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Expression and Function of Myostatin in Obesity, Diabetes, and Exercise Adaptation

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Abstract

Myostatin is a member of the transforming growth factor-beta/bone morphogenetic protein (TGF-β/BMP) super-family of secreted factors that functions as a potent inhibitor of skeletal muscle growth. Moreover, considerable evidence has accumulated that myostatin also regulates metabolism and that its inhibition can significantly attenuate the progression of obesity and diabetes. While at least part of these effects on metabolism can be attributable to myostatin's influence over skeletal muscle growth and therefore on the total volume of metabolically active lean body mass, there is mounting evidence that myostatin affects the growth and metabolic state of other tissues, including the adipose and the liver. In addition, recent work has explored the role of myostatin in substrate mobilization, uptake and/or utilization of muscle independent of its effects on body composition. Finally, the effects of both endurance and resistance exercise on myostatin expression, as well as the potential role of myostatin in the beneficial metabolic adaptations occurring in response to exercise, have also begun to be delineated in greater detail. The purpose of this review is to summarize the work to date on the expression and function of myostatin in obesity, diabetes, and exercise adaptation.

Keywords

GDF-8; Endurance Exercise; Resistance Exercise; Metabolism

INTRODUCTION

In 1997, hypermuscularity was described in mice carrying a targeted inactivation of a novel transforming growth factor-beta/bone morphogenetic protein (TGF-β/BMP) family member, growth/differentiation factor 8 (GDF-8; 39). This gene was named myostatin for its ability to inhibit muscle differentiation and growth (39). Myostatin was subsequently identified as the mutated allele in the highly muscular Belgian Blue breed of cattle (12, 23, 40), and naturally occurring mutations have also been associated with an increase in muscle mass in

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dogs (45) and humans (59). Furthermore, mice in which myostatin protein processing or signalling is disrupted also exhibit increased muscle mass (36, 70, 73), while myostatin over-expression is associated with dramatic muscle atrophy in mice (53, 74). Together these studies have confirmed a central, critical role for myostatin in suppressing muscle growth.

The mechanisms by which myostatin achieves this suppression of muscle growth have been well defined. Like most TGF-β/BMP family members, myostatin is proteolytically cleaved in the endoplasmic reticulum into an amino terminal inhibitory pro-domain and a carboxy terminal active domain (18). Secreted myostatin then circulates as an inactive multi-protein complex consisting of the carboxy dimer non-covalently bound to the pro-domain and/or other inhibitory proteins such as follistatin and follistatin-like-3 (FSTL3; 7). The active myostatin dimer is freed from this inhibitory protein complex most likely by the tolloid and tolloid-like extracellular proteases located in the extracellular matrix of muscle and other tissues (68). Free myostatin then binds to the cell surface activin receptor type II or IIb (ActRII, ActRIIb) which in turn induces the phosphorylation of the SMAD family of transcription factors via the canonical TGF-β signaling pathway (52), although there is also evidence that myostatin can regulate muscle mass independent of SMAD signaling (50). Regardless of the pathway, myostatin inhibits muscle stem cell proliferation (61, 62) and differentiation, (29) and attenuates adult muscle fiber protein accretion, (38, 63, 65) resulting in decreased skeletal muscle mass.

Because myostatin greatly influences skeletal muscle growth and therefore the total amount of metabolically active lean tissue, it can have profound effects on whole body metabolism. This in turn can have consequences for the development of obesity and diabetes. However, some recent work has suggested that myostatin may also affect substrate uptake and utilization independent of its effects on muscle mass. In addition, myostatin appears to have direct metabolic effects on tissues other than skeletal muscle, such as adipose and liver. This raises the possibility that the well-documented changes in myostatin expression in response to exercise training may modulate or in some way contribute to the beneficial effects of exercise through direct effects on skeletal muscle metabolic function as well as through effects on these other tissues.

The purpose of this review is to summarize the work to date on the expression and function of myostatin in obesity, diabetes, and exercise adaptation. Specifically, we will address two related questions regarding the influence of myostatin on metabolism: (1) Are the effects of myostatin signaling on the metabolism of non-muscle tissues/cells (such as adipose or liver) direct due to myostatin signaling in these tissues or indirect due to myostatin signaling in muscle? (2) Are the effects of myostatin on metabolism due solely to its effects on cellular growth or can myostatin also influence cellular metabolism directly via changes in substrate mobilization, uptake, and/or utilization?

