rises to about 18% after insulin activation. It has been suggested (Arch & Newsholme, 1976) that under conditions where there is a high turnover of cyclic AMP then small changes in cyclic AMP phosphodiesterase activity might have important consequences on intracellular cyclic AMP contents. Furthermore, simulation studies (Fell, 1980) indicate that the activity of a low K_m enzyme associated with the plasma membrane might play an important role in regulation of concentrations and gradients within the cell. We should not, however, discount the possibility that rapidly internalized insulin (Desbuquois *et al.*, 1979) might lead to a similar activation of the enzyme associated with intracellular membranes (Marchmont & Houslay, 1980).

Insulin under conditions in the liver where cyclic AMP concentrations have been elevated above basal can affect a number of target proteins by triggering their phosphorylation by a cyclic AMP-dependent protein kinase associated with the plasma membrane

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Metabolic response to starvation in the hypothyroid pregnant rat

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Although development of the rat foetus is influenced by the mother's hormonal balance, it has not yet been clearly established that maternal hypothyroidism can cause anomalies in the offspring (Baksi, 1979). The circulating concentration of thyroid hormones in the foetus is independent of maternal values, owing to the minimal transfer of these hormones through the placenta (Sack, 1979). Thus the negative influence of the hypothyroid mother on her foetus must be mainly exerted by changing the availability of metabolic substrates to the foetus. We have observed that the metabolic balance is considerably altered in hypothyroid unfed animals (Llobera *et al.*, 1978). In the present study we investigated the influence of food deprivation in the hypothyroid mother on specific aspects of the foetus/mother metabolic interactions.

Female Wistar rats were mated when weighing 160–180g. On the day that spermatozoids appeared in vaginal smears, half of the animals were surgically thyroidectomized and the others were sham operated and used as controls. All animals were killed by guillotine on day 21 of gestation, either fed or after 24h starvation. Plasma thyrotropin was measured by radioimmunoassay (NIAMD kit from National Institutes of Health, U.S.A.) and the metabolic parameters were determined as previously described (Aranda *et al.*, 1972; Arola *et al.*, 1976).

Body-weight increase throughout pregnancy was less in thyroidectomized (146 ± 3 g) than in control (173 ± 5 g) rats, and starvation produced a smaller decrease in the former group (decreases of 8.2 ± 0.6g and 10.7 ± 0.8g respectively). Body weight was less in foetuses from thyroidectomized than from control mothers ($P < 0.001$). Starvation produced a greater decrease in foetal body weight with thyroidectomized than with control mothers. As shown in Table 1, plasma thyrotropin

Table 1. *Effects of hypothyroidism and starvation in the pregnant rat*

Rats were thyroidectomized on the day of mating and were killed on day 21 of gestation either fed or after 24h starvation. Sham-operated animals were used as controls. Experimental details are indicated in the text. Values are means ± S.E.M. of 6 rats/group. Statistical comparison between fed and starved animals are denoted by the *P* values in the Table, and comparison between thyroidectomized mothers and their controls are shown by asterisks: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; no asterisk represents difference not significant (N.S.).

	Plasma thyrotropin (μg/ml)	Plasma glycerol (μM)	Plasma total amino acids (μM)	Liver glycogen (%)
Mother				
Control: Fed	0.26 ± 0.03	102 ± 14	6006 ± 614	3.29 ± 0.55
Starved	0.19 ± 0.01	148 ± 10	3173 ± 278	0.01 ± 0.00
P	N.S.	<0.001	<0.01	<0.001
Thyroidectomized: Fed	4.10 ± 0.24***	73 ± 15	5006 ± 328	2.53 ± 0.16
Starved	2.50 ± 0.32***	104 ± 12**	4413 ± 183*	0.03 ± 0.00
P	<0.001	N.S.	N.S.	<0.001
Foetuses				
From controls: Fed	0.02 ± 0.01	34 ± 9	8644 ± 665	6.01 ± 0.22
Starved	0.19 ± 0.01	80 ± 16	8208 ± 834	3.46 ± 0.41
P	N.S.	<0.05	N.S.	<0.001
From thyroidectomized: Fed	0.20 ± 0.02	46 ± 11	10856 ± 325*	4.75 ± 0.21**
Starved	0.23 ± 0.04	54 ± 11	7736 ± 773	0.09 ± 0.01***
P	N.S.	N.S.	<0.01	<0.001

concentrations were much higher in thyroidectomized than in control mothers, and starvation produced a significant decrease in this parameter in thyroidectomized but not in control mothers. Plasma thyrotropin concentrations were not affected in the foetuses of either group. Plasma glycerol concentration was augmented with starvation in control mothers and foetuses, but it did not change in either the thyroidectomized mothers or their foetuses. Plasma total amino acid concentrations decreased in starved control mothers, but the concentrations in their foetuses remained unchanged. In contrast, starvation did not affect this parameter in thyroidectomized mothers, but the value decreased in their foetuses. Liver glycogen concentration was decreased in the foetuses from thyroidectomized mothers as compared with those from controls, and this difference was much greater during starvation.

