

THE INFLUENCE OF ACTH AND DEXAMETHASONE  
ON LEPTIN AND NO RELEASE  
FROM OVINE PITUITARY CELLS *IN VITRO*

INFLUENZA DELL'ACTH E DESAMETASONE SULLA LIBERAZIONE  
DI LEPTINA E OSSIDO NITRICO  
DALLE CELLULE IPOFISARIE OVINE *IN VITRO*

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SUMMARY

The objective of the study was to analyse the effect of ACTH and dexamethasone on leptin and nitric oxide release from ovine pituitary cells *in vitro*. Pituitary cells were cultured in McCoy 5A medium without hormones (control) and with  $10^{-10}$ ,  $10^{-9}$ ,  $10^{-8}$ ,  $10^{-7}$ ,  $10^{-6}$  or  $10^{-5}$  M/l of ACTH or dexamethasone, respectively. After 24 h of cell culture, the media were collected to leptin and NO analysis. The obtained results show that dexamethasone exerts the stimulative effect, both on leptin and NO release from ovine pituitary cells *in vitro*. The influence of ACTH on leptin and NO release, instead, is dose dependent. ACTH in concentration ( $10^{-10}$  -  $10^{-9}$  M/l) increases and in concentration  $10^{-8}$  -  $10^{-5}$  M/l decreases the release both of these compounds. There was the positive correlation between the quantities of leptin and nitric oxide released to the culture medium, both in the experiment with using of dexamethasone and ACTH ( $r = 0.77$  and  $r = 0.89$ , respectively).

Key words: ACTH; Dexametason; Leptin; Ovine Pituitary cells; *in vitro*.

RIASSUNTO

Lo scopo di questa ricerca è stato quello di analizzare l'effetto dell'ACTH e del desametasone sulla liberazione di Leptina e dell'ossido nitrico dalle cellule ipofisarie ovine *in vitro*. Le cellule ipofisarie erano coltivate su di un terreno McCoy 5a senza ormoni (controllo) e rispettivamente con  $10^{-10}$ ,  $10^{-9}$ ,  $10^{-8}$ ,  $10^{-7}$ ,  $10^{-6}$  o  $10^{-5}$  moli/l di ACTH e desametasone.

Dopo 24 ore di incubazione il mezzo di coltura era raccolto per l'analisi della Leptina e dell'ossido nitrico. I risultati ottenuti mostrano che il desametasone esercita un effetto stimolante sulla liberazione di Leptina e ossido nitrico da parte delle cellule ipofisarie ovine.

L'influenza dell'ACTH sulla liberazione della Leptina e dell'ossido nitrico invece è dose

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dipendente. L'ACTH alle concentrazioni di  $10^{-10}$  -  $10^{-9}$  Moli/l aumenta la liberazione di questi due composti mentre alle concentrazioni di  $10^{-8}$  -  $10^{-5}$  M/l ne riduce la liberazione. Una correlazione positiva era osservata tra le quantità di Leptina e ossido nitrico liberati nel mezzo di coltura sia dopo aggiunta di desametasone ( $r = 0,77$ ) che di ACTH ( $r = 0,89$ ).

Parole chiave: ACTH; desametasone; Leptina; cellule ipofisarie ovine; in vitro.

## INTRODUCTION

Stress hormones are involved in the regulation of synthesis and secretion of leptin from adipocytes (Spinedi & Gaillard, 1998).

It has been shown that this influence is dependent on the stage of stress reaction.

High plasma ACTH levels are associated with low circulating leptin levels, and a decrease in plasma corticotrophin leads to a return of leptin values to normal (Norman et al., 2003).

Glicocorticoids, instead, belong to the stimulators of leptin expression and release from fatty tissue (Sliker et al., 1996; Wabitsch et al., 1996). Leptin is a hormone that signals nutritional status to the hypothalamic-pituitary-ovarian axis (Prolo et al., 1998). The effect of leptin on some secretory activity of pituitary cells, like gonadotropin secretion, can be mediated by NO (Yu et al., 1997).

