Aerobic Exercise + Weight Loss Decreases Skeletal Muscle Myostatin Expression and Improves Insulin Sensitivity in Older Adults

A.S. Ryan1,2, G. Li1,2, J.B. Blumenthal1,2 and H.K. Ortmeyer1,2

Objective: To determine whether aerobic exercise training + weight loss (AEX + WL) would affect the expression of myostatin and its relationship with insulin sensitivity in a longitudinal, clinical intervention study.

Design and Methods: Thirty-three obese sedentary postmenopausal women and men (n = 17 and 16, age: 61 ± 1 years, body mass index: 31 ± 1 kg/m², VO2max: 21.9 ± 1.0 mL/kg/min, X ± Standard error of the mean (SEM)) completed 6 months of 3 days/week AEX + WL. During an 80 mU m⁻² min⁻¹ hyperinsulinemic-euglycemic clamp, we measured glucose utilization (M), myostatin, myogenin, and MyoD gene expression by real-time RT-PCR in vastus lateralis muscle at baseline and 2 h.

Results: Body weight (~8%) and fat mass (~17%) decreased after AEX + WL (P < 0.001). Fat-free mass (FFM) and mid-thigh muscle area by computed tomography did not change but muscle attenuation increased (P < 0.05). VO2max increased 14% (P < 0.001). AEX + WL increased M by 18% (P < 0.01). Myostatin gene expression decreased 19% after AEX + WL (P < 0.05). Basal mRNA myostatin levels were negatively associated with M before the intervention (r = −0.43, P < 0.05). Insulin infusion increased myoD and myogenin expression before and after AEX + WL (both P < 0.001) but basal levels did not change. The insulin effect on myostatin expression was associated with the change in M after AEX + WL (r = 0.56, P < 0.005).

Conclusions: Exercise and weight loss results in a downregulation of myostatin mRNA and an improvement in insulin sensitivity in obese older men and women.

Introduction
Myostatin is a member of the transforming growth factor beta family of secreted growth factors and is a significant negative regulator of skeletal muscle development and size (1). Transcription factors that regulate muscle differentiation include myogenic differentiation factor (MyoD), which is responsible for stimulating myoblasts to enter differentiation and join the muscle lineage, and myogenin which mediates terminal differentiation of myoblasts (2). Knock-out in the myostatin (Mstn) gene has led to dramatic increases in muscle mass in animals (3,4) and mutations have been documented in a child (5). In humans, resistive training either acute or chronic reduces basal mRNA myostatin (6-12). One study showed a decrease in myostatin protein levels in middle-aged men after aerobic training (13), whereas two studies report significant reductions in myostatin mRNA levels after severe weight loss by gastric bypass surgery (14,15). Thus, the effects of aerobic training and modest weight loss on these genes in skeletal muscle remain largely unaddressed, especially in older adults.

Animal model studies have demonstrated the importance of myostatin in glucose metabolism (16-18). Agouti lethal yellow and obese (Lepob/ob) myostatin knock-out mice have a reduction in body fat and improved glucose tolerance (16). Mstn−/− mice have increased glucose uptake measured by the hyperinsulinemic-euglycemic clamp (17) and when fed a high-fat diet, myostatin deficient mice also...
have improved muscle insulin sensitivity (17,18). Myostatin knock-out mice have increased Adenosine monophosphate (AMP)-activated protein kinase, Akt, and phosphorylated Akt (19) which may explain the increased insulin sensitivity. Because in vivo insulin increases serine phosphorylation of Akt in healthy controls and men with impaired glucose tolerance (20), it is of interest to study the effect of insulin stimulation on myostatin in vivo. Based on the studies in the myostatin knock-out model and the limited evidence that exercise and weight loss can affect myostatin levels in humans, it was our interest to test the hypothesis that chronic aerobic exercise training + weight loss (AEX + WL) would reduce basal skeletal muscle myostatin protein and gene expression and be associated with improvements in insulin sensitivity. Therefore, the aim of this investigation was to examine glucose utilization and the basal and insulin-stimulated gene expression levels of myostatin, MyoD, and myogenin before and after a 6-month aerobic training + weight loss program in older, obese adults.

