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# Adenosine and the control of lipolysis in rat adipocytes during pregnancy and lactation

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1. The rate of noradrenaline-stimulated lipolysis is lower in fat-cells from lactating than from pregnant rats; this difference is eliminated by the addition of adenosine deaminase [Aitchison, Clegg & Vernon (1982) *Biochem. J.* **202**, 243–247]. 2. The activity of 5′-nucleotidase, and hence the capacity of the cells to synthesize adenosine, was the same in fat-cells and also stromal cells of adipose tissue from pregnant, lactating and male rats. 3. The response and sensitivity of fat-cells to the anti-lipolytic effects of adenosine were measured by incubating cells in the presence of noradrenaline, adenosine deaminase (to remove endogenous adenosine) and various concentrations of the adenosine analogue $N^6$-phenylisopropyladenosine (PIA). 4. PIA caused a greater inhibition of the rate of noradrenaline-stimulated lipolysis in adipocytes from lactating than from pregnant rats. 5. The concentration of PIA required to inhibit by 50% the rate of noradrenaline-stimulated lipolysis fell from over 100nM for fat-cells from pregnant rats to 30nM for fat-cells from lactating rats. 6. The decreased rate of noradrenaline-stimulated lipolysis during lactation was not due to the smaller mean cell volume of adipocytes during this state.

Lactation in rats usually results in a net mobilization of reserves of lipid from adipose tissue (see Vernon & Flint, 1983). It was thus surprising to find that the lipolytic response of rat adipocytes to noradrenaline was diminished during lactation, apparently owing to increased antagonism by adenosine (Aitchison *et al.*, 1982). Adenosine is a local anti-lipolytic agent (Sollevi & Fredholm, 1981; see also Arch & Newsholme, 1978*a*; Fredholm, 1978); it is released from adipose tissue *in vivo* (Fredholm & Sollevi, 1981) and accumulates in the incubation medium when adipocytes are incubated *in vitro* (Schwabe *et al.*, 1973). Adenosine is synthesized from AMP by 5′-nucleotidase, an ecto-cellular enzyme (see Arch & Newsholme, 1978*a*), which is found on both adipocytes and stromal tissue cells of adipose tissue (Green & Newsholme, 1981). Adenosine is metabolized to inosine and AMP by adenosine deaminase and adenosine kinase respectively (see Arch & Newsholme, 1978*a*). Activities of these enzymes of adenosine production and metabolism vary with physiological state (Green *et al.*, 1981). In addition, the response and sensitivity of adipocytes to adenosine can vary (Ohisalo & Stouffer, 1979; Saggerson, 1980). The main objective of the present study was to determine if the diminished lipolytic response of adipocytes from lactating rats was due to a change in their capacity to synthesize or metabolize adenosine or to a change in their sensitivity or response to adenosine.

Abbreviation used: PIA, $N^6$-phenylisopropyladenosine.

## Experimental

### *Animals*

Wistar rats (A. Tuck and Son, Rayleigh, Essex, U.K.) were fed on diet 41B (Oxoid, London E.C.4, U.K.) and water *ad libitum*. They were mated at 2–3 months of age. The number of pups per mother was adjusted to eight by 24h after birth. Female rats were killed at either 12–16 days of pregnancy or 12–16 days of lactation (except for Table 5). Male rats weighed about 200g and were about 8 weeks old.

Rats were killed at about 10:00h by cervical dislocation. The parametrial or epididymal fat-pads were removed, and samples of the tissue were used to prepare adipocytes as described previously (Aitchison *et al.*, 1982). Adipocyte size and number were determined as described previously (Vernon, 1977), except that the lipid content of adipose tissue

(used in calculating the number of fat-cells/g of tissue) was taken to be equal to the dry weight of the tissue.

*Rate of lipolysis*

The rate of lipolysis (glycerol release) of isolated adipocytes was determined as described previously, by using Medium 199 containing Earle's salts, L-glutamine and 25mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid), pH 7.3, and 4% essentially fatty-acid free bovine serum albumin as incubation medium. The albumin was dialysed against 0.15M-NaCl before use (Hanson & Ballard, 1968). Adenosine deaminase was dialysed before use as described by Honnor & Saggerson (1980). PIA was obtained from Sigma (London) Chemical Co. For measurement of the rate of lipolysis of adipose-tissue pieces, pieces of tissue each weighing about 5mg were preincubated for 30min at 37°C in Medium 199 supplemented as described above. Tissue slices were then transferred to flasks containing fresh Medium 199, supplemented as described above, with further additions as shown in the legend to Table 6, and incubated for a further 60min at 37°C; the amount of glycerol released during this period was then measured as described above.

