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Effect of postweaning thyroid status on endocrine and adipose development in rats

MONIQUE Q. ROBERT AND EMILIO HERRERA
Departamento de Bioquímica y Fisiología, Unidad B, Facultad de Biología, Universidad de Barcelona, Barcelona 08028, Spain

Robert, Monique Q., and Emilio Herrera. Effect of postweaning thyroid status on adipose development in rats. Am. J. Physiol. 255 (Endocrinol. Metab. 18): E280–E286, 1988.—We previously reported that 6 wk after thyroidectomy, rat fat cell diameter and number were 86 and 32% of values in age-matched controls. This study aims to 1) determine the time course of these changes and of parallel changes in the levels of several hormones known to participate in adipose differentiation and 2) try to normalize the altered cellularly with thyroxine (T₄) treatment. Rats were thyroidectomized (T₄) or sham-operated and studied 2, 5, or 6 wk later. At 5 wk some T rats received 1-T₄. Epididymal fat cells were sized and used to estimate fat cell number. Triiodothyronine (T₃), T₄, thyroid-stimulating hormone, somatotropin (GH), and insulin plasma levels were assayed. In T rats fat cell hypertrophy was blunted uniformly with time, whereas fat cell hyperplasia was slow for 2 wk and zero thereafter. Insulinemia was less drastically reduced than T₃, T₄, and GH levels. The fat cell number-to-body weight ratio always stayed close to the ratio present at thyroidectomy. In T rats receiving a T₄ substitution dose for 1 wk, fat cell number and body weight increased, but not when the T₄ dose was lower even though the plasma thyroid hormone levels were increased. These thyroid hormone effects on adipose tissue are probably secondary to other endocrine changes that occur in hypothyroidism.

epididymal fat pad; white adipose tissue; fat cell size and number; hypothyroidism; thyroid hormones; growth hormone; insulin

THE DEVELOPMENT of rat white adipose tissue (WAT) has been well documented, mainly the epididymal depot (12); no WAT is observable in newborns, and adipose depots form later with the increase of fat cell size and number. Data concerning the influence of thyroid hormones on WAT cellularity in growing rats are scarce (16, 17, 19). However, it is clear from observations in cultured adipose cell lines that some aspects of in vitro adipose conversion are modulated by the concentration of triiodothyronine (T₃) in the medium (1, 25); adipocytes differentiated without T₃ are smaller, and their enzyme markers have lower specific activities.

When a hypothyroid state overlaps with some phase of WAT development in the rat, cellularity is clearly affected. In rats treated with propylthiouracil (PTU) from birth, retroperitoneal adipose tissue (RPAT) has a low fat cell number at weaning, and it does not further increase with age, whereas fat cell size is not significantly affected (16). Our previous data (19) indicate that, in rats thyroidectomized at weaning and observed 6 wk later, the epididymal fat cell diameter is almost normal (86% of value in age-matched controls), and the low fat cell number is mostly due to the low number of fat cells in the depot. This low fat cell number is proportional to their low body weight. Perhaps fat cell hyperplasia and hypertrophya were uniformly blunted during this 6-wk experimental period, but our data suggest that fat cell hyperplasia might be stopped at the same time as body growth (which stops ~2 wk after thyroidectomy). Therefore, in the present work we studied epididymal fat pad cellularity at the time of thyroidectomy and at different times thereafter. The plasma levels of thyroid axis hormones, pituitary growth hormone [somatotropin (GH)], and insulin were studied alongside with WAT cellularity because correlative endocrinological data are lacking in the literature treating thyroid influence on WAT cellularity.

If the endocrine state is reverted from hypothyroid, some of the altered WAT development parameters normalize. When young rats (5–6 wk old) are no longer treated with PTU, RPAT hyperplasia is reinitiated and fat cell number normalizes (16). In the present work the possibility of reversing altered WAT cellularity was studied in a different depot and in adult hypothyroid rats (10 wk old, when WAT cellular multiplication is usually lower than in younger cats) by treating thyroidectomized animals with thyroxine (T₄) for 1 wk.

In the present study we found that, as a consequence of thyroidectomy at weaning, the epididymal fat depot and the hormone levels were affected as follows: 1) both fat cell hypertrophy and hyperplasia were blunted, but fat cell size was affected to a lesser degree and later than fat cell number; 2) fat cell number remained in proportion to body weight, whatever the group or age; 3) insulinemia was less affected than thyroid axis hormones and GH plasma levels; 4) when hypothyroid adult rats received a week-long T₄ substitution treatment that normalized thyroid axis hormones and GH (but not insulin) plasma levels, body weight and estimated fat cell number were increased.

