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EFFECT OF PREGNANCY ON THE UPTAKE OF LIPOPROTEIN TRIGLYCERIDE FATTY ACIDS BY ISOLATED ADIPOCYTES IN THE RAT

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SUMMARY

To determine whether the reduced lipoprotein lipase activity in adipose tissue in late pregnancy corresponds to parallel changes in the uptake of triglyceride fatty acids, isolated adipocytes from 19- and 21-day pregnant rats and virgin controls were incubated for different periods in the presence of rat plasma triglyceride-rich lipoproteins with their esterified fatty acids of neutral glycerides (triglycerides) labelled with ^{3}H. The hydrolysis of triglycerides and uptake of fatty acids by the adipocytes increased linearly and parabolically with respect to the incubation time and were always lower in cells from pregnant animals than from controls. Addition of heparin to the incubation medium produced similar increases in hydrolysis and uptake in all groups. Results indicate that the diminished uptake of triglyceride fatty acids by adipose tissue contributes to hypertriglyceridemia in late pregnancy which is counteracted by lipogenesis increase to maintain the mother's augmented body fat.

INTRODUCTION

The reduced activity of adipose tissue lipoprotein lipase (EC 3.1.1.3) in late gestation (1,2,3) has been considered responsible for typical maternal hypertriglyceridemia (4,5) related to diminished uptake of triglyceride fatty acids by adipose tissue. Tolerance to alimentary fat is, however, unimpaired (6) and it has recently been suggested that removal of triglyceride from circulation is not delayed (7) in late pregnancy. Triglyceride uptake by adipose tissue in the mother has not yet been specifically studied. This problem deserves attention because inconsistencies have been reported between the activity of lipoprotein lipase in adipose tissue and the uptake of glycerides. For example, mesenteric adipose tissue and fat pad

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pieces have higher lipoprotein lipase activity, respectively, than epididymal tissue and isolated adipocytes (8,9) while triglyceride uptake is lower (9,10). In the present study, the "in vitro" hydrolysis and uptake of rat plasma triglyceride-rich lipoproteins prelabelled with ^{3}H in their fatty acids by adipose tissue from 19- and 21-day pregnant rats was investigated. As these parameters were found to be comparatively greater when using isolated adipocytes than fat pad pieces (11), the former preparation was used here.

MATERIALS AND METHODS

Female virgin Wistar rats (160-180 g) and age matched pregnant rats at 19- and 21 days' gestation (estimated by the appearance of spermatozoids in vaginal smears) were sacrificed by decapitation and lumbar fat pads were immediately placed in Krebs-Ringer bicarbonate buffer, pH 7.4 (12). Adipocytes were isolated by a modification (13) of the method of Rodbell (14) in the presence of an ovomucoid trypsin inhibitor (Sigma) (2 mg/ml) and crude collagenase (Worthington Biochemical Co.) (2 mg/ml) for 30 min. Aliquots of the washed adipocyte suspension were placed in siliconized glass vials containing ^{3}H-labelled triglyceride-rich lipoproteins (coming from 0.4 ml of initial rat plasma and prepared as described below), Krebs-Ringer bicarbonate buffer, 4 mM glucose, 0.8 % purified bovine albumin (15), and 5 µl of 24 h fasted rat serum, in a final volume of 1.25 ml. Where stated, the medium was supplemented with heparin (3 IU/vial). Incubations were performed at 37°C in a Dubnoff metabolic shaker in an O_2/CO_2 (95:5) atmosphere for 30, 60 or 120 min. They were terminated by centrifugating the adipocytes at 200 rpm for 2 min in plastic syringes. Media were allowed to elute while adipocytes were thoroughly washed with plain Krebs-Ringer bicarbonate and successive centrifugations. Adipocytes and aliquots of media were placed in chloroform-methanol (2:1, by vol) for lipid extraction (16) and fractionation (17). In the fractions containing glycerides, phospholipids were eliminated by treatment with activated silicic acid in chloroform. Proteins were measured (18) in aliquots of the adipocyte suspensions. ^{3}H-labelled triglyceride-rich lipoproteins were obtained by i.v. injection in the tails of other virgin female rats (180 g) of 60 uCi of (9-10(n)-^{3}H)-palmitate (230 mCi/mmol) bound to fatty acid free bovine albumin (15). 30 min later, the animals were exsanguinated and plasma was centrifuged under 0.15 M NaCl (d = 1.006) for 18 h at 143.000 xg at 15°C. Supernatants contained both chylomicrons and very low density lipoproteins according to their size under the electron microscope after negative staining with phosphotungstic acid (19). These lipoprotein fractions were further purified by dialysis on 0.9 % NaCl and aliquots used for the incubations and lipid extraction and fractionation as described above. More than 87.8 ± 3.1 % of the label present in these lipoproteins always appeared as esterified