Pituitary gland can also produce and release leptin that may act by auto- or paracrine way. So, the objective of the experiments was to study the effect of ACTH and dexamethasone on leptin and nitric oxide release from ovine pituitary cells.

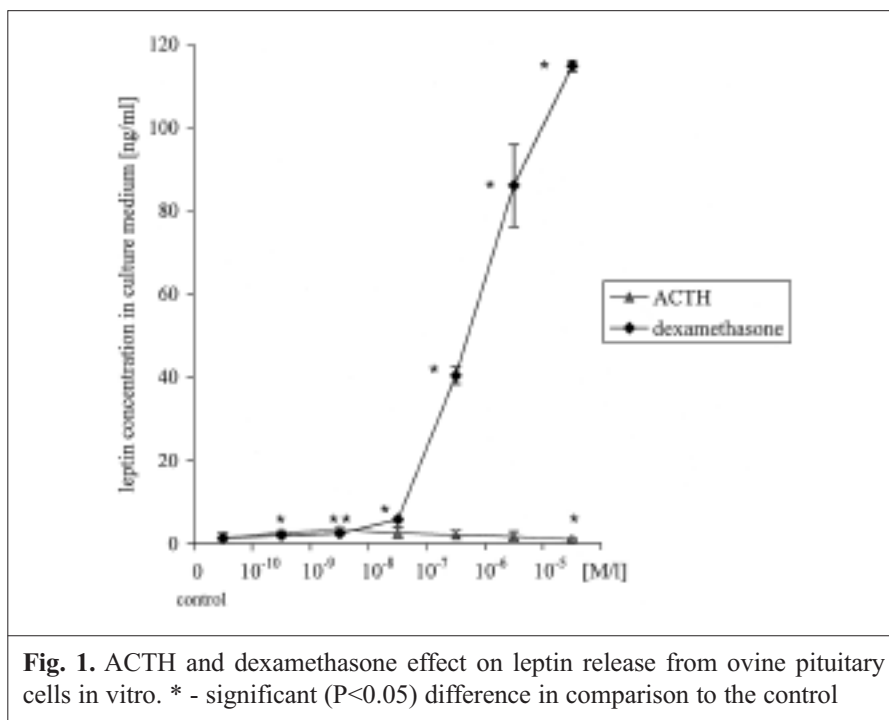
## MATERIALS AND METHODS

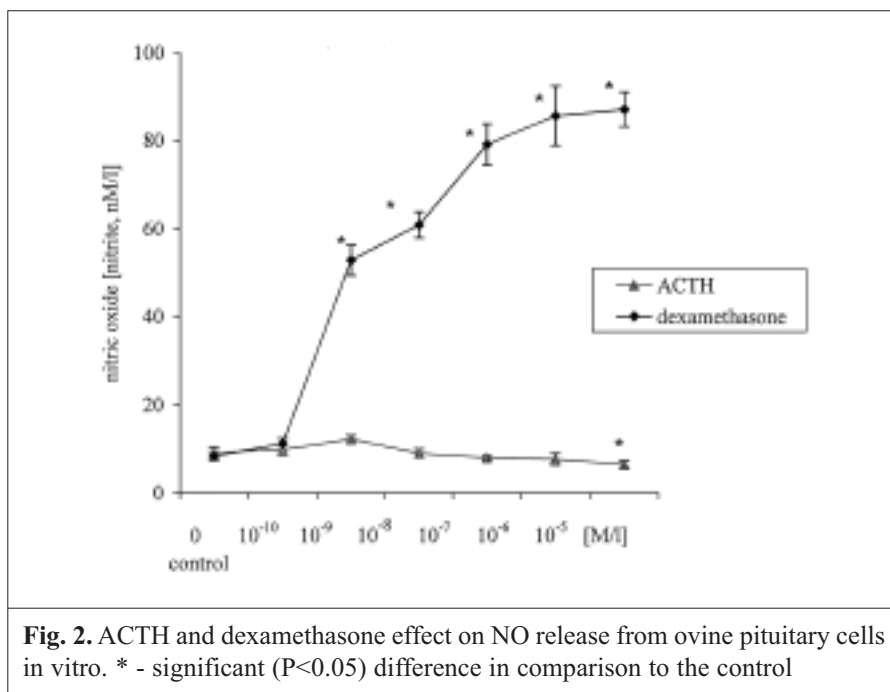
Pituitary glands were obtained from 4 years old, cyclic crossbreed ewes (25% Suffolk + 25% Romanov + 50% Polish Lowland Sheep). After isolation, pituitary cells were cultured at 37°C in atmosphere of 5% CO<sub>2</sub> in McCoy 5A medium (Kosior-Korzecka & Bobowiec, 2004) without hormones (control) and with  $10^{-10}$ ,  $10^{-9}$ ,  $10^{-8}$ ,  $10^{-7}$ ,  $10^{-6}$  or  $10^{-5}$  M/l of ACTH (Cortrosyn Depot<sup>®</sup>, N.V. Organon, Italia) or dexamethasone (Dexasone, ScanVet, Poland), respectively. After 24 h of cell culture, the media were collected to leptin and NO analysis. Leptin concentration in culture medium was analysed by radioimmunoassay (Multi-Species Leptin <sup>125</sup>I Radioimmunoassay KIT, Linco Research, USA). Measuring of the concentrations of nitrite (NO<sub>2</sub><sup>-</sup>) as an indicator of nitric oxide (NO) production was carried out by Griess method. All results are calculated using Statistical PL programme and expressed as a mean ± standard deviation (SD). The comparisons between the results obtained for control and experimental cultures were performed by Student *t*-test.