*Enzyme activities*

*Adipose tissue.* Pieces of adipose tissue were stored in liquid $N_2$ before assay: preliminary experiments showed that storage in this way for at least 3 weeks had no effect on the activities of the enzymes. 5'-Nucleotidase, adenosine kinase and adenosine deaminase activities of adipose tissue were determined as described by Arch & Newsholme (1978*b*) with the following modifications: 2% (w/v) homogenates were prepared with an Ultra-Turrax tissue disintegrator (2 × 20s at 70% of maximum speed at room temperature); homogenates were left at room temperature for 5min and then at 4°C for 15min to allow the fat to float to the surface and congeal, after which the infranatant was removed; substrate concentrations in the assays were 400$\mu$M-AMP for 5'-nucleotidase, 2$\mu$M-adenosine for adenosine kinase, and 200$\mu$M-adenosine for adenosine deaminase (Green & Newsholme, 1981); the adenosine kinase assay included 4mM-$MgCl_2$ and 4mM-ATP. Assays were performed at 37°C for 10min. Separation of [$^{14}$C]inosine from [$^{14}$C]-adenosine for the assay of adenosine deaminase was done by t.l.c. as described by Arch & Newsholme (1978*b*), and separation of [$^{14}$C]AMP from [$^{14}$C]-adenosine for the adenosine kinase assay was performed by descending chromatography on DEAE-cellulose paper for 24h with water as solvent after stopping the reaction with an equal volume of ethanol instead of $HClO_4$. For the 5'-nucleotidase assay, the reaction was stopped by the addition of 25$\mu$l of ethanol followed by 5$\mu$l of carrier solution (20mM-adenosine, 20mM-AMP and 7mM-inosine). A 25$\mu$l sample was taken and 50$\mu$l of Dowex-1 resin (chloride form; 8% cross-linkage; 200–400 mesh) was added. The mixture was agitated for 5s and allowed to settle for 10min; this was repeated three times. Finally, the mixture was centrifuged for 5min in an Eppendorf bench centrifuge and 25$\mu$l of the supernatant was taken for measurement of $^{14}$C content.

*Adipocytes.* Samples (0.3ml) of adipocytes suspended in 0.15M-NaCl (about 120mg dry wt. of cells) were mixed with 0.9ml of homogenization medium (10mM-Tris/maleate, 1mM-EDTA and 0.1% Triton X-100, pH 7.0, for 5'-nucleotidase, and 1mM-$MgCl_2$, 1mM-EDTA and 0.1% Triton X-100, pH 7.0, for adenosine kinase and adenosine deaminase) and homogenized by hand with a ground-glass homogenizer (Jencons Scientific). Activities of 5'-nucleotidase, adenosine kinase and adenosine deaminase were determined as described above. The number of adipocytes in the 0.3ml sample was determined by measuring the dry weight, which was assumed to be all lipid, and the adipocyte mean volume.

*DNA and protein concentrations*

DNA content of adipose tissue was determined by the method of Labarca & Paigen (1980). Lipid was removed from the adipose-tissue homogenates by extraction with an equal volume of diethyl ether at 4°C: preliminary experiments showed that this was adequate, and the method in general was suitable for measuring DNA in adipose tissue. Protein concentration of adipose-tissue homogenate was measured by the method of Wang & Smith (1975).

*Statistics*

Results are expressed as means ± S.E.M. and were analysed by Student's *t* test for unpaired or paired observations as appropriate.

