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Histomorphology, adipocyte size and total fat cell number in the interscapular brown adipose tissue of pregnant and lactating rats

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Abstract. Interscapular brown adipose tissue was studied in pregnant and lactating females and compared with virgin females in inbred Wistar rats, using the histomorphology and quantitation of cell numbers. In pregnant females an increase in the interscapular brown adipose tissue weight, total fat content and total fat cell numbers associated with a decrease in adipocyte diameter was observed when compared with virgin females. The total lipid content and total fat cell number in the interscapular brown adipose tissue of lactating females showed a decrease when compared to the pregnant females. This suggests that pregnancy and lactation are accompanied by changes in the status of interscapular brown adipose tissue which may be related to the changes in energy metabolism in these physiological states.

Keywords. Interscapular brown adipose tissue; adipocyte histomorphology; adipocyte size; adipocyte number.

1. Introduction

Brown adipose tissue (BAT) is an important organ responsible for non-shivering thermogenesis in cold-adapted mammals (Afzelius 1970) and for the maintenance of the core-body temperature (Foster and Frydman 1978). It may also regulate energy balance (Rothwell and Stock 1981) and participate in the regulation of body weight (Cannolly *et al* 1982), since the role of BAT mediated thermogenesis in the regulation of body weight and energy stores is now considered a credible hypothesis (Trayhurn and James 1981). Further support is forthcoming from studies on pregnant and lactating animals which imply that conservation in energy expenditure are achieved in these physiological states by altering the activity of BAT metabolism (Trayhurn *et al* 1982).

In the present study, inbred Wistar rats were used to assess the functional status of interscapular brown adipose tissue (IBAT) in pregnant, lactating and virgin females, using histomorphology, adipocyte size and number. A preliminary study was also carried out to find out whether there were any differences in the IBAT of males and females.

2. Materials and methods

Inbred Wistar rats were used for studying IBAT in the different animal models, i.e., males, virgin females, pregnant and lactating females. Three month old rats, with body weight range between 150–250 g were chosen for the study. The animals were maintained in the animal house at 25 + 2°C and were given food and water *ad libitum*.

The animals were sacrificed using ether and the IBAT was excised. It was trimmed off the surrounding skeletal muscle and white adipose tissue, blotted dry and the weight recorded. One half of the IBAT was used for total lipid estimation using the method of Folch *et al* (1957). From the other half, a portion was fixed in Bouin's fluid, processed and embedded in paraffin wax for histological observations after staining with haematoxylin-eosin (H-E) or Azocarmine-G.

The remaining bit of IBAT was immediately frozen to $-20°C$ in the cryostat. 80–100 μ thick frozen sections were cut for the adipocyte diameter measurement. The sections were taken out from the cryostat knife using a coverslip and mounted on a slide with a drop of glycerine. The diameter of the adipocytes were measured according to the method of Ashwell *et al* (1976) at a magnification of $\times 400$ using an ocular micrometer. From each section 10 adipocytes were measured horizontally and 10 vertically. Diameters were recorded in 20 sections per IBAT specimen; a total of 400 cells were measured. The divisions on the ocular micrometer were calibrated using a Leitz stage micrometer. The adipocyte diameter was measured in the units of the ocular micrometer and then converted into microns.

2.1 *Calculations*

The total fat cell number was calculated using the formula (Trayhurn *et al* 1979):- $MCV = (3SD^2\ MCD + MCD^3)/6$ (MCV = mean cell volume; MCD = mean cell diameter; SD = standard deviation of the adipocyte diameter). The mean fat cell mass (MFCM) was calculated by multiplying MCV by the density of the triglyceride (0·915) that the cell contained. The total fat cell number was derived by dividing the total fat in the depot estimated chemically by the mean fat cell mass.

3. Observations

3.1 *Weight of IBAT*

The weight of the IBAT in males was significantly higher than that of virgin females, however, the IBAT weight per unit body weight was significantly greater ($P < 0·01$) in virgin females. A significant increase ($P < 0·01$) in the total IBAT weight was noticed in the pregnant females as compared to the virgin females, although the IBAT weight per unit body weight did not significantly differ. The IBAT weight of lactating females was less than that of virgin and pregnant females, however, the decrease in weight was more significant when compared with pregnant females (table 1).