Methods and Procedures

Subjects were healthy, overweight, and obese (body mass index, BMI > 25 kg/m²; range of 25-46 kg/m²) aged 54-77 years. They were weight stable (<2.0 kg weight change in past year), sedentary (<20 min of aerobic exercise 2×/week) and screened by medical history questionnaire, physical examination, and fasting blood profile. Individuals with untreated hypertension (blood pressure higher than 160/90 mmHg) or hyperlipidemia (triglycerides ≥ 4.5 mM, cholesterol > 6.2 mM, low density lipoprotein (LDL) cholesterol > 4.3 mM) were referred to their doctor for therapy and allowed to enter if they were treated with an antihypertensive or lipid-lowering drug that did not affect glucose metabolism. All subjects were nonsmokers, showed no evidence of cancer, liver, renal or hematological disease, or other medical disorders. The women in the study were postmenopausal and had not menstruated for at least 1 year. In addition, all subjects underwent a screening Bruce graded treadmill test to exclude those with asymptomatic coronary artery disease.

Thirty-three men (n = 16) and women (n = 17) met all study criteria and enrolled into the AEX + WL intervention. The Institutional Review Board of the University of Maryland approved all methods and procedures. Each participant provided written informed consent to participate in the study.

Study protocol

Subjects received instruction in maintaining a weight-stable, Therapeutic Lifestyle Changes (TLC) diet (21), by a registered dietician (RD) 1 day/week for 6-8 weeks, before baseline testing. Subjects were weight stable on the TLC diet before baseline testing and were instructed to maintain the TLC diet throughout the study.

AEX + WL program

Subjects exercised at the Baltimore VA Medical Center GRECC exercise facility three times a week for 6 months using treadmills and elliptical trainers. Each exercise session included a 5-10 min stretching and warm-up phase and a 5-10 min cool-down phase. For the first 3-4 weeks, subjects exercised on the treadmill at ~50-60% heart rate reserve for 20-30 continuous minutes as tolerated. Initially, time was progressed to 50 min. The duration remained for 50 min throughout the remainder of the intervention. Then, intensity was progressed to >60% VO₂max and up to 80% VO₂max. This progression of intensity typically took 4-6 weeks based on each subject’s exercise tolerance.

Exercise intensity, prescribed as a target heart rate range, was monitored using chest-strap heart rate monitors (Polar Electro, Lake Success, NY). There were no rest periods during the exercise sessions. All sessions were supervised by exercise physiologists.

All subjects also attended weekly weight loss classes led by a RD for instruction in the TLC diet. Compliance was monitored by 7-day food records (or 24 h recalls) using the American Diabetes Association exchange list system. Individuals were instructed to restrict their caloric intake by 500 kcal/day. The average compliance to the exercise sessions and weight loss classes was greater than 75%.

V₀₂max and body composition

V₀₂max was measured using a continuous treadmill test protocol (22). Height (cm) and weight (kg), waist and hip circumference, were measured to calculate BMI and waist-to-hip ratio. Fat mass, lean tissue mass, and bone mineral content (BMC) (fat-free mass = lean + BMC) were determined by dual-energy X-ray absorptiometry (DXA) (Prodigy, LUNAR Radiation Corp., Madison, WI). A single computed tomography (CT) scan at L₂-L₅ region using a Siemens Somatom Sensation 64 Scanner (Fairfield, CT) was used to determine visceral adipose tissue and subcutaneous adipose tissue areas and analyzed using MIPAV (NIH Image Analysis Program). A second scan at the mid-thigh was used to quantify muscle area, total fat area, low-density lean tissue area, and muscle attenuation in Hounsfield units (HU) of both the right and left legs (22).

