Effects of muscle type, castration, age, and compensatory growth rate on androgen receptor mRNA expression in bovine skeletal muscle[1]

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ABSTRACT: The effect of testosterone on sexual dimorphism is evident by differential growth of forelimb and neck muscles in bulls and steers. Divergent hormone sensitivites may account for the differential growth rates of individual muscles. Therefore, the objective of this study was to compare androgen receptor (AR) expression in three different muscles of bulls and steers at various ages and growth rates. Thirty Montbéliard bulls and 30 steers were assigned to four slaughter age groups. Four or five animals of each sex were slaughtered at 4 and 8 mo of age. Animals in the remaining two slaughter groups (12 and 16 mo) were divided into groups of either restricted (R) or ad libitum (AL) access to feed. Five animals of each sex and diet were slaughtered at the end of the restricted intake period at 12 mo of age. To simulate compensatory growth, the remaining animals (R and AL) were allowed ad libitum access to feed until slaughter at 16 mo of age. Total RNA was extracted from samples of semitendinosus (ST), triceps brachii (TB), and splenius (SP) muscles. Androgen receptor mRNA was quantified in 200-ng total RNA preparations using an internally standardized reverse transcription (RT) PCR assay. Data were analyzed using 18S ribosomal RNA concentrations as a covariable. Steers had higher AR mRNA levels per RNA unit than bulls ($P < .01$). Androgen receptor mRNA levels differed between muscles ($P < .05$), with lowest expression in the SP. The pattern of AR expression differed ($P < .05$) for each muscle with increasing age. Between 4 and 12 mo of age, AR mRNA levels increased ($P < .05$) in SP but remained unchanged in the ST and TB. Feeding regimen had no effect on muscle AR expression, but steers exhibiting compensatory growth had higher AR mRNA levels than AL steers ($P < .01$) or bulls ($P < .01$). Our results show that AR expression is muscle-specific and may be modulated by circulating testicular hormones. These data suggest that the regulation of AR expression may be linked to allometric muscle growth patterns in cattle and compensatory gain in steers.

Key Words: Cattle, Skeletal Muscle, Growth, Castration, Androgens, Messenger RNA, Polymerase Chain Reaction

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Introduction

Intact male have relatively greater muscle in the neck and forequarter than females or castrates. Although the presence of testicular hormones is related to greater muscle growth capacity in intact males (Arnold et al., 1997), a conclusive explanation for the allometric growth of certain muscles is lacking. Also, it is unclear whether androgens modulate compensatory growth because previous experiments consisted of only steers and(or) heifers.

The mechanism of how testosterone modulates muscle growth is unclear. Testosterone may exert an effect on muscle growth indirectly through the somatotropin axis (Ford and Klindt, 1989). More specifically, different muscle may possess different IGF-I sensitivities (Boge et al., 1995) and(or) IGF-I synthesis rates (Pfaffl et al., 1998), suggesting that testosterone can modulate these actions indirectly through stimulation of the IGF axes. More likely, however, receptor density accounts for the accelerated growth observed in certain muscle groups

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of growing males. Sauerwein and Meyer (1989) showed that the sensitivity to androgens and estrogens is different among muscles of prepubertal cattle. However, conclusions regarding muscle sensitivity to steroids in intact and castrated males are still a matter of debate because the methods used to quantify receptors are based on either ligand binding studies or immunological recognition with specific antibodies. Although valid, both approaches provide different types of information and yield little information regarding potential gene regulation, which are important in modulating tissue receptor concentrations. We, therefore, decided to quantify AR expression in muscle tissue of growing cattle using a reverse transcriptase (**RT**) PCR assay, which has greater sensitivity than traditional gene expression techniques (Saiki et al., 1988).

Materials and Methods

Animals

Thirty Montbéliard steers (castrated at 2 mo of age) and 30 bulls were allotted to different diets, slaughter ages, and feeding regimens, as described earlier (Brandstetter et al., 1998a,b). Briefly, four bulls and steers each were slaughtered at 4 mo of age and five bulls and steers each at 8 mo of age. The diet, composed of corn silage, concentrates, and hay, was formulated on the basis of live weight (INRA, 1989). At 9 mo of age, the remaining bulls and steers were assigned to either an ad libitum (**AL**; 1,300 g ADG for bulls and 900 g for steers) or restricted intake diet (**R**; 800 g ADG for bulls and 670 g for steers). Restricted-fed animals were targeted to 60 to 70% ADG of AL controls while maintaining similar protein intakes. At the end of this restriction period (12 mo of age), five animals per treatment combination were slaughtered. All remaining animals were then allowed ad libitum access to feed and slaughtered at 16 mo of age. The ADG and feed conversion rates of animals with compensatory (**C**; n = 7 each sex) growth rates were approximately 30 to 40% higher than those of their AL controls (n = 4 each sex). Animals were slaughtered at the experimental facility fo the INRA Research Centre Clermont-Ferrand/Theix, France. Muscle samples from semitendinosus (**ST**), triceps brachii (**TB**), and splenius (**SP**) were collected from the right side of the carcass within 15 min after exsanguination, snap-frozen in liquid nitrogen, and stored at –80°C until they were subsequently analyzed. Hot carcass weights were recorded and carcasses were chilled at 12°C for 2 h and then held at 4°C. At 24 h postmortem, carcasses were reweighed and dissected into muscle, bone, and fat. Individual ST and TB muscle weights from the left side of the carcass were recorded.

