RELATIONSHIP BETWEEN FATTY ACID-BINDING PROTEIN ACTIVITY AND MARBLING SCORES IN BOVINE LONGISSIMUS MUSCLE[1]

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ABSTRACT

Studies were conducted in an attempt to establish a relationship between fatty acid-binding protein (FABP) activity and marbling score in bovine longissimus muscle. Longissimus muscle was obtained from four 20-mo-old Charolais-Hereford crossbred heifers, three 16-mo-old Angus steers, and four 18-mo-old Angus steers. Immediately after slaughter, longissimus muscles were removed for the extraction of FABP. Supernatant (S_{104}) fractions containing 41.3 to 144 mg of protein (depending on animal group) were eluted over Sephadex columns, and elution fractions were analyzed for the binding of radiolabeled palmitoyl-coenzyme A (CoA). Specific activities of FABP were 23, 32, and 101 nmol palmitoyl-CoA bound/mg protein for the Charolais-Hereford, 16-mo-old Angus, and 18-mo-old Angus cattle, respectively. These preliminary results suggested that longissimus muscle FABP activity was positively correlated with marbling score. To test specifically for this possibility, longissimus muscle was obtained at slaughter from each of four Wagyu steers, Angus heifers, and Braford heifers. Marbling scores taken at the 12th-13th rib junction were Sm^{45}, Sm^{43}, and Sl^{50} for the Wagyu, Angus, and Braford cattle, respectively. Interfascicular adipose tissue was exhaustively removed from sections of the 5th to 8th thoracic region of the longissimus muscle to eliminate any contribution of adipose tissue to FABP activity. For each animal, 300 mg of the S_{104} were eluted over Sephadex columns. Specific activities for the Wagyu, Angus, and Braford longissimus muscle FABP were 3.1, 3.8, and 3.9 pmol palmitoyl-CoA bound/mg protein, respectively, and were not different ($P > .05$) among the three animal groups. Furthermore, no correlation ($r = .017$) existed between FABP activity and marbling score for this sample population. The data suggest that correlations between marbling scores and FABP activities in preliminary investigations were the result of contamination by interfascicular adipose tissue FABP.

Key Words: Bovidae, Adipose Tissue, Fatty Acids

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Introduction

This laboratory reported the existence of fatty acid-binding protein (FABP) activity in bovine longissimus muscle and, at the same time, demonstrated that FABP activity was positively and significantly correlated with the incorporation of palmitate into longissimus muscle strips incubated in vitro (Smith et al., 1985). This suggested that FABP activity was

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related to the ability of the muscle to oxidize fatty acids, which was supported by the observation that porcine longissimus muscle, which has a low abundance of type I (oxidative) myofibers, had no detectable FABP activity (St. John et al., 1987).

During initial attempts to purify FABP from skeletal muscle (Smith et al., 1988), it was noted that there seemed to be a positive correlation between longissimus muscle FABP activity and the marbling score of the muscle. If such a correlation existed, it might provide a biochemical means by which carcass quality could be predicted. The findings of the earlier study are reported in detail here and pose the possibility that the FABP activity detected in bovine longissimus muscle was due to interfascicular adipose tissue that was not detected during dissection and homogenization of the muscle. This possibility was addressed in a subsequent investigation of the correlation between marbling scores and longissimus muscle FABP activity. These results have been reported in preliminary form (Kirby and Smith, 1989) and are reported in detail here.

Materials and Methods

Animals and Procedures. A total of 23 steers and heifers were used in two separate experiments. In Exp. 1, longissimus muscle from the 5th to 8th thoracic vertebrae region was obtained from four 20-mo-old Charolais-Hereford crossbred heifers, three 16-mo-old Angus steers, and four 18-mo-old Angus steers. The Charolais-Hereford and older Angus steers were typical slaughter-weight cattle. In Exp. 2, longissimus muscle from the same location was obtained from four 22- to 24-mo-old Wagyu steers, four 10- to 12-mo-old Braford heifers, and four 12- to 14-mo-old Angus heifers. All cattle were adapted at weaning to a corn-milo finishing diet (Schiavetta et al., 1990) and were fed the diet until slaughter. Marbling scores were obtained for the carcasses in Exp. 2 by trained Texas A&M University personnel using the methods outlined in USDA (1987). Feed was withdrawn 14 h before slaughter, but the cattle had free access to fresh water. Care was taken to minimize preslaughter stress to the animals.