MYOSTATIN AND OBESITY

Several lines of evidence demonstrate that obesity is associated with increased myostatin expression. Myostatin mRNA levels are increased in both adipose and skeletal muscle from genetically obese, leptin-deficient ob/ob mice and from wild type mice fed a high fat diet for one month (2). Muscle and circulating myostatin protein levels are also increased in obese human subjects, as is myostatin secretion from myotubes derived from myoblasts isolated from muscle biopsies of obese compared to non-obese women (19). Conversely, myostatin mRNA levels decrease during weight loss: myostatin mRNA levels were decreased in muscle and adipose from ob/ob mice upon two weeks of daily injection of recombinant leptin (2), and also decreased in muscle biopsies from obese human patients following weight loss due to either biliopancreatic diversion (42) or gastric bypass surgery (48).

Moreover, the experimental manipulation of myostatin expression or signaling can dramatically affect the development of obesity in mice. For example, muscle-specific over-expression of myostatin caused a decrease in muscle mass and an increase in epididymal fat pad mass (53). Conversely, several studies have shown that inactivation of myostatin can attenuate the development of obesity in mice. Crossing myostatin null mice with genetically obese ob/ob or agouti lethal yellow mice attenuated the increased adipose mass, hyperglycemia, hyperlipidemia, and hyperinsulinemia typically observed in these genetically obese animals (41). Postnatal transgenic over-expression of the inhibitory myostatin pro domain in mice also increases muscle growth and attenuated the effects of two months of a high fat diet on increasing adipose mass and circulating insulin and glucose levels (71, 72). Injection of mice with a soluble ActRIIb receptor, which disrupts signaling for myostatin and related TFG-β family members by binding them and preventing them from binding to the endogenous cell surface ActRIIb receptor, increased muscle mass and decreased fat mass in mice fed either standard chow or a high fat diet (1). Taken together, these studies clearly demonstrate that the inhibition of myostatin signaling can greatly attenuate the development of obesity and its adverse health consequences in mice.

The anti-obesogenic effects of myostatin inactivation raise the important question of whether this may be due to its direct effects on adipocyte hypertrophy or hyperplasia. In fact, several lines of evidence suggest that adipocytes may respond directly to myostatin signaling. First of all, adipocytes express the ActRIIb receptor both *in vitro* (52) and *in vivo* (2). Furthermore, ActRIIb mRNA expression is increased dramatically in adipocytes of ob/ob mice (2), suggesting that myostatin signaling may be increased in adipocytes in the obese state. However, levels of both myostatin and ActRIIb mRNA in adipose of obese mice remained several orders of magnitude lower than their levels in skeletal muscles of lean or obese mice (2), which suggests that this expression may be too low to evoke biologically meaningful responses in adipose.

Nevertheless, functional studies have shown that myostatin treatment influences adipocyte differentiation *in vitro*. Myostatin treatment inhibits adipogenic differentiation of the 3T3-L1 cell line and of human bone marrow-derived mesenchymal cells *in vitro* (14, 24, 52). In addition, while myostatin treatment promoted the commitment of C3H 10T1/2 mesenchymal cells to an adipogenic lineage *in vitro* (4, 10), it produced smaller, more immature adipocytes (10). Similarly, adipose-specific transgenic over-expression of myostatin *in vivo* also resulted in decreased adipocyte size *in vivo* although body composition was unchanged (10). However, these mice were resistant to dietary obesity due to increased energy expenditure (10). Together these studies have suggested that myostatin may directly inhibit differentiation and/or growth of adipocytes, though this is counter to the beneficial effects of myostatin inactivation on obesity. Moreover, while these studies have demonstrated that treating adipocyte precursors in culture with myostatin, or exogenously elevating myostatin expression within adipose via transgenic means, is sufficient to decrease adipocyte size *in vitro* and *in vivo*, they do not address whether changes in *endogenous* myostatin expression or activity are necessary for and/or contribute to the progression of obesity.