RNA Isolation and Northern-Blot Analysis

Total cellular RNA was extracted using a combined guanidinium isothiocyanate and phenol/chloroform extraction method (AGS RNA-Clean, Heidelberg, Germany). Concentrations of final preparations were calculated from a spectrophotometer reading at A_{260}, with 260/280 nm absorbance ratios > 1.7, and aliquots were adjusted to 200 ng/μL. The RNA integrity and 18S ribosomal RNA (rRNA) quantity was verified with Northern-blot analysis. Denatured RNA (20 μg) was loaded onto a 1% agarose/2.2 *M* formaldehyde gel containing ethidium bromide. Size-fractionated RNA was electrophoretically transferred to nylon membranes (Immobilon-P, Millipore, Bedford, MA), covalently cross-linked to membranes by ultraviolet irradiation (UV crosslinker, Fisher Scientific, Maurepas, France), and dried at 80°C for 2 h. Blots were hybridized with a rat 18S rRNA probe (Chan et al., 1984), which was [γ-^{32}P]ATP 5′ end-labeled using 8 U of T4 polynucleotide kinase (Promega, Lyon, France), then column-purified (Bio-Spin P-6, Bio-Rad, Ivry Sur Seine, France). Detection of the bovine 18S rRNA has been previously reported (Hocquette et al., 1996). Prehybridization was performed at 42°C for 2 h in solutions containing 6× SSC (1× SSC: 15 m*M* sodium citrate, 150 m*M* NaCl, pH 7.0), .5% SDS, 2× Denhardt's solution (1× Denhardt: .02% polyvinylpyrrolidone, .02% BSA, and .02% Ficoll), and 250 μg/mL denatured salmon sperm DNA. Labeled probe (10^4 cpm/mL) was added to the prehybridization solution, and hybridization was performed at 55°C for 18 h. Membranes were washed twice for 20 min at room temperature in 2× SSPE (1× SSPE:150 m*M* NaCl, 10 m*M* NaH_2PO_4, pH 7.4, and 1 m*M* EDTA). Membranes were exposed to phosphoimage screens overnight and signal intensities were quantified using a Storm Imager and Image-Quant software (Molecular Dynamics, Sunnyvale, CA).

Competitive RT-PCR

Androgen receptor (**AR**) mRNA was quantified by competitive co-amplification of native target mRNA and an internal AR standard (**ARSt**) RNA template using RT-PCR. The same primers were used for both transcripts and were designed to produce amplimers spanning RNA splicing sites to control for genomic DNA contamination. This method was previously described by Malucelli et al. (1996). The sequence of interest was a 172-bp fragment within the ligand-binding domain of the AR. The standard contained a 38-bp deletion and was obtained from an amplified bovine AR sequence that was subcloned and transcribed into cRNA.

Dilutions of competitor ARSt cRNA and 200 ng of native total RNA were denatured (65°C, 5 min) in RT-buffer (50 m*M* Tris-HCl, 75 m*M* KCl, 2 m*M* $MgCl_2$, pH 8.3), 175 μ*M* each of dATP, dGTP, dCTP, and dTTP, and 10 m*M* DTT. The RNA were reverse-transcribed after adding 10 pmol of complementary oligonucleotide primer 5′-TTGATTTTTCAGCCCATCCACTGGA-3′, RNAse Inhibitor (10 U; MBI Fermentas, Vilnius, Lithuania), and RT Superscript II (50 U; Gibco BRL, Paisley, Scotland) in a master mixture to a reaction volume of 20

μL. Reverse transcription was performed in a thermal cycler (Perkin-Elmer/Cetus, Norwalk, CT) at 42°C for 1 h, finishing at 99°C for 1 min. Then, 30 μL of a mster mixture were added, containing PCR-buffer (10 m*M* Tris-HCl, 50 m*M* KCl, .1% Triton X-100; final concentration of $MgCl_2$ was .8 m*M*) and 10 pmol of the second primer 5′-CCTGGTTTTCAATGAGTACCGCATG-3′. The PCR was performed with 2 U of Primezyme TM DNA Polymerase (Biometra, Tampa, FL). Thermal cycling conditions were 30× (95°C/l min, 64°C/40 s, and 72°C/40 s) followed by a final incubation of 72°C for 5 min.