Metabolic testing

All subjects were weight stabilized (±2%) for at least 2 weeks before metabolic testing before and after the interventions and were provided with a eucaloric diet for 2 days before the clamp by a RD to control nutrient intake (22). All testing was performed in the morning after a 12-h overnight fast. At the end of the 6-month program, subjects were asked to continue the aerobic training 3 days/week during the final testing period, and the glucose clamps were performed 36-48 h after the last bout of exercise.

Oral glucose tolerance test (OGTT)

Blood samples were drawn before and at 30-min intervals for 2 h after ingestion of 75 g of glucose. Plasma glucose concentrations were measured using the glucose oxidase method (2300 STAT Plus, YSI, Yellow Springs, OH). Plasma insulin was measured by radioimmunoassay (RIA) (Lincro Research, St. Charles, MO). Glucose and insulin total area under the curve (Gₐᵤₜₚₑₜ and Iₐᵤₜₚₑₜ, respectively) were calculated by the trapezoidal method.

Hyperinsulinemic-euglycemic Clamps with skeletal muscle biopsies

Peripheral tissue sensitivity to exogenous insulin was measured using the hyperinsulinemic-euglycemic clamp technique (23). Arterialized blood was obtained from a dorsal heated hand vein (24). For the assessment of basal glucose and insulin levels, three arterialized blood samples were drawn at 10-min intervals. Blood samples were obtained every 5 and 10 min thereafter for the determination of plasma glucose and insulin levels. A 10 min priming and continuous infusion of insulin (80 mU m⁻² min⁻¹, Humulin, Eli Lilly Co., Indianapolis, IN) was performed for 180 min with a continuous infusion of 20% glucose solution starting at 10 min. The plasma glucose levels during each clamp period
Analysis of blood samples. Blood samples were collected for serum or plasma (heparin) by blood draw and placed in test tubes. For the serum samples, blood was allowed to clot by leaving it undisturbed at room temperature for 30 min. For the plasma samples, blood was placed in prechilled test tubes containing 1.5 mg EDTA/mL of blood and centrifuged at 2,000g for 10 min at 4°C. The liquid component (serum or plasma) was immediately transferred into clean polystyrene tubes. Aliquots were stored at −80°C until analysis for plasma insulin by RIA (22) and for serum myostatin by enzyme-linked immunosorbent assay (ELISA). Serum myostatin was measured in duplicate using Myostatin ELISA kit according to the manufacturer’s instructions (ALPCO, Cat# K1012). Samples were read using VERSAMAX microtiter plate reader at 450 nm against 620 nm as a reference. A 4-parameter-algorithm was used to calculate the standard curve and the concentrations of myostatin in human serum were determined. The coefficient of variance between replicates was below 15%.

Skeletal muscle biopsies and analysis. Before the start of the clamp procedure and at 120-min during the glucose clamp, vastus lateralis muscle biopsies were taken from each subject under local anesthesia using a 5 mm Bergstrom needle for the measurement of basal and insulin-stimulated gene expression. Three women had basal and not insulin-stimulated muscle biopsies. Muscle was immediately freeze-clamped and stored at −80°C. Approximately 50-80 mg of muscle was used for RNA isolation and 100 mg of muscle was used for tissue lysate preparation. There was sufficient muscle sample of 15 individuals for the western blot analyses.

RNA extraction and reverse transcription and real-time RT-PCR

Total RNA was extracted from skeletal muscle using TRIzol® Reagent (Invitrogen, Cat# 15596-018) as specified by the manufacturer. The RNA pellet was resuspended in RNAsecure Resuspension solution (Cat# 7010, Ambion) and RNA concentrations were measured in a spectrophotometer.

One microgram of total RNA for each sample was reverse transcribed into first strand cDNA using Transcriptor First Strand cDNA Synthesis Kit (Cat# 04 896 866 001, Roche Applied Science) according to the detailed manufacturer’s protocol. The random primer was used as the primer and the RT reaction was carried out at 10 min at 25°C and then 55°C for 30 min in 20 μl volume. The reaction was inactivated by incubating at 85°C for 10 min and stopped by placing the tube on ice. An RT control (master mix without RT enzyme) was performed.

