Biochem. J. (1979) **182**, 421–427
Printed in Great Britain

Role of Insulin Receptors in the Changing Metabolism of Adipose Tissue during Pregnancy and Lactation in the Rat

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(*Received* 6 *December* 1978)

Changes in the volume, the rates of fatty acid synthesis and synthesis of the glycerol moiety of acylglycerols, the activity of lipoprotein lipase, and the number and affinity of insulin receptors of adipocytes, and concentrations of serum insulin, prolactin and progesterone were determined in virgin rats and in rats at various stages of pregnancy and lactation. Changes in the metabolic activities of adipose tissue appeared to be synchronized and primarily comprised a marked decrease in anabolic activity around parturition. In contrast, the number of insulin receptors (K_d 1.5 nM) per adipocyte doubled during pregnancy before returning to normal values around parturition. It is postulated that the increase in the number of insulin receptors is an adaptation to counteract the effects of insulin-antagonistic hormones during pregnancy and that the decrease in the number of receptors is primarily responsible for the loss of anabolic activity around parturition.

During pregnancy in rats lipid is accumulated within the body; this lipid is then lost during late-pregnancy and lactation (Spray, 1950; Beaton *et al.*, 1954). In adipose tissue, lipoprotein lipase activity and lipogenesis increase during mid-pregnancy, decrease during late-pregnancy and remain low during lactation (Fain & Scow, 1966; Otway & Robinson, 1968; Hamosh *et al.*, 1970; Knopp *et al.*, 1973; Smith, 1973).

As these observations on adipose tissue metabolism were obtained from separate studies, one purpose of the present investigation was to determine whether the changes in lipoprotein lipase activity and the rate of fatty acid synthesis during pregnancy and lactation are synchronized. Further objectives were to confirm that these changes could not be attributed solely to changes in the concentration of insulin in the peripheral circulation, and to investigate the possibility that the changes in the anabolic capability of adipose tissue are due to alterations in the number or affinity of insulin receptors in the adipocyte plasma membrane. Also, to shed more light on the relative roles of different adipose tissue depots, measurements were carried out on both parametrial and subcutaneous adipocytes.

Experimental

Materials

Collagenase (C 2139, 200 units/mg), heparin (158 units/mg), all steroid hormones, dextran (mol.wt. 80000), activated charcoal (acid-washed) and bovine serum albumin (fraction V, essentially fatty acid-free) were from Sigma (London) Chemical Co., London, S.W.6, U.K.; medium 199 with Earle's salts and containing L-glutamine and 25 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] was from Gibco–Bio-cult, Paisley, Scotland, U.K.; insulin-binding reagent was from Wellcome Reagents, Beckenham, Kent, U.K.; progesterone antiserum was from Steranti Research, St. Albans, Herts., U.K.; [U-^{14}C]glucose (sp. radioactivity 2.8 mCi/mmol), [1,2,6,7-^{3}H]progesterone (sp. radioactivity 100 Ci/mmol) and ^{125}I-labelled insulin (sp. radioactivity 120–150 μCi/μg) were from The Radiochemical Centre, Amersham, Bucks., U.K.

Bovine insulin (23.6 units/mg) and the rat prolactin radioimmunoassay kit were kindly donated by The Boots Co., Nottingham, U.K., and the National Institute for Arthritis, Metabolic and Digestive Diseases (N.I.A.M.D.D.), Bethesda, MD, U.S.A. respectively.

Bovine serum albumin was dialysed before use in metabolic studies (Hanson & Ballard, 1968).

Animals

Wistar rats were from A. Tuck and Son, Rayleigh, Essex, U.K.; food (diet 41B; Oxoid, London E.C.4, U.K.) and water were supplied *ad libitum*. At the beginning of the experiment rats weighed between 210 and 220 g and were between 76 and 84 days old. The interval between mating the first and last rats in the series was 50 days.

Control rats and rats at various stages of pregnancy and lactation were killed over a period of 65 days by cervical dislocation between 10:00 and 11:00 h. Blood was collected and centrifuged at 1000*g* for 5 min to obtain serum, which was stored at −20°C

until used for hormone assays. The subcutaneous fat-pads from around the inguinal mammary glands and the parametrial fat-pads were removed and weighed.

