by specific inhibitors or genetic manipulations results in a dramatic increase in skeletal muscle mass.[8,9] Furthermore, inhibiting the effects of myostatin is not just confined to the skeletal muscle, evidence from the study of mice disrupted of myostatin gene indicates that fat mass decrease twice as much as the skeletal muscle does.[10] Loss of myostatin function can prevent obesity due to high fat diet.[11] Also, the disruption of myostatin functions by its propeptide results in the activation of Akt, and the improvement of insulin sensitivity.[12] Inactivating myostatin can decrease cardiovascular risk factors such as LDL, HDL, and improve the insulin sensitivity.[13] In addition, the inhibition of myostatin can increase the expression of adiponectin and peroxisome proliferator-activated receptor-α (PPAR-α),[14] whereas the disruption of myostatin signaling results in the improvement of obesity and insulin resistance indices.[4] These pieces of evidence emphasize: firstly, the close interaction between skeletal muscle and fat tissue in the adjustment of body's metabolic homeostasis, secondly, targeting (inhibition) of myostatin might be an effective therapeutic intervention for the prevention and treatment of metabolism related diseases such as insulin resistance. From another perspective, physical inactivity is one of the major risk factors for cardiovascular diseases. In the last few years, resistance training (or weight training) have turned into such common types of exercise for improving health and increasing muscular mass.[15] Resistance training can lead to increased muscular strength, alleviation of insulin resistance, decreased adiposity, and decreased risk of metabolic syndrome.[16-18] However, there are some contradictions about the results of this type of exercise and

Received on: 08/02/2010
Accepted on: 21/06/2011

Correspondence to: Dr Abbas Saremi, Department of Sport Sciences, University of Arak, Arak, Iran. E-mail: a-saremi@araku.ac.ir

the mechanism of its positive effects on metabolic indices is not crystal clear yet. Hence, this study aimed to determine the effects of resistance training on insulin resistance and the level of serum myostatin in obese-overweight women. Consequently, the main hypothesis of this study was that twelve weeks of resistance training can result in the improvement of obesity related metabolic indices (including insulin resistance) that might be related to alterations (reductions) in myostatin, since it has already been shown that in healthy men, resistance training can reduce the expression and secretion of myostatin.[19]

Material and methods

Participants

Nineteen overweight-obese women (age 23.2±3.4 yr, BMI≥25 kg/m^2) were recruited for the study. The subjects were randomly divided into either obese control (obese CON, n=9), or exercise intervention (obese EX, n= 10) group. The obese EX group participated in 12 week resistance training program, while the obese CON maintained their lifestyle as usual. All subjects had a stable weight for at least 3 months before inclusion. We excluded candidates who smoke, had cardiovascular disease or any other major illness, or were taking medications that could have affected laboratory test results. The physical activity level of subjects was assessed during an interview. The Ethics Committee of University approved the experimental procedures and study protocols, which were fully explained to all subjects. A written consent form was signed by each subject after having read and understood the details of the experiments.

Dietary assessment

The subjects were instructed to follow their usual eating habits during the course of the study. Dietary intakes were registered during the 12-week intervention period using 3-day dietary records. The participants were provided with several practice sessions regarding a measuring cup, spoon, and ruled paper until they learned how to estimate the portions of food items consumed. Then food records were analyzed using the food processor II nutrition System-analysis software Version 3.1 (The Medicine Science University of Tehran, Iran) to determine total daily caloric and macronutrient intake.