## RESULTS

High positive correlation between the dose of dexamethasone and the concentration of leptin released to the culture medium has been observed ( $r = 0.80$ ).

Inversely, ACTH influence was dose dependent. Corticotrophin in the range of  $10^{-8}$  -  $10^{-5}$  M/l caused the suppression in leptin release by ovine pituitary cells ( $r = -0.90$ ) in comparison to the control. However, contrary to the results obtained with using adipose tissue (Norman et al., 2003), there was positive relationship between low doses of corticotrophin ( $10^{-10}$  -  $10^{-9}$  M/l) and leptin ( $r = 0.84$ ) (Fig. 1). Nitric oxide release from pituitary cells was proportionally increased under influence of dexamethasone ( $r = 0.67$ ), whereas in the presence of ACTH it was dose dependent by leptin - like way. Despite of positive correlation between nitric oxide release and lower doses of ACTH ( $10^{-10}$  -  $10^{-9}$  M/l) ( $r=0.99$ ), there was negative relation in a case of higher ACTH concentrations ( $10^{-8}$  -  $10^{-5}$  M/l) ( $r= -0.86$ ) (Fig. 2). It has be pointed out high positive correlation between the quantities of leptin and nitric oxide released to the culture medium, both in the experiment with using of dexamethasone and ACTH ( $r = 0.77$  and  $r=0.89$ , respectively) (Fig. 3). So, probably the stress – induced changes in NO release are mediated by leptin in great measure, especially that glucocorticoids cause the suppression of NO synthase I (NOS I) expression in some types of cells, for example in neurons of rat hippocampus (López-Figueroa et al., 1998).





