Myostatin expression is increased by food deprivation in a muscle-specific manner and contributes to muscle atrophy during prolonged food deprivation in mice

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Submitted 10 May 2010; accepted in final form 25 June 2010

Allen DL, Cleary AS, Lindsay SF, Loh AS, Reed JM. Myostatin expression is increased by food deprivation in a muscle-specific manner and contributes to muscle atrophy during prolonged food deprivation in mice. J Appl Physiol 109: 692–701, 2010. First published July 1, 2010; doi:10.1152/japplphysiol.00504.2010.—During food deprivation (FD), skeletal muscle protein is broken down to produce amino acids for hepatic gluconeogenesis to maintain blood glucose levels. However, it is unclear what role, if any, the secreted antigrowth factor myostatin (MSTN) plays in the muscle atrophy induced by FD. We therefore examined expression and function of MSTN in FD in mice. Two days of FD significantly decreased muscle mass and protein content and increased mRNA levels of ubiquitin ligases MuRF-1 and atrogin-1 in fast-twitch tibialis anterior (TA) muscle but not slow-twitch soleus (Sol) muscle, while 2 days of refeeding returned these to fed values in TA. MSTN mRNA levels were significantly increased approximately threefold after 2 days of FD but not 1 day, of FD and returned to fed levels with 2 days of refeeding in TA but were not significantly affected by FD or refeeding in Sol. TA mass decreased to a similar amount after 1 day of FD in wild-type mice and mice null for the MSTN gene but was decreased to a greater amount in wild-type than MSTN-null mice after 2 days of FD. However, blood glucose levels decreased and corticosterone levels increased approximately threefold by 2 days, but not 1 day, of FD and returned to fed levels with 2 days of refeeding in TA but were not significantly affected by FD or refeeding in Sol. TA mass decreased to a similar amount after 1 day of FD in wild-type mice and mice null for the MSTN gene but was decreased to a greater amount in wild-type than MSTN-null mice by 2 days of FD. In addition, blood glucose levels decreased and corticosterone levels increased to a greater extent in MSTN-null mice after 2 days of FD, but surprisingly muscle MuRF-1 and atrogin-1 mRNA levels were not affected by the lack of MSTN during FD. Similarly, changes in hepatic enzyme expression in response to FD were identical between wild-type and MSTN-null mice. These data are consistent with the hypothesis that MSTN is dispensable for the initial atrophy occurring in response to FD but attenuates the decrease in fast-twitch muscle mass during prolonged FD.

fasting; metabolism; starvation; transgenic mice

DURING PERIODS OF FOOD DEPRIVATION (FD), maintenance of blood glucose levels is of paramount importance to maintaining normal brain function. Food-deprived organisms therefore initiate a series of physiological mechanisms designed to allow them to maintain blood glucose levels in a state of zero intake. For example, organisms typically decrease their activity levels and/or body temperature in an effort to reduce caloric expenditure and glucose utilization by nonessential tissues (54). In addition, energy stores are mobilized from several tissues to provide a continued source of nutrients for energy production. Skeletal muscle in particular plays a critical role in this process: skeletal muscle protein is broken down to amino acids (10), which can then be converted into glucose in the liver by gluconeogenesis (41). This results in a rapid and dramatic atrophy of skeletal muscle during the food-deprived state, and this atrophy is critical to the maintenance of glucose homeostasis during FD (46).

One important protein that regulates muscle size is myostatin (MSTN). MSTN is a secreted factor expressed by skeletal muscle that inhibits muscle growth at least in part by antagonizing the phosphatidylinositol 3-kinase (PI3-kinase)/Akt/mammalian target of rapamycin (mTOR) progrowth pathway (22, 34, 42, 57, 64), which regulates muscle atrophy during FD and other atrophic states (13, 26, 52, 63). MSTN gene inactivation is associated with dramatic muscle growth in mice, dogs, cows, and humans (14, 36, 37, 44, 53), and overexpression of MSTN in transgenic mice is sufficient to induce muscle atrophy in vivo (48). Moreover, expression data from a wide variety of models have suggested that an increase in endogenous MSTN expression is a key aspect of every type of muscle atrophy examined to date (7, 9, 11, 23, 47). Together these data have supported the hypothesis that MSTN is a primary determinant of muscle size.