Resistance training program

The subjects participated in a resistance training program, designed to target both the upper and lower body muscle groups. They were trained three times a week on non-consecutive days for 12 weeks. All sessions were supervised by a member of the research team. Training intensity for the program was determined using one-repetition maximum (1-RM) (the maximum amount of weight that one can lift) for the given movements. The movements consisted of bench press, late pull down, biceps curl, leg press, knee extension, and knee flexion which engaged upper and lower extremities. Each training session included a 10-min warm-up and 50–60 min of resistance exercise. The resistance training exercises were based on the recommendations of the American College of Sports Medicine for obese and insulin resistant individuals.[20] During the first week, the participants performed two sets of 15-20 repetitions with the intensity of 40-50% of IRM. In the second week, the program included three sets of 15-20 repetitions with the intensity of 50-60% of IRM. During weeks 3 to 6, the number of repetitions was reduced to 12-15 while the intensity was increased up to 60-75% of IRM. In the final 6 weeks of training, the number of repetitions was lowered to 8-12 and the intensity of exercises was raised to 75-85% of IRM. The 1RM for chest press and leg press were intended as indices for upper and lower extremity strength, respectively.

Strength testing

Prior to 1-RM assessments, a familiarization session was conducted in order to minimize learning effects on the strength testing protocols. In these sessions specific exercise techniques were toughed and sub-maximal practice for each exercise session determined. Before training and at the end of weeks 2, 4, 6, 8, 10 and 12 the subjects were tested to determine muscular strength using the 1-RM for bench press and leg press to assess absolute upper and lower body strength, respectively.[21] The 1-RM for each lift was reached within five attempts, after a brief warm-up of five to eight repetitions with loads of approximately 50% of the anticipated 1-RM. During the 1-RM process, the attempts were separated by 3–5 min breaks. All strength assessments (pre- and post-testing sessions) followed the same direction (bench press and leg press), and each test was followed by an adequate recovery period of at least 10 min.[22] All lifts were performed according to standardized procedures and were monitored by the staff.

Anthropometric measures

Height (m) and body mass (kg) were measured to calculate body mass index (BMI) as body mass (kg)/ height^2 (m). Waist circumference was measured at the narrowest point superior to the hip using a tape ruler. Lean body mass and fat mass were determined using a lunar DPX-L (Lunar CO, Waukesha, Wisc., USA) dual energy X-ray absorptiometer, software version 4.6 d, at baseline and 12 weeks. Whole-body scans were performed by the same device, and the same licensed operator. Quality assurance was assessed by analyzing a phantom spine and daily calibrations were performed prior to all scans using a calibration block provided by the manufacturer. Intra-class correlation coefficients (R≥0.98) for bone mineral content, lean body mass, and fat mass were obtained from repeated scans on a group of ten women who were tested on 2 consecutive days.

Blood collection and analysis

All subjects reported for blood sampling in the morning after an overnight fast. Post- training blood samples (10 ml) from subjects in the training group were obtained 48 h after their last exercise session. After collection, blood samples were centrifuged (40c, 1500×g, 15min) and plasma- and serum-containing tubes were stored at -70^c until analysis. Plasma glucose was determined by the glucose oxidase method (Pars Azmun, Tehran, Iran). Plasma insulin was determined by chemiluminescent immunometric assay

(Monobind Inc, USA). The homeostasis model assessment (HOMA), insulin resistance index, was calculated by using the formula: fasting glucose (mg dl -1) × fasting insulin (μU ml -1) × 405 -1.[23] Serum myostatin concentrations were assessed using a competitive enzyme-linked immunosorbent assay (ELISA) method. At first, the wells of microtiter plates were coated with recombinant human myostatin (Santa Cruz Biotechnology, SC-6884P) (300 ng/ml) dissolved in phosphate-buffered saline (PBS, pH 7.2, 0.01 M) for 12 h at room temperature. The plates were washed four times with PBS. The wells were blocked with 300 μl of 3% bovine serum albumin (BSA) in PBS to prevent non-specific binding and the plate incubated for 1 h. Sera were mixed with equal volume of mouse anti-human myostatin monoclonal antibody (Santa Cruz Biotechnology, SC-6884) (500 ng/ml) in PBS containing 0.1% BSA for 1 h. Standard curves were constructed by mixing serial dilutions of recombinant human myostatin with equal volume of the same anti-human myostatin antibody (500 ng/ml) in PBS containing 0.1% BSA for 1 h. The mixtures were transferred to the coated well and incubated for 1 h at room temperature. After washing the wells with PBS containing 0.05% Tween 20 (PBST), peroxidase-conjugated rabbit anti-mouse polyclonal antibody was added to the wells and the plate was incubated for 1 h at room temperature. Finally, it was washed six times with PBST and then 100 μl of 3,3′,5,5′-tetramethylbenzidine (TMB) reagent was added and incubated at room temperature for about 10 min. Peroxidase reaction was stopped by adding 100 μl of 200 mM H_2SO_4, and the optical density at 450 nm was determined by a microplate reader. All the standards and samples were assayed in duplicate. The data for the standard curve were fitted to a logistic plot and the levels of myostatin were calculated. The intra-assay coefficient of variation for glucose, insulin, and myostatin were 1.28%, 5.3%, and 4.6%, respectively.

