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Effect of Chronic Administration of Dexamethasone on Growth Hormone and Anthropometric Markers of Obesity in Male Wistar Rats

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ABSTRACT

Background: Dexamethasone (Dex) is a glucocorticoid that is similar to the glucocorticoid hormone produced by the adrenal glands. It is a catabolic and anti-inflammatory steroid that works on the immune system, but indiscriminately used by drug prescribers. The aim of this study is to determine the effect of chronic administration of dexamethasone on growth hormone and anthropometric markers of obesity in male Wistar rats.

Methodology: Twenty-one (21) animals were randomly divided into 3 groups of 7 rats each. Animals in Group A was the control group, while groups B and C were given intramuscular dexamethasone injection at doses of 0.225 mg/kg body weight and 2.25 mg/kg body weight respectively for two weeks. After 2 weeks of administration, the weight and height of the rats were measured and blood samples collected following euthanasia.

Result: There was significant ($p<0.05$) increase in BMI and Lee index of the control group and significant ($p<0.05$) reduction in the low and high dose groups on day 14 when compared to day 0. There was also significant ($p<0.05$) reduction in the BMI and Lee index of the low and high dose groups compared to the control group, while there was no significant ($p>0.05$) difference between the low and high dose groups. There was significant ($p<0.05$) reduction in serum growth hormone in the low and high dose groups compared to the control.

Conclusion: The study showed that Dexamethasone impaired growth, and by extension tissue development perhaps via hypothalamic-pituitary-growth hormone axis.

Keywords: Dexamethasone, Anthropometry, Obesity, Growth hormone

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INTRODUCTION

Dexamethasone (Dex) is a member of the glucocorticoid class of hormones that is similar to the natural glucocorticoid hormone produced by the adrenal glands. It is a catabolic and anti-inflammatory steroid that works on the immune system. It is often used to replace inadequate or deficient endogenous production (1,2). It is a corticosteroid that prevents the release of substances in the body that can cause inflammation (3). Dexamethasone was first synthesized by Philip Showalter Hench in 1957, and was later introduced for medical use in 1961 (4). It is on the World Health Organization list of essential drugs (5). It used to treat rheumatic problems, a number of skin diseases, severe allergies, asthma, chronic obstructive lung disease, croup, cerebral oedema, eye pain following ophthalmic surgery, (6). It is usually only prescribed for short-term use; however, in certain instances, Dex may be prescribed for longer periods. It acts similarly to prednisolone but has more potent anti-inflammatory, hormonal, and metabolic effects. When given at the same dosage, Dex is less likely than hydrocortisone to cause fluid retention (7,8). The effects of dexamethasone are frequently seen within a day and last for about three days. However, the long-term use of dexamethasone may result in constellations of features associated with immunosuppression which include: thrush, bone loss, cataracts, easy bruising, and muscle weakness (1). Dexamethasone is a catabolic hormone which exerts a negative effect on growth and nitrogen balance through an increase in protein breakdown and turnover rate, unlike testosterone which exhibits its anabolic synergistic activity with growth hormone (9,10,11,12).

Obesity is a metabolic disease that develops over time when you consume more calories than you expend. It is a state of energy imbalance when your energy in (calories) does not equal your energy out (calories your body uses for things such as breathing, digesting food, and being physically active). The body uses certain nutrients, such as carbohydrates or sugars, proteins, and fats, from the foods consumed to make and store energy (13). It is of high prevalence and incidence worldwide (14), and is a risk factor that serves as platform for several cascades of disease conditions ranging from diabetes, cardiovascular, orthopaedic to psychosocial disorders (15-17). Studies have shown about 15 genes that influence obesity, showing that some individuals are more predisposed with natural tendency. For people with genetic predisposition, making healthy lifestyle changes can help lower that risk. Currently, one of the criteria generally used for assessment of obesity is to determine the body mass index (BMI) (18) of the subject. Of importance also as assessor of obesity is the Lee index which was established in the work of Stephens to correlate well with increased percentage body fat in three groups of adult rats (19).

