

# Evaluation of body weight in androgenized female rats

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## Summary

**Purpose:** To evaluate the body weight of androgenized female rats or of rats in persistent estrus since these conditions mimic states of chronic anovulation in adult animals.

**Methods:** Forty-four Wistar-Hannover female rats were divided at random into two groups: control group (n = 17) which received only vehicle (0.1 ml corn oil) by the subcutaneous route on the second day of life, and experimental group (n = 27) which was similarly treated with 1.25 mg testosterone propionate diluted in 0.1 ml corn oil. The animals were weighed weekly from the fourth to the 12th week of life. Data were analyzed statistically by analysis of variance and by the Tukey-Kramer multiple comparisons test (p < 0.05).

**Results:** The experimental rats (androgenized or in persistent estrus) presented a significant increase in body weight starting on the fifth week of life compared to control rats.

**Conclusions:** We conclude that androgenized rats present a significant increase in body weight, thus representing an interesting biological model for the study of diseases associated with excess weight and polycystic ovarian syndrome.

**Key words:** Female rats; Obesity; Polycystic ovaries; Persistent estrus.

## Introduction

The nature of the complex interrelationship between excess body fat, hyperandrogenism and insulin resistance is still unclear [1, 2]. Obesity is a common clinical finding in patients with polycystic ovary syndrome [3-5], with approximately 75% of these patients being hyperinsulinemic and insulin-resistant and showing an increased incidence of type II diabetes [6]. Thus, increased obesity in polycystic ovary syndrome is associated with increased hyperinsulinemia, which in turn is significantly correlated with the risk of cardiovascular disease [7, 8].

Antidiabetogenic drugs are being tested with satisfactory results on weight reduction, hyperandrogenism, and most of the metabolic disorders of the polycystic ovary syndrome [9-11]. However, the use of drugs in humans for research purposes has ethical limitations. Thus, since hyperandrogenism and chronic anovulation are the basic characteristics of polycystic ovary syndrome [7, 12, 13], it would be interesting to develop a biological model mimicking states of chronic anovulation presenting excess body weight or obesity for research purposes. In the present study, we evaluated the body weight of androgenized female rats or rats in a state of persistent estrus.

## Materials and Methods

We used 44 Wistar-Hannover female rats divided at random into two groups: control (n = 17) which received only 0.1 ml corn oil (vehicle) by the subcutaneous route on the second day of life, and experimental (n = 27), submitted to induction of per-

sistent estrus with a subcutaneous injection of 1.25 mg testosterone propionate diluted in 0.1 ml corn oil on the second day of life. Confirmation of a state of persistent estrus at the end of the experiment was based on the obliteration of the distal portion of the vagina, which is already a sign of persistent estrus [14], and on the presence of keratinization of the vaginal wall epithelium (Figure 1) and of polycystic ovaries (Figure 2) observed at autopsy by histological examination.

Starting on the fourth week of life, after weaning, the animals, kept under the same conditions and receiving the same supply of water and food, were weighed weekly with a digital scale and with the aid of a plastic beaker up to the 12th week of life. Data were analyzed statistically by analysis of variance and by the Tukey-Kramer multiple comparisons test (p < 0.05).

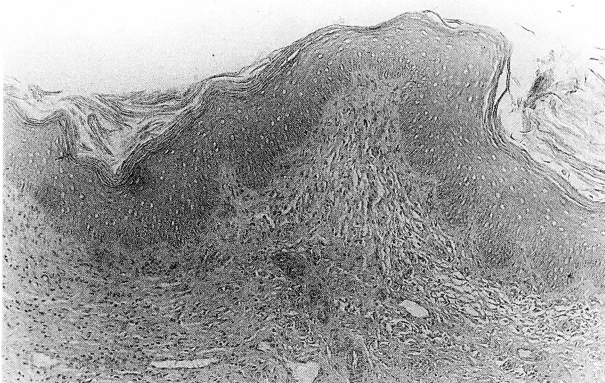


Figure 1. — Photomicrograph of a histological section of the vaginal wall from an androgenized rat. Observe the mature stratified squamous epithelium keratinized by the continuous estrogenic stimulus. H-E staining (140X).