## Results

An initial experiment showed that the rate of lipolysis in the presence of 100$\mu$M-noradrenaline of parametrial adipocytes from lactating rats (125 ± 21nmol of glycerol released/h per $10^5$ cells, mean ± S.E.M. for six observations) was significantly lower ($P < 0.02$) than that obtained with adipocytes from pregnant rats (330 ± 63nmol of glycerol released/h per $10^5$ cells, mean ± S.E.M. for eight observations). In contrast, similar rates of lipolysis were obtained when adipocytes from pregnant and lactating rats were incubated wtih 100$\mu$M-noradrenaline plus 2mM-theophylline (812 ± 73 and 883 ± 89nmol of glycerol released/h per $10^5$ cells, for pregnant and lactating rats respectively). Adipo-

Table 1. *Adipocyte mean cell volume and number/g of adipose tissue, and DNA and protein concentrations of pieces of parametrial adipose tissue from pregnant and lactating rats and of epididymal tissue of male rats*

Experimental details are given in the text. Protein concentrations are for tissue homogenized in the 5'-nucleotidase homogenization medium; protein concentrations obtained with the homogenization medium used for adenosine deaminase and adenosine kinase assays were about 40% lower and are not shown. Values are means ± S.E.M.; *, **, ***, value significantly different from that for pregnant rat ($P < 0.05$, $< 0.01$, $< 0.001$ respectively); †, ††, †††, value significantly different from that for lactating rat ($P < 0.05$, $< 0.01$, $< 0.001$, respectively).

| | Physiological state | | |
|---|---|---|---|
| | Pregnant | Lactating | Male |
| Adipocyte mean volume (pl) | 436 ± 32 | 218 ± 46*** | 95 ± 4*** |
| $10^{-5}$ × No. of adipocytes/g of tissue | 23 ± 2 | 53 ± 9** | 101 ± 4***,†† |
| DNA concentration (μg/g of tissue) | 446 ± 49 | 818 ± 130* | 635 ± 67 |
| Protein concentration (mg/g of tissue) | 7.7 ± 0.5 | 10.2 ± 0.7** | 22.0 ± 0.7***,††† |
| Protein concentration (g/g of DNA) | 18.1 ± 1.2 | 13.7 ± 1.8 | 35.2 ± 3.2***,††† |
| No. of observations | 8 | 6 | 3 |

Table 2. *Enzyme activities of parametrial adipocytes from pregnant and lactating rats and of epididymal adipocytes from male rats*

Adipocytes were prepared and the enzyme activities determined as described in the text. Results are means ± S.E.M.; *, **, ***, value significantly different from that for pregnant rats ($P < 0.05$, $< 0.01$, $< 0.001$ respectively); †, value significantly different from that for lactating rats ($P < 0.05$).

| Physiological state | Enzyme activity (nmol/min per $10^6$ cells) 5'-Nucleotidase | Adenosine deaminase | Adenosine kinase | No. of observations |
|---|---|---|---|---|
| Pregnant | 12.7 ± 1.5 | 3.2 ± 0.3 | 2.8 ± 0.2 | 8 |
| Lactating | 14.2 ± 3.6 | 5.5 ± 1.2 | 1.7 ± 0.1*** | 6 |
| Male | 13.2 ± 1.5 | 8.5 ± 2.4** | 2.3 ± 0.2† | 3 |

cyte mean cell volumes for these rats are given in Table 1.

Samples of the adipocytes from the rats used in the above experiment were also used for enzyme assays. As shown in Table 2, the 5'-nucleotidase and adenosine deaminase activities per adipocyte did not differ significantly for adipocytes from pregnant and lactating rats, whereas the adenosine kinase activity was significantly lower ($P < 0.001$) in adipocytes from lactating rats. 5'-Nucleotidase and adenosine kinase activities were the same in adipocytes from male rats and the pregnant rats, whereas adenosine deaminase activity of fat-cells from male rats was significantly greater ($P < 0.01$) than that of pregnant rats.

Pieces of parametrial or epididymal adipose tissue, again from the same rats as used above, were assayed for the three enzymes (Table 3). When activities were expressed per g of tissue, both 5'-nucleotidase and adenosine kinase activities were higher ($P < 0.05$) in tissue pieces from lactating than from pregnant rats (Table 3). When activities were expressed per mg of protein or per mg of DNA, all three activities did not differ for adipose tissue from pregnant and lactating rats (Table 3). The activities of all three enzymes per mg of protein were lower in adipose tissue from male rats than from pregnant or lactating female rats (Table 3), but these differences were not found when results were expressed per mg of DNA (Table 3). Adipose tissue from the male rats contained significantly ($P < 0.001$) more protein per g of tissue and per g of DNA than did adipose tissue from female rats (Table 1). Assuming that the adipocytes contained about 10 pg of DNA/cell, adipocytes from female rats contributed only 5–6% of the total DNA of the tissue, compared with about 16% in male rats. The contribution of adipocyte 5'-nucleotidase and adenosine deaminase to the total activities of the tissue was much smaller in female than in male rats (Table 3), whereas in both sexes over 70% of the adenosine kinase activity of the tissue was located in the adipocytes (Table 3).