Experiment 1. The longissimus muscle was obtained immediately after exsanguination by cutting through the hide over the muscle. The muscle was transported to the laboratory on ice; elapsed time between slaughter and homogenization was approximately 30 min. For all groups of cattle, the muscle was dissected free of visible interfascicular adipose tissue and supernatant fractions from 104,000 × *g* ultracentrifugation of muscle homogenates were prepared as described by Smith et al. (1985). The muscle was homogenized in .154 *M* potassium chloride[6], .02% sodium azide, .01 *M* potassium phosphate buffer (pH 7.4) at 4°C in a 2:1 (vol:wt) dilution. For Exp. 1, the 104,000 × *g* supernatant fractions (S_{104}) were pooled within each animal group in initial attempts to purify FABP from longissimus muscle (Smith et al., 1988).

The S_{104} from the longissimus muscle of the treatment groups was dialyzed against distilled-deionized H_2O and lyophilized. The dialyzed S_{104} was rediluted in .01 *M* potassium phosphate buffer (pH 7.4) to a final protein concentration of 41.3 mg/ml (Charolais-Hereford heifers), 48.1 mg/ml (16-mo Angus steers), or 36.0 mg/ml (18-mo Angus steers). Because the assay for FABP (see below) detects fatty acids bound to any protein, FABP was separated from albumin by column chromatography. One to 4 ml of concentrated S_{104} were eluted over G-100 (heifers and 16-mo steers) or G-50 (18-mo steers) Sephadex columns[7] and 2-ml fractions were collected as described by Smith et al. (1985). Different sieve sizes were used in an attempt to obtain greater fractionation during attempts to purify FABP. Column dimensions were 2.6 cm × 30 cm. Binding in the elution fractions was measured in a postcolumn assay by the procedure of Glatz and Veerkamp (1983). The assay used .4 ml of sample, 1.99 nmol of palmitoyl-Coenzyme A (CoA), and .01 μCi of ^{14}C-labeled palmitoyl-CoA in a total assay volume of .5 ml. Measurement of radioactivity bound to protein in the column fractions was accomplished with a Beckman liquid scintilla-

[6]Biochemicals were purchased from Sigma Chemical Co., St. Louis, MO. Radioisotopically labeled fatty acids were obtained from Amersham, Arlington Heights, IL. All other chemicals were reagent grade or higher and obtained from commercial sources.

[7]Pharmacia Fine Chemicals, Piscataway, NJ.

tion spectrophotometer[8]. The fatty acid binding in the fractions exhibiting the greatest binding, and corresponding to an approximate molecular weight of 14,000 Da, was summed and taken to represent FABP activity in the sample. Protein content of the S_{104} was measured by a biuret method (Mokrasch and McGilvery, 1956).

Experiment 2. The results of Exp. 1 suggested a relationship between ability to marble and FABP activity. However, carcass data were not obtained for the animal groups used for Exp. 1, and S_{104} fractions were pooled within groups, so that this possibility could not be addressed specifically. To more rigorously address the possibility that marbling score of the longissimus muscle was positively correlated with skeletal muscle FABP activity, longissimus muscle was obtained from Wagyu steers and Braford and Angus steers (described above). Interfascicular adipose tissue was exhaustively dissected to eliminate any contribution of this tissue to longissimus muscle FABP activity. An S_{104} fraction was prepared for each animal and kept separate; preparation of the S_{104} was essentially as described by Smith et al. (1985) except that a commercial food processor[9] was used to grind and blend the muscle. The S_{104} fractions were frozen at –70°C until they were assayed for FABP activity.

Elution of the S_{104} fractions was performed with .01 *M* potassium phosphate buffer (pH 7.4) with .02% sodium azide at 4°C. Samples were equilibrated by dilution with phosphate buffer to a final concentration of 20 mg protein/ml, and 15 ml of each sample were eluted over 2.6-cm × 100-cm Sephadex G-100 columns. Five-milliliter fractions were collected at a flow rate of .4 ml/min, and protein concentrations were determined by a biuret method (Mokrasch and McGilvery, 1956). Fatty acid binding was quantified as described in Exp. 1.

