PE1.45 Hind limb immobilization results in muscle-specific growth inhibition in growing pigs 297.00

Jason Scheffler (1) jmscheff@vt.edu, Steven Kasten(1), David Gerrard (1)
(1)Virginia Polytechnic Institute and State University

Abstract—Rodent models have made significant contributions to our understanding of muscle growth. Yet, it is imperative that large-animal models of muscle growth are developed. Data generated in rodents do not always translate to human medicine, livestock, or poultry production. Therefore our objective was to develop a pig hind limb immobilization model to study effects of unloading on muscle growth. Immobilization for 14 d did not alter body weight gain compared to controls. Soleus and semitendinosus muscle weights were reduced (P<0.05) in immobilized limbs. Importantly, these muscle weights were increased compared to baseline measurements indicating growth inhibition rather than muscle atrophy. Semitendinosus weights were increased (P<0.05) in overloaded limbs. Gastrocnemius muscle weights were not affected by treatment. Taken together, these data suggest loading is an important stimulus for muscle growth. Further development of this model will be useful in delineating specific mechanisms involved in muscle growth in weanling pigs.

J. M. Scheffler is with Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 USA. (e-mail: jmscheff@vt.edu) S. C. Kasten is with Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 USA. (e-mail: skasten@vt.edu) D. E. Gerrard is with Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 USA. (phone: 540-231-9157; fax: 540-231-3010; e-mail: dgerrard@vt.edu).

Index Terms— atrophy, muscle growth model

I. INTRODUCTION

Skeletal muscle is the most abundant tissue in domesticated livestock. From a value standpoint, it is the most important contributor to carcass value. The rate and extent of muscle growth is primarily dictated by the rates of protein synthesis and degradation. The net effect of protein synthesis and degradation determines if muscle mass will increase (hypertrophy) or decrease (atrophy) in size as the total protein content directly parallels muscle growth [1]. These two processes are not independent, but are intricately linked through a variety of signaling pathways. Muscles consisting of primarily white, glycolytic fibers exhibit atrophy in response to different stimuli than those muscles containing predominately red, oxidative fibers. For example, white muscles experience atrophy to a greater extent in response to nutritional deficiency [2], exposure to glucocorticoids [3] and sepsis [4]. By contrast, red muscles readily experience atrophy when the muscle is denervated [5] or unloaded [6, 7]. In response to unloading, mouse soleus muscle can decrease in weight by 25-40% [8]. This reduction in muscle mass is associated with a fiber type-specific reduction in cross-sectional area (CSA) with myosin heavy chain (MyHC) type I and IIA(X) decrease by 33% each, whereas MyHC type IIx/IIb CSA only reduces by 16% [8]. While rodent models have made significant contributions to our understanding of muscle growth, there exists a need to develop large animal models of altered skeletal muscle growth. Many findings in rodent models fail to translate to livestock production or human medicine. Therefore, the objective of this study was to develop a pig-hind limb immobilization model to study muscle specific responses to unloading.

II. MATERIALS AND METHODS

Animals Seventeen crossbred pigs were weaned at 28 d of age and randomly assigned to individual pens. All pigs were provided ad libitum access to a standard pig starter diet containing 18% crude protein and medicated with chlortetracycline hydrochloride, sulfamethizine, and penicillin. Feed offerings were weighed daily to provide an index of feed intake, but feeders were not allowed to go empty. Spilled feed was not weighed back, nor was the weight of remaining feed measured. After 3 d of acclimation to the new environment, pigs were randomly assigned to immobilization (IMB; n=7), sham-taped (Sham; n=7) or baseline (n=3) treatment groups. Baseline pigs were immediately euthanized. Semitendinosus (ST), gastrocnemius (GST), and soleus muscles were dissected and weighed. The right hind leg of IMB pigs was immobilized in a retracted state using a modified Ehmer sling. Elastikon tape was used to make a "figure 8" to flex the hock and the leg was then positioned in close proximity to the abdomen. Sham pigs were taped by wrapping above and below the right hock and around the abdomen without impeding movement of the leg. Pigs were monitored daily for signs of swelling. All tape was replaced at d 7. Body weights were measured every 48 h. At the end of the 14 d immobilization period, all pigs were euthanized and muscles were dissected as described for baseline pigs. All procedures were approved by the Virginia Tech Institutional Animal Care and Use Committee. Statistics Growth performance data were analyzed using the Proc MIXED procedures of SAS. Muscle weights adjusted to carcass weights are reported. Muscle data were analyzed using SAS JMP. Where indicated, contrasts comparing pooled control and sham treated muscles against either overloaded or unloaded muscles were used.

