**Hormone Research**
Editor: J. Girard, Basel
Publishers: S. Karger, Basel
Reprint (Printed in Switzerland)

Hormone Res. *11:* 254–261 (1979)

# Adipose Tissue Cellularity in Hypo- and Hyperthyroid Rats[1]

*A. Muniesa, M. Llobera and E. Herrera*[2]

Cátedra de Fisiología General, Facultad de Biología, Universidad de Barcelona, Barcelona, and Departamento de Investigación, Centro Ramón y Cajal, Madrid

**Key Words.** Adipose tissue cellularity · Hypothyroidism · Hyperthyroidism

**Abstract.** To determine adipose tissue cellularity in hypo- and hyperthyroidism, male rats were thyroidectomized after weaning (T) and injected daily with either 0, 0.1, 1.8, or 25 μg of *L*-thyroxine/100 g body weight for 40 days. They were compared with intact controls (C). Both epididymal fat-pad weight and adipocyte diameter were reduced in T+0, T+0.1 and T+25 animals. When corrected per unit of body weight, the diameters of adipocytes from T+0 and T+0.1 animals were larger than in the other groups. Those same animals have reduced absolute adipocyte number but not when corrected per unit of body weight. The fat-pad protein concentration varied conversely with the fat cell diameter. These findings indicate that thyroid hormone deficiency reduces the proliferation of fat cells in parallel with body growth while hyperthyroidism causes reduction in the size, but not the number, of fat cells which corresponds to its depletion of fat storage.

Regulation of adipose mass is determined by the rates of two processes: the formation and maturation of new fat cells and the turnover of lipid in existing fat cells (15). Adipose tissue cellularity changes with individual growth in normal conditions (10, 14) and growth hormone affects this parameter significantly (2, 12). Retarded growth is a well-known effect of thyroid deficiency as has been widely demonstrated in the rat (1, 6, 16, 20–23) and alterations in adipose tissue metabolism have frequently been described in both hypo- and hyper-

[1] This study was supported in part by a grant from the 'Comisión Asesora de Investigación Científica y Técnica, Presidencia del Gobierno', Spain.

[2] The authors wish to express their gratitude to *Caroline S. Delgado* for her editorial assistance.

thyroid animals (5, 8, 24, 29). Although changes in thyroidal status affect both adipose tissue mass and protein content in the rat (8, 29), no data on adipose tissue cellularity have been reported. In addition to its physiological implications, knowledge of the adipose cell size and number in hypo- and hyperthyroid animals would facilitate intergroup comparisons when studying other metabolic parameters in the adipose tissue of these animals, expressed either per fresh tissue weight or tissue protein content. In the present study we have analyzed adipose tissue cellularity in thyroidectomized rats injected daily with different doses of exogenous thyroxine.

## Materials and Methods

*Animals*

Male Wistar rats were thyroidectomized after weaning at 26–31 days, fed a low iodine diet of the Remington type (26), and injected daily with either 0, 0.1, 1.8, or 25 $\mu$g of *L*-thyroxine/100 g body weight. Age-matched, intact controls were maintained on the same diet but supplemented with 1.7 $\mu$g of $KIO_3$/g, injected daily with saline. Animals were decapitated after 40 days treatment, blood was collected into heparinized beakers, and the epididymal fat-pads were dissected at the level of the epididymal vein. Aliquots of the tissue were immediately frozen in liquid $N_2$ for DNA and protein evaluation and other aliquots were used for cellularity determination.

*Adipose Tissue Cellularity*

The mean cell sizes were determined using the method of *Sjöstrom et al.* (30) with minor modifications. The tissue samples were fixed in 37% formaldehyde for 7 min, rinsed with Krebs-Ringer phosphate buffer, pH 7.4 (33), and embedded in OCT for frozen-cut to yield slices 200 $\mu$m thick. The slices were floated in a closed chamber containing Krebs-Ringer phosphate buffer and examined with a microscope equipped with a Filar Micrometer (American Optical Co., Buffalo, N.Y.). The largest diameter (D) of 200 cells was determined in different tissue slices from each experimental animal with a technique that avoids selection of large cells (25). The number of fat cells was derived from the cell volume (volume = $\pi \times D^3/6$) and the lipid density of rat adipose tissue (0.915) as proposed by *Di Girolano et al.* (10).