Statistical Analysis. For Exp. 2, FABP total and specific activities were determined by summing the four 5-ml fractions exhibiting the greatest fatty acid binding, eluting at an approximate molecular weight of 14,000 Da. Means and SEM were calculated for each animal and mean separations were effected with the Student's *t*-test (Montgomery, 1984). Simple linear regression of the FABP specific activity as a function of marbling score was performed as described by Montgomery (1984).

[8]Model LS 3800, Beckman Instruments, Inc., Irvine, CA.

[9]USA, Inc., Ridgeland, MS.

Results

Experiment 1. The G-100 elution of the S_{104} gave very similar results for the first two experiments (Figure 1). Most of the fatty acid binding was observed in the first peak (putatively representing binding to albumin), with only minor binding in the second peak. Elution of molecular weight markers under the same elution conditions indicated that the second binding peak corresponded to proteins with approximate molecular weights of 14,000 Da, similar to results reported previously (Smith et al., 1985). The elution of myoglobin (indicated as peak absorption at 410 nm) preceded peak FABP activity, which would be predicted from its greater molecular weight (16,000 Da).

As indicated in Table 1, the specific activity of the S_{104} protein and of the binding peaks of the gel filtration (excluding the albumin peak) increased in each succeeding experiment and was especially high in muscle from the third group of animals (older Angus steers). This also is apparent in the elution pattern in Figure 1, in which pronounced absorbance at 280 nm and fatty acid binding are observed in the second peak.

Experiment 2. Palmitoyl-CoA binding was measured only in those tubes surrounding the FABP peak. A typical elution pattern (for an Angus heifer) is indicated in Figure 2. The FABP activities were calculated by summing the binding in the four tubes displaying the greatest activity (Table 2). Unlike the results observed in Exp. 1, FABP specific activities were not different ($P > .05$) among the animal groups (3 to 4 pmol of palmitoyl-CoA bound/mg of protein). The specific activities were substantially less than those observed in the earlier experiment and were three orders of magnitude lower than FABP activities reported for rat heart (Bass and Manning, 1986).

Marbling scores (Table 2) were least ($P < .05$) in the Braford heifers and were not different ($P > .05$) between the Angus heifers and Wagyu steers. No correlation ($r = .017$) existed between FABP activity and marbling score.