However, despite this wealth of information on the expression and function of MSTN during wasting conditions, it is not currently clear whether its expression is increased during FD, nor is it clear whether MSTN plays any mechanistic role in modulating the compensatory mechanisms associated with FD. Both increases (15, 21) and decreases (49, 56) in MSTN expression have been demonstrated during FD, depending on the species and the duration of FD. And while inactivation of the MSTN gene greatly attenuates muscle responsiveness to some forms of muscle atrophy, including glucocorticoid-mediated muscle atrophy (12), not all atropic states appear to be MSTN dependent (35, 43).

The purpose of the present work was to examine the expression of MSTN in both fast- and slow-twitch skeletal muscle in response to FD and to determine whether MSTN may play a role in FD-induced muscle atrophy. We demonstrate that expression of MSTN was increased by FD in the predominantly fast-twitch/glycolytic tibialis anterior (TA) muscle but not in the more slow-twitch/oxidative soleus (Sol) muscle and that this mirrors the changes in mass, protein content, and atrogenic expression experienced by TA but not Sol in response to FD. Moreover, our results show that the influence of MSTN on FD-induced muscle atrophy is subtle and complex: while MSTN is dispensable for the atrophy occurring in response to 1 day of FD, MSTN-null mice showed an attenuated decrease in body and TA muscle mass in response to 2 days of FD. MSTN-null mice also experienced a greater decrease in blood glucose levels and a greater increase in corticosterone levels in response to 2 days of FD than wild-type mice, suggesting that the reduction in MSTN-dependent muscle atrophy in these mice caused an even greater homeostatic shift than that expe-
rienced by wild-type mice in response to prolonged FD. Thus increased muscle-specific MSTN expression is a hallmark of FD, and MSTN appears to play a time- and muscle-specific contributory role to the maintenance of blood glucose levels during prolonged food-deprived states.

METHODS

Experimental animals. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of the University of Colorado, Boulder, and complied with the guidelines of the American Physiological Society on the use of laboratory animals. Male wild-type C57/black6j mice were obtained from our breeding colony in the Department of Integrative Physiology at the University of Colorado, Boulder. Homozygous MSTN-null mice were kindly provided by Dr. Se-Jin Lee of Johns Hopkins University School of Medicine.

Food deprivation studies. For the initial FD studies on wild-type C57 mice to examine the effects of FD on MSTN and atrogin expression, 3-mo-old male C57 mice (n = 7 each) had ad libitum access to food (fed), had the food removed from the wire tops of their cages for 2 days (food deprived), or had the food removed from the wire tops of their cages for 2 days followed by ad libitum access to food for an additional 2 days (food deprived + refeed). All animals had ad libitum access to water throughout the entire experimental period. At the end of the treatment periods, mice were killed and TA and Sol were isolated, weighed, frozen in liquid nitrogen, and stored at −80°C until use.

Similarly, for the subsequent study on the effects of FD on MSTN-null mice, 2.5- to 3.5-mo-old wild-type and homozygous MSTN-null mice (n = 4 or 5 per group) were fed ad libitum, food deprived for 1 day, or food deprived for 2 days. Mice were weighed before FD and then each day to quantify body mass, and at the end of the deprivation time point mice were killed by decapitation after brief (<10 s) inhaled anesthesia exposure. Trunk blood was collected, allowed to sit on ice for 20–30 min, and centrifuged, and serum was isolated, weighed, frozen in liquid nitrogen, and stored at −80°C until use.

In all the experiments reported in this article, animals were killed and blood and tissue samples were collected at the end of the dark cycle, i.e., first thing in the morning, so as to avoid diurnal fluctuations in glucocorticoid and/or glucose levels, and typically completed by 10:00 AM. Moreover, mice were not killed in groups by genotype, but rather were killed in alternating fashion so that no single group was completed before the other.