Growth hormone (GH), also called Somatotropin or human growth hormone is a peptide hormone of about 190 amino acids that is synthesized and secreted by somatotroph cells in the anterior lobe of the pituitary gland which release between one and two milligrams of the hormone each day. It stimulates the growth and differentiation of essentially all tissues of the body, including cartilage, muscle and bone (20). It is a major participant in control of several complex physiologic processes associated with growth and metabolism as seen in protein synthesis and increase in fat breakdown to provide energy necessary for tissue growth. It is also of considerable interest as a drug used in both humans and animals (21). Although also found in other tissues, but as early as the 1920s pituitary extracts were used to elicit the growth promoting effects in rats and dogs that were later ascribed to growth hormone (22,23). It may act directly on tissues, but much of its effect is mediated by stimulation of the liver and other tissues to produce and release insulin-like growth factors, primarily insulin-like growth factor I (IGF-1, formerly called somatomedin) (24). This suggests that in addition to its function as a classic endocrine hormone, growth hormone may also function in an autocrine paracrine fashion. In addition to direct receptor mediated activity, growth hormone stimulates the production of insulin-like growth factors (IGFs) that mediate many growth promoting actions of the hormone (25). The intricate relationship between chronic

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dexamethasone administration and its diverse physiological effects intersects notably with the realms of growth hormone regulation and anthropometric markers associated with obesity, prompting an exploration into its impacts in male Wistar rats. Thus, the aim of this study is to determine the effect of chronic administration of dexamethasone on growth hormone and anthropometric markers of obesity in male Wistar rats.

METHODOLOGY

Source of Animals

Twenty-one (21) male Wistar rats with an average weight of 300g ± 50g were used in this study. These rats were purchased from the Animal Holding Unit of Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo state.

Source of ELISA Kit/Dexamethasone Phosphate

Growth hormone elisa kit was a product of Cloud-Clone Corporation, Export Processing Zone, Wuhan, Hubei 430056, China. It was purchased at 3, Grace Avenue, Rumuigbo 500272, P.O.Box 8196 Port Harcourt, Nigeria.

Dexamethasone injection was a product of Tianjin Kingyork Group, Hubei Tianyao Pharmaceutical Company Limited. No 99, Hanjiangbei Road, Xiangyang, Hubei, China. It was purchased at Onestep Pharmacy, Sawmill, Ilorin, Kwara State, Nigeria. It is prepared in an ampoule of 4mg/ml for intramuscular/intravenous use.

Animal Care and Ethical Approval

The animals were housed in metabolic cages in the animal house of the Faculty of Basic Medical Sciences under standard laboratory conditions. The animals were kept in a well-ventilated room and subjected to artificial day and night cycle of 12 hours by 12 hours, under optimum temperature of $25^0C \pm 2^0C$. They were given pelleted rat feeds and water ad libitum. The research was approved to be in compliance with internationally accepted laboratory animal use and care guidelines, and the guidelines for the institution research Ethical Review Committee of College of Health Sciences, University of Ilorin, Ilorin Nigeria.

Animal Grouping

The experiment was performed in accordance with guidelines for the care and use of laboratory animals. The rats were kept in metabolic cages after purchase, and acclimatized for two weeks in a private Animal House at Lane 2, Ramadan Quarters, Gerewu, Ilorin, Kwara State, Nigeria. They were fed with normal feed and water *ad libitum* throughout the entire period of the experiment after acclimatization for two weeks.

The 21 animals were randomly divided into three (3) groups of 7 rats each. Animals in Group A served as control group. Groups B and C were given intramuscular dexamethasone injection at doses of 0.225 mg/kg body weight (low dose) and 2.25 mg/kg body weight (high dose) respectively (adjudged by human dosage reconciled in our pilot study) for an experimental period of two weeks (14 days).

