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Comparison of Proteolytic Variables in a Lean and Obese Strain of Pig at the Ages of 2.5 and 7 Months

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Abstract | The mode(s) of skeletal muscle protein turnover as well as muscle and animal growth may be studied by using lean and obese animals as models. The objectives of this study were to look at proteolytic variables implicated in these processes. A lean and obese strain of swine from similar genetic lineage (Duroc x Yorkshire, 50:50) have been well established and may prove ideal for this purpose. This study was done in two phases. Phase I included eight lean and eight obese pigs at 2.5 months of age, and phase II was identical, but the pigs were 7 months old. Longissimus muscle samples were processed immediately after euthanasia for activity measurements of μ-calpain, m-calpain, calpastatin, and lysosomal cathepsins B and B + L. Additional samples were taken for DNA, RNA, and total protein determinations. In phase I, total calpastatin activity, total and specific cathepsin B + L activity, and total protein/g muscle were greater in the obese pigs than in the lean pigs. In contrast, DNA and RNA/g muscle were greater in the lean pigs. No other differences were observed in phase I. In phase II, total calpastatin activity and total cathepsin B activity were greater in the obese pigs than in the lean pigs. No other differences were observed in phase II. From phase I to phase II, μ-calpain total activity increased in the lean pigs but not in the obese pigs and calpastatin activity decreased in both lean and obese pigs; however, the phase-II-obese and phase-I-lean total calpastatin concentrations were not significantly different. In both lean and obese pigs from phase I to phase II, μ-calpain activity, DNA/g muscle, RNA/g muscle, cathepsins B and B + L specific activity, and cathepsin B total activity/g muscle decreased. Total cathepsin B + L activity/g muscle increased in the lean pigs from phase I to phase II, but not in the obese pigs. The data obtained in this study suggest that these strains of pigs may be useful models for the study of muscle and animal growth as well as skeletal muscle protein turnover.

Mechanisms that control skeletal muscle turnover, obesity, and growth may be closely linked. Protein accretion within a muscle is controlled by a delicate balance between protein synthesis and degradation. Altered rates of protein degradation can greatly affect the rate of protein accumulation, thus changing muscle growth rates. Greater knowledge is needed regarding proteolytic variables in the growing muscle to help us understand the mechanisms of muscle and animal growth. Lean and obese swine, of similar genetic background, may be ideal as animal models to study protein turnover and mechanisms of obesity and growth.

Strains of obese and lean swine of common ancestry were developed by selecting for backfat thickness in purebred Duroc and Yorkshire populations (1). The subsequent purebred obese Durocs were crossbred with the purebred obese Yorkshires, and the purebred lean Durocs were crossbred with the purebred lean Yorkshires to create the current strain of obese and lean swine. These lean and obese swine have been maintained for over 16 generations (2). Obese swine differ in a variety of ways from lean swine: 1) less skeletal mass, 2) less muscle mass, 3) greater fat mass, 4) smaller internal organs with a lower preprandial heat production (lower energy maintenance requirements), 5) shorter gestation period, 6) lower plasma somatotropin concentration up to the age of 6 months, 7) lower gain-to-feed ratios, and 8) earlier carcass composition maturation (*see* 2).

The objectives of this study were to measure and compare proteolytic enzyme activities as well as to measure total DNA, RNA, and protein content as an index of protein turnover in muscle of lean and obese swine at the ages of 2.5 and 7 months. Calpain/calpastatin (1-3) and lysosomal cathepsins (6-8) are the best-characterized proteolytic systems known to be present in muscle cells. Consequently, the activities of the calpain/calpastatin system and cathepsins B and B + L were examined. These measurements may help determine whether lean and obese pigs can be used as animal models for the study of skeletal muscle protein turnover and growth.

Materials and Methods

This experiment was done in two phases, involving 16 obese and 16 lean pigs. The Roman L. Hruska, U.S. Meat Animal Research Center animal care and use committee ap-

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proved the use and treatment of animals in this study according to guidelines established by the USDA.

Phase I: Sixteen pigs, eight lean and eight obese, were euthanized at the age of 2.5 months. Longissimus muscle samples were removed from each pig immediately after euthanasia and placed on ice. A 50-g portion was taken from each of these samples and processed immediately for analysis of the enzyme activities of the calcium-dependent proteolytic system, μ-calpain, m-calpain, and their endogenous inhibitor, calpastatin, using the ion-exchange method as described by Koohmaraie (3). The remainder of each of these samples was immediately frozen in liquid nitrogen for subsequent analysis of DNA, RNA, and protein content estimations, as well as lysosomal cathepsins B and B + L activity determinations. Protein determinations were performed according to procedures of Gornall et al. (9) but modified by trichloroacetic acid precipitation of the proteins to remove the ß-mercaptoethanol and/or triton x-100 and then redissolved in 1 N NaOH. Muscle DNA and RNA concentrations were determined according to the procedures of Labarca and Paigen (10) and Munro and Fleck (11), respectively. Cathepsins B and B + L enzyme activities were determined by the cystatin removal method as described by Koohmaraie and Kretchmar (12).