The response and sensitivity of fat-cells to adenosine were assessed by removing endogenous adenosine with adenosine deaminase and then by measuring the ability of the adenosine analogue PIA (which is not metabolized by adenosine deaminase) to inhibit noradrenaline-stimulated lipolysis (as described by Ohisalo & Stouffer, 1979). Addition of adenosine deaminase increased the rate of noradrenaline-stimulated lipolysis in adipocytes from both pregnant and lactating rats, but this effect was not observed if 100 nM-PIA was included in the incubation medium (Table 4). As shown in Fig. 1, PIA, at all concentrations used, resulted in a greater inhibition of noradrenaline-stimulated lipolysis in adipocytes from lactating rats than in those from pregnant rats. A concentration of about 30 nM-PIA caused a 50% inhibition of the rate of noradrenaline-stimulated lipolysis of adipocytes from lactating rats, whereas a concentration of over 100 nM

Table 3. *5'-Nucleotidase, adenosine deaminase and adenosine kinase activities of pieces of parametrial adipose tissue obtained from pregnant and lactating rats and of epididymal adipose tissue from male rats*

Tissue was taken from the same animals as used in Tables 1 and 2. Experimental details are given in the text. Results are means ± S.E.M.; numbers of observations are given in Table 1. *, **, ***, Value significantly different from that for pregnant rats ($P<0.05$, $<0.01$, $<0.001$ respectively); †, ††, †††, value significantly different from that for lactating rats ($P<0.05$, $<0.01$, $<0.001$ respectively).

| | | Enzyme | | |
|---|---|---|---|---|
| Activity (nmol/min) | State | 5'-Nucleotidase | Adenosine deaminase | Adenosine kinase |
| Per g of tissue | Pregnant | 683 ± 74 | 189 ± 23 | 8.9 ± 1.1 |
| | Lactating | 936 ± 71* | 271 ± 31 | 12.6 ± 1.0* |
| | Male | 539 ± 76† | 254 ± 14 | 19.5 ± 4.5* |
| Per mg of protein | Pregnant | 86.4 ± 8.3 | 43.3 ± 3.7 | 2.1 ± 0.2 |
| | Lactating | 93.0 ± 5.3 | 42.8 ± 4.1 | 2.0 ± 0.1 |
| | Male | 24.4 ± 2.7**,††† | 17.3 ± 0.7**,†† | 1.3 ± 0.2*,† |
| Per mg of DNA | Pregnant | 1626 ± 212 | 447 ± 64 | 20 ± 2 |
| | Lactating | 1242 ± 130 | 358 ± 46 | 17 ± 2 |
| | Male | 861 ± 148 | 405 ± 37 | 32 ± 9 |
| Percentage of total activity due to activity of adipocytes | Pregnant | 5 ± 1 | 4 ± 1 | 79 ± 8 |
| | Lactating | 7 ± 1 | 9 ± 1** | 71 ± 11 |
| | Male | 25 ± 4***,††† | 33 ± 7***,†† | 134 ± 31*,† |

Table 4. *Effects of $N^6$-phenylisopropyl adenosine (PIA) on the rate of glycerol release by parametrial adipocytes from pregnant and lactating rats*

Parametrial adipocytes were prepared from pregnant and lactating rats, and the amount of glycerol released over 1 h in the presence of the various substances was determined as described in the text. Flasks contained approx. $10^5$ cells/ml. Results are means ± S.E.M. for five and six observations for pregnant and lactating rats respectively. **, ***, value differs from that for pregnant rat ($P<0.01$ and $P<0.001$ respectively).

| | Glycerol released (nmol/h per $10^5$ cells) | |
|---|---|---|
| Additions to medium ... State | Pregnant | Lactating |
| None (basal) | 10 ± 3 | 19 ± 11 |
| Noradrenaline (100 μM) | 713 ± 121 | 215 ± 24** |
| Noradrenaline + adenosine deaminase (0.8 μg/ml) | 1079 ± 99 | 783 ± 96 |
| Noradrenaline + adenosine deaminase + PIA (100 nM) | 760 ± 144 | 245 ± 25** |
| Mean cell volume (pl) | 295 ± 14 | 210 ± 9*** |

was required to achieve the same degree of inhibition of noradrenaline-stimulated lipolysis of adipocytes from pregnant rats (Fig. 1*a*), suggesting that the effective sensitivity of noradrenaline-stimulated lipolysis to inhibition by adenosine was increased during lactation. However, as shown in Fig. 1(*b*), the major differences in adipocytes from pregnant and lactating rats was in the size of the response to PIA, which in both cases appeared to be maximum at about 100 nM-PIA. The concentration of PIA required to achieve a half-maximum effect was about 10 nM and 14 nM for adipocytes from lactating and pregnant rats respectively (assessed from Fig. 1*b*).