3.2 *Histology and histomorphology*

In males and virgin females the IBAT showed some adipocytes with multilocular lipid droplets and others without lipid droplets. The latter cells were mainly observed surrounding the blood vessels (figure 1), and had a peripherally situated spherical nucleus (figure 2). In pregnant females the cells were predominantly brown adipocytes with multilocular lipid droplets. The adipocytes had spherical nuclei which were displaced

Table 1. Showing body weight, IBAT weight and IBAT wt/unit body weight in inbred Wistar rats.

Group	Body weight (g)	IBAT weight (mg)	IBAT weight/ unit body weight
Virgin females (15)	158·0 ±5·6	158·3 ±8·9	101·2 ±5·6
Pregnant females (13)	230·7 ±6.6	212·9 ±9.4[c]	92·9 ±4·2NS
(7)	195·4 ±7·1[e]	203·6 ±7.1[c]	105·3 ±5·8NS
Lactating females (11)	197·7 ±6·1	121·8 ±9·3[a,d]	60·1 ±4·1[c,d]
Males (12)	271·0 ±8·5	199·0 ±10·8[b]	75·0 ±5·3[b]

Number in the parenthesis indicates the number of animals used.
±Standard error of the mean.
[a] $P < 0.01$;
[c] $P < 0.001$—when compared to virgin females.
[d] $P < 0.001$—when compared to pregnant females.
[e] Weight on day 19th excluding weight of pups, amniotic fluid etc.
NS = Not significant.

to the periphery. The adipocytes without lipid droplets were few (figure 3). In lactating females however, the majority of adipocytes were devoid of lipid droplets. The tissue had a classical 'worm-eaten' appearance (Afzelius 1970). The number of adipocytes with lipid droplets were reduced (figure 4).

3.3 *Diameter of the adipocytes*

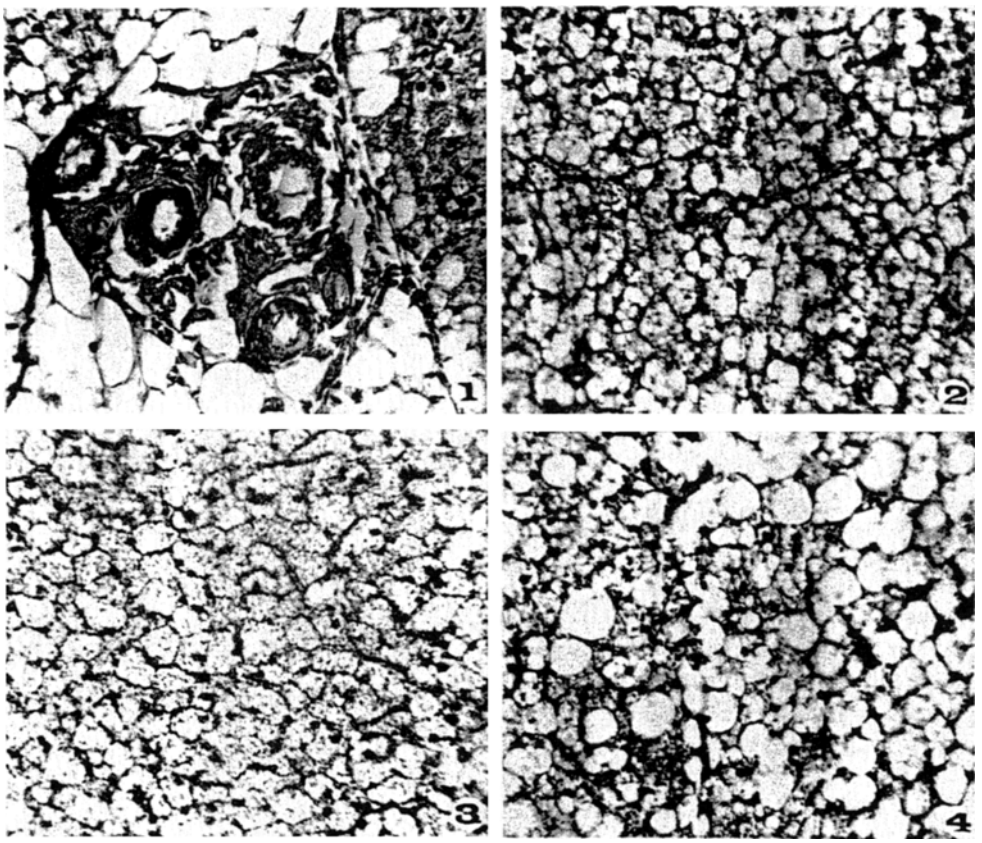
The adipocyte diameter of males and virgin females did not show any significant differences. Pregnant females showed a significant decrease ($P < 0.001$) in adipocyte diameter when compared to the virgin females. The adipocyte diameter of the lactating females was also significantly lower ($P < 0.01$) than in the virgin females. On the other hand, the adipocyte diameter of the lactating females was significantly higher ($P < 0.05$) when compared to pregnant females (table 2).