Phase II: This portion of the experiment was identical to phase I but was conducted on lean and obese swine approximately 7 months old.

Statistical analysis: Data were analyzed by one-way analysis of variance. Outlier tests were performed on all data groups. Rankit plots were used to assess normality of data. When significant differences among treatments were detected, treatment means were compared by using the least significant difference method. The comparison-wise error rate was 0.05 (13).

Results

Phase I: No significant differences were observed in total μ-calpain and m-calpain activities between the 2.5-month-old lean and obese pigs (Figure 1). In contrast, the calpastatin activity was greater (33%, $P < 0.05$) in the obese pigs than in the lean pigs. Total cathepsin B + L activity/g muscle was greater ($P < 0.01$) in the obese pigs than in the lean pigs; however, cathepsin B + L specific activity/mg protein was greater ($P < 0.05$) in the lean pigs than in the obese pigs (Figures 2 and 3). The difference observed in specific and total cathepsin B + L activity was due to the different amounts of extractable protein/g muscle obtained from the lean and the obese pigs. No other significant differences were found in any other catheptic variables in phase I (Figures 4 and 5). Total and specific cathepsin B activity followed the same trend as cathepsin B + L but was not significantly different between lean and obese pigs. Both DNA and RNA content per gram of muscle were greater ($P < 0.05$) in lean pigs than in obese pigs (Table 1). Both mg protein/g muscle and mg protein/mg DNA were greater ($P < 0.01$) in the obese pigs than in the lean pigs (Table 1). The RNA to DNA ratios were similar in muscle from obese and lean pigs (Table 1).

Phase II: In contrast to phase I, only two significant dif-

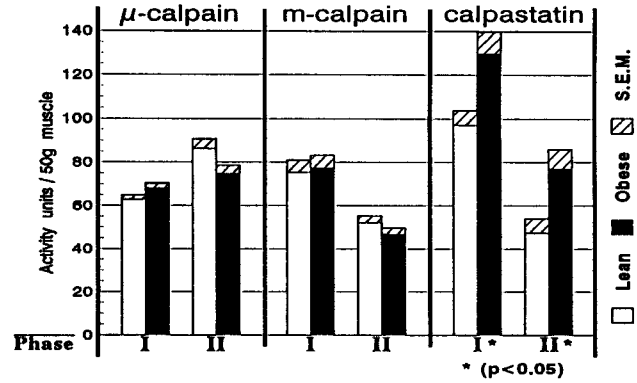

Figure 1. Total calpain and calpastatin activity from 50g longissimus muscle from 2.5-month-old (phase I) and 7-month-old (phase II) lean and obese pigs.

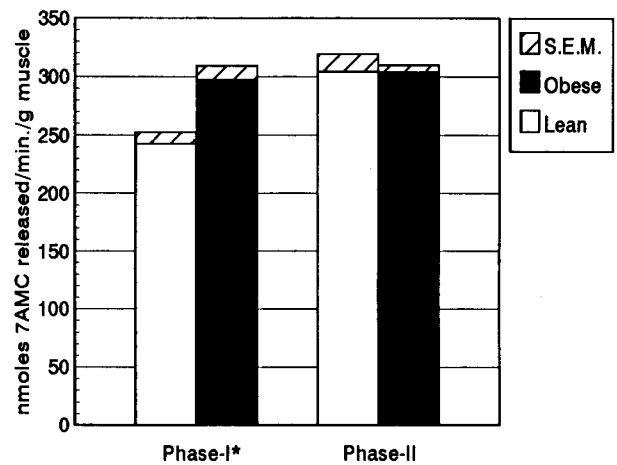

Figure 2. Cathepsin B+L total activity from longissimus muscle of 2.5-month-old (phase I) and 7-month-old (phase II) lean and obese pigs. *($P < 0.05$).

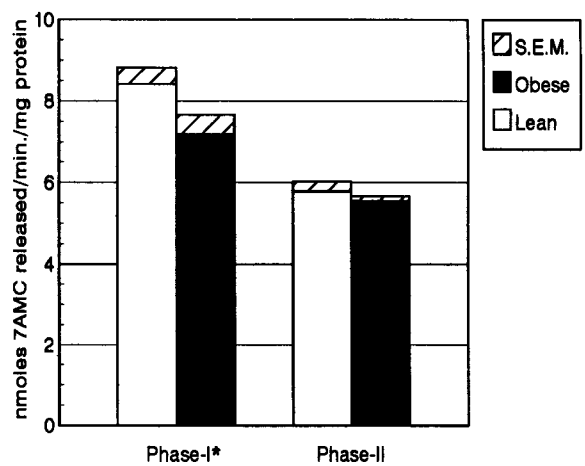

Figure 3. Cathepsins B+L specific activity from longissimus muscle of 2.5-month-old (phase I) and 7-month-old (phase II) lean and obese pigs. *($P < 0.05$).

ferences ($P < 0.05$) were observed in any of the measured variables between the lean and obese pigs at the age of 7 months. Calpastatin activity was still greater (62%) in the obese pigs than in the lean pigs (Figure 1), and total cathepsin B activity/g muscle was greater in the obese pigs than in the lean pigs (Figure 4).