Pregnant rats in general have adipocytes with larger mean cell volumes than do lactating rats, although there is considerable overlap (Table 5). For parametrial adipocytes from rats between days 9 and 20 of pregnancy there was no significant variation in the rates of basal, noradrenaline-stimulated or noradrenaline + theophylline-stimulated lipolysis with mean cell volume over the range of mean cell volumes encountered (Table 5), or with stage of pregnancy. In contrast, both the basal and the noradrenaline + theophylline-stimulated rates of lipolysis of parametrial adipocytes from lactating rats were significantly correlated with mean cell volume ($r=0.28$, $P=0.05$, and $r=0.71$, $P<0.001$, respectively) (Table 5). The rate of noradrenaline-stimulated lipolysis of adipocytes from rats at 2 to 18 days of lactation did not vary with mean cell volume over the range 150–450 pl (Table 5), but the rate was significantly lower for adipocytes with mean cell volumes below 150 pl ($P<0.01$). Adipocytes with mean cell volumes in the range of 50–149 pl were mostly found in rats at 15–18 days of lactation, but the lower rates of lipolysis observed in these relatively small adipocytes would appear to be due to cell size rather than stage of lactation, as adipocytes from rats at 15–18 days of lactation with mean cell volumes over 150 pl had rates of lipolysis similar to those of animals at earlier stages of lactation. For rats with parametrial adipocytes with

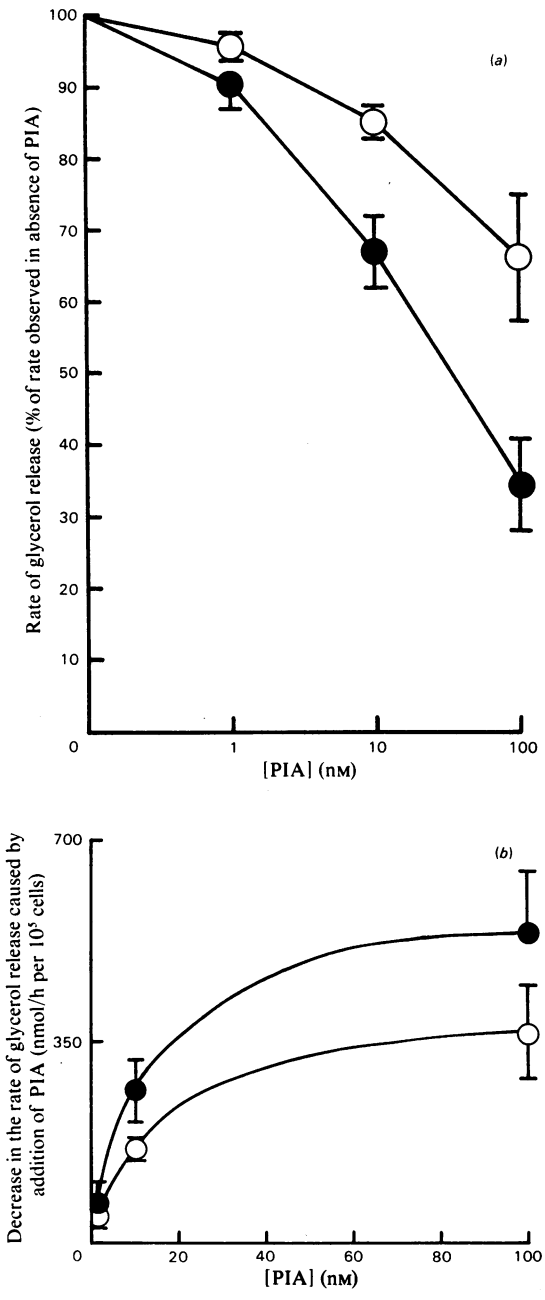


Fig. 1. *Inhibition of noradrenaline-stimulated lipolysis in adipocytes from pregnant* (○) *and lactating* (●) *rats by $N^6$-phenylisopropyladenosine (PIA)*

