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IN VITRO GLYCEROL METABOLISM IN ADIPOSE TISSUE FROM FASTED PREGNANT RATS[1]

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SUMMARY

Lumbar fat pad pieces taken from fed and 48 h starved 19-day pregnant rats and virgin controls were incubated for different times with [U-^{14}C] glycerol, albumin and glucose. The glycerol conversion rates to either CO_2, saponified lipids and glyceride glycerol were higher in the pregnant rat tissue than in the controls. Starvation produces a greater decline in these parameters in pregnant rat tissue than in controls. The lipolysis rate was elevated in pregnant rat tissue. The augmented glycerol utilization by adipose tissue in the mother would contribute to the net deposition of fat, despite augmented lipolysis. In the starved state the enhanced lipolysis of the mother is potenciated by a decreased reutilization of glycerol, allowing a maximal net mobilization of the fat stores.

INTRODUCTION

The hyperlipemia of pregnancy (1-6) seems to be influenced by the active adipose tissue metabolism of the mother. Indeed, it has been shown that the *in vitro* release of glycerol and the esterification of fatty acids are enhanced in the adipose tissue from pregnant rats (7). As adipose tissue is able to metabolize glycerol when incubated *in vitro* (8,9), it is necessary to determine how this is affected during pregnancy, allowing a more precise evaluation of the rate of lipolysis, as well as contributing to a better understanding of the net accumulation of adipose tissue mass of the mother (7,10,11). The rate of glycerol utilization in adipose tissue from 19-day pregnant rats has been studied here.

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The work has been carried out in both fed and 48 h starved animals, as it is known that starvation maximizes the lipidic metabolism of the mother shown by the enormous increase in circulating free fatty acids (FFA), triglycerides and ketone body levels (1,4,5,12), and adipose tissue activity (7,12,13).

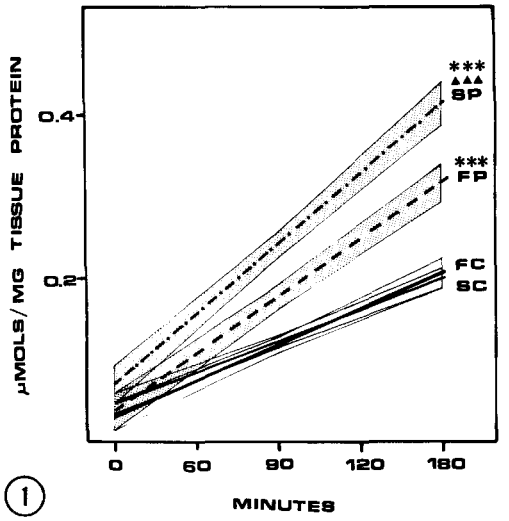
MATERIALS AND METHODS

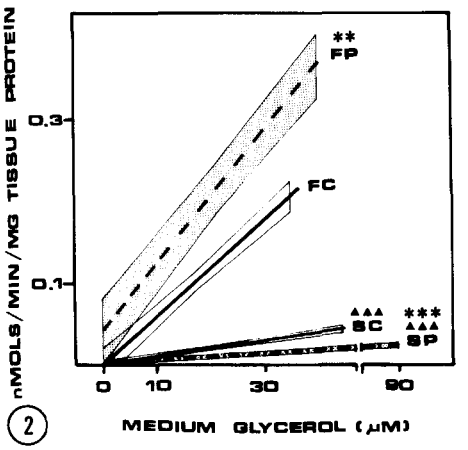
Female Wistar rats were mated at two months of age and maintained in a controlled environment (23°C; light cycle of 12 h on, 12 h off). They were stunned by a blow on the head without prior anesthesia on day 19 of pregnancy, and compared with age-matched virgin female controls. Prior to sacrifice, rats were allowed either continuous access to standard food pellets,or deprived of all food, but not drinking water, for the preceeding 48 h. Right and left lumbar fat pads were excised from both sides (21.3±1.9 mg), incubated in 1 ml of Krebs Ringer bicarbonate buffer , pH 7.4, containing 0.5 uCi of [U-^{14}C]glycerol (46 mCi/mmole), 5 mM glucose and 10 mg of bovine albumin purified by the method of Chen (14). The incubations were carried out for 60, 90, 120 and 180 min at 37°C under O_2/CO_2 (95:5), with shaking (100 cycles/min). Samples were processed as previously described (8,9,15), for the development and analysis of [^{14}C]CO_2, the enzymatic determination of glycerol in the media (16), purification (17), and fractionation of lipids in the tissues (15). The residual pellet after lipid extraction of the tissues was used to estimate protein contentration (18). The radioactivity measures were expressed as μmol by using the specific radioactivity of glycerol in the media incubated in the absence of tissue. The data were adjusted to linear regressions (19), and the standard error of the estimation of Y was calculated for each line (20). The