A recent study has more directly addressed this issue. Specifically, adipose vs. muscle-specific promoters were used to drive expression of a dominant negative ActRIIb gene construct to inhibit myostatin signaling to assess the relative contributions of myostatin inhibition in these two tissues to the progression of dietary obesity. Mice with muscle-specific inhibition of myostatin signaling fed a standard diet had greater muscle mass and decreased adipose depot weights and adipocyte size compared to wild type mice. Furthermore, they gained significantly less weight and had lower fasting blood glucose, insulin, resistin, leptin and triglycerides in response to 10 weeks on a high fat diet compared to wild type mice (13). In contrast, mice with adipose-specific inhibition of myostatin

signaling showed no alterations in lean or fat mass, and no changes in circulating glucose, insulin, or adipokine levels on either a standard or high fat diet compared to wild type mice (13). These data strongly suggest that the beneficial effects of myostatin inhibition on attenuating the adverse consequences of dietary obesity stem predominantly from its effects on skeletal muscle.

A study by Bernardo et al. (6) supports the hypothesis that myostatin inhibition attenuates the adverse metabolic consequences of obesity primarily through its effects on muscle and not through changes in adipose growth. Injection of ob/ob mice with a neutralizing antibody to myostatin decreased serum glucose and fatty acid levels; these changes were accompanied by increases in muscle mass, energy expenditure, and habitual activity levels but were not associated with a change in adipose mass (6). Also consistent with the interpretation that myostatin does not affect adipocyte size via direct changes in fat storage is work showing that administration of recombinant myostatin had no effect on lipid release in adipocytes *in vitro* or on fat mass in mice *in vivo* (60).

Thus the emerging consensus is that the majority of the beneficial effects of myostatin inhibition on limiting the negative metabolic consequences of obesity appear to be an indirect consequence of the loss of myostatin signaling in skeletal muscle. Whether this is a result of increased muscle mass alone, or is a result of other changes downstream of myostatin signaling in muscle that affect metabolism, is not clear. Muscular transgenic mice overexpressing genes in the insulin-like growth factor signaling pathway under control of muscle-specific promoters also have decreased adiposity and improved metabolic parameters (22), suggesting that the increase in muscle mass caused by myostatin inhibition alone may account for the resistance to adipose tissue accumulation found in animals with myostatin inhibition in muscle. Specifically, the improvements in blood glucose, insulin, and/or fatty acid levels with myostatin inactivation may simply reflect an increase in substrate partitioning towards this greater lean muscle mass. In summary, the direct effects of myostatin on adipose growth and metabolism appear to be minimal, at least in the presence of adequate nutrition, and likely produce only a minor contribution to the changes occurring during the obese state.

MYOSTATIN AND DIABETES

Recent evidence suggests that myostatin may play a role in the development of diabetes in addition to, and perhaps independent of, its effect on obesity. For instance, gene chip analysis revealed that myostatin mRNA levels were elevated in skeletal muscle biopsies from type 2 diabetics as well as from non-obese but hyperinsulinemic relatives of type 2 diabetics (47). In addition, both muscle and plasma myostatin protein levels in insulin-resistant middle-aged men were decreased by aerobic exercise training and were strongly correlated with insulin sensitivity (20). These subjects experienced decreases in circulating myostatin, insulin, and glucose levels that were not accompanied by any change in fat mass or body mass index following exercise training, strongly suggesting that these changes were not secondary to a change in adipose mass (20). Furthermore, Bernardo et al. (6) showed improvements in fed and fasted blood glucose levels without any change in adipose mass in ob/ob mice injected for six weeks with a neutralizing antibody to myostatin. Lastly, injection of recombinant myostatin decreased insulin sensitivity in healthy male mice (20) and in mice harboring a loss-of-function mutation to myostatin on a high fat diet (67) without a corresponding change in body mass in either study. Together these studies have suggested that increased myostatin expression is inversely related to insulin sensitivity independent of obesity status.

These mice receiving short-term injections of recombinant myostatin in these latter two studies had decreased insulin sensitivity not only in the absence of a change in adipose mass, but also in the absence of a change in muscle mass (20, 67). This suggests that myostatin might directly regulate skeletal muscle glucose uptake or utilization independent of its effects on muscle mass. In this regard, myostatin has been found to directly influence glucose uptake and utilization in a cell specific manner. For instance, myostatin treatment inhibited glucose uptake of BeWo cells, a choriocarcinoma cell line (3) yet increased glucose uptake by human placental tissue (43). Furthermore, myostatin treatment increased glucose uptake and glycolysis and inhibited glycogen synthesis in cultured skeletal muscle cells *in vitro* via an AMP kinase-dependent mechanism (8). The findings that myostatin increases muscle glucose uptake and glycolysis are at odds with the previously described inverse relationship between myostatin levels and insulin sensitivity, and it is not clear to what extent these *in vitro* results reflect the state of muscle glucose uptake *in vivo*. Regardless, these data suggest that myostatin may influence circulating glucose levels via direct affects on glucose uptake independent of its effects on muscle growth.