III. RESULTS AND DISCUSSION

Initial weights (T0) of baseline, HLI and Sham treatment groups were not different ($P=0.87$; Figure 1, data not shown). Over the course of the study, there were no differences in body weight between HLI and Sham treated pigs at any time point ($P>0.49$). Feed offerings for sham and HLI pigs were 0.86 and 0.73 kg, respectively and were not significantly different ($P>0.24$). These data indicate that immobilization of a hind limb did not inhibit overall animal growth performance.

Immobilization resulted in an 18% reduction ($P<0.05$) in muscle weight compared to control and sham taped limbs (Figure 2a). Overload had no effect ($P<0.05$) on soleus weight. Gastrocnemius weights were not different ($P<0.05$) after either overload or unloading (Figure 1b). Semitendinosus muscle weights were increased ($P<0.05$) by 9% in overloaded limbs and decreased by 11% in unloaded limbs compared to pooled control and sham limbs (Figure 2c). Preliminary data indicated no differences in semimembranosus, gluteus medius, adductor, biceps femoris or combined vastus lateralis, medias and intermedius muscle weights (data not shown). Therefore, we focused on the soleus, GST, and ST muscles because of their inherent differences in muscle fiber type composition. These muscles are readily obtained and have distinct characteristics useful for study. The soleus consists of primarily red type I and IIA muscle fibers. Gastrocnemius has a higher proportion of white type IIX and IIB fibers. Both these muscles are in a stretched position during the immobilization. By contrast, the ST is held in a relaxed state during immobilization. Previous studies have shown red muscles are more susceptible to disuse atrophy than white muscles [5-7]. Indeed, we observed reductions in muscle mass of the soleus, but not the GST. The ST, on the other hand, is particularly interesting. This muscle experienced both overload induced hypertrophy and unload-induced growth inhibition. The 11% reduction in muscle weight compared to pooled control and sham-treated muscles can be explained by the same growth inhibition in red ST observed in soleus, exacerbated by being in a relaxed state. Previous research has shown that passive stretch can prevent atrophy in unloaded rat soleus [9, 10]. The ST experienced overload-induced hypertrophy whereas the GST did not. There are a number of possibilities for this difference. First, functional overload has been shown to cause a fiber type shift in soleus muscle, namely, the appearance of type IIX fibers [11]. With a higher proportion of slow fibers in the ST than GST, overload may have driven a higher percentage of fibers from slow IIA fibers to transition to faster and larger IIX fibers. Alternatively, immobilizing one limb not only overloads the contralateral limb, it also alters the animal's balance. Potentially, the pig may compensate for this reduced balance by using their muscles differently. Specifically, we did observe immobilized pigs resting on their hocks when eating which would overload the ST and not the GST. Preliminary studies used 14 d old pigs instead of 28 d old pigs. These younger, lighter pigs adapted better to immobilization and was associated with a slight, albeit insignificant, increase in GST weight with no change in ST weight (data not shown). Initially, this model was intended to study muscle atrophy in a large animal. However, these data clearly show none of the muscles are undergoing atrophy; rather, growth is being attenuated in immobilized soleus and ST (Figure 2; compare base to rest of treatments). During two week immobilization, control, sham and overloaded muscles weights increased by 38%. By contrast, unloaded muscles only grew 24%. Therefore, growth of muscles with primarily red fiber type is partly dependent on the load placed on that muscle. However, other growth stimulating mechanisms are still intact in weanling pigs.

IV. CONCLUSION

Hind limb immobilization in weanling pigs results in a consistent and repeatable muscle specific growth inhibition. This model has the potential to distinguish between mechanisms associated with load-induced hypertrophy from other growth stimulating mechanisms in young, growing pigs. Specifically, delineating between mechanical and chemical signal transduction pathways will provide insight to how muscle growth is regulated during rapid growth phase of pigs.

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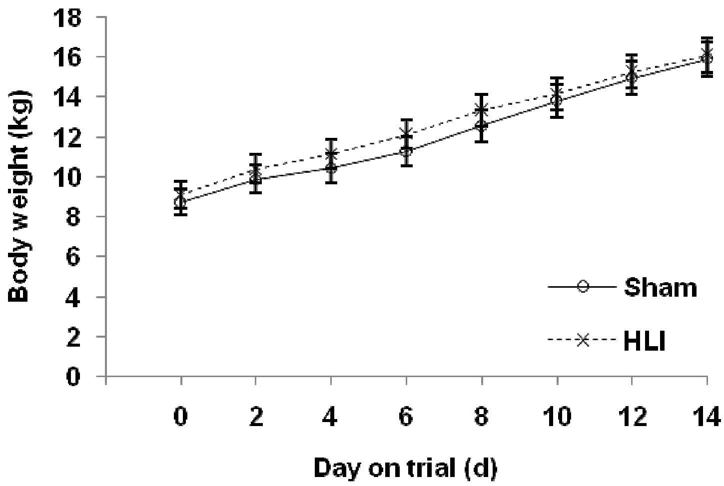

Figure 2. Changes in body weight of pigs during hind limb immobilization. The rear right leg of pigs was either immobilized (HLI) or sham tapped (Sham). No differences (P>0.49) between treatments was observed within any time point.