Quantification of PCR Products with Densitometry

The PCR products were electrophoretically separated on a 4% NuSieve agarose gel (FMC Bio Products, Rockland, ME) and visualized by ethidium bromide staining. Gels images were captured under UV transillumination (Pharmacia Biotech, Freiburg, Germany), and the intensity of the bands was quantified using the NIH Image 2.0 program (NIH, Bethesda, MD). To obtain the relative amount of AR mRNA in each sample, band intensities from the native AR amplificate (172 bp) in a series of four ARSt dilutions were compared to the intensities from the ARSt amplificate (134 bp). The resulting ratios were plotted against the known input of AR cRNA standard after transformation to the $\log_{10}$ scale (Siebert and Larrick, 1992). Using linear regression analyses (SAS, 1987), the number of molecules on the y-axis for ratio = 0 was estimated (Figure 1). This value corresponded to the concentration of ARSt cRNA molecules, which equaled the concentration of native AR mRNA molecules initially present in aliquots of total tissue RNA preparations.

Statistical Analysis

Data for live and carcass component weights were tested for effects of castration and feeding intensity using Student's *t*-tests (SAS, 1987). Additionally, carcass weight was used as a covariable in Type III ANOVA of the GLM procedure (SAS, 1987) to compare least squares means for muscle and adipose tissue content between treatment combinations within age at the same carcass weight.

Androgen receptor expression data were analyzed using the MIXED procedure (SAS, 1987; Littell et al., 1998), based on the assumption that repeated observations on the same animal are likely related. Data were analyzed in a model that contained 18S rRNA levels as a covariable and tested the effects of muscle, age, sex, nutrition and all possible two-way interactions between fixed effects. Unstructured covariance was fitted on the basis of the Schwarz's Bayesian criterion. Muscle was considered repeated on the same animal, which was nested within age, sex, and nutrition. The F-tests for total extractable RNA amounts were obtained by using the same model but omitting the covariable 18S rRNA.