Myostatin might also affect glucose uptake indirectly through its effects on TNF-α expression, which can antagonize the effects of insulin on glucose uptake (44). A loss-of-function myostatin mutation in mice increased glucose tolerance and protected against insulin resistance in response to a high fat diet, and decreased circulating TNF-α levels as well as TNF-α mRNA levels in both muscle and adipose (67). Conversely, treatment with recombinant myostatin increased systemic TNF-α levels in myostatin mutant mice on a high fat diet and resulted in greater insulin resistance (67).

A final question related to myostatin's role in regulating systemic glucose metabolism centers on the effects of myostatin on the liver. Increased hepatic gluconeogenesis is a consequence of hepatic insulin resistance and contributes to elevated blood glucose levels in type II diabetics (55), and several studies have shown that myostatin appears to modulate hepatic insulin resistance and/or glucose production. For instance, injection of mice with a recombinant soluble ActRIIb receptor increased hepatic insulin sensitivity (1). Similarly, mice homozygous for a myostatin loss-of-function mutation fed a high fat diet had reduced hepatic steatosis and increased insulin-dependent suppression of hepatic glucose production compared to wild type mice (67).

It is possible these effects of myostatin on liver glucose metabolism are indirect due to the favorable effects of muscularity on energy partitioning. However, the inactivation of the gene for the myostatin binding and inhibitory protein FSTL3, which would be expected to increase myostatin activity, resulted in greater hepatic steatosis and hypertension in mice in the absence of any change in muscle mass (46). Furthermore, injection of recombinant myostatin in wild type mice on a standard diet decreased insulin-stimulated hepatic Akt phosphorylation without affecting muscle or adipose mass (20), suggesting that insulin signaling might be directly inhibited by myostatin in the liver. In addition, there is evidence that the liver may be directly responsive to myostatin signaling. The mouse liver as well as cultured mouse and human liver cell lines are responsive to activin, which binds to the same receptors as myostatin (7, 69). Consistent with this, myostatin administration can induce reporter gene activity in the HepG2 hepatocyte cell line *in vitro* (54).

Thus several lines of evidence suggest that myostatin may influence liver function. A key question is whether the liver is capable of activating the latent myostatin complex. Future studies in which myostatin signaling has been selectively inactivated or overexpressed in the liver will directly address these issues.

MYOSTATIN AND ENDURANCE EXERCISE ADAPTATION

Studies that have examined the relationship between myostatin expression and endurance exercise adaptation have generally demonstrated changes in myostatin expression complementary to those observed with obesity and/or diabetes, i.e., endurance exercise training is usually characterized by a decrease in myostatin expression. For example, myostatin mRNA levels were decreased by more than half in gastrocnemius and vastus lateralis muscles 24 hours after the last bout of a five-day swim training regimen in rats (34). Similarly, nine weeks of cycling endurance exercise training decreased by over half myostatin mRNA levels from vastus muscle biopsies taken immediately post-exercise from human hemodialysis patients (28). Myostatin mRNA levels decreased by approximately half in vastus lateralis muscle biopsies taken from older women 48 hours after the final bout of a 12-week training regimen of cycle ergometry (27). Moreover, as mentioned above, muscle and plasma myostatin protein levels decreased after 6 months of low intensity aerobic training in pre-diabetic male subjects (20).

Myostatin mRNA levels appear to be decreased in response to a single bout of endurance exercise training as well. Myostatin mRNA levels were decreased approximately 3–4-fold in gastrocnemius biopsies 8 and 12 hours after a single bout of running for 30 minutes at 75% VO_{2max} in physically active men and women (16), and were similarly decreased approximately 3-fold in soleus and vastus lateralis biopsies taken 4 hours after, but were not altered 24 hours after, a running bout of 45 minutes at 75% VO_{2max} in trained men (33). Together with the results above, these data suggest that a decrease in myostatin expression is a hallmark of both the response to chronic exercise training as well as to a single bout of endurance exercise.