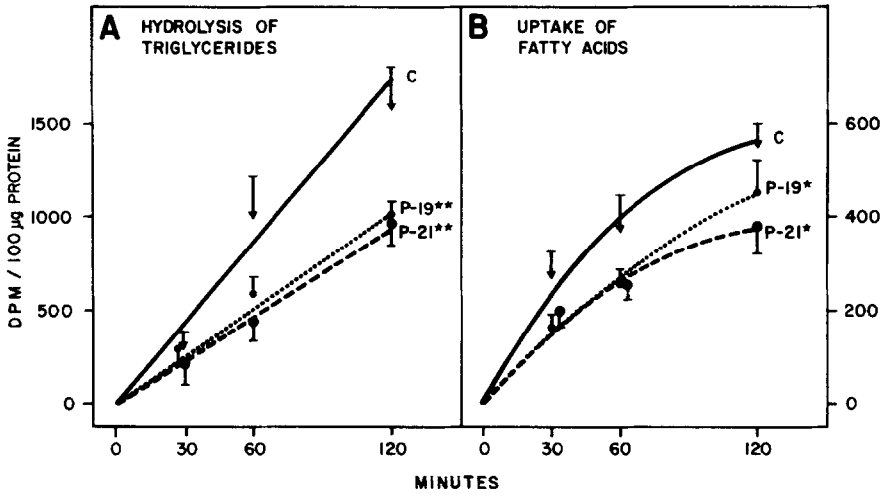

Fig. 1. Loss of saponifiable labelled neutral lipids from the media (hydrolysis) (A) and appearance of labelled fatty acids in the adipocytes (uptake) (B) in isolated adipocytes from 19- and 21-day pregnant rats (P-19 and P-21) and virgin controls (C) incubated in the presence of rat plasma triglyceride-rich lipoproteins labelled with ^{3}H in their esterified fatty acids of neutral lipids (triglycerides), n = 6 rats/group. Regression lines and means ± S.E.M. of the values at each incubation time. Statistical comparisons between lines (P-19 or P-21 vs. C): * = $p < 0.05$, ** = $p < 0.01$.

fatty acids of neutral glycerides (presumably triglycerides). Radioactive measurements were adjusted to 10^4 dpm contained in each vial before incubation. The data were adjusted to linear regressions (20) and the standard error of the estimation of Y was calculated for each line (21). The statistical comparisons between lines were performed by means of an ANOVA test (22,23).

RESULTS

Isolated adipocytes from 19- and 21-day pregnant rats and virgin controls were incubated for different times in the presence of rat plasma triglyceride-rich lipoproteins labelled in their esterified fatty acids. The loss of saponifiable labelled neutral lipids from the media was considered as the hydrolysis of triglycerides. As shown in Fig. 1a, this parameter increased linearly in all groups with incubation time but was significantly lower in adipocytes from 19- and 21-day pregnant rats than in those from controls. A certain proportion of the esterified fatty acids