Preparation of adipocytes

Minced adipose tissue (2–7g) was incubated in 10ml of medium 199 containing 300mg of dialysed albumin and 25mg of collagenase at 37°C for 60min with constant agitation. The resulting cell suspension was diluted with either Krebs–Ringer bicarbonate buffer containing 1mg of glucose/ml (for use in metabolic studies) or Krebs–Ringer phosphate buffer containing 1mg of glucose/ml and 1% albumin (for use in the determination of insulin-receptor numbers). The cell suspensions were filtered through a nylon mesh to remove undigested material and were washed three times with 30ml of the appropriate buffer. Adipocytes were finally suspended in 10ml of Krebs–Ringer bicarbonate incubation medium (see below) or in 10ml of Krebs–Ringer phosphate. Adipocytes were maintained at approx. 37°C during this procedure. The yield of adipocytes was $79 \pm 5\%$ and $91 \pm 6\%$ (means $\pm$ S.E.M.) for parametrial and subcutaneous adipose tissue respectively. Adipocyte size and number were determined as described previously (Vernon, 1977*a*).

Rate of lipogenesis

Samples of adipocytes were incubated in 2.5ml of Krebs–Ringer bicarbonate buffer containing 1mg of glucose/ml and 30mg of dialysed albumin/ml with or without insulin (100ng/ml). After addition of 0.1ml of [U-^{14}C]glucose (2.5μCi/ml) the adipocytes were incubated for 2h at 37°C with constant agitation. After 2h, the lipids were extracted and the amounts of ^{14}C in the fatty acids and in the glycerol moiety of acylglycerols were determined as described previously (Vernon, 1977*b*).

Lipoprotein lipase activity

Homogenates (20–30%, w/v) of adipose tissue in 50mM-Tris/HCl, pH 8.1, containing 0.1mg of heparin/ml were prepared by using hand homogenizers. Lipoprotein lipase activity and protein content of the homogenates were determined by the methods of Nilsson-Ehle & Schotz (1976) and Wang & Smith (1975) respectively.

Binding of ^{125}I-labelled insulin to isolated fat-cells

Isolated fat-cells (approx. 3×10^5 cells) were incubated at 22°C for 60min with 0.4ng of ^{125}I-labelled insulin and unlabelled insulin (1–75ng) in a final volume of 400μl. At the end of this period the incubations were terminated by removing a 300μl sample from the cell suspension and rapidly centrifuging the cells in plastic micro-centrifuge tubes (capacity 400μl) containing approximately 80μl of dinonyl phthalate as described by Gliemann *et al.* (1972). After centrifugation the upper layer, containing the packed fat-cells, was cut off and the radioactivity present determined by gamma counting. All data were corrected for non-specific binding by subtracting the amount of radioactivity still bound in the presence of 10μg of porcine insulin from all other values.

Results were subjected to Scatchard analysis (Scatchard, 1949). The results were interpreted in terms of a component with a high affinity for insulin (K_d about 1.5nM) and a second component with a lower affinity for insulin (K_d about 7nM). The total numbers of both types of insulin receptor were determined from the Scatchard plots.

Insulin degradation was determined by the procedure involving precipitation with trichloroacetic acid described by Freychet *et al.* (1973).

Radioimmunoassays

Radioimmunoassay of rat prolactin involved a double antibody system by using the kit supplied by N.I.A.M.D.D. The assay was carried out according to the standard procedure supplied with the kit and results were expressed as ng of N.I.A.M.D.D. rat prolactin-RP-1/ml (the reference preparation provided with the kit).

Serum insulin was determined by a double antibody assay by using ^{125}I-insulin and insulin-binding reagent. The procedure used was essentially that described in the insulin RIA kit supplied by The Radiochemical Centre. Results were expressed in terms of ng of a bovine insulin standard/ml.