statistical comparisons between lines was done by means of an ANOVA test (21). The rate of glycerol utilization and/or production were calculated following the mathematical analysis previously described (22).

RESULTS

As shown in Fig. 1, the release of glycerol to the incubating media by adipose tissue pieces was linear as function of time for all groups, but higher in the tissues from 19-day pregnant rats than those from virgin controls. This difference was greater when the animals were studied after a 48 h starvation period than when fed. The incubations were carried out in the presence of [U-^{14}C]glycerol. The greater concentration of glycerol in the media of tissues from the pregnant rats obliges one to correct the utilization of the labelled substrate by the different dilution of the tracer among the groups. The calculated values were expressed as rates of glycerol utilization per the actual concentration of glycerol in the medium at each time of incubation studied (60, 90, 120 and 180 min) as it was previously shown that differences in the amount of glycerol in the media could directly affect the ability of the tissue to metabolize it (23). The rate of glycerol uptake is greater in the tissues from fed pregnant rats than in controls (Fig.2). Starvation produced an intense reduction in this parameter, and the fall is greater in the tissues from the pregnant rats than in controls. These changes are complementary to all the parameters in which the glycerol is being converted. Accordingly, the rates of the formation of CO_2 (Fig. 3A), saponifiable lipids (fatty acids) (Fig. 3B), and glyceride glycerol(Fig. 3C) from glycerol are all higher in the adipose tissue from pregnant rats than from the virgin controls





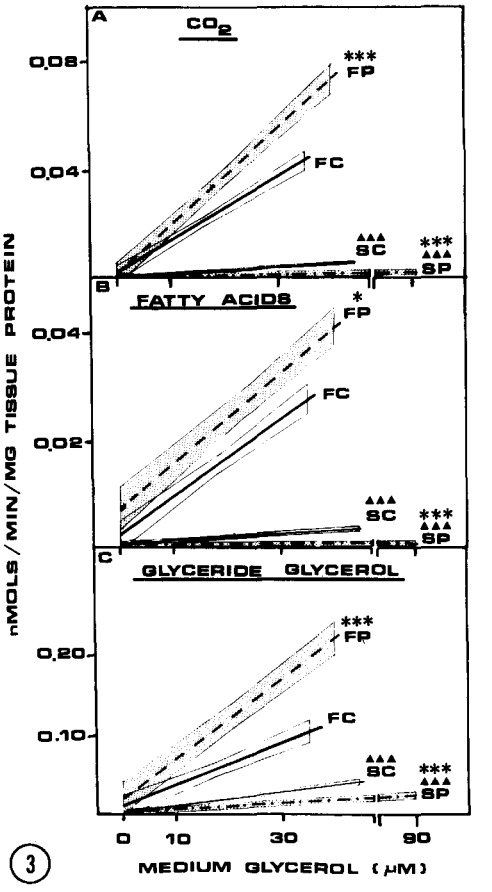


Fig. 1. Glycerol production by adipose tissue pieces from fed and 48 h starved 19-day pregnant rats (FP and SP) and their fed and starved virgin controls (FC and SC) as function of the incubation time. Regression lines ± standard error of the estimation. (n=30 values/group). P vs. C:***=p $<$ .001; S vsF: ▲▲▲ =p $<$ .001

Fig. 2. Rate of glycerol uptake by adipose tissue pieces from the same groups of animals as in Fig. 1, as function of the concentration of glycerol in the incubation medium. Regression lines ± standard error of the estimation have been calculated by using the values of the individual vials incubated at each time. P vs C:***=p $<$ .001**=p $<$ .01, S vs F:▲▲▲=p $<$.001.