Quantitative real-time RT-PCR. RNA was isolated from skeletal muscle and liver samples by the TRIzol method as described previously (1–4). The reverse transcription (RT) reaction was carried out with 0.5 μg of RNA and the cDNA Archive kit (Applied Biosystems) according to the manufacturer’s protocol. Primer and probe sets for MSTN, atrogin-1, MuRF-1, glucokinase, phosphoenolpyruvate carboxykinase (PEPCK), and β-actin were obtained from Applied Biosystems. All real-time PCR procedures were run in triplicate to correct for variances in loading. In addition, a standard curve ranging from 10- to 0.001-μg dilutions of mouse TA (for MSTN, atrogin-1, MuRF-1) or 2-day food-deprived liver (for glucokinase and PEPCK) cDNA was run in duplicate for each assay to produce a standard curve for quantification. All values are expressed as the mean of the triplicate measure for the experimental divided by the mean of the triplicate measure of β-actin for each sample.

Western blotting. TA muscle was homogenized in 1× RIPA buffer (Pierce) containing (in mM) 2 sodium orthovanadate, 200 sodium fluoride, 40 sodium pyrophosphate, and 2 phenylmethylsulfonyl fluoride, with 1× phosphate inhibitor cocktail (Sigma) and 1/4th of a complete EDTA-free protease inhibitor tablet (Roche). After centrifugation at 5,000 g for 15 min, the supernatant was frozen until use. Total protein concentration was determined with the Bradford reagent (Bio-Rad). Because of the small size of the Sol muscle, both solei were homogenized for RNA isolation and thus were not able to be used for protein analysis.

For Western blotting, 40 μg of total protein was loaded per lane of each sample onto a 10% acrylamide gel and run at 100 V for 2 h to separate proteins. Proteins were transferred to polyvinylidene difluoride (PVDF) membrane at 90 V for 3 h with standard techniques. After transfer, membranes were air dried for 30 min and then rinsed briefly in 100% methanol before incubation for 10 min in Tris-buffered saline containing 0.1% Tween 20 (TTBS). Membranes were blocked overnight in TTBS containing 1% bovine serum albumin (BSA) at 4°C and then incubated in this same blocking solution containing primary antibody for 1 h at room temperature. The primary antibody used in this study was a polyclonal rabbit anti-MSTN antibody (Chemicon). After several rinses in TTBS and three 5-min incubations in TTBS, the membrane was incubated in the secondary antibody solution diluted in blocking solution. The secondary antibody was a donkey anti-goat IgG horseradish peroxidase (HRP) conjugate (Santa Cruz). After several rinses in TTBS and three 5-min incubations in TTBS, bands were visualized with the Pierce SuperSignal West Femto chemiluminescence kit and scanned with a chemiluminescent imager. Blots were then stripped with a commercial stripping agent (Restore Plus; Pierce) and reprobed with a rabbit anti-human β-actin antibody at 1/1,000 in TTBS, followed by a goat anti-rabbit secondary at 1/1,000, and visualization was carried out as above. Blots were densitometrically scanned with a gel documentation system and analyzed with ImageJ software, and bands were quantified after subtraction of background. Values are reported as arbitrary densitometric units, and MSTN values were normalized to those of β-actin.

Blood measurements. Corticosterone levels were quantified on 5 μl of plasma diluted in 200 μl of dilution buffer with a commercially available ELISA kit (Assay Designs). Blood glucose levels were quantified from 2 μl of plasma with a OneTouch Ultra glucometer (LifeScan).

Statistical analysis. All in vivo studies represent n = 4–7 animals per condition, and data from these studies are reported as means ± SE. Statistically significant differences between fed, fasted, and refeeding for body mass, tissue mass, protein content, or mRNA expression in wild-type mice were determined by one-way analysis of variance (ANOVA) and Fisher’s post hoc test, with an α-level of 0.05 taken as significant. Differences in the loss in body mass and muscle mass, atrogin-1 and MuRF-1 expression, liver enzyme MRNA levels, and serum corticosterone and glucose levels between wild-type and MSTN-null mice after 1 and 2 days of FD were determined by two-way ANOVA with time (1 and 2 days of FD) and genotype (wild type vs. MSTN null) as the independent measures, and again a P level of <0.05 was taken as significant.