Serum progesterone was determined by radioimmunoassay by using an antibody to progesterone conjugated to bovine serum albumin at the 11 position. Serum samples (50μl) were extracted with 1ml of hexane for 1min. The hexane was dried under N_2 and the residue redissolved in 1ml of assay buffer (0.1%, w/v; bovine serum albumin in 0.1M-phosphate pH 7.0). Portions (100μl) were then assayed in duplicate along with standards in the range 1–100ng/ml. Serum and standards were added to tubes containing 50μl of antibody plus 100μl of assay buffer containing approx. 10000c.p.m. of [1,2,6,7-^{3}H]progesterone. Incubation was performed at 4°C overnight. The next day each tube received 250μl of dextran-coated charcoal (0.625% charcoal, 0.0625% dextran) and was allowed to stand on ice for 10min after which it was centrifuged at 2500rev./min for 10min and a 300μl sample of the supernatant was taken for the measurement of radioactivity by liquid-scintillation spectrometry. Major cross-reactants, as reported by the

supplier, were 5α-pregnanedione (2.5%) and corticosterone (0.1%), but corticosterone is not extracted to any major extent by hexane (Demetriou & Austin, 1971). Cross-reaction with all other steroids tested was less than 1%.

Assay sensitivities (twice the standard deviation of the zero values) were 0.4ng, 60pg and 1ng for prolactin, insulin and progesterone respectively. Inter- and intra-assay coefficients of variation were 11 and 4% respectively for prolactin, 8 and 2% for insulin and 6 and 4% for progesterone.

Results

The experimental design used resulted in each group of rats containing animals of various ages. However, no age-associated changes in the different metabolic and other variables measured were apparent in either the control, virgin rats or in the various groups of pregnant or lactating rats.

Unfortunately, it was not possible to obtain sufficient subcutaneous adipose tissue from late-pregnant or lactating rats to determine all the variables chosen. Therefore different groups of rats were used for measuring the number of insulin receptors and the metabolic activities; adipocyte volume and numbers in both groups of rats did not differ significantly (pooled values are given in Fig. 1).

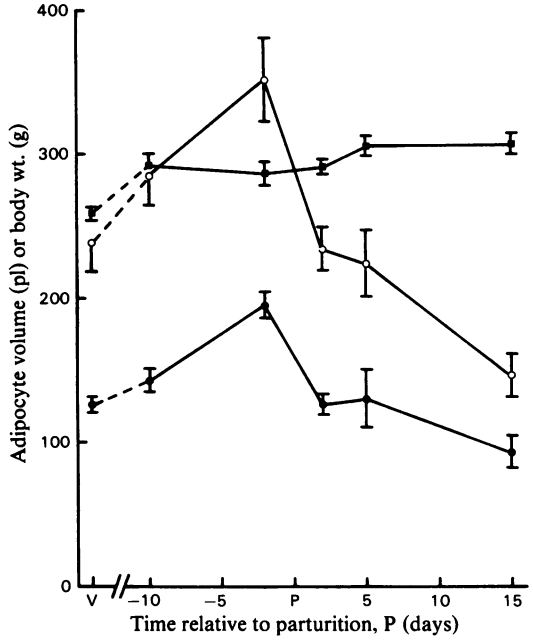

Fig. 1. *Body weight* (■) *and the volumes of parametrial* (○) *and subcutaneous* (●) *adipocytes of virgin control rats* (*V*) *and rats at various stages of pregnancy and lactation.* Results are means ± S.E.M. for eight to 11 observations.

The mean volume of both parametrial and subcutaneous adipocytes increased during pregnancy; maximum values were observed at 2 days before term (Fig. 1). Between 2 days *pre partum* and 2 days *post partum* there was a rapid statistically significant ($P<0.05$) decline in the size of adipocytes from both sources. During lactation there was a further more gradual decrease in adipocyte mean volume that was again statistically significant ($P<0.05$) despite there being no loss of body weight (Fig. 1). Changes in the weights of the parametrial and subcutaneous fat-pads paralleled changes in their respective adipocyte mean volumes: there was no apparent change in the number of adipocytes in either fat-pad during pregnancy and lactation [mean value for all rats $(1.94\pm0.16)\times10^7$ and $(1.26\pm0.07)\times10^7$ adipocytes in the parametrial and subcutaneous fat-pads respectively; results are means ± S.E.M. for 24 observations in each case].