Some recent studies suggest that transgenic myostatin inactivation decreases endurance exercise performance in untrained mice. Untrained myostatin null mice show a 38% lower work output in an involuntary treadmill run to exhaustion compared to untrained wild type mice (58), and a reduced swim time to exhaustion (37), compared to wild type mice. In addition, inducible postnatal myostatin deletion in muscle decreased voluntary wheel running primarily through a decrease in nightly running bout number (49).

The reasons for the decreased endurance performance in myostatin null mice have not been definitively established, but two explanations have been postulated. First, myostatin null mice have a greater percentage of fast/glycolytic fibers compared to wild type mice (11, 17), and thus the decrease in endurance exercise performance with myostatin inactivation may reflect the greater percentage of fatigable fast-twitch glycolytic fibers in the untrained state. Consistent with this interpretation, Matsakas et al. demonstrated that blood lactate levels were significantly elevated 1 and 8 minutes following a swimming bout in myostatin null mice compared to wild type mice (37). Second, the greater skeletal muscle fiber cross-sectional area of untrained myostatin null mice compared to wild type mice (39) may adversely affect diffusion of oxygen or energy substrates into the contracting muscles in the untrained state.

Despite their poorer endurance performance pre-exercise, myostatin null mice nevertheless are able to increase comparably to wild type mice activity of the mitochondrial enzymes citrate synthase in response to run training (58) and succinate dehydrogenase in response to swim training (37), respectively. Myostatin null mice also underwent a similar IIb to IIa shift in myosin heavy chain expression as wild type mice in response to wheel running training (37). Thus myostatin inactivation does not adversely affect the contractile and metabolic fiber type adaptations accompanying endurance exercise training. Moreover, any differences in post-training performance do not appear to be driven by an inability of

myostatin inactivated animals to carry out normal fiber type shifts and/or increases in muscle oxidative potential with endurance training. On the other hand, plantaris muscle fiber cross sectional area were significantly increased in wild type mice but actually decreased in myostatin null mice in response to five weeks of voluntary wheel running training (37). These authors suggested that the decrease in fiber cross-sectional area in myostatin null mice may have contributed to their decreased endurance exercise performance relative to wild type mice by decreasing power output per unit time (37).

Interestingly, myostatin inhibition through injection of a myostatin neutralizing antibody potentiated exercise performance when combined with endurance exercise training in older mice. While four weeks of injection of a myostatin neutralizing antibody without daily treadmill training had no effect on treadmill performance parameters in 24-month old mice, myostatin inhibition combined with treadmill training actually increased treadmill run time and distance to exhaustion compared to untrained control mice and trained mice not injected with the anti-myostatin antibody (30). This improvement in exercise function may reflect the effects of myostatin inhibition on attenuating sarcopenia, the age-associated loss in muscle mass, resulting in decreased muscle mass loss with age that allowed these older animals to maintain greater power output during exercise. Consistent with this, while the anti-myostatin treatment in these aged mice increased muscle mass by less than 15%, it attenuated the loss in muscle mass due to aging alone (30). In addition, unlike myostatin null mice (11), adult mice treated with anti-myostatin approaches do not undergo an increase in glycolytic fiber types compared to untreated mice (15, 35), and this too may have allowed these mice to improve their endurance exercise performance in contrast to the decrement in performance demonstrated for myostatin null mice. Future experiments will need to explore separately the effects of age and adult onset myostatin inhibition on exercise performance to better delineate the effects of myostatin inhibition on endurance exercise performance.

A final point of relevance to the effects of myostatin on endurance exercise performance concerns the expression and function of myostatin in the heart, but here the results have not been consistent. Myostatin is expressed in the heart (5), and myostatin mRNA levels were increased approximately 2-fold in the heart by four weeks of swim training five times per week 90 minutes per bout in rats (35). However, four weeks of twice-daily 30 minute treadmill running had no effect on cardiac myostatin protein levels in rats (32). At present it is not clear whether the conflicting results of these two studies reflect the differences in exercise modality or in mRNA vs. protein evaluation utilized between the two studies. In addition, the function of myostatin in cardiac adaptations to endurance exercise is not clear. Cardiac-specific over-expression of myostatin decreased while myostatin inactivation increased heart mass and left ventricular mass in mice, but neither affected resting cardiac ejection fraction in otherwise healthy mice (5). At present, the interaction between myostatin inactivation and endurance exercise adaptation on cardiac size or function has not been addressed, specifically whether myostatin inactivation affects the physiological hypertrophy of the heart associated with endurance exercise training.