Adipocytes were incubated with 0.1 mM-noradrenaline plus 0.8 $\mu$g of adenosine deaminase/ml (to remove endogenous adenosine) plus various concentrations of PIA: other details are given in Table 4. Results are expressed as (*a*) rate of glycerol released in the presence of PIA as a percentage of the rate observed in the absence of PIA and (*b*) the observed decrease in the rate of glycerol released in the presence of PIA. Vertical bars denote ± S.E.M.

mean cell volumes between 150 and 450 pl, the basal rate of lipolysis was significantly higher during lactation ($P < 0.01$); the rate of noradrenaline-stimulated lipolysis was greater during pregnancy ($P < 0.001$), whereas the rate of lipolysis in the presence of noradrenaline plus theophylline was the same in both states (Table 5).

A diminished lipolytic response to noradrenaline was also observed with pieces of adipose tissue as well as with isolated adipocytes from lactating rats when results for tissue pieces were expressed per cell (Table 6). In this experiment, adipocytes from lactating rats also exhibited a significantly lower rate of lipolysis ($P < 0.05$) when incubated with noradrenaline plus theophylline than did adipocytes from pregnant rats, probably owing to differences in mean cell volume (Table 5). In the presence of noradrenaline, the rate of lipolysis of isolated adipocytes was about 3-fold greater than that of intact tissue (Table 6).

## Discussion

Although this study was primarily concerned with the control of lipolysis in isolated adipocytes, it seemed pertinent to extend the studies to adipose-tissue pieces. In addition, preliminary studies, while establishing and checking techniques, suggest some curious sex differences.

### *Male–female differences*

The activities of 5′-nucleotidase, adenosine deaminase and adenosine kinase of adipose tissue from male and female rats are very similar to those found by Green *et al.* (1981) (allowing for differences in assay temperature). Green *et al.* (1981) noted that activities of the three enzymes were the same in adipose tissue from pregnant and non-pregnant (female) rats. They also found that 5′-nucleotidase activity/mg of protein was higher in adipose tissue from female than from male rats (Green *et al.*, 1981), but the results of the present study, although confirming their observation, show that this sex difference disappears when activities are expressed per mg of DNA.

Green & Newsholme (1981) found that only 32% of 5′-nucleotidase and 40% of adenosine deaminase of adipose tissue from male rats was due to the adipocytes; the results of the present study are in fair agreement with these findings. In female rats even smaller proportions of these enzyme activities of adipose tissue are due to adipocytes; this appears to be due to adipocytes comprising a smaller proportion of the total cell population in female than in male rats. The estimate of 16% of the cells of adipose tissue from male rats being due to adipocytes is in good agreement with previous estimates (14–18%) for young male rats (Kazdova *et al.*,

Table 5. *Relationship between adipocyte mean cell volume and rates of lipolysis of adipocytes from pregnant and lactating rats*

Results from several experiments were pooled. Rats were killed at 9–20 days of pregnancy or at 2–18 days of lactation. Parametrial adipocytes were prepared and the rates of lipolysis were determined as described in the text. Noradrenaline, theophylline and adenosine deaminase, when added, were at concentrations of 100 $\mu$M, 2 mM and 0.8 $\mu$g/ml respectively. Results were grouped according to adipocyte mean cell volume and are means ± S.E.M. None of the animals used had adipocyte mean cell volumes of less than 50 pl or more than 650 pl: values for cells of mean volumes in the range 450–549 and 549–650 pl were pooled. *, **, ***, Value significantly differed from that for pregnant rats ($P < 0.05$, $< 0.01$, $< 0.001$ respectively).

