Biochem. J. (1982) **202**, 243–247
Printed in Great Britain

Lipolysis in rat adipocytes during pregnancy and lactation

The response to noradrenaline

Robert E. D. AITCHISON,* Roger A. CLEGG† and Richard G. VERNON*
**Department of Physiology and †Department of Biochemistry, The Hannah Research Institute, Ayr, Scotland KA6 5HL, U.K.*

(*Received 2 October 1981/Accepted 6 October 1981*)

1. Lipolysis has been measured in parametrial adipocytes from virgin, pregnant and lactating rats. 2. The basal rate and the maximal rate of lipolysis, the latter measured in the presence of noradrenaline and theophylline, remained constant between the three experimental categories, with the exception of a significant transient increase in the basal rate at parturition. 3. The noradrenaline-stimulated lipolysis rate rose above the virgin rate during pregnancy and fell below it during lactation; inclusion of adenosine deaminase in incubations abolished these differences in response to noradrenaline. 4. Cyclic AMP phosphodiesterase activity was lower in adipocytes during pregnancy and lactation than in virgin animals.

Rats tend to accumulate lipid reserves during most of pregnancy and then utilize these reserves during late-pregnancy and during lactation (Spray, 1950; Beaton *et al.*, 1954; Knopp *et al.*, 1970; Bershtein & Aleksandrov, 1977; Flint *et al.*, 1979). The switch from net lipid accumulation to net mobilization, which normally occurs around parturition in the rat, is associated with well documented changes in capacity for lipid synthesis in adipose tissue (Fain & Scow, 1966; Otway & Robinson, 1968; Hamosh *et al.*, 1970; Knopp *et al.*, 1973; Smith, 1973; Flint *et al.*, 1979). The above suggests that changes in the rate of lipolysis may also occur during pregnancy and lactation but there are only a few limited studies of this aspect, mostly comparing lipolysis at one stage of either pregnancy or lactation with that of virgin rats (Knopp *et al.*, 1970; Felber *et al.*, 1972; Smith & Walsh, 1976; Farid *et al.*, 1978). All of these have used adipose tissue slices rather than isolated adipocytes and have expressed results per g of tissue, which, in view of the known changes in fat-cell mean volume during pregnancy and lactation (Bershtein & Aleksandrov, 1977; Flint *et al.*, 1979), complicates interpretation of the results reported.

In the present study we show that there is no change in the apparent maximum rate of lipolysis in isolated fat-cells from rats during pregnancy and lactation but that marked changes in the response to noradrenaline occur.

Experimental

Wistar rats (A. Tuck and Son, Rayleigh, Essex, U.K.) were fed diet 41B (Oxoid, London, U.K.) and water *ad libitum*. They were mated at 2–3 months of age. The number of pups per mother was adjusted to 8 within 24 h after birth.

Rats were killed at about 10:00h by cervical dislocation. The parametrial fat-pads were removed and adipocytes were prepared from these by collagenase digestion as described previously (Flint *et al.*, 1979), except that the cells were washed and resuspended in Medium 199 {containing Earle's salts, L-glutamine, 25mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]; Gibco-Biocult, Paisley, Scotland, U.K.} instead of Krebs–Ringer bicarbonate buffer. Adipocyte size and number were determined as described previously (Vernon, 1977).

Rate of lipolysis

Adipocytes (final cell number approximately 10^5 cells·ml^{-1}) were incubated in 2.5ml of Medium 199 containing the ingredients listed above plus 4% essentially fatty-acid-free bovine serum albumin. The albumin was dialysed against 0.15M-NaCl before use (Hanson & Ballard, 1968). Various amounts of noradrenaline, dissolved in pyrogen-free 0.15M-NaCl, 2mM-theophylline and adenosine deaminase [Boehringer Corp. (London), Lewes, East Sussex,

0306-3283/82/010243-05$01.50/1

U.K.] were added as indicated in the text. Adenosine deaminase was dialysed before use (Honnor & Saggerson, 1980); final concentration was $0.8\,\mu g \cdot ml^{-1}$ ($160\,m\text{-unit} \cdot ml^{-1}$).

Adipocytes were incubated for 60min at 37°C. The reaction was terminated by addition of 0.3ml of 45% (w/v) $HClO_4$. Deproteinization and neutralization were performed as described by Honnor & Saggerson (1980). Glycerol was assayed enzymically (Garland & Randle, 1962).