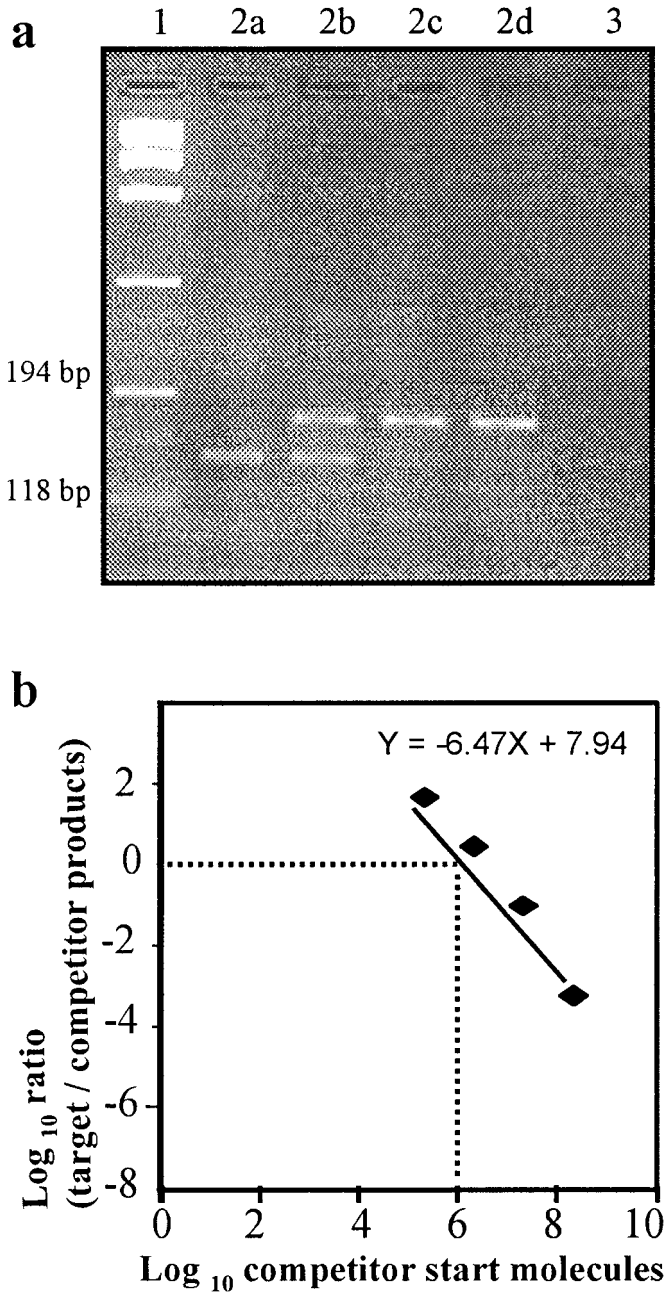

Figure 1. Quantitative reverse transcription (RT)-PCR analyses for androgen receptor (AR) mRNA abundance. (a) Gel electrophoresis of PCR products for wild-type and standard (174 and 134 bp, respectively) DNA templates. Lane 1: DNA molecular weight standard. Lane 2a–d: Native mRNA spiked with decreasing standard cRNA concentrations (2.24×10^8, 2.24×10^7, 2.24×10^6, and 2.24×10^5 molecules, respectively). Lane 3: negative control. (b) Quantification of native AR mRNA according to Siebert and Larrick (1992). Ratios of wild-type over standard products (ordinate) were plotted against known AR standard cRNA (abscissa). AR content corresponds to ratio = 0.

Table 1. Means ± SE for final live weights and carcass components for bulls (B) and steers (S) at different ages and feeding intensities (AL, ad libitum access to feed; R, restricted feed intake; C, compensatory growth rates)

	Age, mo:	4	8	12		16	
Item and sex	Feeding level:	AL	AL	AL	R	AL	C
Final weight, kg							
B		170^{a} ± 5.7	279^{a} ± 11.4	426^{ax} ± 15.1	357^{ay} ± 24.8	559^{ax} ± 0.15	550^{ax} ± 13.0
S		162^{a} ± 5.4	269^{a} ± 10.0	375^{bx} ± 15.5	348^{ax} ± 14.2	496^{bx} ± 18.2	486^{bx} ± 13.4
Carcass weight, kg							
B		82^{a} ± 2.9	131^{a} ± 7.2	226^{ax} ± 8.0	186^{ay} ± 12.8	305^{ax} ± 2.6	304^{ax} ± 6.7
S		82^{a} ± 3.5	128^{a} ± 4.6	196^{bx} ± 8.6	171^{ax} ± 8.5	271^{ax} ± 12.8	267^{bx} ± 6.9
Adipose tissue, kg							
B		7.4^{a} ± .83	15.8^{a} ± .99	46^{ax} ± 2.8	29^{ay} ± 3.3	64^{ax} ± 1.2	69^{ax} ± 2.8
S		7.7^{a} ± .73	17.5^{a} ± 1.98	44^{ax} ± 3.7	32^{ay} ± 3.0	83^{ax} ± 11.0	68^{ax} ± 2.7
Muscle tissue, kg							
B		59^{a} ± 2.3	91^{a} ± 5.5	153^{ax} ± 4.7	126^{ay} ± 8.4	205^{ax} ± 4.0	203^{ax} ± 5.3
S		57^{a} ± 2.4	87^{a} ± 3.5	128^{bx} ± 5.5	115^{ax} ± 5.9	164^{bx} ± 5.2	170^{bx} ± 5.1
Semitendinosus, kg							
B		$.68^{a}$ ± .034	1.19^{a} ± .072	2.0^{ax} ± .12	1.6^{ax} ± .13	2.5^{ax} ± .12	2.6^{ax} ± .08
S		$.68^{a}$ ± .038	1.09^{a} ± .066	1.6^{bx} ± .11	1.5^{ax} ± .14	2.0^{ax} ± .24	2.1^{bx} ± .10
Triceps brachii, kg							
B		$.95^{a}$ ± .044	1.45^{a} ± .082	2.5^{ax} ± .11	2.0^{ay} ± .13	3.5^{ax} ± .03	3.4^{ax} ± .14
S		$.88^{a}$ ± .030	1.42^{a} ± .054	2.2^{ax} ± .16	1.9^{ax} ± .11	2.7^{bx} ± .14	2.8^{bx} ± .15

^{a,b}Means bearing different letters within age and feeding level indicate a sex effect ($P < .05$).
^{x,y}Means bearing different letters within age and sex indicate an effect of feeding level ($P < .05$).

A 5% confidence level was considered statistically significant.

Individual muscle scatter plots were produced to examine the relationship between AR mRNA levels and muscle weights. Using the GLM procedure (SAS, 1987), estimated AR mRNA levels adjusted for 18S rRNA were plotted against individual muscle weights, which were adjusted for age, sex, nutrition, and total muscle mass in a Type I ANOVA analysis. A simple regression line by sex group was fitted to plots to demonstrate trends.

Results

Means for live and carcass component weights for each treatment are shown in Table 1. After 12 mo of age, bulls had higher final live weights, carcass weights, and total muscle weights than steers, with the exception of R animals. Adipose tissue weights were less ($P < .05$) in R than in AL bulls and steers. No sex-related differences in absolute adipose tissue weight were found (Table 1), but, when adipose tissue was adjusted for carcass weight, bulls were leaner ($P < .05$) than steers at 16 mo of age, regardless of feeding regimen. Additionally, muscle adjusted to carcass weight was higher ($P < .05$) for bulls than for steers independent of feeding regimen. Steers experiencing compensatory gains (C) had greater ($P < .01$) muscle weights at a similar carcass weight than AL steers.