Quantitative real-time PCR (qPCR) and data analysis were performed in a LightCycler 480 Real-Time PCR System with LightCycler® 480 software (Roche Applied Science). LightCycler 480 Multiwell plate 384 (Cat# 04 729 748 001), LightCycler 480 Probes Master kit (Cat# 04 887 301 001), and Taqman gene expression primer/probe set (Applied Biosystems, Foster City, CA) were used. Each qPCR reaction was carried out in a final volume of 10 μl, consisting of 2 μl 1:4 diluted template cDNA, 5 μl LightCycler 480 Probes Master, 0.5 μl Taqman gene expression primer and probe mix, and 2.5 μl nuclease-free water. Water instead of cDNA served as the no template control. According to the manufacturer’s instruction, the qPCR protocol was adopted for all samples: after incubation at 95°C for 10 min to activate the DNA polymerase, 45 cycles of 95°C for 10 s and 60°C for 30 s each were performed to facilitate the PCR reaction. 36B4 served as an internal control for normalization. Data acquisition occurred at real time during the annealing/elongation incubation at 60°C. All samples were amplified in triplicate from the same RNA preparation. Gene expression data were analyzed by Roche LightCycler 480 system Software version 1.5 advanced relative quantification program. Data is presented as the mean ± SEM of three determinations for each sample and the normalized ratio of Target/Reference.

Tissue lysate preparation and western immunoblot analyses

In a 15 individuals (n = 7 women and n = 8 men), a fasting/basal muscle samples obtained before and after AEX + WL were lyophilized for 48 h and then dissected free of obvious connective tissue, fat, and blood. Microdissected muscle (~5 mg) was homogenized in complete RIPA buffer (Santa Cruz Cat# sc-24948) for 1 min on ice. The homogenate was incubated on ice with shaking for 30 min and then centrifuged at 14,000 rpm for 15 min at 4°C. Total protein concentration was determined using bichinonic acid (BCA) protein assay kit (Pierce Cat# 23227). Thirty micrograms of protein were denatured at 100°C for 5 min and separated on a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel (Bio-Rad Cat# 345-0009) along with molecular weight markers and subsequently transferred to polyvinylidene difluoride (PVDF) membranes. The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.05% Tween 20 (TBS-T) for 1 h at room temperature, then incubated overnight at 4°C with primary antibodies (MSTN, NVUS Cat# H00002660-M07 and GAPDH, Santa Cruz, Cat# sc-25778) diluted according to the manufacturer. After TBS-T for 15 min 3× washes, the membranes were incubated for 1 h at room temperature with secondary antibody, anti-mouse IgG TrueBlot conjugated with horse-radish peroxidase (HRP) (eBioscience, Cat# 18-8817), and goat anti-rabbit IgG conjugated with HRP. Detection was performed using the ECL Plus Western Blotting Detection System (GE Healthcare, Cat# PRN2132). The emitted light was captured on X-Omattm Blue XB film (Kodak, Cat# 1776699). Blots were imaged using a personal density scanning imager (Molecular Dynamics), and protein band densities were determined using the Image QuanNT software 5.0 (Molecular Dynamics). GAPDH served as an internal reference and was used to correct the difference in sample concentration and loadings. The whole normal skeletal muscle lysate (NVUS Cat# NB820-59169) served as a positive control and an external calibrator to counteract variations in Western blotting efficiency. Target/reference ratio was normalized using the calibrator on each Blot.

Statistical analyses

For the hyperinsulinemic-euglycemic clamps, the mean concentrations of glucose and insulin were calculated for each sample time point. The trapezoidal rule was used to calculate the integrated response over 30-min intervals from 30-180 min for each subject during the clamp. The integrated response was divided by its time interval to compute mean concentrations for glucose and insulin during the clamp. Glucose utilization (M) for 30-min intervals was
calculated from the amount of glucose infused after correction for glucose equivalent space (glucose space correction). For one male who did not undergo post DXA, the FFM change was estimated from the mean change in order to calculate M expressed per FFM. The CT mid-thigh measurements were not different between the right and left legs so the values of the right leg were used in the statistical analyses.