| Mean cell-volume range (pl) | No. of rats with mean cell volumes in each range | | Rate of glycerol release (nmol/h per $10^5$ cells) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Addition ... None (basal) | | Noradrenaline | | Noradrenaline + theophylline or adenosine deaminase | |
| State ... | Pregnant | Lactating | Pregnant | Lactating | Pregnant | Lactating | Pregnant | Lactating |
| 50–149 | 0 | 8 | — | 10 ± 3 | — | 93 ± 14 | — | 653 ± 158 |
| 150–249 | 3 | 20 | 15 ± 6 | 28 ± 7 | 478 ± 134 | 224 ± 32* | 806 ± 111 | 812 ± 51 |
| 250–349 | 17 | 11 | 16 ± 3 | 28 ± 7 | 597 ± 61 | 233 ± 34*** | 975 ± 54 | 1063 ± 72 |
| 350–449 | 11 | 7 | 14 ± 4 | 39 ± 12* | 628 ± 106 | 216 ± 61* | 1009 ± 90 | 1372 ± 84* |
| 450–650 | 7 | 0 | 28 ± 6 | — | 590 ± 104 | — | 1024 ± 66 | — |
| 150–450 | 31 | 38 | 15 ± 2 | 30 ± 5* | 596 ± 51 | 225 ± 22*** | 974 ± 45 | 964 ± 50 |

Table 6. *Comparison of rates of lipolysis of pieces of tissue and isolated adipocytes of parametrial adipose tissue from pregnant and lactating rats*

Pieces of parametrial adipose tissue and isolated adipocytes were prepared and their rates of lipolysis were determined as described in the text. Results are means ± S.E.M. for four observations in each case. *, **, Value significantly different from that for pregnant rats ($P < 0.05$, $< 0.01$, respectively). Adipocyte mean cell volume was 464 ± 99 and 214 ± 17* pl for pregnant and lactating rats respectively.

| Additions to incubation medium | Rate of lipolysis ($\mu$mol of glycerol released/h) | | | | | |
|---|---|---|---|---|---|---|
| | Adipose-tissue pieces | | | | Adipocytes | |
| | (per g of tissue) | | (per $10^6$ fat-cells) | | (per $10^6$ fat-cells) | |
| Preparation ... State ... | Pregnant | Lactating | Pregnant | Lactating | Pregnant | Lactating |
| None | 0.6 ± 0.1 | 0.5 ± 0.2 | 0.3 ± 0.0 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.4 ± 0.2 |
| Noradrenaline (0.1 mM) | 5.3 ± 0.4 | 4.0 ± 0.7 | 3.0 ± 0.2 | 1.3 ± 0.2** | 8.1 ± 1.2 | 2.9 ± 0.8* |
| Noradrenaline + theophylline (2 mM) | 6.9 ± 0.5 | 6.6 ± 0.8 | 3.6 ± 0.2 | 2.3 ± 0.2** | 10.6 ± 0.5 | 7.6 ± 0.7* |

1974; Bjorntorp *et al.*, 1979; Cleary *et al.*, 1979). The contribution of adipocytes to total cell number in adipose of female rats does not appear to have been reported previously. As the percentage contribution of adipocytes to total cell number in the epididymal fat-pads of male rats does not change between about 1.5 and 3.5 months of age (Bjorntorp *et al.*, 1979; Cleary *et al.*, 1979), the smaller percentage contribution of adipocytes to total cell number in the parametrial fat-pads of female rats observed in the present study would appear to be a sex difference rather than being due to differences in age of the male and female rats used. In contrast with the other two enzymes, almost all of the adenosine kinase activity of adipose tissue of both male (Green & Newsholme, 1981) and female rats is located in adipocytes.

*Lipolysis during pregnancy and lactation*

The present study confirms the observation that the lipolytic response to noradrenaline is lower in adipocytes from lactating than from pregnant rats (Aitchison *et al.*, 1982). However, the explanation proposed previously for this observation, namely an increased rate of adenosine production by adipocytes from lactating rats (Aitchison *et al.*, 1982), would not appear to be correct, for the 5′-nucleotidase activity did not differ significantly between adipocytes of pregnant and lactating rats. As

5′-nucleotidase is primarily an ecto-cellular enzyme, a leakage of AMP as well as adenosine from adipocytes contributes to the accumulation of adenosine in the incubation medium. Medium 199 contained 0.5μM-AMP, which is likely to be sufficient to mask differences in the rate of AMP release from adipocytes of pregnant and lactating rats. The ability of adipocytes from lactating rats to metabolize adenosine is less than that of adipocytes from pregnant rats, in view of the lower adenosine kinase activity. Adenosine kinase, rather than adenosine deaminase, probably has the key role in adenosine metabolism within the adipocyte, in view of its much greater affinity for adenosine (see Arch & Newsholme, 1978*a*). However, as the effect of adenosine on lipolysis can be relieved by adding adenosine deaminase to the incubation medium, and as adenosine is thought to exert its effects on adipocyte metabolism via receptors on the cell surface (see Fain, 1980), the significance of changes in intracellular adenosine kinase in modulating such effects of adenosine is uncertain. Rather, the results presented in Fig. 1 and Table 4 suggest that the diminished lipolytic response of adipocytes from lactating rats to noradrenaline is due mainly to an increased response of the cells to adenosine.