RESULTS

Effects of food deprivation and refeeding on tissue mass, total protein content, and atrogene expression. Two days of FD significantly decreased mean body mass compared with fed mice (Table 1). TA mass was significantly decreased by FD compared with the TA of fed mice, while Sol mass was only

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<th>Table 1. Body mass, muscle mass, and total protein values for fed, FD, and RF mice</th>
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Values shown are means ± SE. FD, food deprivation; RF, refeeding; TA, tibialis anterior; Sol, soleus. *Significantly different from fed (P < 0.05).
modestly and nonsignificantly decreased by 2 days of FD in these studies (Table 1). Moreover, TA mass, while only 0.2 mg greater in the refed group than in the FD group, was nevertheless not significantly different from that of the fed or the FD mice (Table 1). Two days of FD also significantly decreased total protein content in TA but not Sol, and protein concentration was not significantly altered in either muscle by FD or refeeding. Bars represent means ± SE of \( n = 4 \) mice/group. *Significantly different from fed muscle \((P < 0.05)\).
In contrast, in Sol MSTN mRNA levels relative to β-actin were unaffected by FD or refeeding (Fig. 3B). The increase in MSTN mRNA with FD was a relatively late event in this process, as MSTN mRNA levels in the TA did not significantly increase after 1 day of FD (Fig. 3C) even though muscle mass of the TA significantly decreased at this time (Fig. 3B). However, the increase in MSTN mRNA with 2 days of FD was accompanied by a significant increase in the unprocessed ~51-kDa form of the MSTN protein as shown by Western blotting (Fig. 3D). We were unable to visualize a band for the mature, processed forms of MSTN (data not shown).

MSTN-null mice experience smaller losses in TA muscle mass with 2 days of FD. As reported elsewhere (37), both body mass (22.4 ± 0.6 and 24.0 ± 0.5 g for wild type and MSTN null, respectively) and muscle mass (35.8 ± 2.2 and 54.7 ± 0.7 mg for wild-type and MSTN-null TA, respectively) were significantly greater for fed MSTN-null mice relative to wild-type mice. To correct for this discrepancy in starting body and muscle masses, all data in Fig. 4 are reported as percentages of the fed condition for wild-type and for MSTN-null mice. Two-way ANOVA revealed both time and genotype effects on body mass loss with FD. Both wild-type and MSTN-null fed animals tended to lose a small and nonsignificant amount of body mass as a consequence of the stress of daily handling across the 2 days of the study (Fig. 4A). Wild-type mice significantly lost body mass relative to starting body mass after 1 day of FD and significantly lost further mass after 2 days of FD. MSTN-null mice lost a relative amount of body mass similar to wild-type mice after 1 day of FD, but the relative percentage of lost body mass was slightly but significantly lower after 2 days of FD, ~25% of starting fed mass for MSTN-null mice compared with nearly 30% of starting fed mass for wild-type mice (Fig. 4A). Similarly, the relative TA muscle mass loss was the same after 1 day of FD, but the loss in relative TA mass was significantly attenuated in MSTN-null mice compared with wild-type mice after 2 days of FD (11.5% vs. 29%; Fig. 4B). In other words, MSTN-null mice lost <40% of the TA mass that wild-type mice lost with 2 days of FD. In this study, wild-type mice lost a small but significant amount of Sol mass, but MSTN inactivation had no effect on this more moderate loss in relative Sol mass with 1 or 2 days of FD (Fig. 4C).

MSTN-null mice experience similar increases in atrogene levels with FD. Two-way ANOVA revealed effects of time but not genotype on atrogene expression. FD was again associated with a significant increase in atrogin-1 and MuRF-1 mRNA levels in wild-type mice (Fig. 5); MuRF-1 mRNA levels significantly increased after 1 day of FD, and atrogin-1 mRNA levels showed a similar trend but were not significantly different at this time point. Both atrogin-1 and MuRF-1 mRNA levels significantly increased further after 2 days of FD relative to fed control and 1 day of FD (Fig. 5). Similarly, both atrogin-1 and MuRF-1 mRNA levels increased significantly in MSTN-null mice after either 1 or 2 days of FD and were not significantly different from those of wild-type mice at either time point (Fig. 5).