Experimental procedure

At the commencement of the procedure on day 0, the weight (g) and length (cm) of each animal were measured for calculation of body mass index (BMI). Dexamethasone was administered intramuscularly using 1ml syringe. Group A (control) was given 0.2ml of normal saline once daily, Group B (low dose) was given 0.225 mg/kg of dexamethasone phosphate daily, while Group C (high dose) was given 2.250 mg/kg of dexamethasone phosphate daily. The administration went on for two weeks (14 days).

After 2 weeks of administration, the weight (g) and height (cm) of the rats were measured again. Blood samples were collected from the heart of each rat by cardiac puncture following euthanasia under intraperitoneal anaesthesia (ketamine 60 mg/kg and xylazine 8 mg/kg). The blood samples were collected in Lithium-heparin bottles and then centrifuged at 3000 revolutions for 10 minutes. The serum was then separated into plain bottles for analysis of growth hormone.

Calculation of body mass index / Lee index

After 14 days of dexamethasone administration, the naso-anal length, the length from nose to anus, was measured with a tape-rule, with the animal in prone position under anaesthesia. The Body Mass Index (BMI) and Lee index were calculated according to the following equations: BMI = body weight (g) divided by the square of the nose-to-anus length (cm) (26), and Lee index calculated by dividing the cube root of the weight in grams and naso-anal length in millimeters and multiplied by 1000 (27).

$$BMI = Weight\ (g) / Length^2\ (cm)$$

$$\text{Lee Index} = \sqrt[3]{\text{Weight}} / \text{Length (mm)} \times 1000$$

Assessment of growth hormone

All reagents, samples and standards were first prepared. Hundred (100µL) microliter standard or sample was added to each well and incubated for 1 hour at 37°C. It was then aspirated and100µL prepared detection reagent A was added and also incubated for 1 hour at 37°C. It was then aspirated and washed 3 times. Hundred (100µL) microliter prepared detection reagent B was then added and incubated for 30 minutes at 37°C. This was aspirated and washed 5 times. Ninety (90µL) microliter substrate solution was then added and incubated for 10-20 minutes at 37°C. Fifty (50µL) microliter stop solution was then added and reading done at 450nm immediately.

Statistical Analysis

Statistical analysis was done with the aid of the statistical package for social sciences (SPSS) version 20. Differences in means were obtained using 2-Way ANOVA and Duncan post hoc test. All values reported in the study were expressed as MEAN +SEM (Standard error of Mean). Differences were taken to be significant at $P<0.05$.

RESULTS

It was evident in table 1 below, that there was significant ($p<0.05$) increase in BMI of the control group on day 14 when compared to day 0, while there was significant ($p<0.05$) reduction in the BMI of the low and high dose groups on day 14 when compared to day 0. There was also significant ($p<0.05$) reduction in the BMI of the low and high dose groups compared to the control group, while there was no significant ($p>0.05$) difference between the low and high dose groups.

Table 1: Effect of Chronic Administration of Dexamethasone on Body Mass Index (BMI) in Male Wistar Rats

Group	Dose	Day 0 (g/cm2)	Day 14 (g/cm2)
A	0.9% NaCl	0.46±0.12	0.60±0.15 *
B	0.225mg/kg	0.43±0.18	0.38±1.43 *a
C	2.250mg/kg	0.41±0.08	0.31±1.71 *a

From table 2 below, there was significant ($p<0.05$) increase in the Lee index of the control group on day 14 compared to day 0, while there was significant ($p<0.05$) decrease in the Lee index of the low and high dose groups administered dexamethasone on day 14 compared to day 0. There was significant ($p<0.05$) reduction in the Lee index of the low and high dose groups administered dexamethasone compared to the control group, while there was no significant ($p>0.05$) difference between the low and high dose groups, though a more reduction was observed at low dose but not statistically significant ($p>0.05$).

