E5

CELLULAR RESPONSE OF BROILER SKELETAL MUSCLE TO THE BETA-ADRENERGIC AGONIST CLENBUTEROL

C. REHFELDT, R. WEIKARD, K. REICHEL
Research Institute for the Biology of Farm Animals, 18196 Dummerstorf, Germany

Keywords: muscle, muscle fibre, protein, nucleic acid, clenbuterol, ß-adrenergic agonist, chicken

Background

Repartitioning agents have been shown to modulate carcass composition of different species by stimulating protein accretion at the expense of fat deposition. The ß-adrenergic agonists have been reported to be efficient in rats, lambs, sheep, cattle, swine and broiler chickens (e.g. Emery et al., 1984, Baker et al., 1984, Beermann et al., 1986, Ricks et al., 1984, Jones et al., 1985, Dalrymple et al., 1984). Additionally there is some evidence that the intensity of responses depends of the endogenous predisposition to a certain body composition, which differs for instance between genotypes or sexes (e.g. Dalrymple et al., 1984, Lamming et al., 1987). The repartitioning process is accompanied by various modifications at the level of the muscle cell. Changes in muscle fibre size, muscle fibre type frequencies and distribution of myonuclei or nucleic acid content in response to ß-adrenergic agonists have been reported based on studies with rats, cattle, lambs and swine (e.g. Maltin et al., 1986, Kim et al., 1988, Vestergaard et al., 1991, Beermann et al., 1987, Kim et al., 1987, Oksbjerg et al, 1990). No results were obtained from experiments with chickens as non-mammals of agricultural importance.

Objective

The effects of chronic administration of the ß-adrenergic agonist clenbuterol on skeletal muscle characteristics, including muscle weight, fibre size, number and type, and DNA, RNA, protein content, in male and female broiler chickens were investigated.

Methods

Broiler chickens of the type 'Tetra8' obtained from VEG(Z) Tierzucht Groß Stieten were used in this study. On day 28 of age males and females were assigned each to 2 groups of 16 birds (control and experimental group). Within each sex all chickens were approximately of the same initial mean live weight. The control groups were fed a finishing diet, containing maize, soybean meal, yeast, fish meal, mineral and vitamin mix. The experimental groups additionally received 1 mg/kg clenbuterol (VEB Arzneimittelwerk Dresden) mixed into the feed. Feed and water were available *ad libitum*. After 3 weeks the broilers were weighed, slaughtered and prepared for analysis. *Biceps femoris (BIC), pectoralis profundus (PEC), gastrocnemius (GAS)* and *extensor hallucis longus(EHL)* muscles were removed and weighed. The latter 2 muscles were prepared for histological analysis for muscle fibre size, number and types, and for nuclear numbers according to Rehfeldt et al. (1993). In addition, the EHL muscle was analysed for protein and nucleic acid content, for cell-free translation activity and for calcium-dependent proteolytic (CDP) activity as described by Weikard et al. (1992a). For statistical analysis the data were subjected to analyses of variance using the GLM procedure (SAS®) considering the effects of clenbuterol treatment and of sex at unequal class size.

Results and discussion

The detailed results of growth performance and carcass composition are given by Reichel (1990). They indicate that the longterm treatment of broilers with clenbuterol caused a repartitioning of energy indicated by improved performance, carcass yield and reduced carcass fat (Reichel, 1990). The effects on carcass were more pronounced in females, which confirms results of Dalrymple et al. (1984). It seems that, based on their higher predisposition to fat, in females more energy becomes available for protein accretion by metabolic repartitioning.