Differences between pre-intervention and post-intervention measures of variables were determined using a paired t-test. Univariate regression analyses were used to determine predictors of glucose utilization. Statistical significance was set at $P < 0.05$. Data were analyzed by SPSS statistical software (SPSS, Chicago) and expressed as mean ± SEM.

**Results**

Body weight decreased 8% ($P < 0.001$) accompanied by an absolute decrease in percent body fat of 4% ($P < 0.001$), a 17% decrease in total fat mass ($P < 0.001$), and no change in FFM or mid-thigh muscle area (Table 1). After AEX + WL, mid-thigh subcutaneous fat decreased ($P < 0.001$), low-density lean tissue area decreased ($P < 0.05$), and muscle attenuation increased ($P < 0.05$). $\text{VO}_{2}\text{max} \ (\text{L/min})$ increased 14% after AEX + WL ($P < 0.001$).

At baseline, 19 subjects had normal glucose tolerance, 11 had impaired glucose tolerance, and 3 had unconfirmed diabetes (25). Fasting plasma glucose concentrations did not change after AEX + WL but fasting insulin levels were reduced ($P < 0.001$, Table 1). Glucose AUC during the OGTT decreased 5% ($P < 0.05$) and insulin AUC during the OGTT decreased 17% ($P < 0.01$). $M$ increased 18% ($P < 0.001$) after AEX + WL.

**TABLE 1** Physical and metabolic characteristics before and after the intervention ($n = 33$)

|                          | Before                | After                 |
|--------------------------|-----------------------|-----------------------|
| Age, y                   | 61 ± 1 years          | 63 ± 1 years          |
| Weight, kg               | 90.8 ± 2.8            | 83.7 ± 2.7*           |
| BMI, kg/m$^2$            | 31.2 ± 0.7            | 28.3 ± 0.7*           |
| % Body fat               | 39.2 ± 1.6            | 34.8 ± 1.8*           |
| Fat mass, kg             | 35.5 ± 1.9            | 29.3 ± 1.8*           |
| Fat-free mass, kg        | 55.4 ± 2.3            | 54.9 ± 2.3            |
| $\text{VO}_{2}\text{max}$, L/min | 2.00 ± 0.11            | 2.26 ± 0.11*          |
| Visceral fat area, cm$^2$ | 163.0 ± 16.9          | 137.8 ± 14.1†         |
| Subcutaneous abdominal fat, cm$^2$ | 357.5 ± 23.5          | 360.8 ± 24.8*         |
| Mid-thigh muscle area, cm$^2$ | 90.7 ± 6.1            | 92.6 ± 5.2            |
| Mid-thigh subcutaneous fat, cm$^2$ | 118.7 ± 11.6          | 102.2 ± 9.6*          |
| Mid-thigh low density lean tissue, cm$^2$ | 22.9 ± 1.5            | 21.5 ± 1.6            |
| Mid-thigh muscle attenuation (HU) | 36.2 ± 2.6            | 38.8 ± 3.0*           |
| Fasting plasma glucose, mmol/L | 5.4 ± 0.1             | 5.2 ± 0.1             |
| Fasting plasma insulin, pmol/L | 87 ± 7                | 69 ± 5*               |
| Glucose AUC, mmol/L/min   | 1.271 ± 0.01          | 1.212 ± 0.01          |
| Insulin AUC, pmol/L/min   | 68.607 ± 5.499        | 57.127 ± 4.521†       |
| $M$, μmol kg$^{-1}$ mm$^{-1}$ min$^{-1}$ | 48.0 ± 3.29            | 56.5 ± 2.9†           |

Values are means ± SEM. Area under the curve from the OGTT. Significantly different before versus after the intervention: *$P < 0.001$; †$P < 0.01$; ‡$P < 0.05$.