Fig. 3. Rate of glycerol conversion to either CO_2 (A), fatty acids (B) or glyceride glycerol (C) by adipose tissue pieces from the same groups of animals as in Fig. 1, as function of the concentration of glycerol in the incubation medium. Regression lines ± standard error of the estimation have been calculated by using the values of the individual vials incubated at each time. P vs C:*=p $<$ .05, ***=p $<$ .001; S vs F:▲▲▲=p $<$.001.

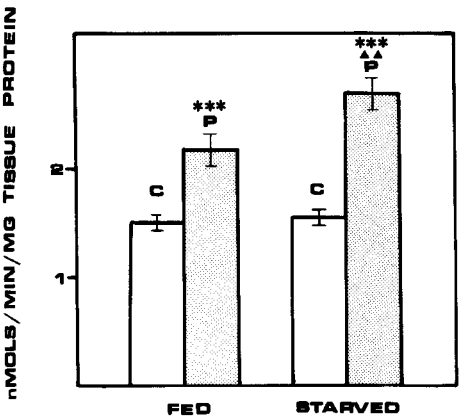


Fig. 4. Rate of glycerol release to the medium (lipolysis) by adipose tissue pieces from fed and 48 h starved 19-day pregnant rats (P) and their virgin controls (C). Mean ± S.E.M. of 6 rats/group. P vs C:**=p < .001; Starved vs fed:▲▲=p < .01.

when fed, while starvation produces a greater fall in all these parameters in the first group than in the second.

The rate of lipolysis is constant as function of time in all the groups, being higher in the tissues from pregnant rats than in those from their controls (Fig.4) both in the fed, as in the starved condition.

DISCUSSION

As previously reported for glucose (7), the present results show that the *in vitro* utilization of glycerol by adipose tissue is also enhanced in the pregnant rat. The incubations were carried out in the presence of glucose in order to allow a maximal rate of lipogenesis from the labelled glycerol (15). The values were expressed in terms of the rates of conversion of the substrate per amount of cold glycerol in the medium , to avoid artifacts due to the different dilutions of the tracer by the glycerol release from the tissues through lipolysis (9,22). This makes

it difficult to quantify the comparative utilization of glucose and glycerol, but it appears that the contribution of the latter may play an important role in the enlargement of the adipose tissues during pregnancy (7). As the glycerol is being used for its conversion to either CO_2 or lipids the process must imply its previous phosphorylation by glycerokinase (24). Thus, the results suggest that the activity of this enzyme is augmented in the adipose tissue from pregnant rats. Actually, an augmented activity of glycerokinase has been reported in situations of high adipose tissue deposition, in the presence of augmented circulating levels of insulin and resistence to this hormone (25-27). These conditions are also present in the pregnant rat (5,12, 13,28,29), which would indicate that the augmented adipose tissue mass in pregnancy is probably influenced by this greater capability to metabolize glycerol. In the presence of the low glucose levels of the pregnant rats (5,6,12), the augmented reutilization of glycerol by adipose tissue allow the mother to maintain a net deposition of fatty acids with a minimum waste of glucose. This picture completely changes in the starved state were the mother must mobilize all her resources, not only to ensure the continuous growth of the fetus, but her own survival, as blood glucose levels fall to intense hypoglycemia (4,5). This fits with the greater reduction of glycerol utilization in the adipose tissue from pregnant rats with fasting. This occurs despite the higher amount of glycerol available in the medium of the tissues from the pregnant rats. Thus, although any extrapolation to the *in vivo* situations must be considered with caution, it seems that in the starved pregnant rat the augmented rate of lipolysis in the presence of a minimum reutilization of glycerol by adipose tissue allow

the mother a rapid mobilization of all her endogenous glycerides to support her high rates of both ketogenesis and gluconeogenesis (5,13).

It was found in the present study that starvation itself does not produce a significant increase in the rate of lipolysis in the issues from the virgin controls, which is similar to previous findings in comparable conditions (7). As starvation produces a smaller effect decreasing the in vitro utilization of glucose (7) than that seen here for the utilization of glycerol, especially in the adipose tissue from pregnant rats, it is suggested that a reduced reutilization of glycerol, more than an enhanced lipolysis, may be responsible for the augmented mobilization of glycerides when food is withheld.

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