MATERIALS AND METHODS

Animals. Male Wistar rats were used. They were housed at 22 ± 1°C on a 12-h light-dark cycle. Before weaning at 21 days of age, the rats were nursed in litters of 10
individuals (litter size was adjusted within 24 h of birth) so that differences in litter size would not affect post-weaning adipose tissue cellularity (4). All rats were fed ad libitum with a pellet diet (from UAR, France; 17% protein, 3% fat, 4% cellulose, 59% other carbohydrates, 5% minerals). Rats aged 30–35 days were surgically thyroidec-tomized or sham operated under ethyl ether anesthesia. Thyroidectomy was completed 24 h later by a 100 μCi 131I-Na intraperitoneal injection. To prevent possible hypocalcemia resulting from loss of parathyroid glands, calcium lactate was added to the drinking water (final concn 10 g/l) for 1 wk. Animals were studied at 2, 5, 6, or 16 wk after surgery. Intact rats killed at the age of 30–35 days were used as a time 0 control group. Five weeks after thyroidectomy, some rats were given a daily intra-peritoneal injection of T3, for 1 wk and studied 24 h after the 7th injection. The dose was either 0.1 or 1.8 μg of T3, 100 g body wt⁻¹ day⁻¹ (sodium salt, Sigma) (T + 0.1 or T + 1.8 rats). All operated rats not receiving T3 were intraperitoneally injected daily with 0.9% NaCl (wt/vol) (same volume per body weight as in T3 injections). Rats were decapitated between 11 and 12 A.M. (light on at 8:30 A.M.) and were considered to be fed because their stomachs were invariably full. Blood collected on heparin was used to assay plasma levels of T3, T4, thyroid-stimulating hormone (TSH), GH, and insulin. WAT from the epididymal site was studied; the entire bilateral depot was excised and weighed. The right fat pad was kept in Krebs-Ringer phosphate buffer at room temperature for fat cell sizing, which was performed on the day of death. The left fat pad was used to determine tissue protein content (18). Each fat pad was divided across its longitudinal axis into three portions of approximately equal size. The median portion was used for protein and cell size determinations.

Adipose tissue cellularity. Fat cell diameter was determined according to the method of Sjöström et al. (24); thick (200 μm) frozen-cut sections were obtained from 20- to 30-mg tissue pieces fixed in formaldehyde. For each rat studied, 100 fat cells were measured after focusing the largest diameter (D) of each cell (50 from each of 2 slices, each slice coming from a different tissue piece) and mean fat cell diameter (D) was calculated. Table 1 shows representative values from one rat of each experimental group. Normal histograms were obtained from the frequency distribution of the 100 diameters measured in each rat. Fat cell volume was calculated for each cell by assuming adipocytes are spheres [volume = (π/6) × D³]. On microscopic examination, all fat cells had a spherical appearance, even the smaller ones found in the youngest animals. Representative values of fat cell volume are also given in Table 1. Lipid content of each fat cell was calculated by fat cell volume times lipid density. Lipid density was assumed to be 0.915, as for lipids extracted from fat epididymal fat pad (6). The contribution of nonlipid components to the fat cell volume was considered to be negligible. The mean fat cell size obtained from the median portion of the fat pad was considered to be representative of the mean fat cell size in the whole depot (11). The number of fat cells in the entire fat depot (fat cell number) was estimated for each rat as the ratio between bilateral fat pad weight and mean fat cell lipid content. (For this estimation of fat cell number, the lipid-to-tissue weight ratio was considered to be 1.)

Assay of plasma hormones. The T3 and T4 plasma concentrations were determined by radioimmunoassay (RIA) (20, 21). Antibodies were from Henning (Berlin, GmbH). Labeled L-T4 and L-T3 were obtained by iodination of L-T4 (3,5-diodothyronine) and L-T3, respectively. Antibody-bound and free hormone were separated by use of polyethylene glycol (Carbowax 6000) and bovine serum. Reagents for RIA of rat TSH and for RIA of rat GH were kindly provided by the Rat Pituitary Hormone Distribution Program (NIAMDD, NIH). Immune complexes were precipitated with the protein A-carrier Staphylococcus aureus (Cowan strain). Reagents for RIA of rat insulin were kindly provided by Novo (Denmark). Rat insulin standard was 20.7 U/mg.

