BODY WEIGHT REGULATION IN FEMALE RATS FOLLOWING NEONATAL TESTOSTERONE

By

Paul U. Dubuc

ABSTRACT

Neonatal testosterone treatment of female rats led to an increase in body weight and skeletal growth and produced evidence of altered ovarian function over a 76 day period following treatment. Bilateral ovariectomy performed eleven weeks after neonatal treatment increased the rate of body weight gain of both testosterone propionate- and oil-treated groups, but no differences in the increased rates of weight gain were evident between groups over a five week post-ovex observation period. Subsequently, long-term oestrogen replacement therapy, via subcutaneous Silastic implants, produced equal reductions in the rates of body weight gain and somatic growth of both early testosterone- and oil-treated ovariectomized groups. In spite of the marked effects of these manipulations on body weight and skeletal growth, no significant differences were noted in the Lee Index of obesity between androgenized and the appropriate non-androgenized control rats at any interval of the experimental period. These results indicate that neither ovarian hormones nor an altered sensitivity to oestrogen of body weight regulatory mechanisms are important in the increased body weight that follows perinatal testosterone treatment. Additionally, the present data add support to previous work which has suggested that a general increase of somatic growth rather than 'obesity' provides the major contribution to the elevated body weights of androgenized female rats.

A single injection of testosterone administered in the immediate post-natal period to the female rat produces (in adulthood) infertility, persistant oestrus, and reduced ovarian weight, coupled with an arrest of corpus luteum develop-
ment at the follicular stage (Barraclough 1961; Harris 1964; Harris & Levine 1965). In addition, the perinatally androgenized female rat exhibits a higher rate of growth and attains a mature body weight well above the untreated control animal (Beatty et al. 1970; Bell & Zucker 1971; Swanson & van der Werff ten Bosch 1963).

Recently a number of investigators have proposed that circulating oestradiol levels contribute to the generation of a 'set point' signal necessary for the normal regulation of body weight in female rats (Mook et al. 1972; Redick et al. 1973; Zucker 1972). This research has demonstrated that a reduction in the level of endogenous oestrogen (e.g., by bilateral ovariectomy) causes an immediate increase in food intake and rate of weight gain that culminates in 'obesity'. In contrast, if oestradiol benzoate (OeB) treatment is begun simultaneously with ovariectomy, no alteration in long-term body weight regulation occurs (Tarttelin & Gorski 1973; Dubuc 1974). Additionally, if after ovariectomy 'obesity' is allowed to develop, OeB replacement therapy will lead to a reduction in the rate of weight gain until the body weight approaches the level of the intact control rat (Dubuc 1974).

As noted earlier, perinatal testosterone treatment results in altered ovarian function and the maintenance of an increased rate of body weight gain. It is possible that the body weight effects of early testosterone treatment are mediated by either a decrease in endogenous oestrogen production or by a decreased sensitivity of oestrogen receptors that govern the level at which body weight is regulated. To explore these possibilities the following study examined the participation of the ovary and ovarian hormones in the altered body weight regulation of female rats following perinatal androgen treatment.

MATERIALS AND METHODS

The experimental animals were the first generation offspring of Sprague-Dawley rats (Horton Labs, Oakland, California) bred in this laboratory. Each litter was reduced to nine animals by removing excess males within 24 h after birth. Litters were maintained in commercial breeding cages over the pre-weaning period with Purina Rat Chow and tap water available at all times. Fresh fruit and vitamins were provided weekly. Litters were weaned at 21 days and thereafter the animals were housed individually with ad libitum access to rat chow and tap water. The colony room was maintained on a 12 h light cycle and an ambient temperature of 23 ± 2°C.

Part 1

Between 60 and 72 h after birth, 30 female animals were randomly selected from sixteen litters and were injected subcutaneously with 100 μg testosterone propionate (TP) suspended in sesame oil (0.5 ml). Littermate control animals (N = 26) received sesame oil alone. From 38 days of age onward, all animals were weighed twice weekly. Approximately eleven weeks (76 days) following birth, bilateral ovariectomies were
performed on all animals. Ovaries were removed via paired dorsolateral peritoneal incisions after Pentobarbital anaesthesia (40 mg/kg). Ovaries were trimmed of fat, weighed, and preserved in Bouin’s fixative. Subsequently the ovaries were sectioned, mounted, and stained (haematoxylin-eosin). Naso-anus lengths were recorded (to 1 mm) at ovariectomy.