In summary, a decrease in myostatin expression in muscle appears to be a hallmark of endurance exercise training in mice and in humans. Interestingly, lifelong myostatin inactivation limits aerobic exercise performance in mice, possibly due to a larger proportion of glycolytic fibers and larger fiber size in untrained myostatin null mice, while myostatin inactivation combined with exercise training in older adult mice appears to increase endurance exercise performance. Moreover, myostatin inactivation does not prevent mice from undergoing the slow-to-fast fiber type transitions accompanying endurance exercise training, but reverses the increase in fiber size with endurance training, which may in turn decrease fiber power output.

MYOSTATIN AND RESISTANCE EXERCISE ADAPTATION

Myostatin inhibition could be considered a resistance exercise mimetic in that myostatin inactivation results in dramatic muscle hypertrophy. Because of this well-characterized effect of myostatin inhibition on muscle mass, it is plausible that a reduction in myostatin expression or function might be required for muscle to hypertrophy in response to resistance exercise. Indeed, several studies have shown that resistance exercise produces a significant decrease in skeletal muscle myostatin mRNA expression. Moreover, like endurance exercise training, this decrease appears to be a hallmark of both chronic (after a training regimen) vs. acute (after a single bout) resistance exercise. For example, a 37% decrease in myostatin mRNA in the vastus lateralis of the dominant leg was observed in biopsies taken from young and old men and women 48–72 hours after the final bout of a 9 week heavy-resistance unilateral knee extension training program using a pneumatic resistancemachine on which subjects trained 3 days per week completing 50 repetitions performed at near maximal resistance (56). With respect to an acute bout of resistance exercise training, Raue et al. observed a 2.2-fold decrease in myostatin mRNA in biopsies of young and old women taken 4 hours after a single bout of 3 sets of 10 repetitions of 70% maximum knee extension (51) while Kim et al. reported a 44% decrease in myostatin mRNA levels in biopsies taken 24 hours after a single bout of knee extensor resistance exercise in young and old male and female subjects (25).

A decrease in myostatin levels in muscle, however, has not been found in all studies on resistance exercise. Willoughby demonstrated a significant *increase* in muscle myostatin mRNA and protein in the vastus as well as increased serum myostatin protein levels in muscle biopsies and blood samples taken 15 minutes after the final bout of a 6 or 12 week lower limb resistance training program involving 3 weekly bouts of 3 sets of 6–8 repetitions of 85–90% of one rep max of leg press and knee extension (66). Also, myostatin mRNA levels were not changed in vastus biopsies taken 4 hours after a single bout of leg extension exercise in either endurance or resistance trained subjects (9). The reason for the discrepancy between these studies and the ones described above is not clear, but may reflect variations in the training regimens employed in terms of rest, repetition number, contraction intensity, training status, etc., as well as differences in biopsy sampling time (48–72 hours vs. 15 minutes-4 hours post-exercise) between the two studies. Consistent with the fact that choice of sampling time may greatly influence interpretation of post-exercise myostatin expression data, Hulmi et al. (21) demonstrated that myostatin mRNA levels were not significantly affected 1 hour following the final bout of a 21-week knee flexion and extension resistance training regimen but were significantly decreased 48 hours post-exercise.

Other authors have attempted to determine whether systemic myostatin protein levels are changed by resistance training. Walker et al. showed that plasma myostatin protein levels were decreased by approximately 20% by Western blot in subjects following 10 weeks of 2 daily bouts of either elbow flexor exercise or whole body exercise, though they did not differ between the two exercise paradigms (64). Saremi and coworkers also reported a modest but significant 10% decrease in serum myostatin levels by enzyme-linked immunosorbent assay (ELISA) following 12 weeks of arm and leg press resistance training that was potentiated by creatine supplementation (57). However, Kim et al. recently reported no change in serum myostatin levels by Western blot following 16 weeks of knee extensor resistance training, and reported high variability in serum myostatin levels even between untrained subjects (26). The differences between these studies may reflect differences in sampling time post-training and/or post-bout as well as the confounding effects of differences in myostatin efflux and/or clearance from the systemic circulation between subjects or between exercise paradigms. Thus it does not appear that circulating myostatin

levels alone can be used as a reliable quantitative marker for training status nor do they appear to accurately reflect the mass of exercised muscle.