Table 1: Least squares means of live weight, muscle weights, histological and biochemical characteristics of *extensor hallucis longus* muscle in controls and clenbuterol treated broiler chickens (*significant treatment effect within sex; $P < 0.05$)

	Males		Females		SEM	Significance of factors (P<)	
	Control	Clenbuterol	Control	Clenbuterol		Clenbuterol	Sex
Live weight (kg)	2.19	2.27*	1.79	1.85	0.03	0.01	0.0001
Weight EHL (g)	3.00	3.53*	2.53	3.09*	0.20	0.0001	0.0001
Weight GAS (g)	9.06	10.20*	7.18	8.25*	0.27	0.0001	0.0001
Weight BIC (g)	9.94	11.09*	7.35	8.79*	0.32	0.001	0.0001
Weight PEC (g)	31.2	34.5 *	29.0	30.8 *	0.74	0.001	0.0001
EHL characteristics:							
Fibre diameter STO (µm)	38.1	41.5	41.3	46.9*	13.5	0.05	0.05
Fibre diameter FTO (µm)	67.8	70.4	61.8	69.8*	13.1	-	-
Fibre diameter FTG (µm)	69.3	71.8	72.4	77.7	7.2	-	0.05
Fibre diameter Mean (µm)	59.2	64.3	60.4	67.4*	11.5	0.01	-
Total fibre number (*10^3)	23.88	22.81	14.54	15.36	1.69	-	0.0001
Fibre number STO (%)	20.6	19.2	21.0	21.1	1.2	-	-
Fibre number FTO (%)	42.1	32.6*	41.4	38.8	2.1	0.01	-
Fibre number FTG (%)	38.0	48.0*	37.8	40.1	1.5	0.001	0.05
Nuclei/fibre	3.93	3.97	4.62	4.66	0.23	-	0.01
Nuclei/mm^2	1461	1259	1662	1330 *	105	0.05	-
DNA (µg/g)	358.8	340.9	403.1	358.8	18.4	0.1	0.1
RNA (µg/g)	952.1	998.5	891.6	885.3	26.3	-	0.01
Protein (mg/g)	178.8	173.5	155.2	159.8	6.4	-	0.01
RNA (mg)	2.87	3.52*	2.28	2.76*	0.13	0.0001	0.0001
RNA/DNA	2.74	3.00	2.30	2.52	0.13	0.1	0.001
Protein/RNA	0.186	0.174	0.175	0.182	0.009	-	-
DNA/Protein	2.14	1.97	2.63	2.28	0.16	0.1	0.05
Translational activity (dpm*10^3)	36.55	31.66	30.86	31.95	2.53	-	-
CDP activity (abs_{334})	0.365	0.345	0.394	0.370	0.010	0.05	0.05

In order to further examine the effect of clenbuterol on skeletal muscle, some biochemical and cellular characteristics of selected muscles were determined. They are summarized in Table 1. The final wet weights of all 4 muscles examined were significantly increased by clenbuterol treatment. The increase was between 6 and 22%. With the exception of PEC muscle the muscles of females showed a somewhat higher relative response than males, which is in accordance with the effects on carcass composition. Other studies with various species have concluded that the efficacy of repartitioning agents is lower in physiologically or genetically lean animals (e.g. Dalrymple et al., 1984, Kanis et al., 1990). This theory might also be true for parts of the body and in this case may be the reason for the very low response of PEC muscle, which is highly influenced by selection.

The response of muscle fibre size to clenbuterol was different between muscles and also slightly dependent on sex. Clenbuterol significantly increased the mean diameters of EHL muscle in males (by 8.5%; $P<0.05$) and females (by 11.5%; $P<0.01$). Both slow (ST, Type I) and fast (FT, Type II) fibres were affected. However, the relative response of fast twitch glycolytic (FTG) fibres was somewhat lower as compared to fast twitch oxidative (FTO) and slow twitch oxidative (STO) fibres. Despite the clear response of GAS weight to clenbuterol feeding the fibres were only slightly larger in GAS muscle (by 2.5%, $P>0.01$; data not shown). From the initial differences in EHL and GAS weights at almost equal fibre size it is suggested that the GAS total muscle fibre number must be markedly larger and that already small increases in fibres size might be sufficient to cause the clear weight response. ß-agonist treatment is reported to enlarge fast and slow contracting fibres in rats (Maltin et al., 1986, Zeman et al., 1986, Kim et al., 1988), sheep (Beermann et al., 1987), cattle (Coleman et al., 1986, Vestergaard et al., 1991) and swine (Sainz et al., 1993). In some cases dominant responses of FT fibres were observed.