Table1. Participant characteristics before and after 12 weeks resistance training.

	Obese EX		Obese CON	
Characteristics	before	after	before	after
Body composition				
Weight (kg)	88.79±4.78	89.05±5.1	89.72±5.91	89.82±5.69
BMI (kg/cm^2)	29.11±1.79	29.17±1.60	29.23±1.88	29.26±1.73
Wc (cm)	90.14±8.54	89.83±8.75	91.24±9.39	91.27±9.51
Fat mass (kg)	33.13±5.31	32.45±6.44	33.53±6.13	33.76±6.41
Fat free mass (kg)	52.55±6.34	53.52±5.01*	53.05±5.59	52.93±5.61
Insulin resistance				
Glucose (mg dl $^{-1}$)	97.26±13.20	92.12±9.33*	100.26±13.12	99.10±15.29
Insulin (μU ml $^{-1}$)	8.26±1.40	8.14±1.56	8.13±1.15	8.07±1.12
HOMA-IR	1.98±0.51	1.84±0.48*	2.01±0.71	1.98±0.83
Muscle strength				
Leg press (kg)	119.25±17.48	140.91±16.51*	118.35±19.35	119.14±18.13
Bench press (kg)	36.14±8.39	42.36±7.38*	36.54±9.61	37.38±10.18

Data are expressed as the mean ± SD. * significant ($p<0.05$) difference between before and after training. BMI= body mass index; WC= waist circumference; HOMA-IR= homeostasis model assessment for insulin resistance.

Data analysis

Data were presented as mean ± SD. Normality assumption of the data was evaluated and confirmed using one-sample Kolmogorov-Smirnov test in each group. Changes in the dependent variables resulting from the exercise intervention were assessed by two-way time-by-group repeated measures analysis of variance. The assumption of homogeneity of variances of groups was tested and confirmed by Levene test for equality of variances. Pearson's correlation was used to analyze various variables. $P<0.05$ was considered significant. The data were analyzed using SPSS (version 16) software.

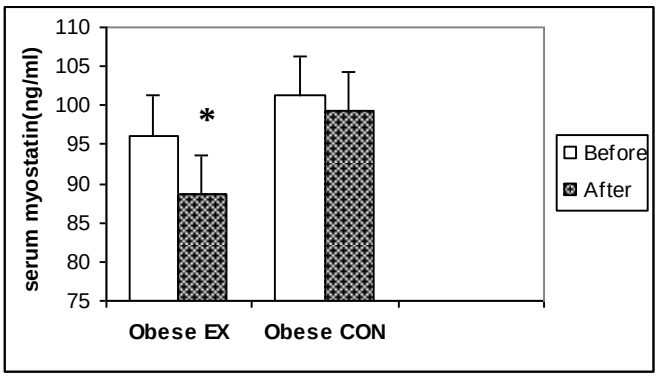

Figure 1: Serum myostatin levels before and after 12 weeks of resistance training. Data are expressed as the mean ± SD. * significant ($p<0.05$) difference between before and after training.