In summary, the effects of resistance exercise on myostatin expression appear to depend upon aspects of the training regimen as well as the sampling time examined, and may differ in either timing or magnitude for muscle mRNA vs. muscle protein vs. serum protein. Moreover, the exact role of changes in myostatin expression in response to resistance exercise is not clear. For example, an experiment in which myostatin over-expressing transgenic mice are resistance exercise trained in order to determine whether myostatin inhibits resistance-induced hypertrophy has not been carried out. But in a recent cluster analysis on resistance trained humans, while myostatin mRNA levels significantly decreased in muscle biopsies 24 hours after a single knee extensor resistance exercise bout and 24 hours after completing the final bout of a 16 week training regimen, the decrease was the same for non-, modest-, and extreme-responders with respect to changes in muscle fiber size, suggesting that the magnitude of decrease in myostatin expression was not associated with greater hypertrophic growth in response to resistance training (26). Thus it is not clear to what extent decreases in myostatin expression may be a prerequisite for the muscle fiber hypertrophy accompanying resistance training in humans.

SUMMARY AND CONCLUSIONS

Since its discovery in 1997, a vast wealth of studies has supported a strong central role for myostatin in the regulation of muscle growth. In addition, much research has established that myostatin has profound effects on metabolism and may promote the progression of obesity and diabetes. In contrast, aerobic exercise attenuates myostatin expression, and myostatin inactivation appears to potentiate the beneficial effects of endurance exercise on metabolism. Many of these effects appear to be indirect consequences of the effects of myostatin inhibition on skeletal muscle growth, increasing the lean tissue metabolic platform available for glucose and fatty acid uptake and utilization. However, myostatin appears to also have effects on skeletal muscle as well as other non-muscle tissues (adipose, liver) that may modulate metabolism independent of or in addition to its effects muscle growth. Future studies will need to explore in more depth the contribution of direct vs. indirect effects of myostatin on these non-muscle tissues.

Furthermore, some strategies for myostatin inhibition, such as FSTL3 and soluble ActRIIB treatment, likely inhibit other TGFβ ligands in addition to myostatin. Several lines of evidence demonstrate that myostatin is not the only TGFβ family member that can inhibit muscle mass. Compared to wild type mice, myostatin null mice expressing a follistatin transgene in muscle have an even greater increase in muscle mass than myostatin null mice, up to 4-fold compared to 2- to 2.5-fold, respectively (31). Different methods of inhibition may therefore have differing effects on metabolism due to their differing effects on substrates other than myostatin. This is not simply an academic question; pharmaceutical therapies targeting myostatin inactivation to prevent muscle atrophy will need to take into consideration possible metabolic consequences of myostatin inactivation on other tissues and the non-specific effects of different modes of myostatin inactivation so as to minimize potentially unwanted side effects. Given the effects observed in mice, the pharmaceutical potential of myostatin inhibitors is huge. Most current efforts have focused on the development of myostatin inhibitors to alleviate muscle atrophy in response to clinical wasting or prolonged muscle unloading, but clearly the beneficial effects of myostatin inhibition on metabolism and attenuation of obesity and diabetes represent another highly lucrative avenue of exploration, particularly given the poor track record of many diabetes drugs in decreasing myocardial infarction risk.

Future studies will undoubtedly refine and extend our current understanding of myostatin's roles, both direct and indirect, in metabolism. While the initial observations of the effects of global myostatin inactivation on the development of obesity and diabetes were obviously critical to our understanding of myostatin's role in these processes, more recent studies using tissue-specific myostatin inactivation have greatly advanced our understanding of the relative contribution of myostatin's effects on skeletal muscle and its effects on adipose. Future/additional studies employing neutralizing antibodies, or inducible, tissue-specific transgenics, will allow researchers to explore the role of myostatin in obesity, diabetes and exercise adaptation without the confound of its effect on prenatal and/or early postnatal development. These and other elegant studies will greatly expand our knowledge of myostatin's role in regulating metabolism.

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