Age effect (phase I vs. phase II): From phase I to phase II, the μ-calpain activity significantly ($P < 0.05$) increased in the lean pigs but not in the obese pigs (Figure 1). The calpastatin activity decreased ($P < 0.05$) within the lean and obese pigs from phase I to phase II (Figure 1); however, the calpastatin activity of the phase-II obese pigs and phase-I lean pigs was not significantly different. From phase I to phase II, within and between the lean and obese pigs, m-calpain activity decreased ($P < 0.05$, Figure 1), DNA and RNA/g muscle decreased ($P < 0.05$, Table 1), total mg of protein/g muscle increased ($P < 0.05$), mg of protein/mg DNA increased ($P < 0.05$), cathepsins B and B + L specific activity/g muscle decreased ($P < 0.05$, Figures 5 and 3, respectively), and cathepsin B total activity/g muscle decreased ($P < 0.05$, Figure 4). The RNA:DNA ratios were not significantly different for the lean and obese pigs from phase I to phase II (Table 1). The total cathepsin B + L activity/g muscle significantly increased ($P < 0.05$) within the lean pigs from phase I to phase II, but no differences were observed within the obese pigs (Figure 2).

Discussion

The purpose of this study was to determine whether a lean and obese strain of swine, of similar genetic background, could be used as an animal model to study skeletal muscle protein turnover and the effects of growth. Except for insulin resistance studies on obese or obese-diabetic animals and humans, no muscle protease data exist, to our knowledge, on lean and obese animals from the same genetic lineage; therefore, this study is extremely useful for establishing the groundwork in this area.

The phase-I data suggest a potential difference in the proteolytic capacity of the lean and obese swine at the age of 2.5 months. The most noticeable change was the increased calpastatin and catheptic B + L activities in the phase-I obese swine, compared with those in the phase-I lean swine. However, these differences in proteolytic variables may be due to the relative rates of maturation of the lean and obese swine. In this breed of swine, the obese pigs reach compositional maturity sooner than do the lean pigs (14, 15). Fat deposition increases and muscle deposition decreases at an earlier age in the obese pigs than in the lean pigs and these differences have been noticed when pigs were 9 to 10 weeks old (14, 15).

The greater DNA and RNA and lower protein concentrations in the phase-I lean pigs as compared with the phase-I obese pigs imply a difference in the growth curves of the lean and obese swine. The growth curve for the obese swine will have a steeper initial slope, suggesting that compositional maturity is reached at a younger age than in the lean pigs. The higher protein:DNA ratios and lower DNA concentrations in the phase-I obese pigs as compared with the phase-I lean pigs support the notion that the obese pigs have greater muscle maturity and physiologic maturity at a younger age, and final muscle development will be less than in the lean pigs. Similar findings have been observed by others, but in genetically different lines of swine (16, 17). Thus the differences observed in the proteolytic capacity during this experiment may be limited to the time frame investigated and,

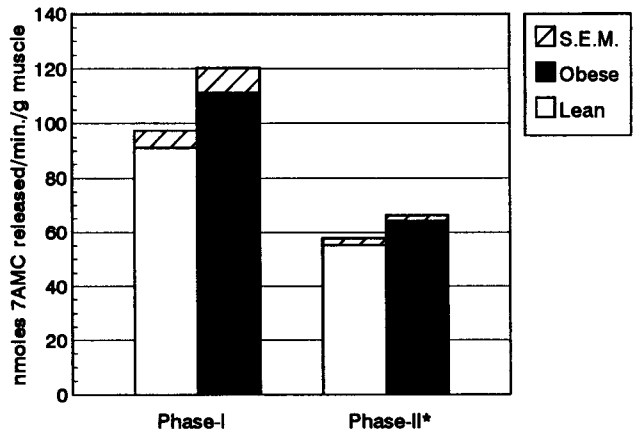

Figure 4. Cathepsin B total activity from longissimus muscle of 2.5-month-old (phase I) and 7-month-old (phase II) lean and obese pigs. *($P < 0.05$).