Statistics. Differences between experimental groups were analyzed with Student’s t test.

**RESULTS**

Effect of thyroidectomy on age-related changes in WAT cellularity and plasma hormone levels. Thyroidectomized (T) rats weighed significantly less than age-matched sham-operated controls at all times studied (Table 2) and as soon as 9 days after surgery. Similar results were obtained for epididymal fat pad weight (Fig. 1), it was always lower in T rats. Although the adipose depot grew continuously in growing control (C) rats, in T rats it grew only during the first 5 wk after surgery. In this group fat pad weight increased between 2 and 5 wk after surgery, although body weight did not. When adipose tissue weights were expressed as a proportion of body weight (Fig. 1, bottom), the difference between T and C rats was initially small but increased with age and became significant at 6 wk after surgery.

As shown in Fig. 2, T rats always had smaller and less numerous fat cells than age-matched C rats, and these differences increased to significant levels with advancing age, but fat cell size was less affected by thyroidectomy than was fat cell number. In Fig. 2 fat cell size is given as the mean fat cell lipid content (the corresponding mean fat cell diameters are shown in Table 3). Statistical differences in fat cell size between T and age-matched C

| TABLE 1. Fat cell measurements in frozen-cut sections of epididymal fat pads from three rats belonging to different experimental groups |
|---------------------------------|-----------------|-----------------|-----------------|
|                                  | Fat Cell Diameter | Fat Cell Volume |                  |
|                                  | μm, pm            | μm, pl          |                 |
| Intact rat (30–35 days old)     | 45.90±0.96        | 2.1             | 57.40±3.73      | 6.5 |
| Thyroidectomized rat (2 wk after surgery) | 56.82±1.07        | 1.9             | 105.97±5.45     | 5.1 |
| Sham-operated rat (6 wk after surgery) | 83.68±1.39        | 1.8             | 332.28±16.35    | 4.9 |

Values are mean of 100 fat cells ± SE. Coefficient of variation (CV) was derived by SE/mean and expressed as percentage. Diameters (D) of 100 fat cells were measured in each rat and, for each fat cell, cell volume was calculated by assuming fat cells are spheres (volume = π × D³/6).
TABLE 2. Effect of thyroidectomy on body weight

<table>
<thead>
<tr>
<th>Weeks After Surgery</th>
<th>Body weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Sham-operated controls</td>
<td>98.2±1.4</td>
</tr>
<tr>
<td>Thyroidectomized</td>
<td>118.2±3.6*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5-8 rats. Male rats, 30-35 days old, were thyroidectomized or sham operated and studied 2, 5, 6, or 16 wk later. Intact male rats, killed at age 30-35 days, were used as time 0 control group. Animals were weighed just before death. * P < 0.001 vs. age-matched controls.

wk after thyroidectomy and did not change thereafter. So, the weight increase in T rat fat pads during the 5th to 6th week after thyroidectomy (Fig. 1) was due to fat cell hypertrophy (Fig. 2).

The pattern of age-related changes in fat cell number (Fig. 2) followed the corresponding body weight pattern. In fact, the ratio between fat cell number and body weight did not differ between T rats and age-matched controls (Table 3). Neither did it change with age in any group. On the other hand, as indicated in Fig. 2, changes in fat cell number (middle) were well paralleled by changes in total epididymal protein (bottom). Therefore, the protein-to-fat cell number ratio was found to be fairly constant (~2 mg/10⁶ fat cells) and independent of thyroidectomy.

Thyroid hormone and TSH plasma levels were summarized in Fig. 3. Two weeks after thyroidectomy, almost undetectable thyroid hormone levels were found, and TSH levels were ~50 times higher than in age-matched sham-operated controls. This same hypothyroid picture was observed later after thyroidectomy. In C rats, plasma levels of thyroid axis hormones varied appreciably with advancing age, T₃ and T₄ being reduced and TSH increased. Insulin and GH plasma levels (Fig. 4) were clearly lower in T rats than in age-matched C rats at all ages.

