

# STUDIES ON HISTOSTRUCTURES OF SOME ORGANS, AS WELL AS ON SOME MORPHOLOGICAL AND LIPID CHARACTERISTICS OF INTERNAL FAT DEPOTS IN HOGGETS RECEIVING DIFFERENT CLENBUTEROL DOSES

DITCHEVA L., BANSKALIEVA V., MARINOVA P. and ANGELOV A.

Institute of Animal Science, Kostinbrod, BULGARIA

S-IVA.21

## SUMMARY

Comparative investigations have been conducted on male hoggets receiving every day clenbuterol 1 mg/kg of food for 6 months (I experiment) and 10 mg/kg of food for 6 weeks (II experiment). Histostructures of the lung, liver and kidney have been studied. Average size of adipocytes from perirenal adipose tissue (PAT), intermuscular adipose tissue (IMAT) and intramuscular adipose tissue (InMAT) as well as fatty acid composition of triacylglycerols from PAT, IMAT, InMAT and caul have been determined. Regardless of both the dose and treating duration, more essential changes were observed in the histostructures of lung only. Both high and low clenbuterol doses induce certain changes in unsaturated/saturated fatty acids ratio for fat depots studied. Changes have been also observed in the size of adipocytes, being more important in IMAT, where maximum of distribution curves in adipocytes average diameter moves to cells of less diameter. In a 7 day withdrawal period (I experiment) and 14 days pause (II experiment) after clenbuterol intake some of observed changes keep on, in other ones a trend to values of control group being observed.

## Introduction

Applying of B-agonists is accompanied with effects on both heart activity and respiratory system (Zimmerli and Blum, 1990), with changes in histostructures of liver, lung and kidney (Ditcheva, 1992), adipocyte cellularity (Schiavetta et al., 1990, Banskalieva et al., 1992, 1993). It is unknown, however, to what extent structural changes in different organs and tissues being connected with changes in lipid metabolism, and how much they depend on both the dose and treating duration with B-agonists. The aim of the present study was at investigating the effect of two different clenbuterol doses, of different treating duration on histostructures of some organs and tissues, as well as on adipocyte cellularity and some lipid characteristics of various internal adipose tissues in hoggets.

## Material and methods

Two experiments have been carried out on male hoggets. Animals of each experiment were divided in control and two experimental groups. For the whole experimental period (6 months - I experiment, 6 weeks - II experiment) animals in all groups received the same diet (Shindarska et al., 1993). To the diet of animals of experimental groups every day 1 mg (I experiment) and 10 mg (II experiment), respectively of clenbuterol per kg of diet has been added. At the end of experiments, the hoggets of both control and the first experimental groups were slaughtered, and these of the second experimental groups - 7 days (I experiment) and 14 days (II experiment) later, having received no clenbuterol for that time. Investigations of histostructures of both lung, liver and kidney have been carried out according a method, being described previously (Ditcheva, 1992). There was determined the average diameter of adipocytes from intramuscular adipose tissue - in m.L.Dorsi (InMAT), intermuscular adipose tissue - in m. Semimembranosus area (IMAT) and perirenal adipose tissue (PAT), as well as fatty acid composition of triacylglycerols (TG) of PAT, MAT, InMAT and caul, described in an our previous investigation (Banskalieva et al., 1992).

## Results and discussion

At the histological studies in liver of animals of experimental group in I experiment have been established hyperemia of blood vessels, dystrophic changes in a part of hepatocytes around v.centralis, as well as groups of quite increase - sized cells. Zones around v.centralis are most vulnerable to disturbance in blood circulation, hypoxia and insufficient providing of nutrients (Damianov, 1982). Increasing or decreasing size of hepatocytes has been observed in investigations studying the effect of different toxic substances on liver (Tasea, 1976). Granulation in cytoplasm of hepatocytes and hyperemia have been established in animals of the second experimental group, a week after withdrawing of clenbuterol supplement to the diet. In histostructures of kidneys at that experiment, no essential changes have been found. Malpighian corpuscles and functional canals were of both normal form and structure. On sites, mainly in pars convoluta dilated blood vessels could be seen. In the first experiment most significant changes were in lungs: overfilled capillaries of blood, in alveolus orifice, oedema fluid and frequently erythrocytes. In both interstitium and alveoli foci of diapedese haemorrhage and macrophagi have been observed, in whose plasma a brown pigment was accumulated. Inter-alveolar walls in those foci are firmed and thickened. A week after stopping of clenbuterol supplement, above described changes - even in a poorer extent - have been observing. In the second experiment, changes in liver are similar to those in the first one. In kidneys, however, except for strongly marked hiperemia of blood vessels, Malpighian corpuscles frequently have been of untypical form, exudate in the space of Bauman - Shumlianski and overfilled capillary ansas of blood. In the lumen of small canals both exudate and erythrocytes have also been observed. Described changes in lungs of the first experiment have been more strongly marked in the second one, with diffuse infiltrates of leucocytes. After a 14 day pause, the condition of histostructures of liver and kidneys significantly return to normal, while in lungs restoration process was more poorly manifested. Changes occurring in organs studied by us may be greatly expressed by the established action of clenbuterol on the organism of animals. Results presented in Fig. 1 show that both at high and low dose, clenbuterol induces increasing of relative part of fat cells of less diameter. Under prolonged treatment, observed changes are more marked in PAT compared to other two depots, while at high dose that holds true of InMAT. In the last adipose tissue, relative part of cells of average diameter 24 m increases over 50%. This is of interest to note that lipid content of this muscle decreases by nearly 50% (Shindarska et al., 1993). Behind 7- and 14-day pauses after clenbuterol intake, a trend is observed to approaching the values of control groups in all four fat depots studied. Regardless of both dose and treatment duration, clenbuterol induces an increase of total unsaturation of TG from internal fat depots studied - PAT, InMAT, IMAT and caul (Fig.2). More significant changes at high dose are observed in PAT and IMAT, and at low one-in IMAT and caul. Averagely for all three depots, however, the effect of both doses is nearly the same. More essential changes in unsaturation correspond to major changes in quantities of perirenal adipose tissue and caul (Banskalieva et al., 1993), i.e. reduced fat deposition is accompanied with increasing of unsaturation of TG. On the other hand, however, no correspondence was observed between the rate of changes in fatty acid composition and average adipocyte size in fat depots studied (Fig. 1, 2). The cause for total unsaturation increase, after B-agonists intake, remain unclear. Presumably, clenbuterol has a direct effect on desaturase activity in fat depots, but biological sense of this effect is not clear. Changes observed are probably the result of complex changes in lipid metabolism, not only in fat depots but also in metabolism of single organs and tissues. It is worth nothing the pauses after clenbuterol treatment have different effect in both experiment (Fig.2). At high dose a trend is observed towards returning to the values of control groups, while at low one, clenbuterol effect persists even after that period. Presumably, more prolonged treatment is of more durable effect on lipid characteristics studied.

## Conclusion

Results obtained show that clenbuterol effect depends on both the dose and treatment duration. Both organs and tissues studied react in a specified manner, but relationship among all those influenced processes remain still unclear.

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