MSTN-null mice experience greater blood glucose and glucocorticoid changes with FD. We next compared both corticosterone and blood glucose levels in fed and food-deprived wild-type and MSTN-null mice. Corticosterone levels significantly increased in wild-type mice after 1 day of FD and increased further after 2 days of FD (Fig. 6A). MSTN-null mice showed a similar pattern, but corticosterone levels were significantly greater than those of wild-type mice at both the 1 and 2 day FD time points (Fig. 6A). Moreover, blood glucose levels decreased significantly in wild-type mice after 1 day of FD and...
remained significantly lower than those of fed mice after 2 days of FD but did not significantly decrease further beyond that observed after 1 day of FD (Fig. 6B). In contrast, blood glucose levels in MSTN-null mice decreased to a extent similar to wild-type mice after 1 day of FD but continued to decrease further after 2 days of FD, at which time they were significantly lower than blood glucose levels in wild-type mice after 2 days of FD (Fig. 6B).

Wild-type and MSTN-null mice experience similar changes to liver gluconeogenesis enzymes with FD. A decrease in blood glucose could come about as a consequence of changes in hepatic enzyme expression in MSTN-null mice during FD. To address this issue, we quantified mRNA levels of glucokinase and PEPCK, two enzymes whose expression is regulated by energy intake status (19, 45). Levels of glucokinase mRNA significantly decreased in response to 2 days of FD in both wild-type and MSTN-null mice, with no difference between the two genotypes (Fig. 7A). Conversely, levels of PEPCK mRNA significantly increased in response to 2 days of FD in both wild-type and MSTN-null mice, and again there was no significant difference between the two genotypes (Fig. 7B).

DISCUSSION

In the present study, 2 days of FD was associated with a significant decrease in body mass, muscle mass, and total protein content and a significant increase in atrogene expression in TA but not Sol muscle (Table 1). This is in marked contrast to the greater susceptibility of Sol to unloading or inactivity-induced atrophy (50), but fast-twitch muscle and/or fast-twitch muscle fibers show greater sensitivity to a number of other loading-independent models of atrophy, particularly those associated with increased stress hormone or cytokine signaling (27, 28, 58). This also suggests that the well-characterized decrease in activity levels due to torpor (54) was probably not the primary determinant of muscle atrophy in the food-deprived state, although previous studies have demonstrated that increased muscle activity tends to suppress MSTN expression (29, 33). In addition, it does not rule out a contribution of decreased blood flow, changes in autonomic nervous system regulation, or other components of the torpor state on MSTN expression or muscle atrophy with FD. The resistance of the more slow-twitch Sol and/or the greater responsiveness of the more fast-twitch TA to FD-induced atrophy may reflect the lower sensitivity of slow compared with fast muscles to glucocorticoid signaling (28), since glucocorticoids are known to play a major role in FD-induced muscle atrophy (62).

Similarly, we observed a muscle-specific effect of FD on MSTN expression in TA and Sol that mirrored the differences in muscle mass, protein content, and atrogene expression in TA but not Sol muscle (Table 1). This finding is consistent with the observation that swimming exercise causes a greater decrease in MSTN expression in the fast-twitch white gastrocnemius muscle than in the more slow-twitch red gastrocnemius muscle in the rat (33). This muscle-specific response of MSTN gene expression to FD is also similar to the fast-specific increase in atrogene (20) and lipid oxidation gene expression with FD (51), and it suggests that the MSTN gene may represent another component of the muscle-specific transcriptome associated with the FD state.
Several previous studies have demonstrated that MSTN expression in skeletal muscle is affected by the energy intake status of the organism, but the results from these studies have not been consistent. For example, MSTN mRNA levels have been shown to increase, decrease, or remain unchanged in various nonmammalian species, depending on the species, the age, and the duration of the FD state (8, 15, 49, 56). In mammals, MSTN mRNA levels significantly decreased in response to a weeks-long regimen of underfeeding in sheep (21), while a recent paper reported that a short-term (40 h) fast was not associated with a change in muscle MSTN mRNA or serum MSTN protein levels in humans (24). These differences may simply reflect the difference in metabolic rate between mice and larger species, in that underfeeding in sheep and/or a 40-h fast in humans may not produce as dramatic effects as 48 h of FD does in the smaller and much more metabolically active mouse, and are therefore consistent with the main finding of the present study that MSTN is dispensable to the atrophy occurring during the early part of FD. In the human study there was also no increase in atrogene mRNA levels (24), consistent with the hypothesis that 40 h of FD in humans is insufficient to produce the changes in atrogene expression seen in the mouse model. Alternatively, it could reflect the fact that human skeletal muscles tend to have more slow-twitch fibers than mouse skeletal muscles, and in the present study the almost exclusively fast-twitch TA demonstrated a robust increase in MSTN mRNA while the more slow-twitch Sol did not.