There were no statistically significant changes in the activity of lipoprotein lipase or rates of synthesis of fatty acid or the glycerol moiety of acylglycerols from glucose in either parametrial or subcutaneous adipocytes during pregnancy (Fig. 2). By 2 days *post partum*, however, the activities of all three variables were lower than in control animals ($P<0.05$). This decrease appeared to begin later than 2 days before parturition in the case of subcutaneous adipocytes, but earlier in parametrial adipocytes. This decrease was greatest for the rate of fatty acid synthesis and least for the rate of synthesis of the glycerol moiety of acylglycerols. Subsequently, there was a general tendency for the three processes to increase between days 2 and 5 of lactation and then decline during the remainder of lactation (except the activity of lipoprotein lipase in subcutaneous adipose tissue; Fig. 2).

The response of fatty acid synthesis and synthesis of the glycerol moiety of acylglycerols to insulin *in vitro* was small (less than a 40% increase in rate) in both parametrial and subcutaneous adipocytes from control rats and rats at various stages of pregnancy and lactation (results not shown). Adipocytes from rats 2 days *post partum* appeared to be least sensitive to insulin, but the response to insulin in these rats was not significantly different from that of control rats. Initial studies showed that insulin had the same effect on the rates of fatty acid synthesis and synthesis of the glycerol moiety of acylglycerols from glucose in both isolated adipocytes and adipose tissue slices from virgin rats of the same age and weight as used in the main study. This indicates that the low response of the isolated adipocytes to insulin was not due to damage during collagenase digestion of the tissue.

Scatchard plots of the insulin binding to parametrial adipocytes from virgin, 20-day pregnant and 2-day lactating rats are shown in Fig. 3. Similar plots were obtained with parametrial adipocytes from rats

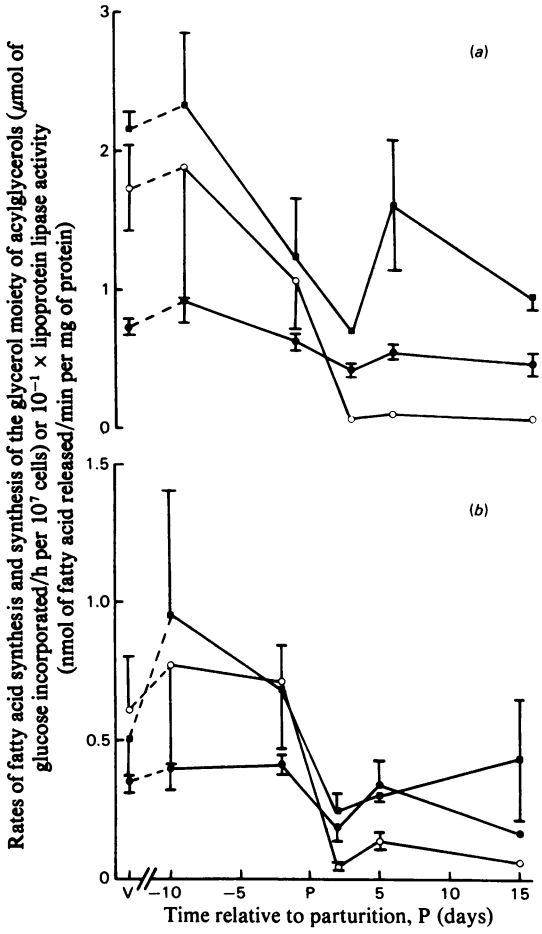

Fig. 2. *Rates of fatty acid synthesis* (○) *and synthesis of the glycerol moiety of acylglycerols* (●) *in adipocytes incubated in the presence of insulin, and lipoprotein lipase activity* (■) *in homogenates of adipose tissue from* (*a*) *the parametrial and* (*b*) *the subcutaneous fat-pads of virgin control rats* (*V*) *and rats at various stages of pregnancy and lactation.* Results are means ± S.E.M. where large enough to record for four or five observations.