Cyclic AMP phosphodiesterase assay

Samples of adipocyte suspensions were frozen and stored in liquid N_2. After thawing, the released triacylglycerol was floated into a coherent cake by centrifugation at 4°C for 10s at $6000\,g_{av.}$ in an Eppendorf micro-centrifuge; no pellet was visible after such brief centrifugation, and the opalescent infranatant was sampled and assayed for cyclic AMP phosphodiesterase (EC 3.1.4.17) by using the anion-exchange-resin column-separation method described by Thompson *et al.* (1979). High- and low-K_m activities were measured at initial cyclic AMP concentrations of 100μM and 0.75μM respectively, and at [8-^{3}H]adenosine 3′:5′-cyclic monophosphate (Amersham International, Amersham, Bucks., U.K.) specific radioactivities of 10.7Ci/mol and 1.4Ci/mmol respectively. Assay incubations were done at a temperature of 30°C.

Results

The rate of glycerol release in the presence and absence of 100μM-noradrenaline was constant for at least 60min.

The response to noradrenaline, in terms of glycerol release, was much smaller in adipocytes from rats at mid-lactation than rats at mid-pregnancy (Fig. 1). The concentration of noradrenaline required for half-maximum stimulation of glycerol release was the same (about 0.5μM; Fig. 1) for both groups of rats.

This diminished response to noradrenaline of fat-cells from lactating rats was corrected by addition of either 2mM-theophylline or 0.8μg of adenosine deaminase/ml (Table 1). Preliminary experiments showed that 0.8μg of adenosine deaminase/ml gave a maximum effect.

The decrease in the lipolytic response to nor-

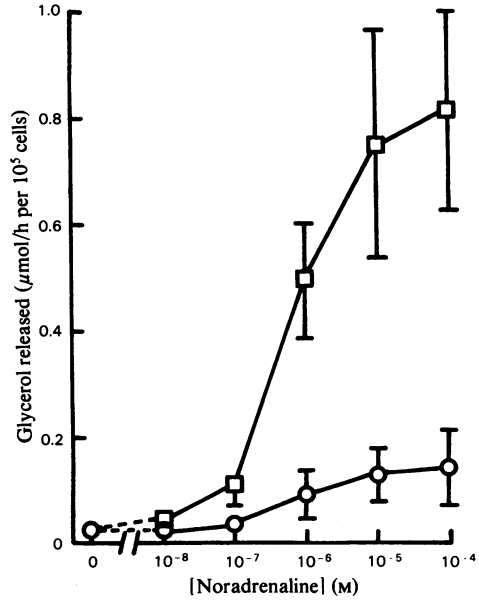

Fig. 1. *The rate of glycerol release from parametrial adipocytes from mid-pregnant* (□) *and mid-lactating* (○) *rats as a function of noradrenaline concentration*
Each value is the mean ± S.E.M. (where large enough to record) of three observations. Flasks contained approx. 10^5 cells/ml.

Table 1. *Effects of noradrenaline, theophylline and adenosine deaminase on the rate of glycerol release by parametrial adipocytes from pregnant and lactating rats*
Parametrial adipocytes were prepared from rats at 10–20 days of pregnancy or 10–16 days of lactation. The amount of glycerol released over 1h in the absence and presence of the various additions was determined as described in the text. Flasks contained approx. 10^5 cells/ml. Results are means ± S.E.M. for the numbers of observations shown in parentheses. * indicates value significantly different from that for pregnant rats ($P < 0.02$) by using Student's *t* test for unpaired samples.

Additions to medium	State ...	Glycerol released (nmol/h per 10^5 cells) Pregnant	Lactating
None (basal)		21 ± 4 (5)	31 ± 13 (5)
Adenosine deaminase (0.8μg/ml)		134 ± 9 (4)	120 ± 43 (5)
Noradrenaline (100μM)		569 ± 144 (5)	134 ± 11 (5)*
Noradrenaline + theophylline (2mM)		1060 ± 78 (5)	1021 ± 137 (5)
Noradrenaline + adenosine deaminase		1134 ± 120 (5)	1108 ± 192 (5)

adrenaline occurred around parturition (Fig. 2). The rate of lipolysis in the presence of noradrenaline at 12 and 20 days of pregnancy (pooled values) was significantly greater than that of virgin and 6-day-pregnant rats (pooled values; $P<0.05$; 32 observations). The rate of lipolysis in the presence of noradrenaline of 12- and 18-day-lactating rats (pooled values) was significantly less than that of virgin and 6-day-pregnant rats (pooled values; $P<0.01$; 35 observations). The apparent maximum rate of lipolysis (measured in the presence of 100 μM-noradrenaline plus 2 mM-theophylline) was the same for virgin, pregnant and lactating rats (mean value of 53 observations was 1137 nmol of glycerol released/h per 10^5 cells). The basal rate of lipolysis did not change significantly, except around parturition, when the rate for cells from rats that had just delivered their pups was significantly greater ($P<0.01$ in each case) than that of cells from either 20-day-pregnant or 2-day-lactating rats.