Basal myostatin mRNA levels decreased 19% after AEX + WL (83.0 ± 9.7 vs. 67.5 ± 6.4 AU, $P < 0.05$, Figure 1) but myostatin protein levels did not change significantly for 26-kDa (1.29 ± 0.10 vs. 1.34 ± 0.10 AU) and 50-kDa (1.44 ± 0.16 vs. 1.26 ± 0.11 AU; Figure 2). Basal myostatin mRNA levels were negatively associated with $M$ before the intervention ($r = −0.43, P < 0.05$, Figure 3). There was a trend toward a difference in the change in myostatin mRNA levels with insulin stimulation before vs. after AEX + WL (−7.2 ± 4.7 vs. 6.0 ± 7.5 AU, $P = 0.10$). The insulin effect on myostatin gene expression was associated with the change in $M$ after AEX + WL ($r = 0.56, P < 0.005$). Serum myostatin tended to decrease after AEX + WL (7.25 ± 3.71 vs. 6.10 ± 2.44 ng/mL, $P = 0.07$). In an examination of each gender, we observed a decrease in skeletal muscle 50-kDa myostatin protein levels in women (1.75 ± 1.22 AU, $P < 0.05$) and a decrease in serum myostatin in men (9.02 vs. 6.48 ng/mL, $P < 0.05$). There were no associations between age and BMI and changes in skeletal muscle protein or gene expression.

Basal MyoD (2.59 ± 0.11 vs. 2.60 ± 0.09 AU) and myogenin (2.39 ± 0.09 vs. 2.39 ± 0.10 AU) mRNA levels did not change significantly after AEX + WL. However, insulin infusion increased myoD mRNA before (+2.62 ± 0.19 AU) and after (+2.71 ± 0.25 AU) AEX + WL (both $P < 0.001$). Likewise, hyperinsulinemia increased myogenin gene expression before and after AEX + WL (+0.71 ± 0.09 and +0.84 ± 0.16 AU, respectively, both $P < 0.001$). The increase in myoD and myogenin expression during hyperinsulinemia was similar before and after AEX + WL.

**Discussion**

Our results indicate that a downregulation of skeletal muscle mRNA content of myostatin occurs after an AEX + WL program in obese men and women. Basal MyoD and myogenin mRNA and myostatin protein expression did not significantly change after the intervention.

Satellite cells are important in the mechanisms involved in the muscles’ response to exercise training. Once the cells are activated, they proliferate and fuse together with pre-existing fibers to regenerate muscle tissue. Activated satellite cells can express the myogenic factors including MyoD, myogenin, and myostatin. Myostatin, a negative regulator of muscle cell growth, represses myoD and
myogenin protein expression (26). Because of the importance of these genes in muscle growth, it is conceivable that an exercise training program could modify the expression of these genes.

To our knowledge, there are no studies examining the effects of aerobic training and weight loss on skeletal muscle basal myostatin, MyoD, or myogenin levels in older men and women. We found a significant decrease in myostatin gene expression after AEX + WL. Although we found a 10% decrease in 50-kDa myostatin protein levels, this was not significant but is similar in magnitude to the 12% decrease in myostatin gene expression. We did not observe any significant changes in 26-kDa myostatin protein, MyoD or myogenin mRNA expression after AEX + WL. Others report downregulation of myostatin mRNA without changing myostatin protein levels in conditions such as sepsis in rodents (27). A 6-month aerobic training program in 10 middle-aged men resulted in a decrease in myostatin protein levels (13). An acute cycling bout increased mRNA myostatin by 2-fold, MyoD by 3-fold, and myogenin by <1-fold in endurance trained subjects (28). The significant decrease in basal myostatin mRNA after the AEX + WL in our study is consistent with studies using resistive exercise (RT) as the mode of training whereby acute bouts of resistive exercise (6-10) and 9 weeks of RT in older men (11) reduce muscle myostatin mRNA. We have also observed a significant decrease in myostatin mRNA expression in the paretic muscle of stroke survivors after 12 weeks of high intensity RT (12). One study has reported an increase in myostatin expression after an acute resistive session (29). In addition, an acute bout of RT can reduce (6) or not change (8) myogenin levels and increase MyoD mRNA expression (30) in young but not elderly men.
subjects (31). Although MyoD and myogenin gene expression increased after 6 weeks of RT in young males (32), we found no change in MyoD or myogenin after a longer aerobic exercise program.