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Figure 2. — Photomicrograph of a histological section from the ovary of an androgenized rat. Multiple cortical follicular cysts surrounded by abundant ovarian stroma, covered with granulosa cells can be observed, with early stages of antrum formation and hyperplasia of the internal theca with luteinization. H-E staining (140X).

## Results

The weight of the experimental animals, androgenized or in a state of persistent estrus, was significantly higher than the weight of control rats starting on the fifth week of life (\* $p < 0.05$ ; \*\* $p < 0.001$ ; \*\*\* $p < 0.0001$ ). The mean weights of the control and experimental groups are listed in Table 1.

Table 1. — Mean weight (g) of the control and experimental groups from the fourth to the 12<sup>th</sup> week of life ( $p < 0.05$ ).

Weeks	Control	Experimental
4 <sup>th</sup>	79.94	88.85
5 <sup>th</sup>	106.04	122.59*
6 <sup>th</sup>	136.12	152.89*
7 <sup>th</sup>	153.12	175.11**
8 <sup>th</sup>	174.43	199.56***
9 <sup>th</sup>	171.76	202.70***
10 <sup>th</sup>	186.94	219.74***
11 <sup>th</sup>	196.50	246.20***
12 <sup>th</sup>	202.29	250.50***

\* $p < 0.05$ ; \*\* $p < 0.001$ ; \*\*\* $p < 0.0001$

## Discussion

Many investigators are devoting their attention to the study of the association of obesity, polycystic ovary syndrome and insulin resistance since insulin resistance with hyperinsulinemia and an increased incidence of diabetes is a prominent characteristic of polycystic ovary syndrome [7]. The increase in excess weight, so common in states of chronic anovulation, is associated with increased hyperinsulinemia [11,15], which in turn seems to induce hyperandrogenemia [9].

Thus there is a correlation between obesity, hyperan-

drogenism, polycystic ovary syndrome and insulin resistance which has not yet been fully clarified [1, 2, 16]. In this respect, antidiabetogenic drugs are being tested with satisfactory results on hyperandrogenic women with excess weight and chronic anovulation in order to reduce body weight, hyperandrogenism and the associated metabolic disorders [7, 9, 10]. However, drug research on humans is limited by ethical reasons.

Since the basic characteristics of polycystic ovary syndrome are hyperandrogenism and chronic anovulation [7, 12], in the present investigation we used androgenized rats or rats in persistent estrus as an experimental model mimicking a state of chronic anovulation in order to evaluate the body weight of the animals.

Barraclough [17] was the first to identify the period of highest hypothalamic sensitivity in female rats since, after applying a single injection of 1.25 mg testosterone propionate up to the fifth day of life, he induced persistent estrus in all animals. The drug, administered during the first days of life during a critical stage of hypothalamic differentiation, damages the preoptic suprachiasmatic area, which is responsible for the cyclic release of gonadotrophins and for ovulation, while the arcuate nucleus, which regulates tonic secretion, remains active, with the consequent occurrence of an acyclic pattern of gonadotrophin release [17, 18]. During adulthood, the animals present chronic anovulation, sterility, polycystic ovaries, and aggressive behavior when in contact with males [17, 19].

In the present study, starting during the fourth week of life, after weaning, the animals, which were submitted to the same living conditions and which received the same supply of water and food, were weighed weekly until the 12<sup>th</sup> week of life, when they were sacrificed. Starting on the fifth week of life, the weight of androgenized rats was significantly higher than the weight of control rats. This model of rats in a state of persistent estrus manifested with polycystic ovaries, anovulation, high food intake, elevated body weight, and obesity, as also reported by Sun and Yu [20]. These anovulatory rats, which are under constant stimulation by estrogen, are being used for the study of antiestrogenic drugs [21]. However, this is an interesting experimental model that could be useful in the investigation of metabolic disorders associated with obesity and polycystic ovary syndrome.

## Conclusion

The most important event observed in the present investigation was the confirmation of the occurrence of excess body weight and obesity in this experimental model of female rats that received androgen at birth and manifested hyperandrogenism, sterility, chronic anovulation and polycystic ovaries when adult, thus representing an interesting biological model that could be useful for the study of diseases associated with excess weight and polycystic ovary syndrome.

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