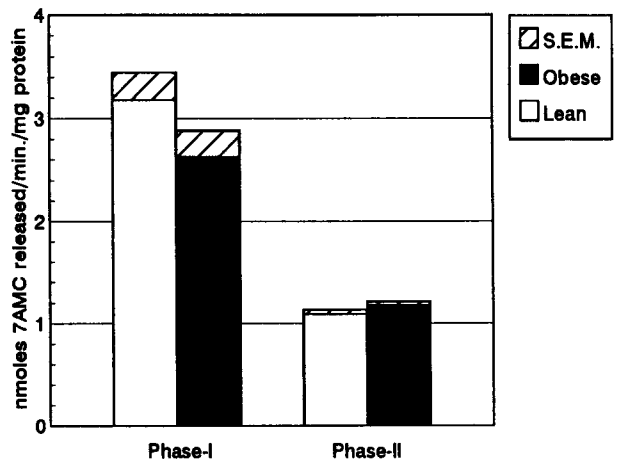

Figure 5. Cathepsin B specific activity from longissimus muscle of 2.5-month-old (phase I) and 7-month-old (phase II) lean and obese pigs.

Table 1. Protein, DNA, and RNA concentrations and protein:DNA and RNA:DNA ratios from longissimus muscle of 2.5-month-old (phase I) and 7-month-old (phase II) lean and obese pigs (Mean ± SE)

	Phase I		Phase II	
	Lean	Obese	Lean	Obese
Protein (mg/g muscle)	186.4 ± 3.0[a]	198.0 ± 2.0[b]	234.9 ± 4.8[c]	221.8 ± 6.3[c]
DNA (μg/g muscle)	1067.4 ± 15.0[a]	998.9 ± 19.0[b]	506.7 ± 9.7[c]	511.0 ± 12.8[c]
RNA (μg/g muscle)	975.4 ± 21.0[a]	888.4 ± 28.0[b]	420.1 ± 7.8[c]	459.0 ± 21.7[c]
Protein:DNA (mg/mg)	174.9 ± 3.7[a]	198.5 ± 2.7[b]	466.3 ± 10.8[c]	435.9 ± 16.4[c]
RNA/DNA (μg/μg)	0.91 ± 0.02[a]	0.89 ± 0.02[a]	0.83 ± 0.02[a]	0.90 ± 0.05[a]

[a,b,c]Means in the same row with different superscripts differ ($P < 0.05$).

therefore, should be viewed with caution. However, these data, particularly the alterations in proteolytic variables and most notably the difference in calpastatin activity, may prove useful in understanding the mechanisms of muscle growth.

Feeding of a ß-adrenergic agonist to meat-producing animals has been shown to increase calpastatin activity and decrease skeletal muscle protein turnover, which increases muscle mass (18, 19). Higher concentrations of calpastatin may thus be associated with lower levels of skeletal muscle protein turnover. The calpains, which are regulated by calpastatin and Ca^{2+}, may initiate the turnover of myofibrillar proteins in muscle (4). Calpastatin thus plays an important role in regulating muscle growth and skeletal muscle protein turnover. The lean and obese pigs provide a model to study the actions of calpain/calpastatin interactions and their effects on skeletal muscle protein turnover. This, in turn, may elucidate a portion of the mechanisms of muscle growth.

In contrast to the greater calpastatin activity in the obese pigs, no other proteolytic variable was significantly different in both phases I and II. The relevance of cathepsins B and B + L in muscle and animal growth has yet to be elucidated. These enzymes probably play an important role in the degradation of sarcoplasmic proteins as well as fragments of myofibrillar proteins released by sarcoplasmic proteases (20). Lambs fed a ß-adrenergic agonist have increased cathepsin B and B + L activity when the cystatins are removed from muscle homogenates (21). The cystatins are a family of cytosolic catheptic inhibitors that can be removed from assay conditions by affinity chromatography (12). In contrast, when cystatins are not removed from muscle homogenates from ß-adrenergic agonist-treated lambs, cathepsin B and B + L activities are decreased (18). Thus the cystatins may play a role similar to that of calpastatin in regulating catheptic enzyme activity and muscle growth.

From phase I to phase II, in both the lean and obese pigs, longissimus DNA and RNA concentrations decreased and protein concentration increased, which implies hypertrophic growth has occurred. Additionally, only calpastatin and total cathepsin B activities were significantly different between the lean and obese swine in phase II. This implies that the level of compositional maturity is much closer in the lean and obese swine at the age of 7 months than at the age of 2.5 months (2, 16, 17). The phase I data may prove useful for genetic selection of a specific metabolic trait in selecting leaner animals. These data also suggest that lean and obese swine are useful models to study both growth and skeletal muscle protein turnover.

Additional research needs to be undertaken to determine whether a divergence in protein turnover variables stays consistent with the current findings prior to the age of 2.5 months or beyond 7 months. If similar differences are found, specifically the difference in calpastatin activity, these swine will prove useful as models for studying skeletal muscle protein turnover and help in understanding the mechanisms of growth.

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