Total RNA extraction yields per gram of muscle tissue varied between muscle types ($P < .001$; Table 2). As shown in Figure 2, highest extractable total RNA amounts were observed for TB and lowest amounts for SP, without any age-related variation in these muscles. Extractable total RNA decreased with increasing age in ST, especially between 4 and 12 mo of age ($P < .01$). Castration or feeding regimen did not affect extractable total RNA amount in any of the muscles studied.

Androgen receptor mRNA levels differed ($P < .01$) with muscle type and sex-group (Table 2). Age and nutrition did not contribute to the variation in AR mRNA levels. However, there was muscle × age interaction ($P < .05$) and sex × nutrition interaction ($P < .05$).

Androgen Receptor mRNA Levels in Bulls and Steers

Pooled over muscles and feeding levels, AR expression was not different between bulls and steers at 4 mo (8.04 and 8.87 × 10^6 molecules), 8 mo (7.63 and 8.21 × 10^6 molecules), or 12 mo of age (8.65 and 8.96 × 10^6 molecules). At 16 mo, AR message abundance tended to be less ($P = .07$) in muscle of bulls than in muscle of steers (8.76 × 10^6 vs 9.36 × 10^6 molecules, respectively).

Table 2. *P*-values obtained in (co)variance analyses of androgen receptor (AR) mRNA levels and extractable total RNA amounts

Dependent variable	Covariable 18S rRNA	Age	Sex	Muscle	Nutrition	Age × muscle	Age × sex	Sex × muscle	Muscle × nutrition	Sex × nutrition
AR mRNA	.13	.12	.01	.0001	.25	.03	.78	.34	.41	.04
Total RNA	—	.05	.84	.0002	.23	.01	.58	.53	.97	.95

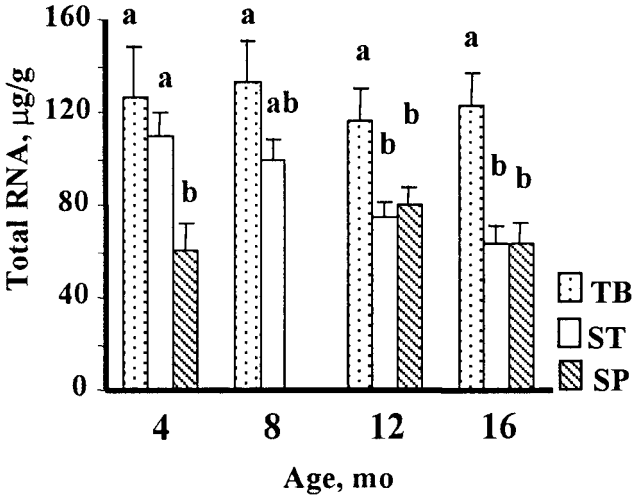

Figure 2. Amount of total RNA extracted from 1 g of muscle tissue (semitendinosus, ST; triceps brachii, TB; splenius, SP). Least squares means and SE of least squares means by age and muscle. Means bearing different letters differ ($P < .05$).

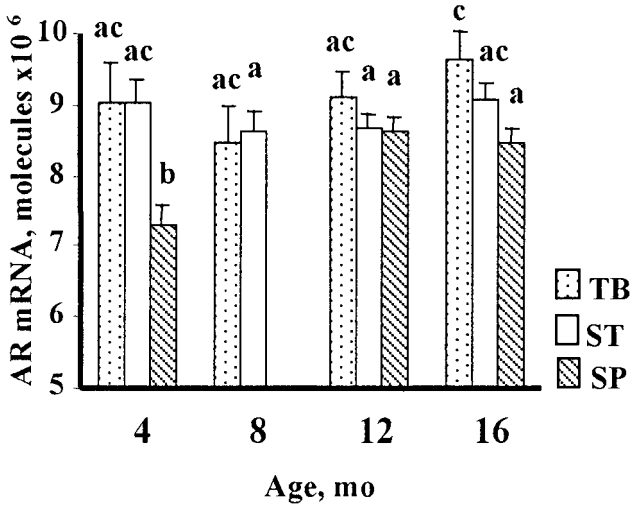

Figure 3. Androgen receptor mRNA levels in semitendinosus (ST), triceps brachii (TB), and splenius (SP) at different ages (least squares means and SE of least squares means). Means bearing different letters differ ($P < .05$).