**Part II**

Following ovariectomy, all animals were returned to the colony room and body weights were recorded over a five week post-ovex period. At the end of this period, equal groups of TP- or oil-treated animals from Part I, closely matched in body weight, were further divided into OeB treatment or sham treatment sub-groups. Oestradiol treatment was accomplished by the subcutaneous placement of Silastic capsules containing 1.5–2.0 mg crystalline oestradiol-17β benzoate. These capsules have been demonstrated in previous work (Dubuc 1974) to provide effective long-term OeB replacement therapy in ovariectomized rats. Following OeB capsule implantation, body weight and naso-anus lengths were recorded for an additional nine weeks.

Changes in the rate of body weight gain between groups were statistically assessed according to a multivariate profile analysis described by Morrison (1967). Group differences in other variables were assessed using Student’s *t*-test for independent groups.

**RESULTS**

**Part I**

Of the 30 animals receiving neonatal injections of TP, the ovaries of 25 animals clearly demonstrated the typical polyfollicular appearance with an absence of corpus luteum development (Fig. 1) noted in previous studies (Barraclough 1961; Harris 1964; Harris & Levine 1965; Swanson & van der Werff ten Bosch 1963). The data of the TP-treated animals not having ovaries exhibiting the morphological changes associated with androgen sterility were not analyzed.

In addition to the morphological changes noted above, ovaries from TP-treated animals were significantly reduced in absolute and relative weight (*P* < 0.01) as indicated in Table 1. Using ovarian morphology as a criterion of the efficacy of early TP administration, the body weight of the TP-treated group was marginally higher than the oil-treated group at 38 days of age (*P* < 0.05). These body weight differences increased in magnitude over subsequent intervals and statistical significance was maintained (*P* < 0.01) over the remainder of the observation period. Early TP-treated animals also demonstrated an increased rate of skeletal growth (*P* < 0.05) at the end of the nine

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1) OeB containing capsules were constructed of 0.5 cm lengths of ‘Silastic’ tubing (0.078" x 0.25", Dow Corning) sealed at each end by Silastic Type ‘A’ Adhesive. Implants were loosely placed subcutaneously in the dorsal cervical region under ether anaesthesia.
Fig. 1.
Photomicrographs of ovaries from animals having neonatal TP (left) or early oil injection (× 5).

Table 1.
Pre-ovariectomy body weight changes and length, ovarian weight, and Lee Index values of early TP- or oil-treated animals recorded at the time of ovariectomy (76 days).
All values presented as group mean ± se.

<table>
<thead>
<tr>
<th>N</th>
<th>Body weight (g)</th>
<th>Naso-anus length (cm)</th>
<th>Paired ovarian wt. (mg)</th>
<th>Ovarian wt/B. W. (mg/g)</th>
<th>Lee Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38 d</td>
<td>57 d</td>
<td>76 d</td>
<td>38 d</td>
<td>57 d</td>
</tr>
<tr>
<td>TP-treated (polyfollicular ovaries)</td>
<td>25</td>
<td>166.2*</td>
<td>243.1*</td>
<td>291.9*</td>
<td>20.9*</td>
</tr>
<tr>
<td></td>
<td>± 3.5</td>
<td>± 3.5</td>
<td>± 5.1</td>
<td>± 0.1</td>
<td>± 3.0</td>
</tr>
<tr>
<td>Untreated</td>
<td>22</td>
<td>156.7</td>
<td>224.8</td>
<td>266.7</td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td>± 1.9</td>
<td>± 2.5</td>
<td>± 4.4</td>
<td>± 0.1</td>
<td>± 4.2</td>
</tr>
</tbody>
</table>

* P < 0.05 (Student's t)
week observation period (Table 1). However, the Lee Index\(^1\), frequently used as a measure of obesity (Bernardis 1970; Lee 1929; Szentogothai et al. 1968), was not different between the TP- and oil-treated groups over the same observation period (Table 1).

**Part II**

The effects of ovariectomy on body weight regulation of animals having either early TP- or oil-treatment are presented in Fig. 2. Consistent with previous studies that have shown an increase in the rate of body weight gain following ovariectomy (Wade 1972), both groups demonstrated an elevated rate of weight gain over the five week post-ovex observation period. However, the differences in body weight between groups that existed before ovariectomy (Table 1) were maintained over this post-surgical period; and the increase in body weight of the early TP-treated animals was virtually identical to that of oil-treated animals at each interval of the post-ovex period (\(F = 0.62, 3\) and 36 df, \(P > 0.05\); multivariate profile analysis).