*DNA and Protein in Tissue and Plasma Protein-Bound Iodine Evaluations*

The frozen aliquot of the tissue was delipidized with chloroform-methanol (2:1, v/v) and the lipidic-free precipitates were digested with 2.5 ml of 1 *N* NaOH at 36 °C for 24 h. Separate aliquots of the digestives were used for protein evaluation by the Lowry procedure (18) and for DNA purification (28). Phosphorous of DNA precipitates was estimated (9) after digestion in 72% $HClO_4$. Plasma protein-bound iodine was determined by the method of *Benotti and Benotti* (3).

**Table I.** Effect of thyroidectomy (T) and treatment with *L*-thyroxine on body and epididymal fat-pad weights and plasma protein-bound iodine (PBI) in the rat

| | Body weight, g | | Epididymal fat-pad weight | | Plasma PBI μg/100 ml |
|---|---|---|---|---|---|
| | initial | final | total mg | mg/100 g b.w. | |
| Controls | 56.3 ± 2.2[1] | 192.9 ± 4.1 | 1,343 ± 361 | 593 ± 127 | 4.59 ± 0.25 |
| T+0[2] | 54.9 ± 1.5 | 103.2 ± 6.7 | 222 ± 27 | 272 ± 30 | 0.23 ± 0.36 |
| p[3] | NS | <0.001 | <0.05 | <0.05 | <0.001 |
| T+0.1 | 55.1 ± 2.1 | 163.5 ± 7.8 | 449 ± 78 | 314 ± 49 | 0.68 ± 0.28 |
| p | NS | <0.001 | <0.05 | NS | <0.001 |
| T+1.8 | 54.5 ± 1.9 | 201.6 ± 7.8 | 752 ± 149 | 396 ± 40 | 4.46 ± 0.28 |
| p | NS | NS | NS | NS | NS |
| T+25 | 58.6 ± 2.6 | 171.7 ± 4.8 | 446 ± 87 | 219 ± 32 | 9.55 ± 0.71 |
| p | NS | <0.001 | <0.05 | <0.05 | <0.001 |

[1] Means SEM of 5–7 rats/group.
[2] Daily dosis of *L*-thyroxine/100 g body weight.
[3] Statistical comparisons versus controls (NS = not significant: $p > 0.05$).

## Results

Although at the onset of the experiment there were no differences in body weight among the groups, 40 days after thyroidectomy the rats had body weights 46.5% below controls (table I). The daily injection of 0.1 μg of thyroxine in the thyroidectomized animals (T+0.1) produced partial recuperation of body weight and treatment with 1.8 μg (T+1.8) produced complete recuperation (table I) while in animals receiving 25 μg (T+25) there was a slight decrease in body weight as compared with controls. The changes in epididymal fat-pad weight were similar to those of body weight in the different groups, although the differences did not disappear when corrected per unit of body weight and the values in the T+0 and T+25 groups were significantly reduced. These changes are similar to those previously shown in animals under the same experimental conditions (20) and correspond to the different thyroidal status of the animals, as shown by the plasma protein-bound iodine concentrations (table I), T+0 and T+0.1 being hypothyroid and T+25 hyperthyroid, while the values in the T+1.8 rats did not differ from those in the controls.

Fat cell size was determined in the epididymal fat-pads of the animals. To validate methodology, the fat cell size was also determined in normal rats of different body weights and it was found to increase as the animals matured – the cell diameter in tissues from rats of 51, 203, and 390 g were 40.2 ± 0.7, 64.9 ± 1.1, and 97.1 ± 1.3, respectively, values which are in agreement with those reported by other investigators using different methods (4, 10, 13).

The diameter of adipocytes from the T+0, T+0.1 and T+25 rats was significantly smaller than in the controls, while diameter in the T+1.8 animals did not differ from controls (table II). These relationships contrast with those found when calculated per unit of body weight, in which the highest values are found in the T+0 rats followed by the T+0.1 group, while values of the T+1.8 and T+25 rats were similar to controls (table II). The number of adipocytes per total epididymal fat-pad was much lower in the T+0 group than in controls (table II); the values in the T+0.1 animals were also low, while those of the T+1.8 and T+25 rats did not differ significantly from controls. The smaller cell number in the T+0 and T+0.1 groups versus controls was observed even when expressed per unit of body weight (table II), although the differences with the controls were no longer significant.

