THE EFFECTS OF CASTRATION ON BODY COMPOSITION, ADIPOSE TISSUE CELLULARITY AND LIPID AND CARBOHYDRATE METABOLISM IN ADULT MALE RATS

By

John G. Kral and Lars-Eric Tisell

ABSTRACT

Gonadal hormones affect body composition, food intake, weight gain and serum lipids in numerous species including man. In this study, mature male Sprague-Dawley rats were castrated or sham-operated at 16 weeks of age. During the 6-week observation period with weekly records of food intake and weight gain, these parameters were significantly lower in the castrated group. The decrease in food intake in this group could not account for the difference in body weight between the groups, indicating a lower feed utilisation in the castrates.

At sacrifice accessory reproductive organs, the levator ani muscle, thymus and adrenals were dissected for determination of organ weight and histology, revealing significant reductions in the accessory reproductive organs and levator ani of the castrates. The thymus was significantly heavier in the castrated animals. No differences were found in the adrenals. Two of the sham-operated animals had signs of accidental functional castration. The proportion of body cell mass and total lipid of the carcass was the same in both groups. Significant reductions in adipocyte weights were found in the epididymal depots of the castrated rats.

Blood samples taken at sacrifice in pentobarbital anaesthesia were analysed for glucose, insulin, triglycerides, cholesterol, FFA, glycerol and protein. Statistically significant reductions in triglycerides and protein were recorded in the castrated animals without any significant changes in the other parameters studied.

The results are discussed with reference to the age of castration and the importance of the reduced food intake in castrated animals.
Evident sex differences in growth and gross morphology in several species, at an early stage prompted studies on effects of gonadal hormones on growth and body composition (Stotsenburg 1913). Retarding effects of oestrogen and promoting effects of testosterone on growth have been shown in both sexes in different species including the rat (Wade 1972). Accordingly castration of male rats decreases body weight (Swanson & van der Werff ten Bosch 1963; Kakolewski et al. 1968) while added testosterone increases it (Wade 1972).

The adipose tissue mass is a function of the size and/or number of fat cells and is influenced by gonadal steroids (Vague et al. 1968; Krotkiewski & Björntorp 1975). Reports on body composition of castrated adult rats are sparse and discordant (Scow 1952; Kakolewski et al. 1968; Mukherjee & Chakraburty 1973) and seem to be lacking with respect to a characterisation of the cellularity of the adipose tissue mass.

In man the observation of sex differences in vulnerability to atherosclerotic diseases initiated research on the influence of gonadal steroids on lipid metabolism, especially serum lipids and lipoproteins (Furman et al. 1958). Many such studies have also been performed on the albino rat, using different experimental approaches such as addition of hormones to intact animals, castration, and different degrees of hormone substitution in castrated animals. Important effects of androgens on cholesterol metabolism in the rat liver in vivo and in vitro (Denef & De Moor 1970; Einarsson et al. 1973; Fillios 1956) have been shown as well as on serum lipids (Laron & Kowadlo-Silberfeld 1965; Raheja & Reber 1974). In this study of fat depots after castration of adult rats it was also considered of importance to study serum lipids, free fatty acids (FFA) and glycerol to gain information about lipid mobilisation in the absence of testicular steroids.

In view of the significant interrelationships between lipid and carbohydrate metabolism and the importance of carbohydrate for the adipose tissue mass, blood glucose and insulin were also registered. Effects of gonadal steroids on these parameters have been studied previously, but so far only few and limited reports on the effects of castration on blood glucose and insulin have been presented (Bailey & Matty 1972; Legrele & Sutter 1972). More data has been presented on local actions of castration on carbohydrate metabolism in accessory reproductive organs, muscle preparations and the brain (Mann & Parsons 1950; Adolfsson 1973; Schiaffini & Scacchi 1974).