Table 2: Effect of Chronic Administration of Dexamethasone on Lee Index in Male Wistar Rats

Group	Dose	Day 0 (g/cm2)	Day 14 (g/cm2)
A	0.9% NaCl	0.26±0. 27	0.32± 1.41 [a]
B	0.225 mg/kg	0.28± 1.35	0.21 ±1. 23 [a,b]
C	2.250 mg/kg	0.29±0. 11	0.23±0.39 [a,b]

Values are expressed as Mean ± SEM. Level of significance was considered as $p<0.05$.
[a] Comparing day 14 to day 0
[b] Comparing test groups to control.

The study revealed from table 3 below, that there was significant ($p<0.05$) reduction in serum growth hormone in the groups administered low and high doses of dexamethasone compared to the control. However, there was no statistical significant ($p>0.05$) difference in serum growth hormone between the low and high doses, although there was increase in the growth hormone of the high dose group, but not statistically significant ($p>0.05$).

Table 3: Effect of Chronic Administration of Dexamethasone on serum growth hormone in Male Wistar

Parameter/Dose	Control	LD (0.225 mg/kg)	HD (2.250 mg/kg)
Growth hormone (ng/ml)	5.43 ± 0.22	2.91 ± 0.04 [a]	2.10 ± 0.13 [a]

Values are expressed as Mean ± SEM. Level of significance was considered as $p<0.05$.
[a] Comparing test groups to control

DISCUSSION

The findings of this study revealed that chronic administration of dexamethasone phosphate has a dose-dependent effect on growth hormone and BMI levels. The serum growth hormone level was observed during the period of this experiment and was noticed that administration of dexamethasone phosphate shows a dose-dependent significant decrease in the growth hormone level. The low and high dose groups showed remarkable reduction in the serum levels of growth hormone, this could be because of effect of dexamethasone on the hypothalamic-pituitary-growth hormone axis which perhaps had led to inhibition or reduction of growth hormone secretion.

The result is in tandem with previous studies that showed that chronic excess glucocorticoid causes an inhibition of spontaneous growth hormone secretion in man as well as in rats (28, 29,30), and also the work of Giustina and Wehrenberg (1992) (31,32), which revealed that chronic glucocorticoids administration impairs growth in humans and laboratory animals. The result is also in harmony with the work of Kemp et al., in 2003 (33) though a human study that concluded that Dex treatment has a suppressive effect on serum GH levels in preterm ventilated infants, without an acute decline in serum IGF-I levels.

The body mass index level was observed after the administration and the result shows a remarkable decrease in the body mass index in low dose group and high dose group. This is in support of the work of Gibson et al., (1993) (34) who showed that growth retardation was associated with excess dexamethasone administration.

With respect to the effect of Dexamethasone on growth hormone levels and BMI level, the result suggested that the decrease in growth hormone levels in the low and high doses had significant reduction effect on BMI and Lee index factors. This is not surprising because growth is characterized by variations in food consumption and energy intake. Therefore, the decrease in BMI and Lee index could have been precipitated by the observed poor food consumption and energy intake in the rats which is in tandem with the work of Thomas et al. in 2002 (35). In a broad term, the growth and survival of almost all tissues invariably depend on growth hormone. Thus, by extension, chronic administration of dexamethasone especially injectables should be cautiously used among corticosteroids to avoid untoward systemic effects; particularly in the presence of underlying background illnesses such as heart failure, renal failure, and hepatic damage which are associated with myriads of chronic inflammatory changes making drug prescribers to indiscrimately recommend it.

CONCLUSION

Dexamethasone on growth hormone levels and BMI assessment suggested that the decrease in growth hormone levels in the low and high doses had significant reduction effect on BMI and Lee index factors. The study showed that Dexamethasone impaired growth, and by extension tissue development perhaps via hypothalamic-pituitary-growth hormone axis. This is not surprising because growth is characterized by variations in food consumption and energy intake. Thus, in a broad term, it is evident that growth and survival of almost all tissues invariably depend on growth hormone.

We hereby recommend that chronic administration of dexamethasone, especially injectables should be cautiously used among corticosteroids to avoid untoward systemic effects; particularly in the presence of underlying background illness such as heart failure, renal failure, and hepatic damage.

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