We recently reported (2) that expression of MSTN by both adipose tissue and skeletal muscle was greatly increased both genetically and by dietary obesity in mice, a finding supported by work showing greater MSTN protein secretion from myotubes formed from myoblasts isolated from obese individuals (17). The increase in MSTN expression during high-food-intake states would appear to be in conflict with the results from the present study demonstrating a significant increase in MSTN expression during FD and a decrease with refeeding. It may be that in both situations MSTN expression increases as a secondary consequence of increased glucocorticoid signaling, since glucocorticoid levels are known to be increased in both cases (32, 41), and MSTN expression has been shown to be increased by glucocorticoids (30, 31). Another possibility, posited by Hittel and coworkers (17), is that skeletal muscle from obese organisms becomes insulin resistant and therefore is in a reduced glucose- and lipid-uptake state analogous to that experienced by true FD, and this may also explain the similar response of the MSTN gene to both increased and decreased food availability.

Previous studies have demonstrated that MSTN-null mice experience attenuated muscle mass loss in response to glucocorticoid injection (12), but a central function of MSTN in all forms of muscle atrophy has not been reported by all.
MSTN inactivation had no significant effect on the induction of atrogen expression in response to FD. Both atrogin-1 and MuRF-1 mRNA levels were significantly increased by 1 or 2 days of FD in the TA of both wild-type and MSTN-null mice, with no significant difference between the two genotypes. Thus the attenuation in muscle mass loss by MSTN inactivation is not sufficient to induce muscle atrophy in these mice, as both atrogin-1 and MuRF-1 mRNA levels increased in the TA of MSTN-null mice after 2 days of FD, but TA muscle mass did not decrease further at this time point. It also demonstrates that increased atrogen expression can occur independently of MSTN signaling, a finding consistent with a recent paper showing that glucocorticoids can act directly on the promoter of MuRF-1 and activate its expression (59).

The exact relationship between MSTN signaling and muscle protein degradation is not clear. In an in vitro model of cancer cachexia, McFarlane et al. (34) showed that treatment of C2C12 myotubes with recombinant MSTN increased MuRF-1 and atrogin-1 mRNA levels as well as protein ubiquitination. However, total protein degradation was unaffected by recombinant MSTN treatment in C2C12 cells (55), while myofibrillar protein half-life was similarly unaffected by transgenic inactivation of MSTN in vivo (61). In addition, MSTN inactivation in vivo is actually associated with an increase in atrogin-1 expression (42). In the present study, we did not observe a significant increase in atrogin-1 mRNA levels in MSTN-null mice, but neither did MSTN inactivation attenuate the increase in atrogin expression with FD. Thus while it appears that MSTN can induce atrogin expression under some atrophic conditions, neither increases in atrogin expression nor changes in protein degradation rates appear to be necessary for MSTN-induced muscle atrophy.

Effects of MSTN on protein synthesis are much better documented and appear to be the more likely mechanism by which MSTN inactivation attenuated the loss in muscle mass with prolonged FD. MSTN treatment decreases total protein synthesis in C2C12 myotubes in vitro (55), and MSTN inactivation in vivo increases myofibrillar synthesis (61). MSTN inhibits signaling through the PI3-kinase/Akt/mTOR pathway (5, 42) and decreases phosphorylation of S6 and 4E-BP1, two downstream targets of this pathway involved in regulating protein synthesis (13). Thus one possible explanation of the results from the MSTN-null mouse studies is that MSTN inactivation increased protein synthesis rates in the muscles of these mice and the greater protein synthesis rate offset at least partially the increased protein degradation induced through MSTN-independent means, resulting in attenuated muscle mass loss during prolonged FD.