Results

Throughout the study no significant differences were observed in the amount of calories consumed (~33 kcal/kg/day) or the percentage of calories obtained from carbohydrate (~56%), protein (~21%), and fat (~23%) between groups during the course of the 12-week resistance training ($p>0.05$).

Obese EX group completed at least 93% of the exercise sessions. After 12 weeks of resistance training, the weight, fat percent, BMI and waist circumference did not change significantly ($p>0.05$), While fat-free mass ($p<0.04$), bench

press strength ($p<0.001$), and leg press strength significantly increased ($p<0.001$) (Table 1). After 12 weeks of resistance training blood glucose ($p<0.03$) and HOMA-IR ($p<0.04$) were significantly decreased in the obese EX group (Table 1). Also, myostatin concentrations were significantly decreased ($p<0.04$) after 12 weeks of resistance training (Table 1). Additionally, at baseline a positive correlation was found between myostatin and HOMA-IR ($r=0.49$, $p<0.04$).

Discussion

Insulin resistance is one of the most important consequences of obesity which plays a key role in the development of obesity associated complications (such as type 2 diabetes). Hence, knowing the interventions that may lead to the improvement of insulin resistance is essential for prevention and treatment of at risk individuals (e.g. the obese).[24,25] In the present study, 12 weeks of aerobic training decreased insulin resistance when comparisons were made between pre- and post intervention levels, and this is in agreement with previous investigations.[16,26] Reduction of insulin resistance in the absence of any alterations in the body adiposity (including weight, fat percentage, and waist circumference) and diet restrictions was one of the interesting findings of this study. Thus, this finding which is in accordance with other studies [27-29] suggests that regular physical activity can decrease insulin resistance independent of changes in the body adiposity and diet restrictions. Also, some recent researches show that fitness, in comparison to fatness, is a more reliable indicator of insulin resistance and probably physical activity improves insulin resistance through a mechanism other than changing the body fat mass.[30]

Therefore, there exists a hypothesis, compatible with the present study, stating that early changes in insulin resistance following physical activity (at least in the first twelve weeks) can be associated with the quality and quantity of skeletal muscle.[31] As observed by Atlantis et al. (2009), muscle strength and mass has a positive relationship with insulin resistance in obese individuals.[32] In the present study, it was revealed that an increase in muscular strength and fat free mass is accompanied by a decrease in insulin resistance. Hence, it is suggested that every intervention that improves strength and muscle mass is likely to result in the improvement of insulin resistance.[33]

Myostatin is a cytokine secreted from skeletal muscle which negatively affects the growth of skeletal muscles in a way that its inactivation or inhibition can increase the skeletal muscle mass and strength.[34] The findings of this study show that following twelve weeks of resistance training and along with the increase of fat free mass and muscle strength, serum myostatin levels decrease in obese women. This finding is compatible with the results of other research[19,35] which have reported a decrease in myostatin concentrations in young individuals of normal weight following resistance training. In fact, the present study shows a reverse relationship between changes in myostatin level and the status of muscle mass and strength, which is in line with the negative theoretical function of myostatin in the regulation of skeletal muscle mass.[6] Yet, in this study, in contrast to some studies[10] and in agreement with some others,[33] it is indicated that despite decrease in myostatin, fat mass indices (such as fat percentage and waist circumference) do not change significantly. Here exist two possibilities: myostatin alterations time course is not concomitant with adiposity changes, which means that more time is needed for myostatin inhibition effects to lead to the reduction of fat mass. [4,36] Another important possibility is that in this study the decrease in myostatin has been within the physiological range while it has been suggested that for decreasing fat mass, the reduction of myostatin should be beyond the physiological boundaries.[37]