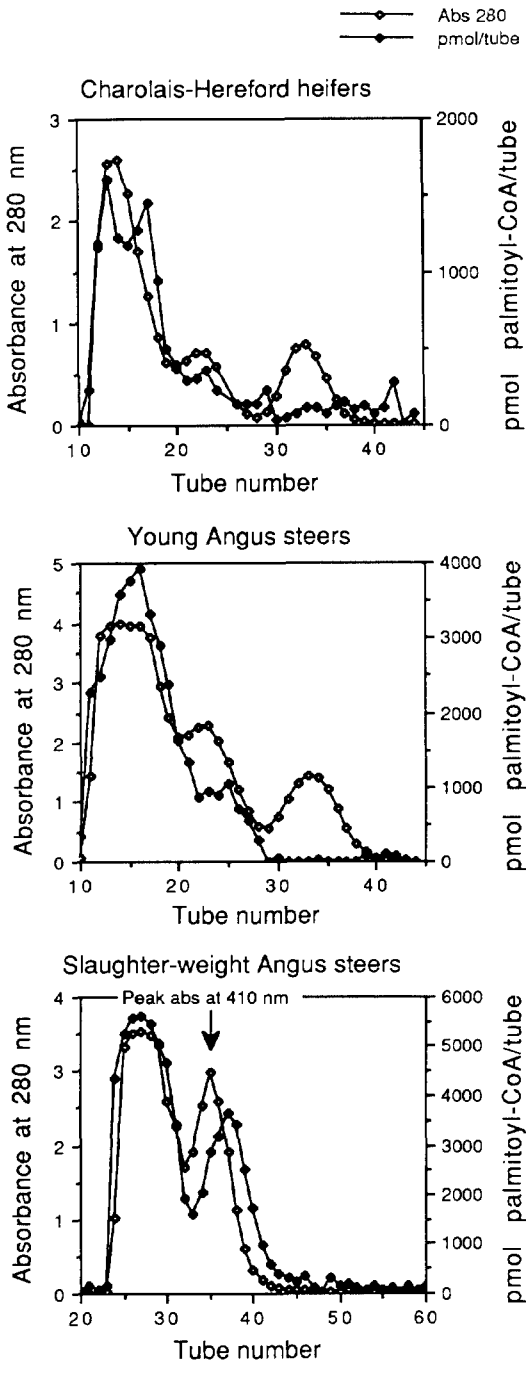

Figure 1. Sephadex chromatography of pooled 104,000 × *g* supernatant fractions (S_{104}) from three sets of animals. Top panel: Elution of 41.3 mg of longissimus muscle S_{104} from three Charolais-Hereford crossbred heifers over Sephadex G-100. The supernate had been lyophilized previously. Flow rate was 1 ml/min and fraction volume was 2 ml. Open symbols, absorbance at 280 nm; closed symbols, binding of ^{14}C-labeled palmitoyl-coenzyme A (CoA) in each tube, determined by a postcolumn procedure. Middle panel: Elution of 96.2 mg of longissimus muscle S_{104} from three 16-mo-old Angus steers over Sephadex G-100. Flow rate was 1 ml/min. Bottom panel: Elution of 144 mg of longissimus muscle S_{104} from four 18-mo-old Angus steers over Sephadex G-50. Flow rate was 1 ml/min and fraction volume was 2 ml. The arrow indicates the peak absorbance at 410 nm (assumed to represent myoglobin).

TABLE 1. FATTY ACID-BINDING PROTEIN ACTIVITIES IN LONGISSIMUS MUSCLE OF CHAROLAIS-HEREFORD HEIFERS AND ANGUS STEERS[a]

Animal group	Total protein eluted per column, mg	Total FABP activity, pmol palmitoyl-CoA[b] bound/column	Specific activity, pmol palmitoyl-CoA bound/mg protein
Charolais-Hereford	41.3	933	23
Angus (16 mo)	96.2	3,078	32
Angus (18 mo)	144.0	14,544	101

[a]Values are for pooled 104,000 × *g* supernatant fractions from four Charolais-Hereford heifers, three 16-mo-old Angus steers, and four 18-mo-old Angus steers.

[b]CoA = coenzyme A.

Discussion

The marbling scores were not measured for the cattle used for Exp. 1. However, on a typical finishing diet, Angus steers increase at least one full marbling score during the last 1 to 3 mo before achieving slaughter weight. The marbling scores for 16-mo-old Angus steers typically range from small to modest, whereas marbling scores for 18-mo-old Angus steers range from modest to moderate (Smith and Crouse, 1984; Miller et al., 1989, 1990). The marbling scores for the Charolais × Hereford heifers have been published previously (Miller et al., 1988a), and were slight[90]. Thus, our previous experience with these and similar cattle convinced us that there would be very real differences in marbling scores among the three groups of cattle used in Exp. 1.

The possibility exists that a portion of the fatty acid binding in centrifugal fractions of bovine longissimus muscle represents contamination by interfascicular adipocytes. An adipose tissue FABP has been characterized in