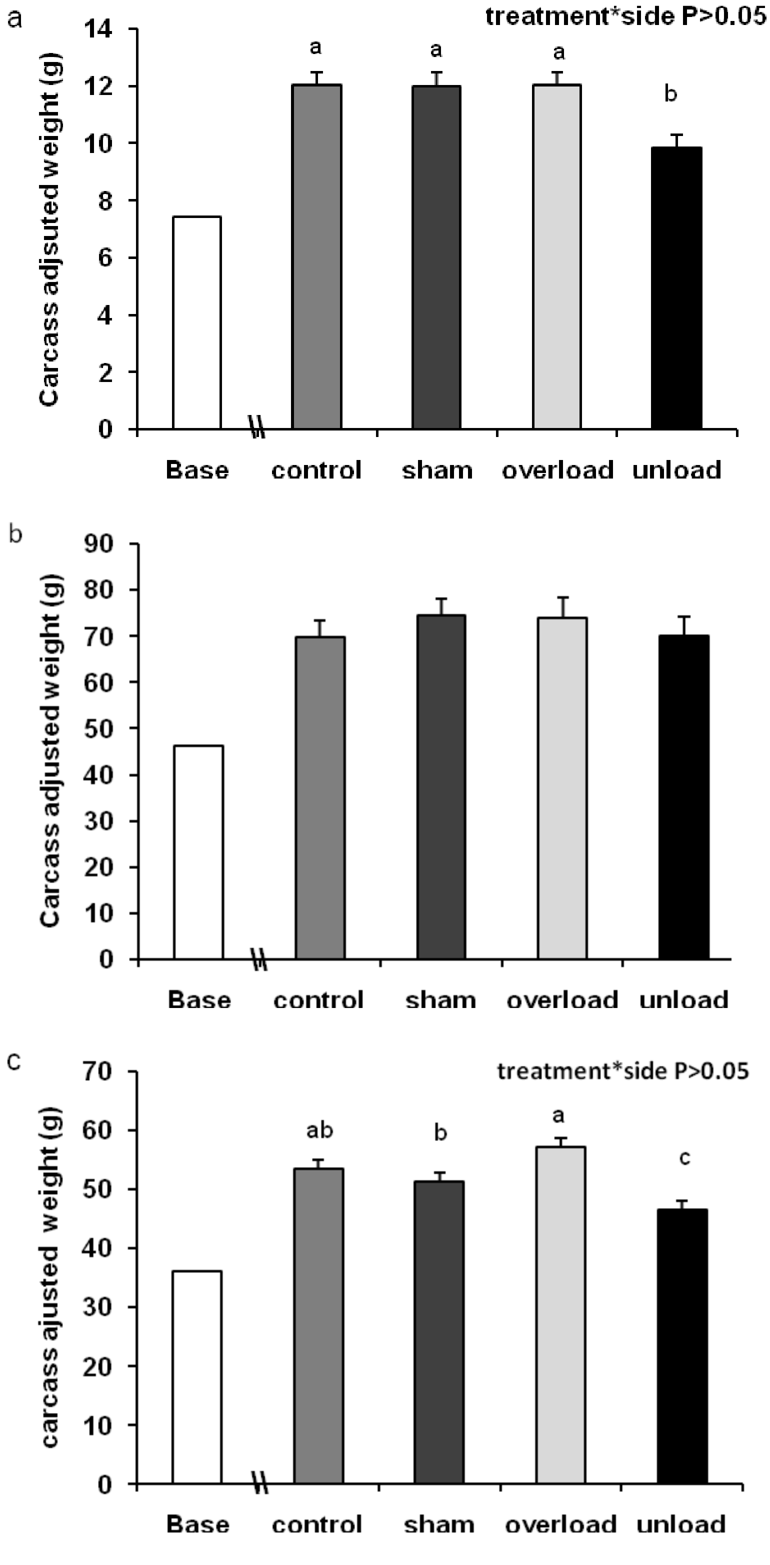

Figure 3. Changes in soleus (a), gastrocnemius (b), and semitendinosus (c) muscles weights at T0 (base) and after 14 days of hind limb immobilization. Right-rear legs were immobilized (unload) or sham-taped. The contralateral limb was a control or overloaded, respectively. Different letters indicate significance (P<.05)