Also, for the first time, it was demonstrated that in obese women, following twelve weeks of resistance training, reduction of myostatin occurs with the improvement of insulin resistance. Recent studies indicate that myostatin, apart from its key function in the regulation of muscle mass [4], also has a role in the adjustment of metabolism [38] in a way that in the present study, a positive correlation was found between serum myostatin concentrations and insulin resistance in the baseline levels. This is in agreement with the findings of other studies that have shown that high levels of myostatin in obese and old individuals are associated with metabolic-cardiac risk factors (e.g. insulin resistance).[38,39] In this realm, some believe that the role of myostatin in the regulation of metabolism is exerted directly. For instance, it has been shown that myostatin can inhibit the activation of such key enzymes as Akt in glucose metabolism, [12] the inhibition of myostatin can increase the expression of PPAR-γ and adiponctin, and can lead to the improvement of insulin resistance[4,14] or in vitro condition, increasing the expression of myostatin can result in the reduction of adiponctin and the increase of resistin and leptin.[40] On the other hand, some others state that the effect of myostatin on metabolism is indirect and it is asserted that the elimination of myostatin is followed by an increase in the muscle mass which results in metabolic substrates steal.[33]Therefore, it is suggested that the positive effects of myostatin elimination or inhibition on metabolic indices such as improvement of insulin resistance and reduction of dyslipedemia might be applied in this way. [12,13]

Overall, other research, [33, 40]verifying the findings of this study, show that the inhibition or reduction of myostatin function can result in the improvement of body metabolic conditions (such as insulin resistance). However, in this study, although it is not made clear whether the effects of myostatin reduction (due to resistance training) on the improvement of insulin resistance in obese individuals is direct or indirect, along with other studies,[12,13,33,40] it is shown that targeting myostatin can be a new strategy for treatment of metabolic induced diseases such as obesity, diabetes, and metabolic syndrome.

In conclusion, the present study shows that twelve weeks of resistance training, independent of changes in body adiposity, improves insulin resistance, and at the same time reduces serum myostatin levels in obese-overweight women. These findings suggest that myostatin decreases in

obese women might be associated with positive impacts of exercise (including the improvement of resistance to insulin). Yet, further studies are needed for clarifying the mechanism of myostatin inhibition effects on the improvement of metabolic indices following exercise.

Acknowledgements
The authors thank the subjects who participated in this study and the laboratory assistants in the clinical laboratory center of Arak university hospital who assisted with data collection and analysis.