The mechanisms for the effects of gonadal steroids, on body composition and growth are so far not known though they are known to influence feeding (Wade 1972). The present study is an attempt to elucidate some of the important mechanisms of the gonadal steroids’ effects on body composition and food intake by a broad approach including adipose tissue cellularity and lipid and carbohydrate metabolism.
METH ODS

Twenty male 16 week old (body weight: 351–420 g) Sprague-Dawley rats (Anticimex, Stockholm, Sweden) were acclimatised in separate wire cages (40 x 25 x 10 cm) in a room kept at 21–23°C with a humidity of 55% on a schedule of 10 h of electric light and 14 h of darkness. The animals had free access to standard laboratory pellets (22.5% protein, 5% fat and 55% carbohydrate) 1) and tap water throughout the experiment. Rats of this age were chosen because of data suggesting that the development of new adipocytes has ceased at this age (Greenwood & Hirsch 1974).

One group of 10 rats was orchiectomised in pentobarbital anaesthesia by an abdominal approach sparing the epididymis and its fat pad. The remaining rats were sham-operated by the same abdominal approach with exposure of both testes and epididymal fat pads on the abdomen after removal from the abdominal cavity. Small incisions in the epididymal fat were also performed to simulate the experimental procedure. Food consumption and body weight were registered once a week for 6 weeks. The rats were sacrificed by carotid ex-sanguination in a standardised intraperitoneal pentobarbital (Mebumal®, ACO) narcosis (Kral 1974) 6 weeks after the operation.

Blood chemistry

Analyses of blood glucose (Levin & Linde 1962), heparin plasma insulin (Hales & Randle 1963), triglycerides (Carlson 1959), cholesterol (Cramér & Isaksson 1959), free fatty acids (FFA) (Trout et al. 1960), glycerol (Laurell & Tibbling 1966), and protein (Lowry et al. 1951) were performed on the samples obtained by ex-sanguination.

Organ weights and histology

Since orchiectomy causes removal of most androgens in the rat (Amatayakul et al. 1971; Coyotupa et al. 1973) it was considered of interest to assess the effects on specific target organs. This also afforded the opportunity to study any adverse effects of the sham procedure on the testicular androgen production.

At sacrifice the ventral and dorsolateral prostate, coagulating glands, seminal vesicles and testicles (in intact rats) as well as the levator ani muscle, the thymus and adrenals were immediately carefully dissected and weighed in a tared humid chamber. They were then fixed in Bouin’s solution. After dehydration the organs were embedded in paraffin and cut into 5 μm sections which were stained prior to microscopic investigation (Tisell & Angervall 1969).

Body composition and adipose tissue cellularity

At sacrifice minute samples of adipose tissue were removed from the proximal epididymal fat pad, the perirenal depot (at the caudal pole of the left kidney) and the inguinal subcutaneous depot (adjacent to the femoral nerve and artery) for adipocyte sizing according to the morphometric method of Sjöström et al. (1971). Both perirenal depots, consisting of all freely dissectable adipose tissue from the diaphragm to the seminal vesicles after removal of the kidneys, were removed in toto and weighed as well as both epididymal fat pads.

After replacement of the fat depots the carcass was ground in a conventional electric household meat-grinder, lyophilised to constant weight and pulverised in a Waring

1) EWOS, Södertälje, Sweden.
blendor. Powder aliquots in duplicate were dissolved in fuming hydrochloric acid, for determination of body potassium by an Eppendorff flame photometer. The body cell mass (BCM) was calculated from body potassium by multiplication with a factor of 8.33 (Moore et al. 1963). Other powder aliquots were taken for gravimetric determination of lipids extracted in (2:1, v/v) chloroform-methanol (1:10, w/v) separated according to Folch et al. (1957). The difference between total body weight and the weight of the lyophilised carcass was considered to be the total body water. As a control three other groups of rats were either castrated (n = 7), sham-operated (n = 6) or left intact (n = 4) and sacrificed after 11 days. The whole carcass of each of these animals was homogenised directly by dissolution in fuming hydrochloric acid (1:1, w/v). Aliquots in duplicate were taken for determination of total body potassium by flame photometry.

Statistics

The means and standard errors of the mean of the different variables were calculated for each group. The significance of the difference between means is studied by Student's t-test.