Body weight changes of early TP- or oil-treated animals over a five week period following ovariectomy and during a nine week period of oestradiol replacement therapy. Data presented as mean body weight (g) ± se

\(^1\) Body weight (g) \(1/3 \cdot 10^5\)

Naso-anus length (cm)
Table 2.
Pre- and post-treatment body weights, naso-anus lengths and Lee Index values for early TP- and early oil-treated groups having OeB or sham replacement therapy. All values presented as group mean ± se.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Pre-treatment Body wt. (g)</th>
<th>Post-treatment Body wt. (g)</th>
<th>Naso-anus length (cm) at Ovex</th>
<th>Δ Naso-anus Length (cm)</th>
<th>Post-treatment Lee Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early TP + OeB</td>
<td>10</td>
<td>371.4 ± 9.6</td>
<td>376.0* ± 11.0</td>
<td>20.9 ± 0.3</td>
<td>23.1 ± 0.3</td>
<td>312.4 ± 1.5</td>
</tr>
<tr>
<td>Early TP + Sham</td>
<td>10</td>
<td>366.9 ± 10.7</td>
<td>419.1 ± 13.5</td>
<td>20.9 ± 0.3</td>
<td>23.7 ± 0.2</td>
<td>315.7 ± 2.8</td>
</tr>
<tr>
<td>Early Oil + OeB</td>
<td>10</td>
<td>346.8 ± 10.3</td>
<td>358.2* ± 12.1</td>
<td>20.6 ± 0.2</td>
<td>22.5 ± 0.2</td>
<td>314.6 ± 1.9</td>
</tr>
<tr>
<td>Early Oil + Sham</td>
<td>10</td>
<td>347.5 ± 8.4</td>
<td>392.3 ± 9.3</td>
<td>20.3 ± 0.1</td>
<td>23.1 ± 0.2</td>
<td>316.9 ± 2.3</td>
</tr>
</tbody>
</table>

* P < 0.05 vs. appropriate control group (Student's t).

Also included in Fig. 2 are the effects of oestradiol benzoate or sham treatment on the body weight of ovex animals having either early TP- or oil-treatment. Consistent with earlier reports (Mook et al. 1972; Redick et al. 1973; Tarttelin & Gorski 1973; Dubuc 1974), OeB treatment of all ovex animals led to a marked reduction in the rate of body weight gain over the treatment period. The body weight differences that were evident before OeB treatment remained during OeB treatment and the absolute body weight changes at each three week interval were not statistically different between early TP- and oil-treated groups (F = 0.89, 3 and 16 df, P > 0.05; multivariate profile analysis). Sham implanted animals of both early treatment groups maintained the elevated rate of growth noted in the immediate post-ovex period (Fig. 2). Again, no differences between TP- or oil-treated groups were apparent over this nine week sham implant period (F = 0.37, 3 and 16 df, P > 0.05; multivariate profile analysis).

OeB treatment following ovariectomy, in addition to reducing the rate of body weight gain, led to a significant reduction in skeletal growth over the post-ovex period of both early TP- and oil-treated groups (Table 2). The Lee Index of obesity, although generally lower in the OeB treated animals of both major experimental groups, was not significantly different between any sub-group pair. Additionally, no differences were noted in the Lee Index at sacrifice.
between combined OeB-treated (early TP- and early oil-) vs. combined sham-treated sub-groups \( (P > 0.05, \text{Student's} \ t) \), suggesting a relatively insignificant effect of OeB replacement therapy on 'obesity' as defined by the Lee Index.

However, it should be noted that the early TP-treated group, by virtue of their increased rate of weight gain in Part I, had a higher absolute body weight when OeB treatment was begun. Since the capsules used in the present study were identical for both the TP-treated and oil-treated groups, these weight differences resulted in the administration of a slightly lower dose (per unit body weight) of OeB to the TP-treated animals. It appears, however, that these small differences in dose between groups were of little importance since the TP-treated animals were, if anything, more sensitive to the weight depressing effects of OeB (Fig. 2). The slightly increased sensitivity to OeB noted in this group may reflect the higher pre-treatment weight, as OeB's effectiveness to reduce body weight has been shown to be inversely related to the pre-treatment weight \( (\text{Mook et al. 1972; Redick et al. 1973; Zucker 1972; Dubuc 1974}) \).