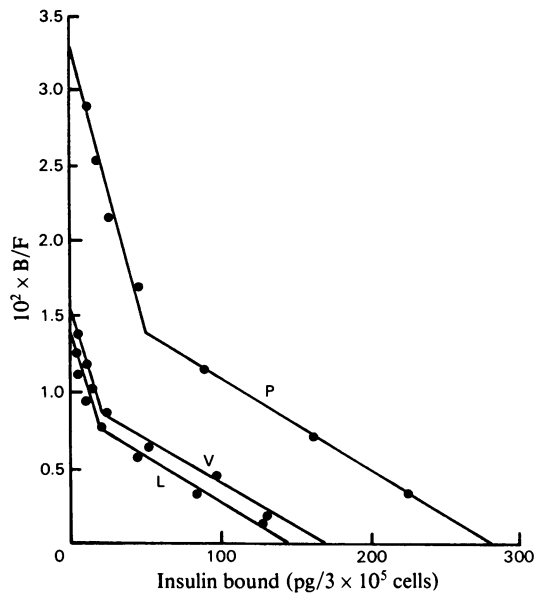

Fig. 3. *Scatchard plots of the insulin binding to parametrial adipocytes from virgin* (*V*), *20-day pregnant* (*P*) *and 2-day lactating* (*L*) *rats*

^{125}I-labelled insulin binding was measured and corrected for non-specific binding as described in the text. Each point is the mean value obtained from separate determinations with adipocytes from five rats. B/F is the ratio of insulin bound to adipocytes: free unbound insulin.

at other stages of pregnancy and lactation and with subcutaneous adipocytes from rats in the various states examined (results not shown). The Scatchard plots were interpreted as comprising two components, one with a K_d for insulin of about 1.5 nM and a second with a K_d for insulin of about 7 nM. Non-specific binding of insulin to the adipocytes amounted to 10 ± 1% of the total insulin bound to the adipocytes (mean ± S.E.M. for 48 observations). Insulin degradation amounted to 12 ± 1% (mean ± S.E.M. for 42 observations) of the insulin added per 3×10^5 adipocytes (the approximate number of adipocytes used in the insulin-binding assays). The non-specific binding of insulin to adipocytes and the amount of insulin degradation were the same for both parametrial and subcutaneous adipocytes, and were not altered significantly by the stages of pregnancy and lactation examined, hence pooled values are quoted above.

Changes in the surface density (number per unit area) of receptors with a high affinity for insulin (K_d 1.5 nM) during pregnancy and lactation are shown in Fig. 4: similar changes were observed in the surface density of receptors with a low affinity for insulin (K_d 7.0 nM; results not shown). The number of insulin receptors per adipocyte showed a very similar pattern over the period of study (results not shown). There was a significant increase ($P < 0.05$) in the numbers of both high- and low-affinity insulin receptors per cell and per unit surface by day 12 of pregnancy in both parametrial and subcutaneous adipocytes. These elevated numbers of insulin receptors were maintained until at least 2 days before parturition. However, by 2 days *post partum* the numbers of both groups of receptors, when expressed on either basis, had fallen significantly ($P < 0.05$) and were again similar to their values in virgin control rats.

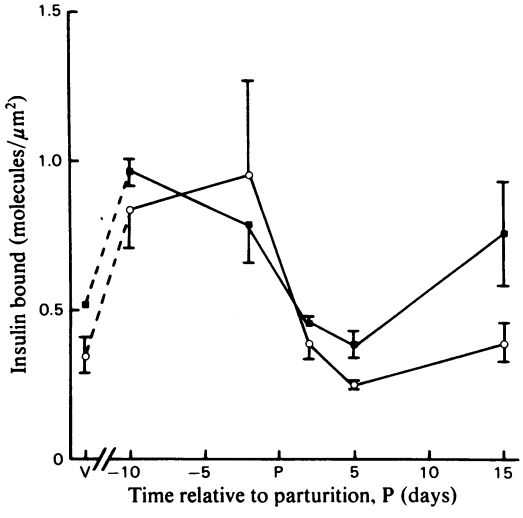

Fig. 4. *Number of insulin receptors with an affinity for insulin of* 1.4 ± 0.1 *nM per unit surface area of parametrial* (■) *and subcutaneous* (○) *adipocytes from virgin control rats* (*V*) *and rats at various stages of pregnancy and lactation*
Results are means ± S.E.M. where large enough to record for five observations.