RESULTS

One of the 10 animals in the castrated series died of post-operative haemorrhage reducing the number to 9 (n = 9). Of the 10 rats in the sham-operated series 1 died in narcosis. Two animals in this group were excluded because of significantly reduced wet weights of the accessory reproductive organs at sacrifice. In 1 of the 2 animals these organs exhibited histological signs of

![Fig. 1.](image)

Mean body weights (g ± 1 sn) of male Sprague-Dawley rats. Sham-operated group (broken line, n = 7) and adult castrated (solid line, n = 9). Rats weighed weekly for 6 weeks after operation.
castration. In view of the absence of histological changes in these organs in the other animal the reduced weights are interpreted as being effects of a transient disturbance of the testes accidentally induced by the sham procedure. The exclusion of these animals leaves a total of 7 (n = 7). All dead or accidentally castrated animals are excluded from all calculations.

Body weight and food consumption (Fig. 1)

Body weight in anaesthesia at the time of operation was 383 ± 7 g in the castrated group compared to 396 ± 6 g in the sham-operated group (difference not statistically significant = n. s.). In the post-operative phase there was a decrease in weight to a minimum body weight of 349 ± 9 g in the castrates and 369 ± 4 g in the sham-operated controls (n. s.). The weight gain is defined as the difference between the minimum body weight and the weight at sacrifice and is 56 ± 5 g in the castrated group compared to 90 ± 7 g in the controls (P < 0.01). At sacrifice 6 weeks after operation the castrated rats had increased to 405 ± 7 g while the sham-operated controls reached a final body weight of 459 ± 9 g which was significantly greater than the castrates (P < 0.01). If one takes into account the total weight reductions of the accessory reproductive organs and the weight of the testes there is still a significant weight difference of 48 g between the groups.

Total food consumption for the 6 week period was 1140 ± 30 g/rat in the castrated group compared to 1290 ± 24 g/rat in the control group. The difference between the groups was statistically significant (P < 0.01). The control rats had a ratio of 15 ± 1 of feed per gram weight increase compared to 22 ± 2 in the castrates (P < 0.05).

Table 1. Body weight at sacrifice and cellularity of adipose tissue depots. Means ± sem.

<table>
<thead>
<tr>
<th></th>
<th>Castrated (n = 9)</th>
<th>Sham-operated (n = 7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight g</strong></td>
<td>405 ± 7</td>
<td>459 ± 9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Perirenal depot</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>weight g</td>
<td>3.94 ± 0.25</td>
<td>3.92 ± 0.36</td>
<td>n. s.</td>
</tr>
<tr>
<td>Cell weight μg TG</td>
<td>0.23 ± 0.07</td>
<td>0.29 ± 0.07</td>
<td>n. s.</td>
</tr>
<tr>
<td><strong>Epididymal depot</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>weight g</td>
<td>355 ± 0.41*</td>
<td>4.05 ± 0.56*</td>
<td>n. s.</td>
</tr>
<tr>
<td>Cell weight μg TG</td>
<td>0.28 ± 0.02</td>
<td>0.37 ± 0.03</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Inguinal cell</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>weight μg TG</td>
<td>0.16 ± 0.03</td>
<td>0.22 ± 0.03</td>
<td>n. s.</td>
</tr>
</tbody>
</table>

* (n = 4).
Body composition and adipose tissue cellularity

The amounts of lipid, total body water and body cell mass (BCM) were lower in the castrated animals corresponding to their lower body weights. The relative content of the different components, however, was the same in both groups, viz. lipids 11 %, body water 65 % and BCM 46 %. In the short-term control series the BCM was 49 % of the body weight in all groups using a different method of homogenisation.

The adipose tissue cellularity (Table 1) does not reveal statistically significant differences between the groups in the perirenal or inguinal depots though there is a trend toward smaller fat cells in the castrates. In the epididymal depot the cell weights were significantly lower in the castrated rats ($P < 0.05$).

Blood Chemistry (Table 2)

The plasma triglyceride level in the castrated rats was significantly lower than the sham-operated controls ($P < 0.01$). The serum protein was also significantly reduced in the castrates ($P < 0.05$). There were no statistically significant differences in the other parameters.

Organ weights (Table 3)

The ventral and dorsolateral prostate, the coagulating glands and the seminal vesicles were all significantly ($P < 0.001$) lighter in the castrated animals. A significantly reduced organ weight was also found in the levator ani muscle of castrated rats ($P < 0.001$). The thymus weight was significantly greater in the castrated animals ($P < 0.001$) while there was no significant difference in the weights of the adrenals.