In the absence of a muscle × sex interaction, the trend for lower AR mRNA levels in bulls than in steers was more pronounced in ST ($P < .01$; 8.50×10^6 vs 9.22×10^6 molecules) and TB ($P = .06$; 8.71×10^6 vs 9.40×10^6 molecules). Bulls and steers had similar AR mRNA levels in SP ($P = .25$).

Androgen Receptor mRNA Levels in Different Muscles

Pooled over ages and sex groups, AR mRNA levels per unit of total RNA measured in TB were 14% greater than those in SP ($P < .001$) but not different from those in ST (9.05×10^6, 7.92×10^6, and 8.86×10^6 molecules, respectively). The AR mRNA levels in ST were higher than in SP ($P < .001$). To a great extent, the variation between muscles was age-dependent, indicated by an interaction between age and muscle ($P < .05$). As shown in Figure 3, the greatest variation in AR mRNA levels between muscles was observed at 4 mo of age. In the absence of sex × muscle or sex × age interactions, AR mRNA levels from bulls and steers were pooled. The AR mRNA levels in ST and TB were approximately 20% higher ($P < .01$) than those measured in SP at 4 mo of age. The AR mRNA levels were similar in ST and TB at 4 and 8 mo of age. No data were recorded for SP at 8 mo of age. At 12 mo of age, similar AR mRNA levels were found in the three muscles studied, which were equal to those measured in ST and TB at 4 and 8 mo. Androgen receptor mRNA levels in SP were 20% higher at 12 mo than at 4 mo of age ($P < .001$). Between 12 and 16 mo of age, no significant variation of AR mRNA levels was observed in any of the three muscles. However, at 16 mo of age, AR message was more ($P < .01$) abundant in TB than in SP. At each of the developmental stages investigated, ST and TB expressed similar amounts of AR mRNA. The AR expression in ST and TB remained constant with age.

Androgen Receptor mRNA Levels at Different Feeding Intensities

Feeding regimen had no effect on AR mRNA abundance (Table 2). Interactions between feeding regimen and muscle type were not evident. However, a significant interaction between sex and feeding regimen was observed ($P < .05$). As depicted in Figure 4, there was no difference in AR mRNA expression between R and

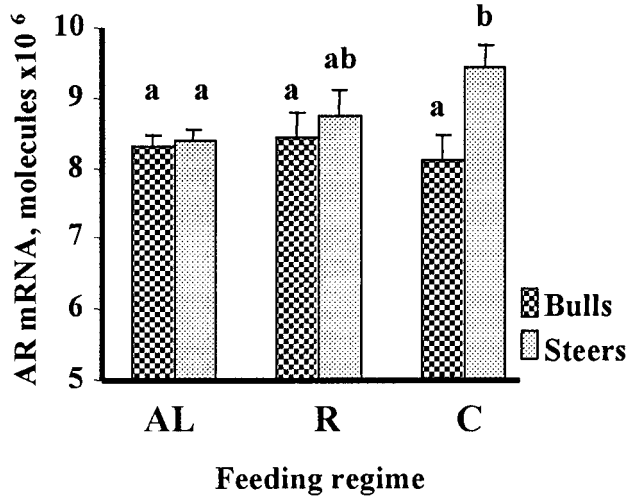

Figure 4. Androgen receptor mRNA levels in muscle from bulls and steers with ad libitum access to feed (AL), restricted feed intake (R), or compensatory (C) growth rates (least squares means and SE of least squares means). Means bearing different letters differ ($P < .05$).

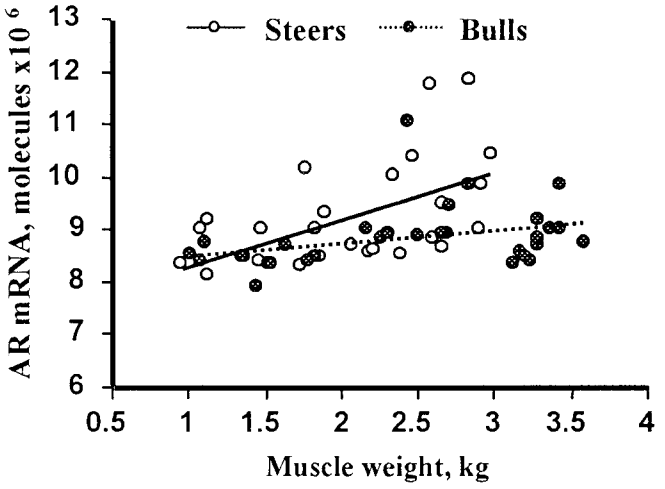

Figure 5. Association between molecules of androgen receptor (AR) mRNA and triceps brachii (TB) muscle weight (wt). Regression equations were as follows: steers = $(7.73 \times 10^6) + (7.73 \times 10^4) \times$ wt, r = .54 (P = .004); bulls = $(8.25 \times 10^6) + (2.05 \times 10^4) \times$ wt, r = .30 (P = .11).