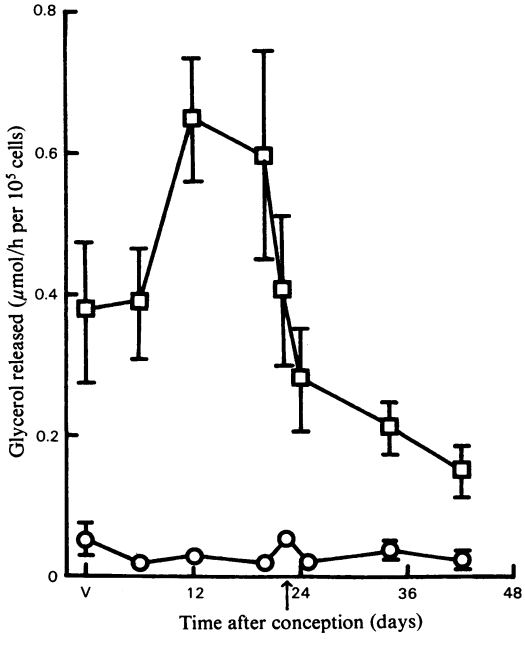

Fig. 2. *The rate of glycerol release from parametrial adipocytes, incubated in the absence* (○) *or presence of 100 μM-noradrenaline* (□), *from virgin rats and rats at various stages of pregnancy and lactation*

Results are means ± S.E.M. (where large enough to record) of at least four observations. Flasks contained approx. 10^5 cells/ml. Abbreviation used: V, virgin. The arrow indicates the time of parturition. A statistical analysis of the results is given in the text.

Cyclic AMP phosphodiesterase activity was higher in adipocytes from virgin animals than in those from pregnant or lactating animals (Table 2). The high-K_m phosphodiesterase decreased significantly ($P<0.05$) from 589 ± 128 pmol/min per 10^5 cells in virgin to 327 ± 46 in pregnant animals. The activity in lactating animals fell still further to 233 ± 42. Although this was not significantly different from the value in pregnant animals, it was ($P<0.02$) from that in virgin animals. A broadly similar picture emerged for the low-K_m phosphodiesterase, although the absolute activities were, as expected, only 10–20% of their high-K_m counterparts. In this case the difference between pregnant and virgin groups was not significant, whereas activity in lactating animals was significantly lower than in both virgin ($P<0.01$) and pregnant ($P<0.001$) groups.

The adipocyte mean volume fell significantly ($P<0.02$) from 375 ± 35 pl at 20 days of pregnancy to 198 ± 41 pl at 16–18 days of lactation (results are means ± S.E.M. of six and seven values respectively).

Discussion

The results show that the lipolytic response of rat fat-cells to noradrenaline *in vitro* changes during pregnancy and lactation. Surprisingly, the response was lowest during lactation when net mobilization of lipid was occurring.

Comparison of the results of the present study

Table 2. *Cyclic AMP phosphodiesterase activity in homogenates of parametrial adipocytes from virgin, pregnant and lactating rats*

Parametrial adipocytes were prepared from virgin rats and rats at 6–20 days of pregnancy and 10–18 days of lactation. High- and low-K_m phosphodiesterase activity was measured in fat-free homogenates of adipocytes as described in the text. Results are means ± S.E.M. for the numbers of observations in parentheses. Significantly different value from that of virgin group: *, $P<0.05$; **, $P<0.02$; ***, $P<0.01$. Significantly different value from that of pregnant group: †, $P<0.001$.