References

1. Antuna Puente B, Feve B, Fellahi S, Bastard JP. Adipokines: the missing between insulin resistance and obesity. Diabetes Metab 2008; 34: 2-11.
2. Hajer GR, van Haeften TW, Visseren FL .Adipose tissue dysfunction in obesity, diabetes and vascular diseases. Eur Heart J 2008; 29: 2959-2971.
3. Nieto Vazquez I, Fernández Veledo S, Krämer DK, Vila Bedmar R, Garcia Guerra L, Lorenzo M. Insulin resistance associated to obesity: the link TNF-alpha. Arch Physiol Biochem. 2008; 114: 183-194.
4. Akpan I, Goncalves MD, Dhir R, Yin X, Pistilli EE, Bogdanovich S, Khurana TS, Ucran J, Lachey J, Ahima RS. The effects of a soluble activin type IIB receptor on obesity and insulin sensitivity. Arch Physiol Biochem 2009; 162: 1-9.
5. McPherron AC, Lawler AM, Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member. Nature 1997; 387: 83-90.
6. Fedoruk MN, Rupert JL. Myostatin inhibition: a potential performance enhancement strategy. Scand J Med Sci Sports 2008; 18:123-131.
7. McFarlane C, Hennebry A, Thomas M, Plummer E, Ling N, Sharma M, Kambadur R. Myostatin signals through Pax7 to regulate satellite cell self-renewal. Exp Cell Res 2008; 314: 317-329.
8. Matsakas A, Foster K, Otto A, Macharia R, Elashry MI, Feist S, Graham I, Foster H, Yaworsky P, Walsh F, Dickson G, Patel K. Molecular, cellular and physiological investigation of myostatin propeptide-mediated muscle growth in adult mice. Neuromuscul Disord 2009; 19: 489-499.
9. Schuelke M, Wagner KR, Stolz LE, Hübner C, Riebel T, Kömen W, Braun T, Tobin JF, Lee SJ. Myostatin mutation associated with gross muscle hypertrophy in a child. N Engl J Med 2004; 350: 2682-2688.
10. McPherron AC, Lee SJ. Suppression of body fat accumulation in myostatin-deficient mice. J Clin Inves 2002; 109: 595-601.
11. Hamrick MW, Pennington C, Webb CN, Isales CM. Resistance to body fat gain in 'double-muscled' mice fed a high-fat diet. Int J Obes 2006; 30: 868–870.
12. Zhao B, Wall RJ, Yang J. Transgenic expression of myostatin propeptide prevents diet-induced obesity and insulin resistance. Biochem Biophys Res Commun 2005; 337: 248-255.
13. Tu P, Bhasin S, Hruz PW, Herbst KL, Castellani LW, Hua N, Hamilton JA. Guo W. Genetic disruption of myostatin reduces the development of proatherogenic dyslipidemia and atherogenic lesions in Ldlr null mice. Diabetes 2009; 58: 1739-1748.
14. Suzuki ST, Zhao B, Yang J. Enhanced muscle by myostatin propeptide increases adipose tissue adiponectin, PPAR-alpha, and PPAR-gamma expressions. Domest Anim Endocrinol 2007; 33: 269-280.
15. Hawley JA. Exercise as therapeutic intervention for the prevention and treatment of insulin resistance. Diabetes Metab Res Rev 2007; 20: 383-393.
16. Ahmadizad S, Haghighi AH, Hamedinia MR. Effects of resistance versus endurance training on serum adiponectin and insulin resistance index. Eur J Endocrinol. 2007; 157: 625-631.
17. Tresierras M, Balady GJ. Resistance training in the treatment of diabetes and obesity: mechanisms and outcomes. J Cardiopulm Rehabil Prev 2009; 29: 67-75.
18. Ibanez J, Izquierdo M, Arguellezs I, Fprga L, Larrion JL, GaricaUnciti M, Idoate F, Gorostiaga EM. Twice-weekly progressive resistance training decreases abdominal fat and improves insulin sensitivity in older men with type 2 diabetes. Diabetes Care 2005; 28: 662-667.
19. Saremi A, Gharakhanloo R, Sharghi S, Gharaati MR, Larijani B, Omidfar K. Effects of oral creatine and resistance training on serum myostatin and GASP-1. Mol Cell Endocrinol 2010; 317: 25-30.
20. Levinger I, Goodman C, Hare DL, Jerums G, Selig S. The Effect of Resistance Training on Functional Capacity and Quality of Life in Individuals with High and Low Numbers of Metabolic Risk Factors. Diabetes Care 2007**;** 30: 2205-2210.
21. Willoughby DS, Rosene J. Effects of oral creatine and resistance training on myosin heavy chain expression. Med Sci Sports Exerc 2001; 33: 1674-1681.
22. Bemben MG, Bemben DA, Loftiss DD, Knehans AW. Creatine supplementation during resistance training in college football athletes. Med Sci Sports Exerc 2001**;** 33: 1667-1673.
23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-419.
24. Tresierras MA, Balady GJ. Resistance training in the treatment of diabetes and obesity: mechanisms and outcomes. J Cardiopulm Rehabil Prev. 2009; 29: 67-75.
25. Venables MC, Jeukendrup AE. Physical inactivity and obesity: links with insulin resistance and type 2 diabetes mellitus. Diabetes Metab Res Rev 2009; 25: 18-23.
26. Misra A, Alappan NK, Vikram NK, Goel K, Gupta N, Mittal K, Bhatt S, Luthra K. Effect of supervised progressive resistance-exercise training protocol on insulin sensitivity, glycemia, lipids, and body composition in Asian Indians with type 2 diabetes. Diabetes Care 2008**;** 31: 1282-1287.
27. Nassis GP, Papantakou K, Skenderi K, Triandafillopoulou M, Kavouras SA, Yannakoulia M,