Because muscle plays a role in helping to maintain blood glucose levels during the food-deprived state, we compared blood glucose responses to FD in wild-type and MSTN-null mice. We observed no significant difference in blood glucose levels between wild-type and MSTN-null mice for either the fed or 1-day fasted conditions, similar to what has been reported previously (16, 38). But consistent with the attenuation in TA muscle mass loss in MSTN-null mice after 2 days of FD, we observed a significantly greater decrease in blood glucose levels after 2 days of FD. Thus attenuation of the muscle mass loss by MSTN inactivation...
may have compromised the ability of mice to maintain blood glucose homeostasis during prolonged FD states.

Decreases in glucokinase and increases in PEPCk mRNA levels have been previously demonstrated in response to FD as the liver shifts from glucose absorption to gluconeogenesis and glucose efflux (19, 45). Hepatic mRNA expression of glucokinase and PEPCk, which regulate glucose phosphorylation during uptake and gluconeogenesis, decreased and increased, respectively, after 2 days of FD in both wild-type and MSTN-null mice, with no difference between the two lines. While not a definitive measure of enzyme activity or glucose flux through the liver, these data are at least consistent with the assertion that major defects in either basal or FD-induced hepatic enzyme expression were not the predominant source of the shifts in blood glucose levels during prolonged FD in the MSTN-null mice, and that the changes in blood glucose were more likely a result of MSTN-dependent perturbations to skeletal muscle atrophy during prolonged FD.

However, MSTN inactivation may have had additional affects on glucose handling that may also have influenced blood glucose homeostasis during prolonged FD. For example, MSTN inhibits glucose uptake in the BeWo choriocarcinoma cell line, while treatment of these cells with the MSTN binding and inhibiting protein follistatin blocks this effect (6), and injection of mice with recombinant MSTN decreases skeletal muscle insulin sensitivity (18). Thus MSTN may inhibit glucose uptake, and MSTN inactivation may have prevented this from happening in the MSTN-null mice during prolonged FD, resulting in greater glucose uptake of the MSTN-null mice after 2 days of FD. Along with the greater overall muscle mass of MSTN-null mice, this could have resulted in a greater level of muscle glucose uptake, which may have contributed to the decrease in blood glucose observed in these mice during prolonged FD. Consistent with this finding, MSTN-null mice show greater glucose uptake in response to insulin treatment and moreover show a higher respiratory exchange ratio (RER), which suggests that carbohydrate oxidation is greater in these mice (16). While insulin levels were probably very low in the 2-day FD mice, the greater insulin sensitivity of MSTN-null mice as well as their greater glucose utilization and larger overall muscle mass may also have contributed to the shifts in blood glucose observed in these mice during prolonged FD in the present study. MSTN-null mice also experienced a significantly greater elevation in blood corticosterone levels after both 1 and 2 days of FD. This demonstrates that the attenuation in TA muscle mass loss in the MSTN-null mice after 2 days of FD is not a consequence of a defect in the synthesis or secretion of glucocorticoids in these mice. The significantly greater increase in systemic glucocorticoid levels in MSTN-null mice is likely a compensatory mechanism resulting from the consequence of the lower blood glucose and the inability of key muscles of the MSTN-null mouse to respond appropriately to glucocorticoid signaling by increasing MSTN expression (30, 31).

Summary and conclusions. Our data support a model in which the initial changes in muscle mass occurring during FD are MSTN independent but MSTN contributes to the loss of muscle mass during prolonged FD states. Moreover, through its inhibitory influence on muscle growth and/or glucose uptake, MSTN appears to influence blood glucose levels during prolonged FD states.

ACKNOWLEDGMENTS

D. L. Allen thanks the Department of Integrative Physiology for providing funds to support this research and for purchasing the quantitative real-time PCR system that made much of the data collection possible in the present study and Dr. Leslie Leinwand, Ann Robinson, and Margeret Ihenhit for breeding the MSTN-null mice.

GRANTS

This work was supported in part by two University of Colorado Innovative Seed Grants given to D. L. Allen, J. M. Reed and S. F. Lindsay were supported by the University of Colorado Undergraduate Research Opportunity Program.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