Recent papers (17,18) provide evidence that myostatin knockout mice have increased insulin sensitivity and glucose uptake. Mstn−/− mice have increased muscle mass, lower adipose tissue mass, and lower basal skeletal muscle, and whole body lipid oxidation with improved insulin signaling by increased Akt phosphorylation during insulin (17). Myostatin mice with the loss of function of both alleles (MstnLoLo) have improved glucose disposal rate compared with Mstn−/− on a high-fat diet (18). In addition, TNFα expression is lower in both muscle and adipose tissue and treatment with recombinant myostatin increases TNFα and results in insulin resistance (18). Treatment of mice with a soluble activin receptor type IIB (an endogenous signaling receptor for myostatin) suppresses hepatic glucose production and increases glucose uptake (33). These animal models provide clear mechanistic evidence of the detrimental effects of myostatin on insulin sensitivity. We are the first to provide human evidence that skeletal muscle myostatin mRNA may be associated with insulin resistance in obese adults although circulating myostatin levels have been examined (13). Our results are consistent with a study in middle-aged men whereby plasma myostatin protein levels were inversely correlated with insulin sensitivity measured during an intravenous glucose tolerance test (13). Some potential molecular mechanisms responsible for increased insulin sensitivity in myostatin knockout mice are increases AMP-activated protein kinase activity in skeletal muscle, increased Glut4 levels, Akt and phosphorylated Akt (19). Treatment of human myoblasts with myostatin for 24 h decreases the phosphorylation of Akt by ~50% (34). During insulin stimulation in vitro, p-Akt increases in adults as a mechanism for increased insulin sensitivity (20), which could suggest that myostatin might change during hyperinsulinemia. Our results also show that the insulin effect on myostatin gene expression was associated with the change in insulin sensitivity after the intervention suggesting that those individuals with the greatest change in myostatin mRNA during hyperinsulinemia had the greatest improvement in insulin sensitivity after AEX + WL. Thus, alterations in insulin signaling may be one mechanistic link with changes in myostatin during hyperinsulinemia in humans, although this cannot be addressed in the present investigation. Furthermore, myostatin-knock out mice have an increase in carbohydrate utilization for energy and increase in respiratory exchange ratio (17). To-gether, these would also suggest that the increased carbohydrate oxidation and decrease in fat oxidation during a clamp (22,35) would align with a potential change in myostatin during insulin. More work is needed to further advance our understanding of myostatin’s role in insulin action in vivo.

There are several limitations in our study that deserve discussion. First, we recognize that our study cannot distinguish whether aerobic exercise alone (described above in one study) or the weight loss contributed to the changes in myostatin that we observed. There are only two papers that have examined the effects of weight loss on myostatin (14,15) but neither examined MyoD or myogenin. Both groups of investigators measured myostatin gene expression before and after severe weight loss induced by gastric bypass surgery (body weight decreased by 38% in n = 6 and 45% in n = 3) in morbidly obese subjects. They reported significant reductions in myostatin mRNA levels (14,15). Given the large degree of weight loss in these two studies, it is unknown whether the modest (8%) decrease in weight in our current study would have such an effect on myostatin. Additional studies of weight loss alone and aerobic exercise training alone on myostatin would be valuable. Although we observed a 10% decrease in myostatin protein levels, the reduced sample size in this measure could have limited our ability to detect a statistical significant change after AEX + WL. In addition, our study results are only applicable to older overweight and obese adults.

We found that myostatin mRNA expression is significantly reduced concomitant with improved insulin sensitivity after a 6-month aerobic exercise training program combined with modest weight loss in obese adults. Further studies are necessary to directly address changes in fuel utilization and the relationship to myostatin signaling. The role of exercise training and myostatin inhibition in the mechanism for improvements in insulin sensitivity also warrants additional investigation.

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