



# Serum leptin in children with asthma treated with inhaled budesonide

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Leptin, a 167-amino-acid peptide, is a recently discovered hormone which is believed to play a major role in the regulation of body weight. Systemic administration of exogenous glucocorticoids has been found to increase circulating leptin levels.

In this study, we aimed to assess serum leptin in children with asthma treated with inhaled budesonide 800  $\mu\text{g day}^{-1}$ .

Ten boys and three girls with asthma, all adolescents aged from 12.9 to 16.6 years, were studied in a randomized double-blind two-period cross-over trial with 4-week treatment periods and a 1-week wash out. Placebo was given during one period and 800  $\mu\text{g}$  budesonide during the other via a 750 ml volume spacer (Nebuhaler<sup>®</sup>, Astra Draco, Lund, Sweden). On the last day of the placebo and budesonide periods blood samples were taken and serum leptin was measured by a specific radioimmunoassay.

The difference in mean (SEM) leptin concentration between the budesonide and placebo period was 0.2 (0.4)  $\mu\text{g l}^{-1}$  ( $P=0.71$ ;  $t=-0.4$ ;  $df=12$ , 95% confidence interval  $-0.9-0.7 \mu\text{g l}^{-1}$ ).

Inhaled budesonide 800  $\mu\text{g}$  per day from a Nebuhaler<sup>®</sup> does not influence circulating leptin levels, suggesting that regulation of body weight is unaffected.

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## Introduction

The recently discovered hormone leptin, a 167-amino-acid peptide secreted by adipose tissue, is believed to influence appetite, energy homeostasis and body weight through regulation of food intake by a negative feedback signal involving the hypothalamic-pituitary-adrenal axis (1–3). The suppressive effect of leptin on fat accretion is thought to be accompanied by stimulatory effects on energy expenditure (2,4–7). Little is known about the regulation of leptin; however, exogenous glucocorticoids have been found to increase leptin levels in adults (8–10) and in children (11). Since high doses of inhaled glucocorticoids may cause systemically detectable effects the aim of the present study was to assess serum leptin in asthmatic children treated with inhaled budesonide 800  $\mu\text{g}$  per day (12,13).

## Patients and Methods

The protocol was part of a clinical study of systemic activity of inhaled budesonide in adolescents (14). Ten boys and three girls with asthma, aged 12.9–16.6 (mean 14.8) years from an outpatient secondary referral center participated in the study. All had mild to moderate asthma needing treatment as required with inhaled  $\beta_2$ -agonists or low doses of inhaled glucocorticoids during intervals of the year. None had received treatment with inhaled or oral glucocorticoids during the month before the study, and no other drugs were taken during the study period. All were pubertals according to the Tanner rating of puberty (15). Genital development in the boys and breast development in the girls varied from stage III to V (stage III:  $n=3$ , IV:  $n=3$ , V:  $n=4$ ) and from stage II to III (stage II:  $n=1$ , III:  $n=2$ ), respectively. Pubic hair development in the boys varied from stage III to V (stage III:  $n=1$ , IV:  $n=7$ , V:  $n=2$ ), and in the girls from stage II to IV (stage II:  $n=1$ , III:  $n=1$ , IV:  $n=2$ ). Height at study entry varied from 151.4 to 179.5 (mean 165.5) cm, height standard deviation score from  $-0.8$  to 0.7 (mean  $-0.02$ ), weight from 40.5 to 67.0 (mean 52.7) kg, and body mass index from 16.0 to 22.3 (mean 18.9). The study was approved by the local ethics committee, and informed consent was obtained from all children and their parents.

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The design was a randomized double-blind, placebo-controlled two-period cross-over trial. After a 1-week run-in during which no other medication except inhaled  $\beta_2$ -agonists was allowed, the children were allocated to two 4-week treatment periods with a 1-week wash-out in between. During one period placebo was given and during the other 400  $\mu\text{g}$  budesonide was given twice daily. The treatment order was allocated by a computerized randomization scheme prepared in balanced blocks. A conventional pressurized metered dose inhaler was delivered at the start of each period. One actuation of the budesonide aerosol delivered 200  $\mu\text{g}$ . The medicine was taken via a 750 ml volume spacer (the Nebuhaler<sup>®</sup>, Astra Draco, Lund, Sweden) at approximately 0730 h and 2130 h.

On the last day of the placebo and budesonide periods blood samples were taken at roughly the same time (that is, within 1 h) in the afternoon (between 1300 and 1800 h). After centrifugation at 4000 rpm for 10 min within 1 h the samples were stored at  $-80^\circ\text{C}$  and batch assayed at the completion of the study. Serum leptin was measured by a commercially available radioimmunoassay (Linco Research Inc., St. Louis, MO, U.S.A.) (16). The assay has a detection range of 0.22–100  $\mu\text{g l}^{-1}$ . Intra- and interassay coefficients of variation were <5%.

## Statistics

Data were tested for normal distribution and were found to fulfil conditions for parametric analysis. Paired Student's *t*-test was used for comparison of the data and a 5% level of significance was applied.

## Results

Individual serum leptin concentrations are shown in Fig. 1. During budesonide and placebo treatment mean serum leptin (SEM) was 4.2 (0.9) and 4.0 (0.9)  $\mu\text{g l}^{-1}$ , respectively. The mean difference of 0.2 (0.4)  $\mu\text{g l}^{-1}$  was not statistically significant ( $P=0.71$ ;  $t=-0.4$ ;  $df=12$ ; 95% confidence interval  $-0.9-0.7 \mu\text{g l}^{-1}$ ).