**Effect of a T₄ substitution dose on WAT cellularity and endocrine state of hypothyroid rats.** As shown in Fig. 5, when rats received T₄ substitution 5 wk after thyroidectomy (T + 1.8), their body growth was reinitiated (mean body weight increased 27 g in 1 wk) at a rate that was close to the one observed in sham-operated C rats throughout the 0- to 6-wk experimental period (33 g/wk). Thus, after 6-7 days of treatment, T + 1.8 rats reached significantly greater body weights than did age-matched hypothyroid rats not receiving T₄ (Fig. 5). The T₄ replacement treatment significantly changed the plasma level of T₃, T₄, TSH, and GH away from the values found in untreated T rats (Table 4), and the new levels reached in T + 1.8 rats were comparable to those found in sham-operated C rats. The T₃-to-T₄ ratio of T + 1.8 rats (0.017 ± 0.001%, wt/wt) was also comparable to that of sham-operated rats (0.020 ± 0.002, 0.016 ± 0.001, 0.015 ± 0.001%, wt/wt, at 2, 5, and 6 wk after surgery). The only hormonal parameter that was not significantly changed by T₄ substitution was insulinemia (Table 4): its values remained close to those found in age-matched T rats.

T₄ treatment did not change epididymal fat pad weight.
TABLE 3. Effect of thyroideotomy on mean fat cell diameter and fat cell number-to-body weight ratio in epididymal fat pads

<table>
<thead>
<tr>
<th>Weeks After Surgery</th>
<th>Mean fat cell diameter, μm</th>
<th>Fat cell no. per body wt, millions/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>48.4±0.7</td>
<td>60.9±3.9</td>
</tr>
<tr>
<td>2</td>
<td>57.4±1.5</td>
<td>65.9±3.6</td>
</tr>
<tr>
<td>5</td>
<td>67.8±4.3*</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>67.8±4.3*</td>
<td></td>
</tr>
</tbody>
</table>

Sham-operated controls

Sham-operated controls

Thyreoidectomized rats are compared with age-matched controls. *P < 0.05. Other differences are not significant (P > 0.05).

cellularity and endocrine state of hypothyroid rats. As shown in Fig. 5, body weight did not differ at any time between hypothyroid rats receiving a low T₄ dose (T + 0.1) and age-matched hypothyroid rats not receiving T₄. When body growth was calculated for each animal as the percentage of body weight increase during the week of treatment over initial body weight, the growth in T + 0.1 rats was 5%, which was significantly higher than the −1% value of T rats (P < 0.05).

In T + 0.1 rats, treatment significantly raised T₃ and T₄ plasma levels above values in T rats (Table 4) and the T₃-to-T₄ ratio was very high (0.037 ± 0.007%, wt/wt). This did not significantly change TSH, GH, or insulin levels from the values found in hypothyroid rats not receiving T₄. In fact, thyroid hormone levels in T + 0.1 rats were still very significantly lower than the normal levels present in T + 1.8 or sham-operated C rats. As compared with age-matched T rats not receiving T₄, no significant change was observed in fat pad weight of T + 0.1 rats. Their fat cell number, fat cell size or total epididymal protein did not significantly differ from values in untreated T rats (Table 5).

DISCUSSION

Thyreoidectomy in rats aged 30–35 days resulted in a typical hypothyroid state (present as soon as 2 wk after thyroideotomy), which markedly reduced the age-related increases in body weight, epididymal fat pad weight and fat cell number, in agreement with earlier data (16, 19). Fat cell size was reliably measured, as indicated by the acceptable coefficients of variation for fat cell diameter and volume (2 and 5–6%, see Table 1). Fat cells were smaller in T rats than in age-matched controls, a common observation for epididymal fat cells whenever hypo­thyroidism is produced during the postweaning growth (10, 13, 19). Fat cell size is not always affected by hypothyroidism in the same way, since rat parametrical fat cell size is unaffected by this state (5), and in the subcutaneous depot of adult hypothyroid patients fat cell size is normal (7) and remains stable with L-T₄ substitution therapy (22, 26).

In spite of the drastic and early reduction in levels of thyroid hormones and GH (and, probably also of thyroid...
hormone- and/or GH-dependent growth factors), fat cell size increased steadily during the whole experimental period in T rats, the increase being somewhat slower in T than in C rats. This quite normal fat cell hypertrophy in T rats may be related to the maintenance of insulinaemia at levels like those existing at the time of thyroidectomy, even though these levels are lower than in age-matched controls. [In other situations fat cell size correlates with both in vivo (8) and in vitro (23) insulin

FIG. 4. Effect of thyroidectomy on plasma levels of growth hormone and insulin in growing rats. See Fig. 1 for additional information.