**Table 2.**

<table>
<thead>
<tr>
<th>Glucose mg/100 ml</th>
<th>Castrated (n = 9)</th>
<th>Sham-operated (n = 7)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin µU/ml</td>
<td>122 ± 4</td>
<td>112 ± 6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Triglycerides mm/ml</td>
<td>50 ± 6</td>
<td>54 ± 6</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cholesterol mg/100 ml</td>
<td>0.84 ± 0.11</td>
<td>1.42 ± 0.20</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>FFA µm/l</td>
<td>86 ± 7</td>
<td>90 ± 4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Glycerol mm/l</td>
<td>257 ± 13</td>
<td>290 ± 34</td>
<td>n.s.</td>
</tr>
<tr>
<td>Protein mg/100 ml</td>
<td>61 ± 5</td>
<td>68 ± 7</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

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**Table 3.**
Organ weights (mg). Means ± sem.

<table>
<thead>
<tr>
<th></th>
<th>Castrated (n = 9)</th>
<th>Sham-operated (n = 7)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral prostate</td>
<td>71.8 ± 12.4</td>
<td>812.3 ± 40.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dorsolateral prostate</td>
<td>90.2 ± 7.1</td>
<td>412.4 ± 24.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Coagulating glands</td>
<td>36.4 ± 2.9</td>
<td>234.9 ± 17.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Seminal vesicles</td>
<td>124.8 ± 7.0</td>
<td>1470 ± 64</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Levator ani</td>
<td>139.7 ± 10.3</td>
<td>283.3 ± 7.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Adrenals</td>
<td>53.4 ± 1.3</td>
<td>54.7 ± 2.4</td>
<td>n. s.</td>
</tr>
<tr>
<td>Thymus</td>
<td>685 ± 21</td>
<td>507 ± 39</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Testes</td>
<td>–</td>
<td>3089 ± 268</td>
<td>–</td>
</tr>
</tbody>
</table>

**DISCUSSION**

*Body weight, food intake, body composition and adipose tissue cellularity*

The lower body weight after castration found in this study agrees with the findings of most other investigators (Kakolewski et al. 1968; Scow 1952) as does the decreased food intake (Wade 1972). The increased requirement for feed per gram weight gain in the castrated rats (Nutrient Requirements of Domestic Animals 1972) indicates a lower feed utilisation in these animals. The present data do not permit any conclusions about the mechanisms responsible for the reduced food intake and lower feed utilisation in testosterone deficiency. The lack of effects of castration on cholesterol, FFA, glycerol and glucose and insulin in this study would seem to rule out any interaction of these parameters with the regulation of appetite. The decreases in adipocyte weight shown here may just as soon be an effect of reduced lipid storage due to lowered food intake as to increased mobilisation of fat, especially considering the lack of raised levels of FFA or glycerol. Lower feed utilisation in animals with altered levels of gonadal hormones as in the present study, and indications of an increased absorption of nutrients during treatment with added gonadal hormones have been described in some species (Gassner et al. 1958).

There were uniformly distributed reductions in body fat, body cell mass and total body water after castration, not revealing any shifts in relative body composition. Concerning body fat, this finding is at variance with a recent study (Mukherjee & Chakraburty 1973) using smaller, and probably younger rats. In their study the rats were observed for a shorter period of time (11 days)
and had a lower fat content in pair-fed controls (6%/s) which, however, were under the influence of food restriction.

Analysis of the changes in fat cell size (weight) reveal decreases in the epididymal depot after castration. The weight of the perirenal adipocytes in castrates did not reach statistically significantly lower levels than in the controls and no significant differences were registered in the subcutaneous inguinal depot. These results are at variance with a recent study on castrated 6 week old mice (Lemonnier & Alexiu 1974). These mice were not adults, however, and the body weight of the castrates were significantly higher than in the control group. The decrease in the epididymal depot might be a specific action due to lack of testosterone stimulation or a persistant effect of the operative procedure. The localisation of the epididymal fat pad suggests a role in the reproductive function of the testis and epididymis which could explain a greater sensitivity of the adipocytes in this depot to a lack of testicular steroids.