## CONCLUSIONS

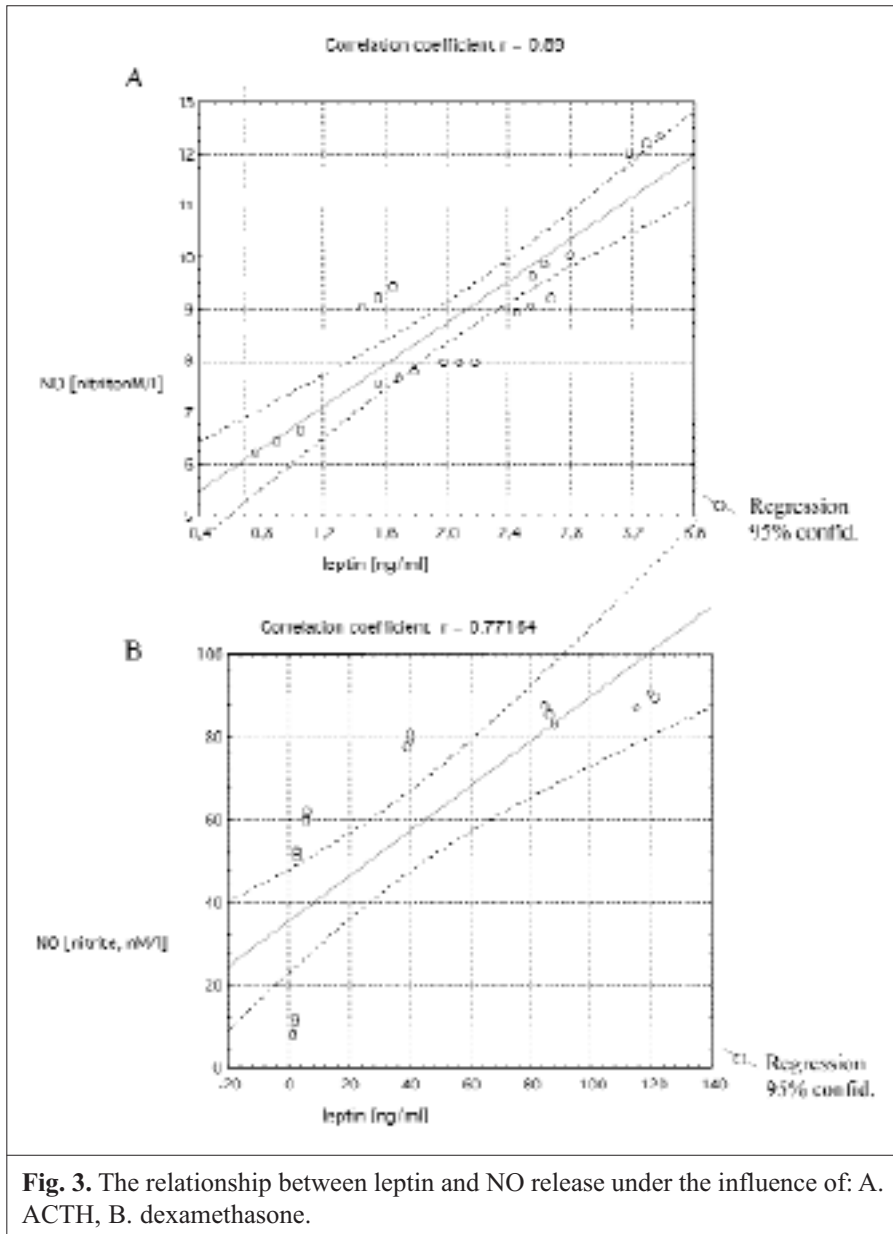
Dexamethasone exerts the stimulative effect, both on leptin and NO release from ovine pituitary cells *in vitro*.

The influence of ACTH on leptin and NO release is dose dependent. ACTH in concentration ( $10^{-10}$  -  $10^{-9}$  M/l) causes the increase and in concentration  $10^{-8}$  -  $10^{-5}$  M/l the decrease in release of both of these compounds.

The positive correlation exists between the quantities of leptin and nitric oxide released to the culture medium both under the influence of dexamethasone and ACTH ( $r = 0.77$  and  $r = 0.89$ , respectively).

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