Chrousos GP, Sidossis LS. Aerobic exercise training improves insulin sensitivity without changes in body weight, body fat, adiponectin, and inflammatory markers in overweight and obese girls. Metabolism 2005**;** 54: 1472-1479.
28. Shaibi GQ, Cruz ML, Ball GD, Weigensberg MJ, Salem GJ, Crespo NC, Goran, MI. Effects of resistance training on insulin sensitivity in overweight Latino adolescent males. Med Sci Sports Exerc 2006; 38: 1208-1215.
29. Bell LM, Watts K, Siafarikas A, Thompson A, Ratnam N, Bulsara M, Finn J, O'Driscoll G, Green DJ, Jones TW, Davis EA. Exercise alone reduces insulin resistance in obese children independently of changes in body composition. J Clin Endocrinol Metab 2007; 92: 4230-4235.
30. Messier V, Malita FM, Rabasa,Lhoret R, Brochu M, Karelis AD. Association of cardiorespiratory fitness with insulin sensitivity in overweight and obese postmenopausal women: a Montreal Ottawa New Emerging Team study. Metabolism 2008**;** 57: 1293-1298.
31. Karelis AD, Tousignant B, Nantel J, Proteau Labelle M, Malita FM, St-Pierre DH, Brochu M, Doucet E, Rabasa Lhoret R. Association of insulin sensitivity and muscle strength in overweight and obese sedentary postmenopausal women. Appl Physiol Nutr Metab 2007**;** 32: 297-301.
32. Atlantis E, Martin SA, Haren MT, Taylor AW, Wittert GA. Inverse associations between muscle mass, strength, and the metabolic syndrome. Metabolism 2009**;** 58: 1013-1022.
33. Guo T, Jou W, Chanturiya T, Portas J, Gavrilova O, McPherron AC. Myostatin inhibition in muscle, but not adipose tissue, decreases fat mass and improves insulin sensitivity. PLoS One 2009; 4: 4937-4943.
34. Whittemore LA, Song K, Li X, Aghajanian J, Davies M, Girgenrath S, Hill JJ, Jalenak M, Kelley P, Knight A, Maylor R, O'Hara D, Pearson A, Quazi A, Ryerson S, Tan XY, Tomkinson KN, Veldman GM, Widom A, Wright JF, Wudyka S, Zhao L, Wolfman NM. Inhibition of myostatin in adult mice increases skeletal muscle mass and strength. Biochem Biophys Res Commun 2003; 300: 965-971.
35. Roth SM, Martel GF, Ferrell RE, Metter EJ, Hurley BF, Rogers MA. Myostatin gene expression is reduced in humans with heavy-resistance strength training: a brief communication. Exp Biol Med 2003; 228: 706-709.
36. Stolz LE, Li D, Qadri A, Jalenak M, Klaman LD, Tobin JF. Administration of myostatin does not alter fat mass in adult mice. Diabetes Obes Metab 2008; 10: 135-142.
37. Awano H, Takeshima Y, Okizuka Y, Saiki K, Yagi M, Matsuo M. Wide ranges of serum myostatin concentrations in Duchenne muscular dystrophy patients. Clin Chim Acta 2008; 139: 115-117.
38. Hittel DS, Berggren JR, Shearer J, Boyle K, Houmard JA. Increased secretion and expression of myostatin in skeletal muscle from extremely obese women. Diabetes 2009; 58: 30-38.
39. Welle S, Bhatt K, Shah B, Thornton C. Insulin-like growth factor-1 and myostatin mRNA expression in muscle: comparison between 62-77 and 21-31 yr old men. Exp Gerontol 2002; 37: 833-839.
40. Zhao B, Wall RJ, Yang J. Transgenic expression of myostatin propeptide prevents diet-induced obesity and insulin resistance. Biochem Biophys Res Commun 2005; 337: 248-255.