State ...	Enzyme activity (pmol/min per 10^5 cells)		
	Virgin	Pregnant	Lactating
High-K_m phosphodiesterase	589 ± 128 (7)	327 ± 46 (13)*	233 ± 42 (8)**
Low-K_m phosphodiesterase	75 ± 16 (7)	55 ± 6 (13)	23 ± 4 (10)***†

with other reports is complicated by the use of tissue slices and, in some cases, markedly different incubation conditions. Smith & Walsh (1976), Farid *et al.* (1978) and Gillon (1981) reported higher basal rats of lipolysis in adipose tissue slices from rats in mid-lactation than in those from virgin rats; the increases were small and would appear to be due to decreases in fat-cell volume during lactation and hence increases in the number of cells per g of tissue as confirmed by Gillon (1981). Farid *et al.* (1978) and Gillon (1981) noted that the lipolytic response to noradrenaline appeared to be smaller in adipose-tissue slices from lactating rats than in those from virgin rats, but the results of Smith & Walsh (1976), on the other hand, suggested an increased response. Knopp *et al.* (1970), but not Gillon (1981), found a higher basal rate of lipolysis in adipose tissue slices from 19-day-pregnant rats than in those from virgin rats. The reasons for the differences between the various studies are not clear. Some effects observed in tissue slices may be lost during preparation of adipocytes by collagenase digestion. The response to noradrenaline was generally much greater in the present study using isolated fat-cells than in the other reports discussed above, but a greater lipolytic response of isolated fat-cells than tissue slices to hormones is usually observed (see Fain, 1977; Hales *et al.*, 1978). It would seem probable that the lipolytic capacity of rat fat-cells does not change during pregnancy and lactation but the actual rate *in vivo* will depend on the hormonal environment and the activity of the sympathetic nervous system. This would account for the apparent discrepancies noted above and also the fact that net mobilization of lipid occurs during lactation despite no apparent change in the basal rate of lipolysis.

The factors responsible for the net lipolysis during lactation have not been defined fully. In the rat serum insulin concentration falls around parturition (Kuhn, 1977; Flint *et al.*, 1979). During the second half of lactation the serum insulin concentration (Robinson *et al.*, 1978; Flint *et al.*, 1979) and the serum insulin/glucagon molar ratio (Robinson *et al.*, 1978) are both lower than in virgin rats; these changes favour lipolysis. In addition the rate of acylglycerol glycerol synthesis of parametrial adipocytes decreases during lactation (Flint *et al.*, 1979) and this should decrease the rate of fatty acid re-esterification. The results of Smith & Walsh (1976) also suggested that the rate of re-esterification was lower in adipose tissue from rats at mid-lactation compared with virgin animals.

Lipolytic response to β-adrenergic agents is mediated by an elevation of intracellular cyclic AMP concentration, brought about by an increase in the activity of adenyl cyclase; this is modulated by cyclic AMP phosphodiesterase, which hydrolyses cyclic AMP to 5'-AMP. The lower cyclic AMP phosphodiesterase activities of fat-cells from mid-pregnant and mid-lactating rats compared with those from virgin control animals would be expected to result in a greater response to noradrenaline in these physiological states, as was, in fact, observed with cells from mid-pregnant rats. Changes in cyclic AMP phosphodiesterase activity cannot, however, account for the diminished response to noradrenaline of fat-cells from lactating rats.

Adenosine is released from adipocytes when incubated *in vitro* (Schwabe *et al.*, 1973) and from adipose tissue *in vivo* (Fredholm & Sollevi, 1981). Adenosine antagonizes the lipolytic effects of noradrenaline and this can be overcome by addition of adenosine deaminase or theophylline (see Schwabe *et al.*, 1975; Fain, 1977; Fredholm, 1978). This suggests that the diminished lipolytic response to noradrenaline of fat-cells from lactating rats is due to a relatively high rate of adenosine production. A similar conclusion has been reached for the poor lipolytic response of fat cells from adrenalectomized (Fernandez & Saggerson, 1978) and hypothyroid (Ohisalo & Stouffer, 1979) rats to noradrenaline. The factor(s) responsible for this apparent increased adenosine production by fat-cells from lactating rats is not certain.

Increased production of adenosine by adipocytes from lactating rats appears paradoxical in view of the net lipolysis that is known to occur in this state as evinced by the decrease in mean cell volume. Adenosine is rapidly metabolized and removed by the blood *in vivo* (Fredholm & Sollevi, 1981) and so may not accumulate to the extent that lipolysis is impaired. Adenosine is also a vasodilator in adipose tissue (Sollevi & Fredholm, 1981) and this may be its primary role *in vivo*. The rate of blood flow through the tissue becomes an increasingly important determinant of the rate of fatty acid release, and hence lipolysis, as the plasma non-esterified fatty acid concentration rises (Scow, 1965). In the rat, plasma non-esterified fatty acid concentration increases during late-pregnancy (Knopp *et al.*, 1973) and concentrations of about 1 mM have been found in plasma from animals near to term (Lorenzo *et al.*, 1981) and during lactation (Burton & Wells, 1977). Thus an increased production of adenosine in adipose tissue around parturition and during lactation may facilitate lipolysis *in vivo* by expediting the rate of removal of fatty acids from the tissue.