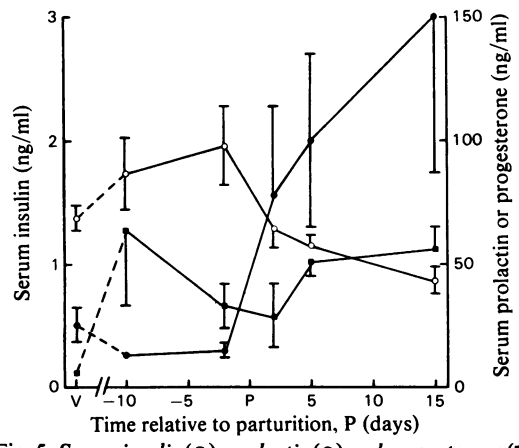

Fig. 5. *Serum insulin* (○), *prolactin* (●) *and progesterone* (■) *concentrations in virgin control rats* (*V*) *and rats at various stages of pregnancy and lactation*
Results are means ± S.E.M. for four or five observations.

There was no apparent change in the affinity for insulin of either the high- or low-affinity receptors on adipocytes during pregnancy and lactation; mean values of K_d for all rats being 1.4 ± 0.1 nM and 7.3 ± 0.3 nM respectively for the high- and low-affinity receptors (means ± S.E.M. for 48 observations).

Serum insulin concentration increased during pregnancy (Fig. 5). Although neither of the mean values at days 12 and 20 of pregnancy were significantly greater than the value for virgin rats, the overall mean value for pregnant rats was significantly higher ($P < 0.05$). There was a significant decrease ($P < 0.05$) in serum insulin concentration between day 20 of pregnancy and day 5 of lactation, and by day 15 of lactation the value was significantly lower ($P < 0.05$) than that of the virgin rats.

Serum prolactin concentrations were decreased during pregnancy and then increased during lactation (Fig. 5), whereas at all stages of pregnancy and lactation examined progesterone concentrations were significantly higher than in the serum of control rats (Fig. 5).

Discussion

Role of the various fat-pads

The present study demonstrates that hypertrophy of both subcutaneous and parametrial adipocytes occurs during pregnancy, whereas no evidence of hyperplasia was found in either fat-pad. These results are in agreement with those of previous studies with perirenal adipose tissue (Knopp *et al.*, 1970*a*; Bershtein & Aleksandrov, 1977).

The decreases in the size of parametrial and subcutaneous adipocytes during late-pregnancy and lactation found in the present study are similar to those found in rat perirenal adipocytes (Bershtein & Aleksandrov, 1977). Also Elias *et al.* (1973) observed a decrease in the size of subcutaneous adipocytes in mice during lactation. Only the finding by Hamosh *et al.* (1970), that rat parametrial fat-pad size remained constant during pregnancy and lactation, is contrary to the overall picture that adipocytes from all the various fat-pads of the body are involved in the fat economy of the rat during pregnancy and lactation.

Metabolic adaptation

The changes in lipoprotein lipase activity and rates of fatty acid synthesis and synthesis of the glycerol moiety of acylglycerols in parametrial and subcutaneous adipocytes observed in the present study are in general similar to those found in perirenal and parametrial adipose tissue in previous studies (Otway & Robinson, 1968; Hamosh *et al.*, 1970; Knopp *et al.*, 1973; Smith, 1973). There are some differences: Knopp *et al.* (1973) found a significant increase in the rate of fatty acid synthesis in perirenal adipocytes by mid-pregnancy, but this was not found in parametrial adipocytes (Smith, 1973) or in the present study. Hamosh *et al.* (1970) found much greater increases in the activity of lipoprotein lipase

during pregnancy and a greater decline during late-pregnancy than the increase observed by Otway & Robinson (1968) or in the present study. A peak in the rate of synthesis of the glycerol moiety of acylglycerols was found on day 21 of pregnancy by Knopp *et al.* (1973), but no evidence for such a peak was observed on day 20 of pregnancy by Smith (1973) or in the present study. The trough in the rate of synthesis of the glycerol moiety of acylglycerols at 2 days of lactation was missed in previous studies.