## Discussion

Leptin is exclusively secreted by differentiated adipocytes and serum concentrations correlate with fat mass or body mass index (17). Data on the biological effects of leptin mainly come from animal experiments showing that leptin acts by both suppressing food intake and stimulating energy expenditure, including thermogenesis (18). In addition to its metabolic effects leptin may interact with hormones such as insulin, gonadotrophins, androgens and catecholamines and leptin receptors have been identified in numerous organs such as the central nervous system, kidney, liver, heart and lungs (19,20). The importance of the receptors in the lungs, or in any peripheral tissue, is not known; however, it has been suggested that leptin may act

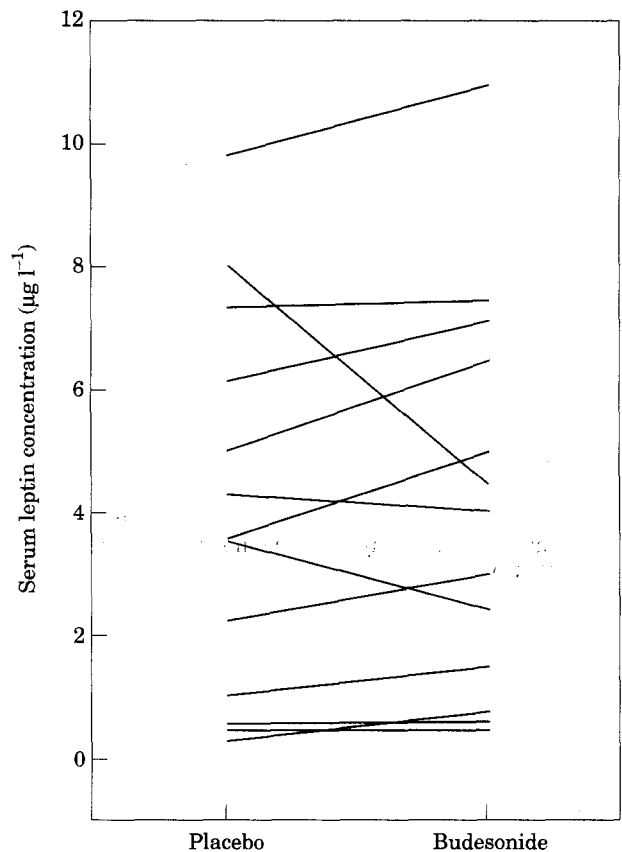


FIG. 1. Serum leptin concentrations in 13 children with asthma during treatment with placebo and inhaled budesonide 800  $\mu\text{g}$  daily.

as a signal from adipose tissue telling the organism how much energy is available from fat stores (21).

Females have higher leptin levels than males (22). Furthermore, a significant diurnal variation in leptin levels has been found showing relatively constant levels from approximately 1000 h to 1800 h followed by increased levels during the night with peak levels between approximately 2200 h and 0200 h (23,24). In the present study possible influences of diurnal variations in serum leptin were avoided since blood was consistently collected from each individual child within 1 h during the afternoon.

The nocturnal rise in leptin levels precedes the early morning rise of adrenocorticotrophic hormone (ACTH) and cortisol, and short-term variations in endogenous cortisol levels do not appear to cause changes in leptin levels (24,25). Administration of dexamethasone and methylprednisolone, however, were associated with increased circulating leptin levels (8–11; unpublished data). The stimulating effect was similar in lean and obese individuals. The mechanism of glucocorticoid stimulation of serum leptin may involve peripheral effects on adipocytes and central effects mediated by hypothalamic secretion of the neurosecretory agent neuropeptide Y (26). It has been suggested that increased leptin secretion may be a counter-regulatory response to limit glucocorticoid-induced hyperphagia and weight gain (26,27).

Few studies have assessed lipid metabolism in children treated with inhaled glucocorticoids. An increase in high-density lipoprotein cholesterol in children treated with inhaled budesonide 800  $\mu\text{g}$  has been reported; however, clinical implications remain to be clarified (28). Besides case reports of weight gain as part of a Cushingoid appearance presumably due to an increased sensitivity to exogenous glucocorticoids, there are no available data to suggest that inhaled glucocorticoids may influence body weight (29). In accordance with that we found no effect of inhaled budesonide 800  $\mu\text{g}$  on circulating leptin levels. Theoretically, this finding could be taken to suggest that the systemic bioavailability of inhaled budesonide is very small or unimportant. However, the systemic bioavailability of inhaled budesonide has been well documented (30). Adverse effects of 800  $\mu\text{g}$  budesonide on short-term growth and markers of collagen turnover in children have been found in studies using comparable designs (13,14). Considering that a similar assay was used in studies of dexamethasone (8–11) and, furthermore, that we recently used the present assay in a study of methylprednisolone in eight children and found elevated leptin levels (unpublished data), we do not believe that the present results could have been influenced by poor sensitivity in assay or study design. So, though long-term effects may not be ruled out from the present data we find it safe to conclude that inhaled budesonide 800  $\mu\text{g}$  from the Nebuhaler<sup>®</sup> does not influence circulating leptin levels in children, suggesting that the regulation of appetite and body weight is not affected. Whether that may also apply to adults in whom the hepatic metabolization rate of glucocorticoids is slower than in children, or to dry powder administration of budesonide which causes a greater intrapulmonary deposition resulting in a greater systemic bioavailability of the drug, remains to be evaluated.

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