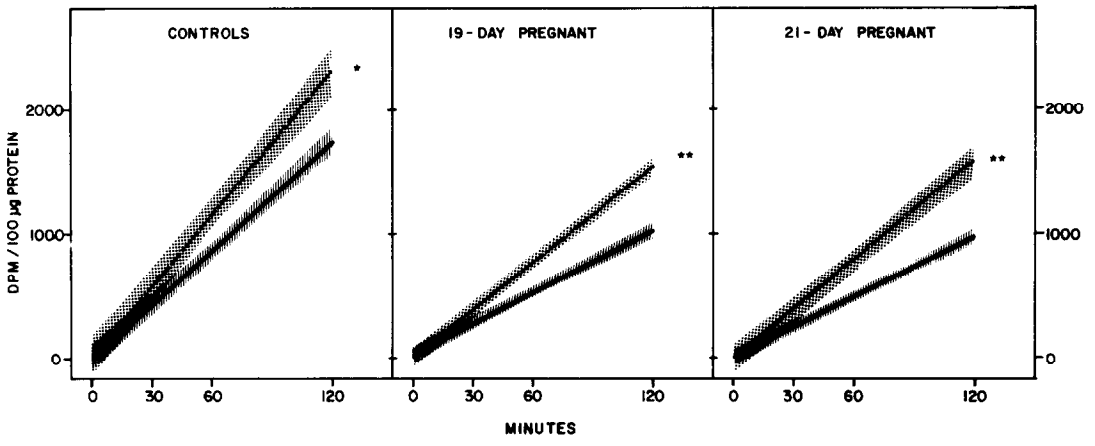

Fig. 2 Effect of heparin in the incubation media (3IU/vial) on the loss of saponifiable labelled neutral lipids from the media (hydrolysis) in adipocytes from 19- and 21-day pregnant rats and virgin controls. Incubations without heparin:——— and with heparin: ..... n = 6 rats/group. Regression lines and standard error of the estimation of Y. Statistical comparisons between lines: * = $p < 0.05$; ** = $p < 0.01$.

(always above 30 %) disappeared from the media and became incorporated into the adipocyte lipids. This parameter was considered as the uptake of fatty acids by fat cells and its value is shown in Fig. 1b. It was observed that this uptake increased parabolically with incubation time and its value in the adipocytes of either pregnant group was significantly lower than in controls. Most labelled fatty acids taken up by adipocytes appeared as esterified fatty acids, this amount being over 85 % of the total lipid content in all experimental conditions and at all incubation times.

To determine whether the decreased triglyceride-lipoprotein hydrolyzing activity in the pregnant rat was affected by heparin, incubations were performed in its presence and absence. As seen in Fig. 2, heparin produced a similar and significant enhancement in the hydrolysis of labelled triglyceride-rich lipoprotein glycerides when incubated in the presence of adipocytes from pregnant rats and virgin controls. Heparin in the media, however, did not affect the comparative reduction of either the hydrolysis of triglycerides or the uptake of fatty acids by the adipocytes of pregnant rats versus controls (data not shown).

DISCUSSION

Present results demonstrate that isolated adipocytes from pregnant rats in late gestation have a reduced capacity to hydrolyse triglyceride fatty acids from triglyceride-rich lipopeoteins and consequently a reduced uptake of these substances. The nonlinearity of the fatty acid incorporation into cells during incubation may reflect the progressive dilution of labelled free fatty acids taken up with the nonlabelled ones continuously being released from the cells by lipolysis. If this interpretation is correct, the dilution effect must be greater in cells from pregnant rats than in those from virgin controls, as lipolysis is known to be more active in the former (24,25). While it is not possible to determine here the quantitative contribution of this effect, it does not appear to influence the hydrolysis of lipoprotein triglycerides in the media, as indicated by its linearity with the incubation time, which was always reduced in cells from pregnant rats. Our findings are in agreement with the reductions in adipose tissue lipoprotein lipase activity observed in late pregnant rats (1,3). Heparin was found to enhance those parameters in a similar way in adipocytes from both pregnant rats and controls. Since the effect of heparin on lipoprotein lipase isolated fat cells corresponds to activation of the enzyme in association with stimulation of its release (26), the observed response in adipocytes from pregnant rats suggests that their reduced triglyceride fatty acid uptake is not a consequence of a defect in the enzyme activation process (27).

On the basis of "in vivo" kinetic studies, it has recently been proposed that delayed removal of triglycerides from circulation may not influence the hypertriglyceridemia of late pregnancy (7). If this were true, an enhancement in lipoprotein lipase activity in other maternal tissues should exist to compensate for its reduction in adipose tissue. This has not yet been confirmed (1) and total post-heparin lipoprotein lipase activity is actually reduced in the late pregnant rat (5). Thus it still seems valid to maintain that the diminished

uptake of triglyceride fatty acids by adipose tissue contributes to the hyperglyceridemia of pregnancy. This conclusion does not invalidate the proposed involvement of other factors such as an increased entry of triglycerides into the circulation (28,29). The specific participation of all factors remains to be determined.

Augmented food intake in the mother (5) in the presence of enhanced adipose tissue glyceride synthesis (30, 31,32,33) appears to counteract the reduced uptake of triglyceride fatty acids in adipose tissue, allowing her to maintain increased body fat until late gestation (34). This situation provides the mother with augmented circulating and stored lipidic resources with greatly increased catabolism in the fasting state, when the products of fatty acid breakdown are maximally enhanced in her blood (4,35). In this way, the mother spares other substrates such as glucose and amino acids, and ensures their adequate supply to the fetus to maintain its continuous growth.

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