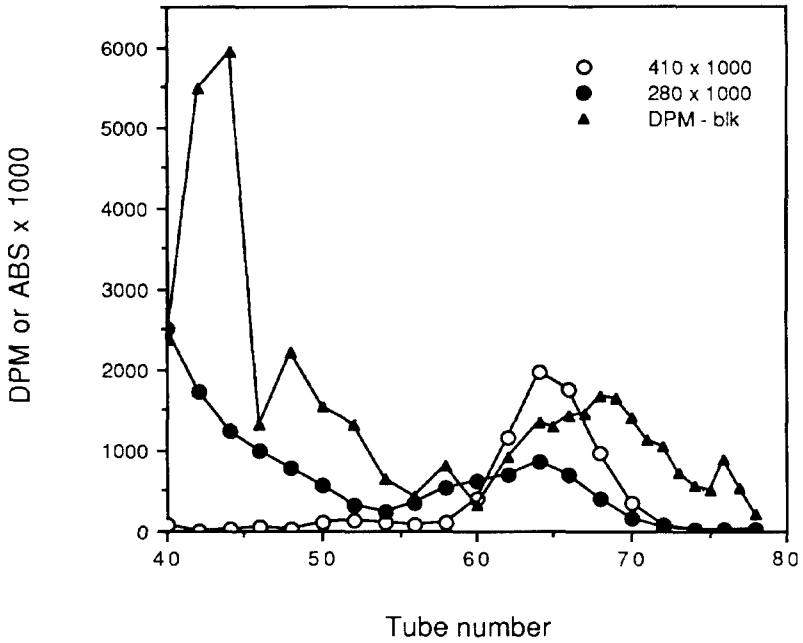

Figure 2. Gel filtration chromatograph of longissimus muscle 104,000 × *g* supernatant fraction (S_{104}) protein from an Angus heifer (Exp. 2). Protein (300 mg) was eluted over Sephadex G-100. Flow rate was .4 ml/min and fraction volume was 5 ml. Peak absorbance at 280 nm occurred at tube number 40. DPM - blk: radioactivity (disintegrations/min) in each fraction after correction for blank values.

TABLE 2. FATTY ACID-BINDING PROTEIN ACTIVITIES IN LONGISSIMUS MUSCLE OF WAGYU STEERS AND BRAFORD AND ANGUS HEIFERS

Animal group	Total protein per column, mg	Total FABP activity, pmol palmitoyl-CoA[a] bound/column	Specific activity, pmol palmitoyl-CoA bound/mg protein	Marbling score[b]
Wagyu	300	931 ± 64[c]	3.1 ± .7[c]	1.45 ± .54[c]
Braford	300	1,175 ± 217[c]	3.9 ± 2.4[c]	.50 ± .03[d]
Angus	300	1,130 ± 111[c]	3.8 ± 1.2[c]	1.43 ± .59[c]

[a]CoA = coenzyme A.

[b]0 = slight, 1 = small, 3 = modest.

[c,d]Means (± SEM; n = 4) with different superscripts within a column are different ($P > .05$).

murine 3T3 fibroblasts (Cook et al., 1985, 1988) that has high homology to the cardiac isoform of the protein (Sacchettini et al., 1988). Furthermore, lipogenic enzyme activities in intramuscular adipose tissue increase two- to fivefold between 16 mo and 18 mo of age in Angus steers (Smith and Crouse, 1984), which would correspond to the threefold increase in FABP specific activity observed in Angus steers in Exp. 1. Although visible interfascicular adipose tissue was removed from the longissimus muscle in Exp. 1, a substantially more thorough dissection of adipose tissue was attempted in Exp. 2. Consequently, FABP specific activity was at least an order of magnitude lower in centrifugal fractions from Exp. 2, and any correlation between FABP activity and marbling score (as suggested by Exp. 1) disappeared.

The existence of FABP was first reported by Ockner et al. (1972) and Mishkin et al. (1972). Fatty acid binding-protein in bovine skeletal muscle was first reported by Smith et al. (1985). In rats, skeletal muscle FABP is distinct from the cardiac form of FABP (Said and Schultz, 1985). The activity of skeletal muscle FABP seems to be dependent on muscle fiber type; muscles consisting primarily of type II (glycolytic) myofibers exhibit little detectable FABP activity (St. John et al., 1987), whereas bovine (Miller et al., 1988a) and rat (Miller et al., 1988b) muscles containing type I myofibers exhibit readily detectable fatty acid binding.

At this point, the existence of an FABP unique to longissimus muscle myofibers has not been demonstrated unequivocally. We have demonstrated a protein of approximately 14,000 Da molecular weight that binds fatty acids (Smith et al., 1988). Amino acid composition and partial sequencing of bovine skeletal muscle FABP indicate that it is similar to the murine 3T3 FABP (Kirby and Smith, 1989). Additionally, myoglobin contamination accounts for much of the fatty acid binding in low molecular weight fractions of muscle homogenates (Gloster and Harris, 1977; Smith et al., 1988), and it has not yet been possible to purify a bovine skeletal muscle FABP that is not contaminated with myoglobin (Kirby and Smith, 1989). However, the presence of skeletal muscle FABP in preparations from rat skeletal muscle (Said and Schulz, 1985; Miller et al., 1988b) and in cardiac muscle (Bass and Manning, 1986) indicates that a protein that binds fatty acids is likely to reside in bovine muscles containing type I myofibers.

Implications

The activity of fatty acid-binding protein in bovine longissimus muscle fibers seems to be a poor index of an animal's ability to accumulate marbling adipose tissue. It is possible that animals that differ widely in their longissimus muscle myofiber composition (e.g., pigs vs cattle) exhibit detectable differences in FABP activity in that muscle. However, this remains to be demonstrated within a species.

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