Concentrations of both proteins and DNA-phosphorous were enhanced in the epididymal fat-pads from T+0 and T+0.1 rats, while they were normal in the T+1.8 group as compared with controls. In the T+25 animals there was a significant increase in protein concentration but no change in the concentration of DNA-phosphorous. These values did not differ significantly among the groups when expressed per adipocyte (table II).

## Discussion

The present study indicates that the size of epididymal fat-pads is reduced in both hypo- and hyperthyroid rats. In hypothyroid animals, the smallest adipose tissue mass is due more to a decreased number of adipocytes than to their small size. Fat cell diameter expressed per unit of body weight is actually greater in the T+0 and T+0.1 animals than in controls. The change of fat cell volume is known to be lineally associated with its lipidic content (13). Thus the preservation of fat cell size in the hypothyroid animals should be associated with their capacity of lipid accumulation (29) which also corresponds to their normal lipogenetic activity in adipose tissue (19). The low GH of these animals (7, 27, 31) would favor the situation as augmented GH produces a reduction in fat cell size due to depletion of fat stores (2).

The reduction in number of fat cells in hypothyroid animals practically disappeared when the values were related to body weight, suggesting that cell number varies according to the limited growth rate of these animals. Thus the presence of fewer fat cells could be either a direct consequence of the reduced availability of thyroid hormones or could be secondary to their GH deficiency, and further research should clarify this point.

The hypothyroid rats were thyroidectomized after weaning and their reduced, absolute adipocyte number supports the recent theory that adipose cellularity is not definitively determined during the early postnatal period (11, 14), as previously proposed (32).

The smaller fat cell size, without change in the total number of adipocytes in hyperthyroid animals, is in agreement with another recent study (17) and is consistent with the reported depletion of fat stores in hyperthyroid animals due to their enhanced adipose tissue lipolysis (24). This effect parallels the reduced body weight in these animals, as shown by the fact that fat cell size decrease

**Table II.** Effect of thyroidectomy (T) and treatment with *L*-thyroxine on fat cell size and number and protein and DNA-phosphorous concentration in the epididymal fat-pad in the rat

| | Average fat cell diameter | | Average fat cell number | |
|---|---|---|---|---|
| | μm | μm/100 g b.w. | n/whole tissue × $10^6$ | n/100 g b.w. × $10^6$ |
| Controls | 68.8 ± 1.0[1] | 32.1 ± 0.9 | 8.63 ± 2.25 | 3.68 ± 0.44 |
| T+0[2] | 59.2 ± 2.0 | 72.6 ± 4.1 | 2.73 ± 0.40 | 2.44 ± 0.13 |
| p[3] | <0.01 | <0.001 | <0.001 | NS |
| T+0.1 | 64.6 ± 1.3 | 43.1 ± 1.3 | 4.73 ± 1.02 | 2.36 ± 0.33 |
| p | <0.05 | <0.001 | <0.01 | NS |
| T+1.8 | 64.2 ± 2.0 | 33.3 ± 1.3 | 6.21 ± 1.04 | 2.73 ± 0.26 |
| p | NS | NS | NS | NS |
| T+25 | 63.6 ± 1.9 | 30.5 ± 1.5 | 5.95 ± 1.38 | 3.24 ± 0.47 |
| p | <0.05 | NS | NS | NS |

[1] Means SEM of 5–7 rats/group.
[2] Daily dosis of *L*-thyroxine/100 g body weight.
[3] Statistical comparisons versus controls (NS = not significant: $p > 0.05$).

disappears when corrected per unit of body weight. Rats under similar conditions have normal body size and pituitary GH content (27), which suggests that adipocyte size in hyperthyroid animals is not directly related to those parameters.