The present study also strongly suggests that changes in lipoprotein lipase activity and the rate of fatty acid synthesis within a given adipose tissue depot are synchronized. These changes may occur at different times in different fat-depots since it appears from the present results that changes in the anabolic activities of parametrial adipose tissue may precede such changes in subcutaneous adipose tissue.

The small response of the rates of fatty acid synthesis and synthesis of the glycerol moiety of acylglycerols to insulin *in vitro* observed in the present study is probably due to the age of the rats (Salans & Dougherty, 1971; Di Girolamo *et al.*, 1974).

Role of insulin and insulin receptors

Reports of changes in serum insulin concentrations during pregnancy and lactation vary. Knopp *et al.* (1973) found a 3-fold increase in serum insulin concentration during pregnancy, which is markedly greater than that observed by Bourne *et al.* (1975) and in the present study. A decrease in the serum insulin concentration around parturition, as shown in the present study, was previously found by Sutter-Dub *et al.* (1974) and Kuhn (1977), but not by Bourne *et al.* (1975). However, during early lactation serum insulin concentrations appear to be similar to those of virgin rats of the same age (Sutter-Dub *et al.*, 1974; Bourne *et al.*, 1975; the present study). Sutter-Dub *et al.* (1974) found that serum insulin concentration increased during lactation, whereas in the present study there was a decrease. Robinson *et al.* (1978) also found lower serum insulin concentrations in 10–18-day lactating rats than in virgin rats. Changes in serum insulin concentrations then cannot account for the changes in anabolic activity of adipose tissue at all stages of pregnancy and lactation, particularly the very low amounts of anabolic activity during early lactation.

The properties of the insulin receptors determined in the present study, namely their number per adipocyte and their division into two groups with apparent affinities for insulin of about 1.5 and 7 nM are similar to those described in previous studies with isolated adipocytes (Olefsky & Reaven, 1975; Ip *et al.*, 1976; Kasuga *et al.*, 1977).

In addition to changes in serum insulin concentration, variations in the number or surface density of insulin receptors of adipocytes during pregnancy and lactation provide another mechanism for modulating the anabolic capacity of adipose tissue. But, as shown above for serum insulin concentration, changes in the number or surface density of insulin receptors in themselves do not explain the low amounts of anabolic activity in adipocytes during early lactation. Furthermore, despite the increase in both serum insulin concentration and insulin-receptor number during pregnancy found in the present study, there was little change in the anabolic capacity of adipose tissue at this time. This suggests that some factor may be antagonising the effects of insulin on adipose tissue metabolism during pregnancy.

Insulin resistance develops during pregnancy (Knopp *et al.*, 1970*b*; Tyson & Felig, 1971; Freinkel & Metzger, 1975), probably due to the high amounts of placental lactogen in the serum (Tyson & Felig, 1971) and the increased concentrations of serum insulin during pregnancy may be a response to this insulin resistance (Knopp *et al.*, 1973). Also, the high serum prolactin concentrations of lactation are thought to be responsible for the low lipoprotein lipase activity of adipose tissue during this period (Zinder *et al.*, 1974). Therefore we suggest that the increase in the number of insulin receptors of adipocytes during pregnancy is an adaptation to counteract the insulin-antagonistic effects of lactogenic hormones and so permit lipid deposition to occur. Thus a decrease in both the number of insulin receptors of adipocytes and the serum insulin concentration around parturition permits prolactin to dominate adipose tissue metabolism during lactation, causing a suppression of anabolic activity.

The factor that increases the number of insulin receptors during pregnancy must be sufficiently potent to overcome the normal regulatory mechanism whereby increased plasma insulin concentrations appear to decrease the number of insulin receptors [the 'down' hypothesis of Gavin *et al.* (1974)]. Progesterone is a possible candidate since decreases in lipoprotein lipase activity in adipose tissue during late-pregnancy were prevented by treatment with progesterone (Spooner *et al.*, 1977). Thus it may well be that progesterone has a role not only in blocking the effects of lactogenic hormones on the mammary gland during pregnancy, preventing premature lactogenesis (see Kuhn, 1977), but also deterring these same hormones, the probable insulin-antagonists, from preventing lipid accumulation in adipose tissue during pregnancy.

We thank Mrs. L. K. Dunabie and Mrs. E. Taylor for skilled technical assistance.

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