AL animals, for either sex. Androgen receptor mRNA amounts were higher ($P < .01$) in muscle of C steers than in muscle of AL controls. In bulls, no difference in muscle AR mRNA expression was observed between C and AL bulls, being similar to AR expression measured in muscle of AL steers. Consequently, when comparing bulls and steers within feeding regimens (Figure 4), a castration effect was only found in animals with compensating growth rates. Muscle AR mRNA levels in C bulls were 16% lower ($P < .01$) than those measured in C steers.

In Figure 5, AR expression in the TB was plotted against TB muscle weight to illustrate growth differences between bulls and steers. Linear regression analysis indicated an association between AR mRNA content and muscle growth in steers ($P < .01$), but not in bulls. A similar association was found in ST of steers ($P < .05$), whereas AR mRNA expression and muscle weight was unrelated in ST of bulls.

Discussion

The AR mRNA levels we determined in three muscles of growing cattle ranged between 5.77×10^6 and 12.48×10^6 molecules of AR mRNA (10 to 20 fg) per 200 ng of total RNA, suggesting that local differences in AR expression exist between different muscle types and(or) modulation with age, androgen status, and growth rates.

Methodological Considerations

Because of its sensitivity, RT-PCR offers a powerful tool to quantify small amounts of mRNA and to measure small but physiologically relevant changes in gene expression, provided an internal standard is coamplified along with the native template (Bouaboula et al., 1992; Lessard et al., 1998). The AR sequence targeted in our experiment codes for the entire F exon within the ligand-binding domain and was identical to the sequence used by Malucelli et al. (1996). This internally standardized RT-PCR assay system allows for controlling differences in amplification efficiency, because both templates are affected similarly.

To normalize AR data for differences in total RNA input, the risk for which increases by lowering initial RNA quantity in the system, the 18S rRNA content was determined. This ribosomal RNA fraction is relatively constant in total RNA (Davies et al., 1997). Assuming a nonlinear relationship with the AR mRNA level, we opted for a regression-based approach with 18S rRNA as a covariable to remove variation in AR mRNA levels explained by differences in 18S rRNA. This data-normalization procedure has been reported by many researchers (Toth et al., 1993; Poehlman and Toth, 1995). Because the contribution of 18S rRNA would very likely not be constant over the full range down to a theoretical zero intercept of AR mRNA, the use of the ratio AR mRNA to 18S rRNA would introduce an arithmetic error (Weinsier et al., 1992). Thus, after appropriately accounting for 18S rRNA level in a covariance model, the variation in AR mRNA level would be due to differences in the expression of this particular gene.

Testicular Steroids as Regulators of Androgen Receptor mRNA Expression

Regulation of AR mRNA expression was investigated earlier in a variety of cell types and was shown to be both complex and tissue-specific. Autologous down-regulation of AR mRNA levels after androgen exposure, as exhibited in male reproductive tissues (Quarmby et al., 1990; Blok et al., 1992; Mora et al., 1996) and the larynx of frogs (Fischer et al., 1995), is the most common mode of regulation. Ligand-dependent up-regulation of AR mRNA has been reported in human bone cells (Wiren et al., 1997) and human adipocytes (Dieudonne et al., 1998). Increasing evidence suggests that complex regulatory loops between a steroid hormone and its receptor gene may exist (Sakeda et al., 1988; Supakar et al., 1993; Katzenellenbogen et al., 1996).

Using intact and castrated males as a model is more complicated because testicular-derived estrogens and(or) aromatization of testosterone in peripheral tissues could affect AR regulation. In most studies, the presence of testicular steroids was related to decreased AR mRNA concentrations when compared to castrates in bovine skeletal muscle (Malucelli et al., 1996), rat prostate (Mora et al., 1996), or rat pancreas (Diaz-Sanchez et al., 1995). In contrast, orchidectomy has also been demonstrated to down-regulate AR mRNA in hamster facial motoneurons (Drengler et al., 1996). We found greater AR expression in steers than in bulls. This was particularly evident for animals exhibiting compensatory growth rates. Malucelli et al. (1996) also

showed an increase in muscle AR expression of 11-mo-old castrates compared to intact males, and our present results support these findings and indicate that testicular steroids may negatively regulate AR expression. Our results, however, are in contrast to those reporting AR protein data, primarily because castration leads to a severe reduction of nuclear AR immunoreactivity in male excurrent ducts of goats (Goyal et al., 1998) or rat prostate (Mora et al., 1996; Suzuki et al., 1997). In ligand binding studies in which nuclear and cytosolic AR protein were differentiated, commonly a decrease of nuclear AR was reported after castration (e.g., in a broad variety of tissues in guinea pig; Choate and Resko, 1996). In contrast, the levels of cytosolic AR in castrates were reported to increase in rat striated muscle (Rance and Max, 1984), in bovine skeletal muscle (Sauerwein and Meyer, 1989), and in various guinea pig tissues (Choate and Resko, 1996). Ligand-dependent responses on the AR may be more evident at the protein level than at the mRNA level. In view of this, one might speculate that testicular steroids more likely regulate posttranscriptional events in AR gene expression.