FIG. 5. Effect of thyroxine administration on body weight of hypothyroid rats. Rats were thyroidectomized (T) at the age of 30–35 days and were daily intraperitoneally injected with NaCl 0.9% (wt/vol) for 5 wk. Then they were divided in 3 groups: some continued with the same treatment (T) and others received either 0.1 or 1.8 μg l-T₄/100 g body wt⁻¹·day⁻¹ (T + 0.1 and T + 1.8). Values are means ± SE of 5–8 rats. T + 1.8 or T + 0.1 rats are compared with age-matched T rats:

* P < 0.05; ** P < 0.01.
levels.] No significant fat breakdown (nor fat cell size reduction) is to be expected in T rat fat cells as a consequence of their high TSH levels, since lipolytic effects of TSH on WAT in vitro are found at far higher concentrations (15).

The fat cell number values obtained in our C rats were close to those reported by other authors for epididymal fat pads in the normal growing rat (2, 3, 12) despite the differences in methodological approach (they either estimated fat cell number from mean fat cell size by using the lipid-to-tissue weight ratio, or directly counted the isolated fat cells). We found that the estimated fat cell number was much lower in T rats than in age-matched C rats, and the difference between both groups increased with advancing age. At 5–6 wk after surgery, the magnitude and level of significance of this difference was such that it seems most improbable that this difference would disappear if lipid-to-tissue weight ratios were used in the estimation of fat cell number: lipids are expected to represent 70–80% of control rat fat pad weight, so only much higher (and unexpected) percentages in T rat fat pads would appreciably reduce this difference in fat cell number between T and age-matched C rats. The evolution of fat cell number in T rats seems to be independent of stable insulin levels and, from timing and magnitude, more related to changes in the thyroid hormone and GH plasma levels.

The stability, in a wide range of body weights and fat cell numbers, of the fat cell number-to-body weight ratio may simply be an indication that the same growth factors that regulate fat cell number also regulate growth in other tissues and, hence, body size.

The fact that our hypothyroid rats treated with a T4 substitution dose started to grow again was in accordance with their normalized GH levels. Because their insulin levels remained at low hypothyroid levels, some hormonal imbalance may have existed that might be responsible for the lack of increase in fat pad weight and fat cell size. The estimated fat cell number of these T4-treated animals was higher than in age-matched untreated T rats. Admittedly, because we did not determine the lipid-to-tissue (wt/wt) ratio for the estimation of fat cell number, a lower percentage of lipids in the fat pads of the treated vs. the untreated T rats could account for the observed increase in estimated fat cell number, and further work would clarify this point. However, there is an evident recuperation process at play in fat pads of these treated rats, since total epididymal protein is very significantly increased, proportionately to the increase in estimated fat cell number. Other reports find that the low fat cell number present in hypothyroid rat adipose tissue can be normalized with return to euthyroidism, but this recuperation was observed at an earlier age than the one studied in this paper (16). Increase in fat cell number may be more related to normalization of GH rather than to normalization of thyroid hormone plasma levels. Arguments in this sense are that new fat cells appear when GH-deficient children receive GH replacement therapy (9), and in rats treated with GH cell replication becomes more active in adipose precursor cells (14).

When rats were treated with a T4 dose one-eighth of that used for substitution, the almost imperceptible body growth agreed with the lack of normalization in GH levels. Because thyroid hormone levels were very significantly elevated by this treatment, the fact that no change in fat pads was detectable indicates that the effects observed in T4-substituted rats were not the result
of direct actions of thyroid hormones on WAT.
In the many works on WAT development in the normal growing rat, there is little information on parallel age-related endocrine changes. Therefore, the results obtained here in the sham-operated control group are briefly commented. Both fat cell size and insulinemia increased steadily throughout the experimental period. Fat cell hyperplasia was high in the younger animals with high thyroid hormone plasma levels, whereas no fat cell number increase was evident in the older rats (aged 62–72 days), in whom both T3 and T4 levels were appreciably reduced as a consequence of age. These observations may therefore be related to the results of the present work showing that hypothyroidism prevents fat cell hyperplasia.

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Address for reprint requests: E. Herrera, Servicio de Bioquímica, Hospital Ramón y Cajal, Ctra. Colmenar km 5, 28034-Madrid, Spain.
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