Regulation of androgen receptor mRNA expression in three different skeletal muscles during postnatal development in bulls and steers.

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INTRODUCTION

The effect of testicular steroids on muscle growth is evident from the sexual dimorphism in muscularity: intact males have more muscle, especially in the neck and forequarter, than females and castrates. Varying steroid hormone sensitivities, i.e., receptor densities of muscles might account for the differential growth rates.
To be able to look at the entire actual androgen receptor (AR) synthesis, the receptor mRNA was quantified. The present study thus compares AR mRNA expression rates in allometrically growing muscles during postnatal development in presence or absence of testicular steroids.

MATERIAL AND METHODS

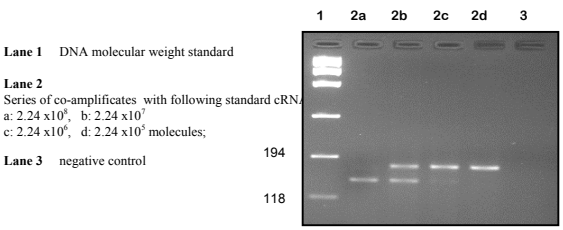
Animals: 12 Montbéliard bulls and 13 steers, slaughtered at 4, 12, 16 months of age (4 or 5 per group).

Muscles: ***Semitendinosus*** (ST), ***Triceps brachii*** (TB) and ***Splenius*** (SP)

Tissue RNA extraction and Northern blots: After homogenization of the tissues according to Chirgwin et al. (1979), total cellular RNA was isolated using RNA-Clean™ (AGS, Heidelberg, Germany) and quantified by OD_{260} readings. RNA integrity and ribosomal RNA quantity was verified by Northern-blot analysis, using a [γ-^{32}P]ATP 5' end-labeled 18S ribosomal RNA probe. Intensity of resulting bands was quantified with the ImageQuant software (Molecular Dynamics).

AR mRNA quantification: AR mRNA was measured with a competitive reverse transcription polymerase chain reaction (RT-PCR) test system, using 200 ng of tissue RNA and known dilutions of internal standard cRNA mutant coding for the ligand binding domain and derived from the bovine AR sequence (Malucelli et al., 1996) .

Figure 2. Separation of co-amplified specimen by gel electrophoresis
PCR co-amplificates from wild-type (174bp) and standard (134bp) DNA templates.



Lane 1 DNA molecular weight standard

Lane 2
Series of co-amplificates with following standard cRNA:
a: 2.24 x10^8, b: 2.24 x10^7
c: 2.24 x10^6, d: 2.24 x10^5 molecules;

Lane 3 negative control

The yields of a series of amplification products were compared by plotting their ratio against the $\log_{10}$ of the known input of internal standard template (Siebert & Larrik, 1992). In a linear regression analysis, the number of competitor molecules for ratio = 0 was estimated, corresponding to the concentration of AR standard cRNA molecules which equaled the concentration of native AR mRNA molecules initially present in aliquots of tissular RNA.

Statistical comparisons: Resulting data was analyzed by utilizing the GLM procedure of Statistical Analysis Systems (1987). 18S mRNA level was used as a covariate. The animal effect was nested within age and sex and was set random. The age and sex effects were tested against animal. The residual mean square was used as an error term for the other effects. $P < .05$ was considered statistically significant.

RESULTS

Table 1. Analysis of covariance of AR mRNA levels per unit of total RNA and per gram of muscle tissue

Item	*18S rRNA*	*Animal*	*Sex*	*Age*	*Muscle*	*Age*Muscle*
AR mRNA (units per 200ng total RNA)	**.53**	**.11**	**.76**	**.15**	**.04**	**.05**
AR mRNA (units per gram of tissue)	**.63**	**.10**	**.85**	**.93**	**.0001**	**.05**

* denotes an interaction

- AR mRNA levels in bulls were similar to those in steers.
- AR mRNA levels per RNA unit varied between muscles, SP showing lower levels than TB.
- Variations in AR mRNA expression rates with age were muscle-dependent, increasing in SP but not being affected in TB and ST.

Figure 1. AR organisation and AR cRNA standard construction

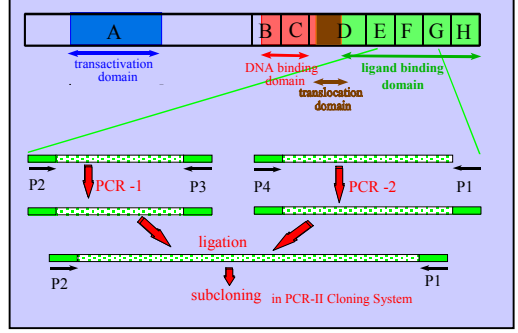
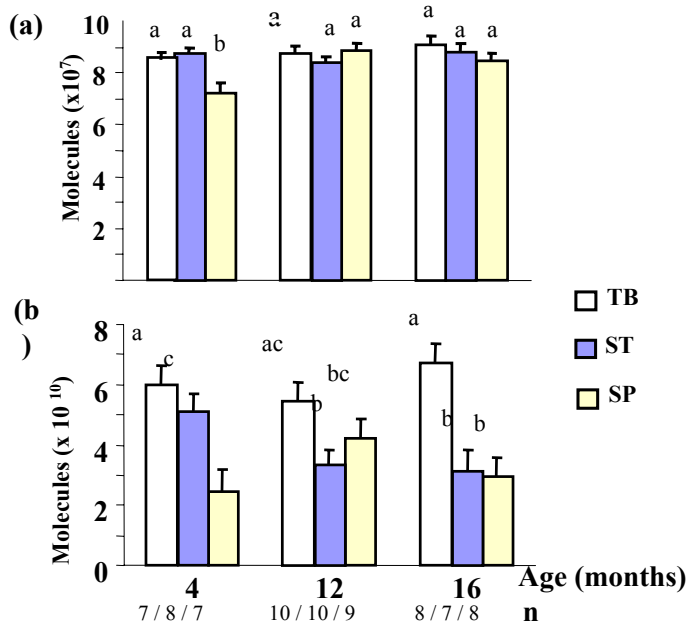


Figure 3. AR mRNA levels measured in 200 ng of tissue RNA (a) and AR mRNA levels expressed per gram of tissue RNA (b) Bulls and steers, by age and muscle type (least squares means)



- When expressed on a gram tissue basis, AR mRNA levels in TB were 60 % to 100 % higher ($P < .01$) than in ST and SP at comparable ages.
- An age-dependent decline of AR mRNA levels per gram of tissue was observed in ST, due to decreasing RNA extraction yields ($P < .01$).

CONCLUSIONS

These data indicate that the AR mRNA level is muscle-individually modulated. The rate of expression of the AR gene seems to follow an age-dependent pattern, which could play an important role for allometric growth phenomena. Androgens are not relevant effectors of AR mRNA expression in skeletal muscle.
The direct effects of androgens on skeletal muscle are regulated at the ligand level rather than at the receptor level, but might depend on receptor expression varying among muscles.

References:
CHIRGWIN, J. M., E. A. PRZYBYLA, J. MACDONALD & W. J. RUTTER (1979). J. Biochem. **18,** 5291-5299.
MALUCELLI, A., H. SAUERWEIN, M. PFAFFL & H. H. D. MEYER (1996). J. Steroid Biochem. Mol. Biol. **58,** 563-568.