We thank Mrs. Eleanor Taylor for skilled technical assistance and Mr. John McDill for the care and management of experimental animals.

References

Beaton, G. H., Beare, J., Ryu, M. H. & McHenry, E. W. (1954) *J. Nutr.* **54**, 291–304

Bershtein, L. M. & Aleksandrov, V. A. (1977) *Bull. Exp. Biol. Med.* (*U.S.S.R.*) (*Eng. Transl.*) **84**, 1177–1180
Burton, L. E. & Wells, W. W. (1977) *J. Nutr.* **107**, 1871–1883
Fain, J. N. (1977) *Cyclic 3',5'-Nucleotides: Mechanisms of Action* (Cramer, H. & Schultz, J., eds.), pp. 207–228, John Wiley and Sons, London
Fain, J. N. & Scow, R. O. (1966) *Am. J. Physiol.* **210**, 19–25
Farid, M., Baldwin, R. L., Yang, Y. T., Osborne, E. & Grichting, G. (1978) *J. Nutr.* **108**, 514–524
Felber, J.-P., Zaragoza, N., Benuzzi-Badoni, M. & Genazzani, A. R. (1972) *Horm. Metab. Res.* **4**, 293–296
Fernandez, B. M. & Saggerson, E. D. (1978) *Biochem. J.* **174**, 111–118
Flint, D. J., Sinnett-Smith, P. A., Clegg, R. A. & Vernon, R. G. (1979) *Biochem. J.* **182**, 421–427
Fredholm, B. B. (1978) *Med. Biol.* **56**, 249–261
Fredholm, B. B. & Sollevi, A. (1981) *J. Physiol.* (*London*) **313**, 351–367
Garland, P. B. & Randle, P. J. (1962) *Nature* (*London*) **196**, 987–988
Gillon, K. R. W. (1981) Ph.D. Thesis, University of St. Andrews, pp. 49–61
Hales, C. N., Luzio, J. P. & Siddle, K. (1978) *Biochem. Soc. Symp.* **43**, 97–135
Hamosh, M., Clary, T. R., Chernick, S. S. & Scow, R. O. (1970) *Biochim. Biophys. Acta* **210**, 473–482
Hanson, R. W. & Ballard, F. J. (1968) *J. Lipid Res.* **9**, 667–668
Honnor, R. C. & Saggerson, E. D. (1980) *Biochem. J.* **188**, 757–761
Knopp, R. H., Herrera, E. & Freinkel, N. (1970) *J. Clin. Invest.* **49**, 1438–1445
Knopp, R. H., Saudek, C. D., Arky, R. A. & O'Sullivan, J. B. (1973) *Endocrinology* **92**, 984–988
Kuhn, N. J. (1977) in *Comparative Aspects of Lactation* (Peaker, M., ed.), pp. 165–192, Academic Press, London
Lorenzo, M., Caldés, T., Benita, M. & Medina, J. M. (1981) *Biochem. J.* **198**, 425–428
Ohisalo, J. J. & Stouffer, J. E. (1979) *Biochem. J.* **178**, 249–251
Otway, S. & Robinson, D. S. (1968) *Biochem. J.* **106**, 677–682
Robinson, A. M., Girard, J. R. & Williamson, D. H. (1978) *Biochem. J.* **176**, 343–346
Schwabe, U., Ebert, R. & Erbler, H. C. (1973) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **276**, 133–148
Schwabe, U., Ebert, R. & Erbler, H. C. (1975) *Adv. Cyclic Nucleotide Res.* **5**, 569–584
Scow, R. O. (1965) *Handb. Physiol. Sect. 5: Adipose Tissue*, 437–453
Smith, R. W. (1973) *J. Dairy Res.* **40**, 353–360
Smith, R. W. & Walsh, A. (1976) *Lipids* **11**, 418–420
Sollevi, A. & Fredholm, B. B. (1981) *Acta Physiol. Scand.* **112**, 293–298
Spray, C. M. (1950) *Br. J. Nutr.* **4**, 354–360
Thompson, W. J., Terasaki, W. L., Epstein, P. M. & Strada, S. J. (1979) *Adv. Cyclic Nucleotide Res.* **10**, 69–92
Vernon, R. G. (1977) *Biol. Neonate* **32**, 15–23