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 ^3H 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)-^3H]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 ^3H-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 ^3H 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					
^3H	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					
^3H	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

After incubation, the triacylglycerol-rich lipoproteins seen under the electron microscope appeared as partially degraded structures with atypical, discoid, flattened and lamellar forms. As shown in Table 1, heparin produced a significant increment in the hydrolysis of ^{3}H-labelled esterified fatty acids and ^{14}C-labelled acylglycerol glycerol from the ^{3}H- and ^{14}C-labelled triacylglycerol-rich lipoproteins in the medium. A significant proportion of the hydrolysed labelled lipids appeared incorporated into the tissue lipids at the end of incubation. Most of the ^{3}H-labelled fatty acids appeared in their esterified form, whereas the ^{14}C taken up by the tissue appeared distributed in unesterified fatty acids, esterified fatty acids and acylglycerol glycerol.

The present results show that adipose tissue *in vitro* is able to utilize not only the fatty acids but also the glycerol moiety released by the action of lipoprotein lipase on the lipoprotein triacylglycerol. This effect is quantitatively small but effective, agreeing with the reported ability of adipose tissue to metabolize glycerol (Chaves & Herrera, 1978; Herrera & Ayanz, 1972). It may be significant in situations of hyperlipidaemia in the presence of augmented adipose-tissue lipoprotein lipase and glycerokinase activities, as in obesity (Rath *et al.*, 1974; Treble & Mayer, 1963).

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Methionine metabolism via the transamination pathway in rat liver

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The substrate specificity of rat-liver leucine (methionine) transaminase (Ikeda *et al.*, 1976) and bacterial leucine dehydrogenase (Livesey & Lund, 1980) suggested that some steps in the catabolism of methionine and branched-chain amino acids may share common enzymes. Transamination then decarboxylation of the 2-oxo acid products initiates the catabolism of these amino acids (see Krebs & Lund, 1977; Mitchell & Benevenga, 1978; Steele & Benevenga, 1978). The methionine-transamination pathway operates independently of the formation of *S*-adenosyl-L-methionine, and flux through the transamination pathway probably exceeds that through the trans-sulphuration pathway (Mitchell & Benevenga, 1978). The enzymes of the transamination pathway have not, however, been identified.

The specificity of the methionine transaminase activity was investigated by using intact hepatocytes. The cells (25 mg wet wt.), isolated as described by Krebs *et al.* (1974) from 48 h-starved female Wistar rats, were incubated for 30 min in 2 ml of Krebs–Henseleit (1932) saline containing 1 mM-L-[1-^{14}C]methionine. Incubation was in 25 ml Erlenmeyer flasks with a centre well to collect CO_2 after stopping the reaction with $HClO_4$. The amount of metabolic $^{14}CO_2$ was 1.6 μmol/h per g of cells. The amount of 2-oxo [1-^{14}C]acid accumulating (measured as $^{14}CO_2$ after H_2O_2 treatment) was equal to 0.5 μmol/h per g of cells. The latter value provides a crude estimate of the rate of methionine transamination and was increased in the presence of the following amino-group acceptors (1 mM): 4-methylthio-2-oxobutyrate, the 2-oxo acid analogue of methionine, (80-fold); pyruvate and the 2-oxo acids of leucine, valine and isoleucine (less than 12-fold); phenylpyruvate (40-fold). Metabolic $^{14}CO_2$ production in the presence of these 2-oxo acids was within $\pm$2 μmol/h per g of cells of the rate from [1-^{14}C]methionine alone (1.6 μmol/h per g of cells).

When 0.05 mM-4-methylthio-2-oxo[1-^{14}C]butyrate was the substrate, the rate of production of L-[1-^{14}C]methionine plus 4-methylthio-2-hydroxy[1-^{14}C]butyrate (the latter produced by the action of lactate dehydrogenase, both measured as H_2O_2-stable radioactivity) was approx. 5 μmol/h per g of cells during a 10 min incubation. L-Methionine (1 mM) and L-glutamine (1 mM) each increased this value 4-fold, and 1 mM-phenylalanine increased it 2-fold, but L-leucine, L-valine and L-isoleucine (1 mM) each had no effect.

The specificity of the methionine aminotransferase activity in the intact hepatocytes parallels that of highly purified glutamine 2-oxo acid aminotransferase (Cooper & Meister, 1972) and not that of leucine (methionine) aminotransferase (Ikeda *et al.*, 1976) or other liver branched-chain amino acid aminotransferases (Ichihara *et al.*, 1973).

Transamination appears to be extramitochondrial and decarboxylation intramitochondrial; 1 mM-2-cyano-4-hydroxycinnamate, a specific inhibitor of the mitochondrial pyruvate carrier (Halestrap & Denton, 1974) did not affect the rate of transamination of 4-methylthio-2-oxo[1-^{14}C]butyrate in either the presence or absence of 1 mM-L-methionine, but the rate of $^{14}CO_2$ formation was decreased by 90%. This also suggests that 4-methylthio-2-oxobutyrate enters mitochondria on the pyruvate carrier and thus explains, in part at least, the inhibition by this 2-oxo acid of gluconeogenesis from pyruvate in kidney (Krebs & de Gasquet, 1964) and isolated hepatocytes (G. Livesey, unpublished work). Metabolic $^{14}CO_2$ formation from 1 mM-L-[1-^{14}C]methionine was also inhibited by 1 mM-2-cyano-4-hydroxycinnamate.

Metabolic $^{14}CO_2$ formation from 0.05 mM-methylthio-2-oxo[1-^{14}C]butyrate (and from 1 mM-L-[1-^{14}C]methionine) was markedly inhibited by branched-chain 2-oxo acids (1 mM), possibly by competition for the pyruvate carrier, which is known also to transport 4-methyl-2-oxovalerate, the 2-oxo acid analogue of leucine (Williamson *et al.*, 1979; G. Livesey, unpublished work). Substrate specificity of the decarboxylation reaction was therefore investigated by using a preparation containing disrupted mitochondria.

A rat liver homogenate was prepared in 0.01 M-K_2HPO_4/KH_2PO_4, pH 7.2, containing 1% (v/v)-Triton X-100 and freeze(liquid N_2)–thawed (at 30°C) three times. 4-Methyl-2-oxo[1-^{14}C]valerate (0.2 mM) and 4-methylthio-2-oxo[1-^{14}C]-butyrate (1 mM) were decarboxylated by the preparation at rates of 60 and 29 μmol/h per g fresh wt. of liver respectively (K_m values were 10 and 160 μM respectively) during a 2–10 min period at 38°C when these substrates were incubated with the equivalent of 2.5 and 10 mg of liver respectively in 1.0 ml of 20 mM-K_2HPO_4/KH_2PO_4, pH 7.2, containing 1.8 mM-$MgSO_4$, 0.4 mM-thiamin pyrophosphate, 0.4 mM-coenzyme A, 2.8 mM-NAD^+ [i.e. conditions similar to those for the assay of branched-chain 2-oxo acid dehydrogenase complex (Parker & Randle, 1978)]. Omission of either $MgSO_4$, thiamin pyro-