With regard to fibre type composition in male EHL muscle a marked shift to FTG fibres (+10% units; $P<0.001$) occurred at the expense of FTO fibres. In GAS muscle and in female EHL muscle FTG percentages only tended to be larger after clenbuterol treatment. No conversion from slow to fast fibres was found. Slow to fast conversion appears to be associated with very long term ß-agonist treatment (Beermann et al., 1987, Zeman et al., 1986). Oxidative to glycolytic fibre conversion was previously found by Maltin et al. (1986), Oksbjerg et al. (1990), Vestergaard et al. (1991) or Rehfeldt et al. (1994). Thus metabolic properties of chicken muscle can be expected to be changed by clenbuterol towards glycolytic pathways, whereas the contractility presumably remains unaffected.

The total number of EHL muscle fibres was clearly different between sexes but unaffected by clenbuterol treatment. The latter was previously found for rat *soleus* and *extensor digitorum longus* muscles (Maltin et al., 1986, Rehfeldt et al., 1994). Consequently, the hypertrophic response of muscle to clenbuterol is based on muscle fibre hypertrophy and not on muscle fibre multiplication. There was no difference in the number of nuclei per fibre cross section between control and clenbuterol groups in EHL and GAS muscle. On the other hand the nucleus-cytoplasm-ratio (nuclei/mm² fibre area) was markedly decreased (by 20%; $P<0.05$) in EHL muscle of females because of the disproportional changes in fibre size and nuclear numbers. Accordingly, the DNA concentration and DNA/protein ratio declined by 11% and 13%, respectively ($P<0.1$). Again this effect was less pronounced in males. Consequently, clenbuterol stimulated fibre growth without additional incorporation of nuclei by satellite cell proliferation, but considerably enhanced protein accretion per nucleus. The same could be suggested from previous studies with rats (Kim et al., 1988, Eadara et al., 1989, Rehfeldt et al., 1994), lambs (Kim et al., 1987, Beermann et al., 1987) and swine (Johnson et al., 1987, Grant et al., 1993). The RNA and protein concentrations of EHL muscle were unaffected by clenbuterol. However, from the elevated total RNA content (by 22%; $P<0.0001$), decreased RNA/DNA ratio (by 10%; $P<0.1$) and unchanged protein/RNA ratio it is suggested that clenbuterol stimulated protein synthesis at the pretranslational level. The capacity for protein synthesis is increased. No changes were found in the cell-free translational activity of EHL muscle cell sap fraction in response to clenbuterol treatment. The calcium-dependent proteolytic (CDP) activity, however, was somewhat decreased by clenbuterol (by 6%; $P<0.05$). On principle, the latter findings are in agreement with prevoiusly obtained results for rat muscle (Weikard et al., 1992b).

Conclusions

Longterm treatment of broiler chickens with clenbuterol stimulates skeletal muscle growth, and this is more pronounced in females. The clenbuterol-enhanced muscle growth is realized through the hypertrophic response of muscle fibres (mainly STO and FTO), but not through the formation of new fibres or the additional incorporation of myonuclei by satellite cell proliferation. Clenbuterol may induce metabolic shifts towards glycolytic pathways indicated by fibre type conversions from fast twitch oxidative to fast twitch glycolytic fibres. The enhanced protein accretion may result from increased gene expression at the pretranslational level of protein synthesis and from decreased proteolytic activity, whereas translation appears to be unaffected.