The body composition data presented here, show a decrease in protein represented by the diminished BCM in the castrates which is well known from other studies in rats as well as other species (Scow 1952). Starvation reduces both fat and protein (Oscai et al. 1974) as in the present study. The decrease in BCM shown after castration was not greater than that of the other components in the 6-week observation period and agreed with the weight reduction.

Blood chemistry

Administration of testosterone to intact animals of different species has been shown to increase albumin synthesis and decrease the synthesis of all globulin fractions without any net effect on total serum protein levels (Solyom et al. 1971). The reduced serum proteins after castration in the present study were not analysed with respect to the different protein fractions.

Decreased levels of serum triglycerides were found in this study after castration. An increase in triglycerides, and to a lesser extent cholesterol, might rather be expected in view of the known decreased locomotor activity of castrated rats (Wade 1972). After an exhaustive exercise program in rats in a previous study we found significant reductions in serum triglycerides without any differences in cholesterol values (Kral et al. 1974). The decreased triglycerides and protein in this study presumably are an effect of the decreased food intake after castration, these variables being the most sensitive to alimentary manipulation.

Several studies have shown a hypocholesterolaemic effect of testosterone depending on the dose and mode of administration (Raheja & Reber 1974). There is less data on the effect of castration on plasma cholesterol. One study showed a definite increase in serum cholesterol in castrated male rats on
both a normal and cholesterol-cholate diet (Fillios 1956). Unfortunately the
time of castration was not stated nor was any information presented on food
intake or body weight. The failure to exhibit any effect of castration on
plasma cholesterol in adult castrated rats indicate that these effects can not
be brought about after the neonatal masculine differentiation of cholesterol
metabolism (Denef & De Moor 1970) has occurred.

In studies on female and castrated male fasting rats Laron & Kowadlo-
Silbergeld (1964, 1965) showed a fat mobilizing effect of large doses of testo-
sterone as evidenced by increased levels of FFA. In the present study, as
was discussed above, no significant serum FFA changes could be demonstrated
after castration in agreement with the data of Taylor et al. (1973). A clear
analysis of the net flux of FFA is complicated by the sensitivity of this para-
meter, as well as glycerol, to stress (Kral 1974). In this study it was noted
that the group of sham-operated controls showed more signs of aggressiveness
and excitement than the castrates, as is known from other studies. Thus a
temporary elevation in the control group in connection with sacrifice could
possibly be surmised to mask a basal level lower than that of the castrates.

Concerning the effects of gonadal hormones on carbohydrate metabolism, a
recent brief communication described an increase in glucose without any
change in insulin in unanaesthetised castrated fasted rats 20 days after castra-
tion (Legrele & Sutter 1972). Another study on glucose tolerance in castrated
untreated and testosterone-substituted and control adult male rats did not
reveal any difference in glucose tolerance but slightly lower insulin levels
in the untreated castrates (Bailey & Matty 1972). In the present study there
were no statistically significant differences in the levels of blood glucose
concurring with a previous study (Taylor et al. 1973). Also there were no
significant differences in insulin levels between the groups.

In conclusion this study confirmed the decrease in food intake and weight
gain in castrated male animals known from other studies. A decreased feed
utilisation by unknown mechanisms was also shown. The diminished food
intake seems to account for many of the results in this study. Thus the pro-
portional reductions of different body compartments in castrated animals com-
pared to sham-operated controls at least to a certain extent can be ascribed
to the reduced food intake and feed utilisation.

Evidently castration did not alter the proportion of body fat and protein
so often described in castrates of different species. This apparent discordance
is interpreted as being due to the fact that the animals were adults at the
time of castration and already had developed a masculine differentiation of
body composition and lipid metabolism. The lack of changes in serum chole-
sterol after castration in this experiment is in agreement with this hypothesis
and emphasizes the importance of time of castration.

Concerning cholesterol as well as the other metabolites the interpretation
of the net blood levels is complicated by the reduced food intake which may mask several more complex effects of an altered metabolism after castration. The significant decreases in serum triglycerides and protein, however, appear to be mainly effects of the reduced food intake.

ACKNOWLEDGMENTS

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