An increased antilipolytic response to PIA has been observed in adipocytes from hypothyroid rats (Ohisalo & Stouffer, 1979) and adrenalectomized rats (Saggerson, 1980). The dose–response curves to PIA observed for rats in the present study are very similar to those reported previously for male rats (Ohisalo & Stouffer, 1979; Saggerson, 1980) and also for adenosine itself (Schwabe *et al.*, 1973): these previous studies also showed that for untreated male rats a maximum inhibitory effect was observed with about 100nM-PIA. The effects of lactation on the response to PIA are similar to those of adrenalectomy, in that there is a marked increase in the response and an apparent small increase in sensitivity to PIA (Saggerson, 1980); lactation, however, is associated with an increase in the glucocorticoid concentration in the blood (see Cowie *et al.*, 1980). The overall effect of lactation in this respect is markedly to decrease the concentration of PIA, and presumably adenosine, required to inhibit noradrenaline-stimulated lipolysis by 50%.

Despite the increased response to adenosine, the rate of noradrenaline-stimulated lipolysis is still sufficient to account for the rate of fat mobilization observed *in vivo* during lactation, which, from differences in the mean cell volume at day 20 of pregnancy and days 15 or 20 of lactation, is about 60–75nmol of triacylglycerol hydrolysed/h per $10^5$ cells (calculated from data of Flint *et al.*, 1979; Steingrimsdottir *et al.*, 1980). The estimate does not allow for re-esterification, but, in the presence of adrenaline, this appears to amount to only 12–15% of the fatty acids produced in adipose tissue from lactating rats (Smith & Walsh, 1976).

The lipolytic response of adipocytes from growing rats to noradrenaline is proportional to mean cell volume (Zinder & Shapiro, 1971). Changes in the lipolytic response to noradrenaline during pregnancy and lactation (Aitchison *et al.*, 1982) roughly parallel changes in mean cell volume (Flint *et al.*, 1979), suggesting that the low rate of noradrenaline-stimulated lipolysis found in adipocytes from lactating rats was the result of a decrease in mean cell volume (Vernon & Flint, 1983). However, the present study shows that it is a change in physiological state rather than mean cell volume which is primarily responsible for the low rate of noradrenaline-stimulated lipolysis during lactation.

*In vivo* and also in pieces of adipose tissue *in vitro*, adipocytes are exposed to adenosine produced by stromal elements of the tissue as well as that produced by the adipocytes themselves. Stromal cells in fact contribute about 95% of the 5′-nucleotidase activity of the adipose tissue in female rats. However, the activity per cell did not change significantly with lactation, hence the capacity of adipose tissue as a whole to produce adenosine would not appear to change with lactation, or, as shown by Green *et al.* (1981), with pregnancy. In addition, the activity of adenosine deaminase, which is also located primarily in the stromal cells of adipose tissue, did not change with lactation, or with pregnancy (Green *et al.*, 1981). Thus the decreased lipolytic response to noradrenaline of pieces of adipose tissue from lactating as compared with pregnant rats, which has also been reported by Gillon (1981), is again probably due to an increased response of the adipocytes to adenosine.

The present study provides a mechanism for the lowered rate of noradrenaline-stimulated lipolysis during lactation, but the physiological significance of the phenomenon remains uncertain. The fall in the rate of noradrenaline-stimulated lipolysis occurs around parturition (Aitchison *et al.*, 1982), a time when there is a marked increase in the rate of lipolysis *in vivo*, as evinced by the rise in the plasma unesterified fatty acid concentration (Knopp *et al.*, 1973; Lorenzo *et al.*, 1981) and by the fall in adipocyte mean cell volume (Flint *et al.*, 1979). The increased response to adenosine may thus be a reaction to a rapid increase in the rate of lipolysis and could be a mechanism for ensuring that the reserves of lipid are not depleted too rapidly during lactation, so that some lipid is conserved for the latter part of lactation and also in case of a period without food.

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