Muscle-Specific Androgen Receptor mRNA Expression

Divergent densities of cytosolic AR in individual bovine muscles have been reported previously (Sauerwein and Meyer, 1989), but these investigations were limited to comparisons within animals of a given endocrine status and age. The present study provided data on developmental regulation of AR mRNA expression in three muscles that differed in muscle fiber composition, metabolic activity, and growth pattern (Brandstetter et al., 1998 a,b). The ST from the hind leg exhibits a high growth rate during the 1st yr of life, followed by a significantly lower growth rate. In this muscle, constant AR mRNA levels per unit of total RNA were measured. The ST growth is related to an overproportional hypertrophy and dilution of myofiber nuclei and mitochondria that was reflected in a drastic decrease of extractable total RNA amounts. In this context, Arnold et al. (1997) also found a similar decrease of total RNA content in ST of growing lambs. In order to evaluate the potential for direct androgen action on muscle growth, total RNA yield should be taken into account, although this method of measuring RNA content is somewhat variable. Provided that AR mRNA concentrations reflect androgen sensitivity, the dilution of total RNA and AR mRNA as a constant proportion in ST might be related to the observed decrease in relative growth intensity. The low AR mRNA level in SP at 4 mo of age and subsequent increase might be related to the biphasic growth pattern of this sexually dimorphic muscle (Arnold et al., 1997). We speculate that maximum growth rates of SP would even occur later than at 16 mo of age and may explain the relatively low AR mRNA levels compared to TB and ST. As shown previously (Brandstetter et al., 1998a), TB exhibited a continuous age-related increase in allometric growth coefficients. We attempted, therefore, to associate TB muscle weight with AR mRNA content over age, which possibly reflected increased androgen sensitivity. Because this relationship in bulls did not reach significance, these results call into question the role of ligand activation of receptors in modulating muscle growth. However, these data support, rather than contradict, a relationship between AR mRNA concentration and allometric muscle growth.

It is still unclear which components of muscle tissue would express AR mRNA. Connective tissue was found to increase with age and to be at greater amounts in TB than in ST (Damergi et al., 1998), but a direct relationship with AR expression needs to be demonstrated. Using immunohistological approaches, many have reported that all myonuclei do not contain immunoreactive AR protein (Dorlochter et al., 1994; Hyyppa et al., 1997; Jordan et al., 1997). It is, therefore, tempting to hypothesize that AR expression could be differentially regulated in different fiber types. Previously, we observed age-related changes in muscle fiber type profiles in cattle (Brandstetter et al., 1998a); thus, is it possible that AR expression is also muscle fiber type-dependent.

Different Feeding Intensities and Androgen Receptor mRNA Expression

Reduced energy intake was reported to prolong the androgen-responsive state of the rat liver by delaying the age-dependent loss of an immunoreactive, androgen-related protein (Chatterjee et al., 1989). Song et al. (1991) showed that this change in hepatic androgen sensitivity was indeed due to a delay in the decline of the AR mRNA level. In our study, feed restriction between 9 and 12 mo of age had no effect on AR mRNA levels in skeletal muscle. We can assume that reduced growth during feed restriction was not androgen-related, but more probably was mediated by other regulatory systems. During the 4-mo compensation period following restricted feeding, the bulls and steers had an advantage in muscle growth compared to their AL counterparts. Though our results on AR expression need further investigation, we hypothesize there might be a positive relation between compensatory muscle growth and AR mRNA concentrations and AR density in steers. However, the mechanism by which AR mRNA levels are up-regulated and might mediate a direct effect of extratesticular androgens on muscle growth in compensating steers remains speculative. Considering the remaining synthesis of sex steroids in the adrenal glands, the marked sexual dimorphism of adrenocortical function in cattle (Verkerk and Macmillan, 1997), and the relatively high concentration of androgen precursors in muscle tissue of steers (Fritsche and Steinhart, 1998), numerous effectors of AR mRNA expression are possible.

Implications

Differences in androgen receptor mRNA concentrations between muscles with different fiber type composi-

tions and growth impetus indicate a positive relationship with the individual growth patterns. However, the importance of age and castration for the androgen sensitivity of skeletal muscle needs further investigation in terms of androgen receptor protein concentration and cellular distribution. Moreover, the role of estrogens in regulating androgen receptor density and affecting muscle growth through estrogen receptors should be considered in future studies.

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