3.4 *Total lipid content*

No significant difference was seen in the total lipid content of IBAT of males and virgin females. In pregnant females the total lipid content was significantly higher ($P < 0.02$) than that of virgin females. The total lipid content in lactating females did not show any significant difference when compared with virgin females, but showed a significant decrease when compared to pregnant females (table 2).

3.5 *Total fat cell number*

There was no significant difference in the estimate of total fat cell numbers of males and virgin females. A statistically significant 3-fold increase in the total fat cell number was



Figures 1–4. Photomicrographs of IBAT in the different animal models. Bouin, 5μ Azocarmine-G. **1.** Section of IBAT in male-adipocytes around the blood vessels depleted of lipid droplets. × 250. **2.** Section of IBAT in virgin females-showing adipocytes with lipid droplets and without lipid droplets. × 250. **3.** Adipocytes in pregnant females with multilocular lipid droplets and spherical nucleus. × 250. **4.** Adipocytes in lactating females devoid of lipid droplets. × 250

Table 2. Showing the cell diameter, total lipid content and total fat cell number in IBAT in inbred Wistar rats.

Group	Cell diameter (μ)	Total lipid quantity (mg)	Total fat cell number $\times 10^6$
Virgin females (15)	36·52 ± 1·35	60·73 ± 5·07	2·2 ± 0·38
Pregnant females (7)	28·34 ± 0·56[d]	90·05 ± 0·95[b]	6·7 ± 0·7[d]
Lactating females (9)	31·01 ± 0·86[c, e]	50·61 ± 6·86[f]	3·1 ± 0·33[a, g]
Males (12)	34·74 ± 1·56	60·04 ± 8·96	2·8 ± 0·37

Numbers in the parenthesis indicate the number of animals used.

± = Standard error of the mean.

[a]$P < 0.05$; [b]$P < 0.02$; [c]$P < 0.01$; [d]$P < 0.001$—when compared with virgin females.

[e]$P < 0.05$; [f]$P < 0.01$; [g]$P < 0.001$—when compared with pregnant females.

observed in pregnant females when compared with virgin females. However, the total fat cell number was lower in lactating females than in pregnant females (table 2).

4. Discussion

Brown adipose tissue is known to undergo changes in the two physiologically hyperphagic situations of pregnancy and lactation. Rothbard (1958) reported the hypertrophy of brown fat in pregnant mice shortly before term and a rapid depletion of lipid in the fat cells just prior to and at the time of delivery. In bats, *Tadarida b. Mexicana* it was reported that the lipid in the brown fat cells start accumulation in early pregnancy and reaches a maximum level late in the gestation period. After parturition, the brown adipose tissue is essentially depleted of lipid (Sims *et al* 1962). The findings of this study are consistent with these reports, since maximum lipid accumulation was observed during late pregnancy which resulted in hyperplasia of the IBAT. Histomorphology too, revealed that the majority of the adipocytes were filled with lipid droplets. On the other hand, depletion of total lipid was observed in the lactating females accompanied by the reduction in the total fat cell number. There was a tendency for the adipocyte number to revert back to the level of that of virgin females.

The increase in IBAT parameters in pregnancy is said to be due to the effects of adrenal hypertrophy (Sims *et al* 1962). However, other endocrine glands may be involved since a gradual depletion of lipid accompanied by weight reduction of brown fat of hamsters was observed as the gestation period advanced (Teodoru and Grishman 1959). Agius and Williamson (1980) reported that lipogenesis increased 5-fold during pregnancy and decreased at parturition and mid-lactation. Trayhurn *et al* (1982) have pointed out that in pregnant and lactating mice an increase in the IBAT weight resulted as there was 'suppression of thermogenesis', and further stated that this might be an adaptation for energy conservation.

On the basis of these two reports (Agius and Williamson 1980; Trayhurn *et al* 1982) the present study suggests that there is either a decreased rate of lipolysis or an increased rate of lipogenesis in pregnancy. In lactating females the depletion of lipid stored during pregnancy may be due to its utilization for the production of milk and to keep the pups warm by way of increased heat production. The present study also gives the histomorphological and quantitative evidence for the physiological status of the IBAT during pregnancy and lactation.

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