The comparative changes in fat cell size among the groups are consistent with the observed values of protein concentration but not with those of DNA-phosphorous which supports the proposal (32) that adipose tissue DNA is derived from other sources such as blood vessels, fibrous tissue, and mast cells, rather than from fat cells.

In summery, our results indicate that in thyroid hormone deficiency there is a reduction in proliferation of fat cells but a preservation of their size which is even enhanced when corrected by the body mass, while the reduced adipose tissue mass in hyperthyroid rats is due to a decrease in the size of adipocytes without change in cell number. These modifications may be the result not only of alterations in circulating thyroid hormone levels but of the other endocrine dysfunctions that appear in both hypo- and hyperthyroidism.

| | Protein | | DNA-phosphorous | |
|---|---|---|---|---|
| | mg/100 mg fat-pads | mg/$10^6$ fat cells | μg/100 mg fat-pads | μg/$10^6$ fat-cells |
| Controls | 0.85 ± 0.12 | 1.48 ± 0.18 | 30 ± 3 | 52.28 ± 6.55 |
| T+0[2] | 1.76 ± 0.23 | 1.65 ± 0.30 | 98 ± 20 | 65.49 ± 13.15 |
| p[3] | <0.01 | NS | <0.01 | NS |
| T+0.1 | 1.76 ± 0.21 | 2.23 ± 0.35 | 85 ± 14 | 82.90 ± 13.40 |
| p | <0.01 | NS | <0.01 | NS |
| T+1.8 | 1.04 ± 0.10 | 1.48 ± 0.15 | 28 ± 5 | 67.62 ± 9.59 |
| p | NS | NS | NS | NS |
| T+25 | 1.66 ± 0.24 | 1.34 ± 0.19 | 38 ± 6 | 42.10 ± 6.52 |
| p | <0.05 | NS | NS | NS |

## References


1 Aranda, A.; Montoya, E., and Herrera, E.: Effects of hypo- and hyperthyroidism on liver composition, blood glucose, ketone bodies and insulin in the male rat. Biochem. J. *128:* 597–604 (1972).

2 Batchelor, B.R. and Stern, J.S.: The effect of growth hormone upon glucose metabolism and cellularity in rat adipose tissue. Hormone metabol. Res. *5:* 37–41 (1973).

3 Benotti, J. and Benotti, N.: Protein-bound iodine, total iodine and butanol-extractable iodine by partial automation. Clin. Chem. *9:* 409–416 (1963).

4 Booth, M.A.; Booth, M.J., and Taylor, A.W.: Rat fat cell size and number with exercise training, detraining and weight loss. Fed. Proc. *33:* 1959–1963 (1974).

5 Bray, G.A. and Goodman, H.M.: Effects of epinephrine on glucose transport and metabolism in adipose tissue of normal and hypothyroid rats. J. Lipid Res. *9:* 714–719 (1968).

6 Castro, M. and Herrera, E.: Effect of thyroidectomy on circulating components and liver metabolism in fed and fasted rats. Hormone Res. *4:* 357–366 (1973).

7 Contopoulos, A.N.; Simpson, M.E., and Koneff, A.A.: Pituitary function in the thyroidectomized rat. Endocrinology *63:* 642–653 (1958).

8 Fisher, J.H. and Ball, E.G.: Studies on the metabolism of adipose tissue. XX. The effect of thyroid status upon oxygen consumption and lipolysis. Biochemistry *6:* 637–647 (1967).

9 Fiske, C.H. and Subbarow, Y.: The colorimetric determination of phosphorous. J. biol. Chem. *66:* 375–400 (1925).

10 Di Girolano, M.: Mendlinger, S., and Ferting, J.W.: A single method to determine fat cell size and number in four mammalian species. Am. J. Physiol. *221:* 850–858 (1971).

11 Dodds, P.F.; Brindley, D.N., and Gurr, M.I.: The effects of diet on the esterification of glycerol phosphate, dihydroxyacetone phosphate and 2-hexadecylglycerol by homogenates of rat adipose tissue. Biochem. J. *160:* 701–706 (1976).

12 Goldman, J.K.: *In vivo* effects of growth hormone on *in vitro* adipose tissue metabolism. Hormone Res. *4:* 207–212 (1973).