References

BAKER, P.K., DALRYMPLE, R.H., INGLE, D.L., RICKS, C.A.: *J. Anim. Sci.* **59**, 1256 (1984)

BEERMANN, D.H., BOYD, R.D., FISHELL , V.K.,. ROSS, D.A.: *Fed. Proc.* **46**, 1020 (1987)

BEERMANN, D.H., HOGUE, D.E., FISHELL, V.K., DALRYMPLE, R.H., RICKS, C.A.: *J. Anim. Sci.* **62**, 370 (1986)

COLEMAN, M.E., EKEREN, P.A., LUNT, D.K., SMITH S.B.: *J. Anim. Sci.* **63**, Suppl. 1, 3 (1986)

DALRYMPLE, R.H., BAKER, P.K., GINGHER, P.E., INGLE, D.L., PENSACK, J.M., RICKS, C.A.: *Poultry Sci.* **63**, 2376 (1984)

EADARA, J.K., DALRYMPLE, R.H., DELAY, R.L., RICKS, C.A., ROMSOS, D.R.: *Metabolism* **38**, 883 (1989)

EMERY, P.W., ROTHWELL, N.J., STOCK, M.J. , WINTER, P.D.: *Biosci Rep.* **4**, 83 (1984)

GRANT, A. L., SKJAERLUND, D. M., HELFERICH, W. G., BERGEN, W. G., MERKEL, R. A.: *J. Anim. Sci.* **71**, 3319 (1993)

JOHNSON, S.E., BERGEN, W.G., MERKEL, R.A., STACHIW, M.A.: *J. Anim. Sci.* **65**, Suppl.1, 277 (1987)

JONES, R.W., EASTER, R.A., MC KEITH, F.K., DALRYMPLE, R.H., MADDOCK, H.M., BECHTEL, P.J.: *J. Anim. Sci.* **61**, 905 (1985)

KANIS, E.; NIEVWHOF, G.J.; DE GREEF, K.H.; VAN DER HEL, W.; VERSTEGEN, M.W.A.; HIUSMAN, J.;VAN DER WAL, P.: *J. Anim. Sci.* **68**, 1193 (1990)

KIM, Y.S., LEE, Y.B., ASHMORE, C.R.: *Growth, Development & Ageing* **52**, 41 (1988)

KIM, Y.S., LEE, Y.B., DALRYMPLE, R.H.: *J. Anim. Sci.* **65**, 1392 (1987)

LAMMING, G.E.: In: Beta-agonists and their effects on growth and carcass quality in farm animals. Ed. Hanrahan, J.P. 186 (1987)

MALTIN, C.A., DELDAY, M.I. , REEDS, P.J.: *Biosci. Rep.* **6**, 293 (1986)

OKSBJERG, N., BLACKSHAW, A., HENCKEL, P., FERNANDEZ, J.A., AGERGAARD, N.: *Acta Agric. Scand.* **40**, 397 (1990)

REHFELDT, CH., ENDER, K.: *Meat Sci.* **34**, 107 (1993)

REHFELDT, CH., WEIKARD, R., REICHEL, K.: *Arch. Anim. Nutr.* **45**, 333 (1994)

REICHEL, K.: PhD Thesis, Univ. Rostock, Germany, 157 pp (1991)

RICKS, C.A., DALRYMPLE, R.H., BAKER, P.K., INGLE, D.L.: *J. Anim. Sci.* **59**, 1247 (1984)

SAINZ, R. D., KIM, Y. S., DUNSHEA, F. R., CAMPBELL, R. G. : *Aust. J. Agr. Res.* **44**, 1441 (1993)

VESTERGAARD, M., PURUP, S., HENCKEL, P., KLASTRUP, S., SEJRSEN, K.: *Proc. 42nd Ann. Meeting EAAP, Satellite Symp.* Dummerstorf, Germany, 69(1991)

WEIKARD, R., REHFELDT, Ch., ENDER, K.: *Arch. Tierz.* **35**, 273 (1992)

WEIKARD, R., REHFELDT, Ch., ENDER, K.: *Arch. Tierz.* **35**, 421 (1992)

ZEMAN, R.J., LUDEMANN, R. EASTON, T.G., ETLINGER, J.D. : *Am. J. Physiol.* **254**, E726 (1988)