The above findings confirm that the thyroid status of the foetus is independent of the mother's. Although it has been proposed that, unlike their mothers, foetuses of hypothyroid mothers incorporate increased amounts of available labelled glucose into glycogen (Porterfield & Hendrich, 1975), we show here that the liver glycogen built up during late gestation, which is necessary for successful early neonatal life, is severely impaired in foetuses of hypothyroid mothers. This is most evident when the mother is starved and indicates that the decreased response to starvation in the hypothyroid mother interferes with the continuous availability of substrates to the foetus, causing its intense developmental retardation.

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Incubation of labelled triacylglycerol-rich lipoproteins with rat adipose tissue in the presence of heparin

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The metabolism of triacylglycerol-rich lipoproteins (chylomicrons and very-low-density lipoproteins) requires the hydrolysis of triacylglycerols by lipoprotein lipase in extrahepatic tissues (Smith *et al.*, 1978). In adipose tissue, after hydrolysis the lipoproteins attain higher density and released unesterified fatty acids are deposited in the tissue (Scow *et al.*, 1972). The fate of the glycerol moiety of these triacylglycerols after hydrolysis has not been established. In the present study, we investigated the utilization *in vitro* of rat plasma triacylglycerol-rich lipoproteins, prelabelled with ^{3}H in the esterified fatty acids and with ^{14}C in the acylglycerol glycerol by pieces of epididymal fat-pads. The presence of heparin in the incubation medium enhanced the release of lipoprotein lipase into the medium (Stewart & Schotz, 1974), allowing more efficient contact of the enzyme with its substrate.

Female Wistar rats were injected intravenously with 60μCi of sodium [9,10(n)-^{3}H]palmitate and 30μCi of [U-^{14}C]glycerol and bled 30min afterward. Triacylglycerol-rich lipoproteins were purified from plasma by flotation after centrifugation at 143000g for 18h at 15°C in 0.15M-NaCl (d = 1.006) and dialysis. Electron-microscopic study of these lipoproteins revealed that most had a diameter of 15–60nm, corresponding to very-low-density lipoproteins, and some chylomicrons were also evident. This preparation contained more than 87% of the ^{3}H-labelled lipids as esterified fatty acids and more than 97% of the ^{14}C-labelled lipids as acylglycerol glycerol of neutral lipids. The prelabelled lipoproteins were incubated for 120min in the presence of pieces of epididymal fat-pads from fed male Wistar rats (180–190g) in Krebs–Ringer bicarbonate buffer containing 4mM-glucose, 0.8% purified bovine albumin and rat serum (5μl/1.25ml), and some were supplemented with heparin (3units/1.25ml).

Table 1. *Effect of incubation in vitro of rat epididymal fat-pad pieces on the utilization of prelabelled triacylglycerol-rich lipoproteins*

Incubations were carried out for 120min in the presence of rat triacylglycerol-rich lipoproteins with their esterified fatty acids labelled with ^{3}H and their acylglycerol glycerol with ^{14}C. The media were supplemented or not with heparin (3 units/1.25ml). Hydrolysis values correspond to the radioactivity (adjusted to 10^{4} d.p.m.) disappeared from the medium as either esterified fatty acids or acylglycerol glycerol, and uptake correspond to the percentage of this radioactivity appearing in the tissue lipids. Values are means ± S.E.M. Statistical comparisons between heparin-supplemented and basal vials are shown by asterisks: * $P<0.05$, ** $P<0.01$.

	Hydrolysis (d.p.m./100μg of protein)	Uptake (% of hydrolysis)	Percentage distribution of labelled tissue lipids: Unesterified fatty acids	Esterified fatty acids	Acylglycerol glycerol
Basal					
^{3}H	733 ± 52	18.8 ± 3.6	15.8 ± 7.3	90.0 ± 5.0	—
^{14}C	540 ± 42	4.1 ± 0.4	23.5 ± 4.8	51.9 ± 5.8	24.5 ± 5.5
Heparin					
^{3}H	2084 ± 31**	17.5 ± 1.4	6.5 ± 2.8	97.6 ± 10.4	—
^{14}C	2102 ± 227**	2.5 ± 0.3*	25.5 ± 16.7	54.5 ± 3.8	24.8 ± 13.7