**DISCUSSION**

Consistent with earlier studies \( (\text{Barraclough 1961; Harris 1964; Harris & Levine 1965; Swanson & van der Werff ten Bosch 1963}) \), the results of Part I demonstrated the effectiveness of neonatal TP treatment to produce the anovulatory syndrome in female rats. Using ovarian morphology as a criterion of the effectiveness of TP treatment, the early androgen-treated females also displayed significantly elevated rates of weight gain and somatic growth over the first eleven week post-natal period. The effectiveness of early TP treatment to increase body weight in female rats has been frequently reported \( (\text{Barraclough 1961; Beatty et al. 1970; Bell & Zucker 1971; Harris & Levine 1965; Swanson & van der Werff ten Bosch 1963}) \) and; except for the slightly lower doses of TP employed here, the present body weight data merely add support to earlier work. However, recent studies that have discussed the effects of early TP treatment on female rats have emphasized the interpretation that TP alters a mechanism important in the regulation of long-term body weight \( (\text{Beatty 1973; Beatty et al. 1970; Bell & Zucker 1971; Wade 1972}) \). Implicit in this interpretation is the contention that early TP induces an upward shift in 'energy balance' (i.e., obesity) to the exclusion of an alteration of long-term growth patterns. Clearly, the data presented in Part I indicate that early TP treatment slightly though significantly increased somatic growth without a major effect on a measure of obesity. Consistent with these data is the conclusion of \( \text{Swanson & van der Werff ten Bosch (1963)} \) who reported a general increase in all carcass constituents following neonatal TP treatment. Additionally, \( \text{Beatty et al. (1970)} \) reported that increased fat deposition, especially of the inguinal depot, followed neonatal
TP treatment. However, it was clear from the data provided by Beatty et al. (1970) that the increased fat deposition noted in the TP-treated animals did not provide a significant contribution to the overall increase in body weight noted in their animals. Thus, in support of previous work, the results presented here indicate that early TP treatment of female rats causes both an elevation of body weight gain and a significant increase in somatic growth. Furthermore, because the present data show that the TP induced increases of skeletal growth were in proportion to the added body weight (since the Lee Index of obesity was not significantly altered) it is apparent that it is this somatic growth rather than “obesity” that provides the major contribution to the increased body weight reported to follow early androgen treatment.

The second part of the experiment examined the participation of the ovary, oestradiol, and/or an altered sensitivity of oestrogen receptors governing body weight regulation, in the increased body weight and growth that follows neonatal androgenization. Ovariectomy resulted in a marked increase in the rate of weight gain of both TP- and oil-treated animals. However, since the body weight increases were of similar magnitude in both TP- and oil-treated groups, the data indicate that ovarian hormone production did not significantly contribute to the body weight effects of neonatal TP-treatment. Generally, similar data and conclusions regarding the role of the ovary in mediating the effects of early TP on body weight have been reported by others (Beatty et al. 1970; Bell & Zucker 1971). It is clear, both from these early studies and the present work, that a diminished ovarian production of oestradiol or altered production of other ovarian hormones is not responsible for the elevation of body weight and growth that follows early TP treatment.

The last aspect of the present study investigated the effects of exogenous oestradiol benzoate on the regulation of body weight and growth in ovariectomized androgenized female rats.

Previous studies have shown that oestrogen treatment following ovariectomy depresses the rate of body weight gain in the post-ovex period (Mook et al. 1972; Redick et al. 1973; Tarttelin & Gorski 1973; Zucker 1972; Dubuc 1974) and the effect is approximately proportional to the level of ‘obesity’ that is allowed to develop following ovariectomy (Dubuc 1974). In the present study long-term OeB treatment led to essentially equal decrements in weight gain in both early TP-treated and oil-treated groups. Non-OeB-treated animals maintained a high level of growth over the entire post-ovex period regardless of neonatal treatment. Thus, in contrast to the effects of early TP on the oestrogen sensitivity of gonadotrophin regulatory mechanisms (Harris & Levine 1965; Neill 1972), and mechanisms of sexual behaviour (Edwards & Thompson 1970; Harris 1964; Harris & Levine 1965), there is no evidence from the present work that early TP treatment affects the sensitivity to oestradiol of mechanisms regulating body weight or growth. Consequently, the increased body growth
and body weight attained in the mature, androgenized female rat are apparently independent of impaired ovarian function, a reduction in systemic oestradiol level, or a reduction in sensitivity to oestradiol of weight-growth regulatory mechanisms.

From the current data it is apparent that no aspect of ovarian function contributes to the growth effects of early TP administration. As an alternative mechanism, previous investigators (Harris 1964; Neumann et al. 1970) have suggested that neonatal TP may permanently alter the release or metabolism of endogenous growth hormone (GH). Since food intake is not elevated in the androgenized female rat (Bell & Zucker 1971), the maintenance of an increased body mass in the post-puberal period is likely a function of an altered metabolic state. An organizing effect of early TP on central GH mechanisms, analogous to the effects of early TP on gonadotrophin regulation (Harris 1964), may chronically alter the metabolic state of the animal and result in the growth effects noted here. Some support for this possibility is provided by work showing the presence of elevated levels of pituitary GH in rats following neonatal androgenization (Kurcz et al. 1968). However, the potential role of GH in mediating the increased body weight and growth of the early androgen syndrome requires further investigation.

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