13 Goldrick, R.B.: Morphological changes in the adipocyte during fat deposition and mobilization. Am. J. Physiol. *212:* 777–782 (1967).

14 Häger, A.; Sjöström, L.; Aroidsson, B.; Björntorp, P., and Smith, U.: Body fat adipose tissue cellularity in infants: a longitudinal study. Metabolism *26:* 607–613 (1977).

15 Hollenberg, C.H.; Vost, A., and Patten, R.L.: Regulation of adipose mass: control of fat cell development and lipid content. Recent Prog. Horm. Res. *26:* 463–503 (1970).

16 Lenzen, S.; Foost, H.G., and Hasselblatt, A.: Thyroid function and insulin secretion from the perfused pancreas in the rat. Endocrinology *99:* 1–125 (1976).

17 Lewis, B.M. and Hayes, T.M.: Adipose tissue cellularity: effect on insulin and thyroxine. Hormone metabol. Res. *10:* 386–389 (1978).

18 Lowry, O.H.; Rosenbrough, N.J.; Farr, A.L., and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. biol. Chem. *193:* 267–275 (1951).

19 Llobera, M.; Muniesa, A., and Herrera, E.: Effects of hypo- and hyperthyroidism on *in vivo* lipogenesis in fed and fasted rats. Hormone metabol. Res. (in press).

20 Llobera, M.; Seibel, M.J., and Herrera, E.: Metabolic response to short periods of starvation in hypo- and hyperthyroid male rats. Hormone metabol. Res. *10:* 319–324 (1978).
21 Malaisse, W.J.; Malaisse-Lagae, F., and McCraw, E.F.: Effects of thyroid function upon insulin secretion. Diabetes *16:* 643–646 (1967).
22 Matsuzaki, S.: Difference in effects of thyroidectomy and thyroxine supplement on hepatic and anterior pituitary enzymes in the rat. Endocr. jap. *15:* 223–228 (1968).
23 Menahan, L.A. and Wieland, O.: The role of thyroid function in the metabolism of perfused rat liver with particular reference to gluconeogenesis. Eur. J. Biochem. *10:* 188–194 (1969).
24 Montoya, E. and Herrera, E.: Effect of thyroid status on glycerol metabolism in adipose tissue of fasted male rats. Hormone Res. *5:* 6–12 (1974).
25 Muniesa, A.: Celularidad y lipogenesis *in vivo* en epididimo graso de ratas hipo e hipertiroideas (Tesina de Licenciatura, Univ. Barcelona, 1977).
26 Remington, R.E.: Improved growth in rats on iodine deficient diets. J. Nutr. *13:* 223–235 (1936).
27 Salas, P.; Llobera, M., and Herrera, E.: Growth hormone and prolactin pituitary content and growth in hypo- and hyperthyroid rats. Endocrinologia (in press).
28 Schmidt, G. and Thannhauser, S.J.: A method for determination of deoxyribonucleic acid, ribonucleic acid and phospho-proteins in animal tissues. J. biol. Chem. *161:* 83–89 (1945).
29 Scow, R.O.: Development of obesity in force fed young thyroidectomized rats. Endocrinology *49:* 522–529 (1951).
30 Sjöstrom, L.; Björntorp, P., and Vrana, J.: Microscopic fat cell size measurement on frozen-cut adipose tissue in comparison with automatic determinations of osmium-fixed fat cells. Lipid Res. *12:* 521–530 (1971).
31 Solomon, J. and Greep, R.O.: The effect of alterations in thyroid function on the pituitary growth hormone content and acidophil cytology. Endocrinology *65:* 158–162 (1959).
32 Stern, J.S. and Greenwood, M.R.C.: A review of development of adipose cellularity in man and animals. Fed. Proc. *33:* 1952–1955 (1974).
33 Umbreit, W.W.; Burris, R.H., and Stawffer, J.F.: Manometric techniques; 4th ed., p. 132 (Burgess, Minneapolis 1964).

Received: November 24, 1978; revised: March 2, 1979; accepted: March 14, 1979

Prof. Emilio Herrera, Departamento de Investigación, Centro Ramón y Cajal, Ctra. de Colmenar km. 9, Madrid 34 (Spain)