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Evidence that use of Triton WR1339 underestimates the triacylglycerol entry rate into the plasma of lactating rats owing to continued accumulation of lipid in the mammary gland

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Measurement of the entry rate of an intragastric load of [^{14}C]triolein into the plasma in the presence of Triton WR1339 gave similar values for virgin and weaned rats, but significantly lower values for lactating rats. This decreased entry rate (65%) in lactating compared with virgin rats was due to a failure of Triton WR1339 to inhibit the accumulation of [^{14}C]lipid in the mammary gland. This is further evidence that mammary-gland lipoprotein lipase behaves differently from that in white adipose tissue or muscle.

INTRODUCTION

Intravenous administration of the detergent Triton WR1339 to experimental animals results in a progressive increase in the concentration of triacylglycerols in plasma (Friedman & Byers, 1953; Otway & Robinson, 1967). This effect is generally assumed to be the result of the inability of the lipoprotein lipase present in extra-hepatic tissues to hydrolyse the plasma triacylglycerols (chylomicrons from the intestine or very-low-density lipoproteins from the liver and intestine) in the presence of Triton WR1339 (Otway & Robinson, 1967). The inhibition of lipolysis is in part due to the coating of the lipoproteins by the detergent, which prevents the interaction of the substrate and the active enzyme situated on the lumenal surface of the capillaries (Robinson, 1963; Scanu, 1965). In addition, there is evidence that Triton WR1339 has a direct inhibitory effect on the lipoprotein lipase in muscle and white adipose tissue (Borensztajn *et al.*, 1976). This property of Triton WR1339 to inhibit the action of lipoprotein lipase is the basis of an extensively used method for measuring the entry rate of triacylglycerols into the plasma (see, e.g., Otway & Robinson, 1967; Lovo & Hustvedt, 1975; Agius *et al.*, 1981; Duerden & Gibbons, 1988). In experiments designed to compare the entry rate of triacylglycerols into the plasma in response to standard meals of different fatty acid composition in virgin and lactating rats, we have consistently found that the increment of triacylglycerols after administration of Triton WR1339 was lower in lactating rats. The lactating mammary gland of the rat has high activity of lipoprotein lipase (Scow & Chernick, 1987) and accumulates [^{14}C]lipid from ^{14}C-labelled chylomicrons *in vivo* (Scow *et al.*, 1977; Oller do Nascimento & Williamson, 1986) and *in vitro* (Zinder *et al.*, 1976). It is generally assumed that this activity is responsible for the hydrolysis of triacylglycerol before entry of the constituent diacylglycerols, monoacylglycerols and non-esterified fatty acids into mammary tissue (Zinder *et al.*, 1976). We have therefore investigated whether Triton WR1339 effectively inhibits the accumulation of [^{14}C]lipid in the lactating mammary gland after an intragastric load of [1-^{14}C]triolein.

EXPERIMENTAL

Animals

Female Wistar rats were housed at 22±2 °C in a 12 h-light/12 h-dark cycle (light period starting at 08:00 h) with free access to water and pelleted stock diet (by weight 52% carbohydrate, 21% protein, 4% fat and 21% non-digestible material; Special Diet Services, Witham, Essex, U.K.). Lactating rats with 8–10 pups (weight gain at least 1 g/day per pup) were used 10–12 days *post partum*. Weaned rats had their litters removed 48 h before the start of the experiment.

To allow intravenous injections in conscious rats, all animals had a 0.9%-NaCl-filled polyethylene cannula (internal diameter 0.63 mm; external diameter 0.96 mm) inserted into the jugular vein and exteriorized between the scapula under anaesthesia 2–3 days before the experiment (Jones *et al.*, 1984). The procedure caused no appreciable effect on food intake, body weight or pup weight gain, all of which were monitored daily.

Chemicals

[1-^{14}C]Triolein was obtained from Amersham International (Amersham, Bucks., U.K.), Triton WR1339 (Tyloxapol) from Sigma Chemical Co. (Poole, Dorset, U.K.), Lumasorb from May & Baker (Dagenham, Essex, U.K.), and all biochemicals were from Boehringer Corp. (London) Ltd. (Lewes, E. Sussex, U.K.).

Measurement of the oxidation and tissue accumulation of [1-^{14}C]triolein

All experiments were commenced at 09:00 h. Conscious rats were given a weighed dose of [1-^{14}C]triolein (about 0.6 ml, equivalent to 690 μmol, 0.30 μCi) by gastric intubation, and 0.9% NaCl or Triton WR1339 (100 mg/ml of water per 200 g body wt.) via the indwelling jugular-vein cannulae. The rats were then placed individually in a large desiccator, and $^{14}CO_2$ was collected over the next 5 h (Oller do Nascimento & Williamson, 1986). During the CO_2 collection, the rats had free access to water and the pups were allowed to suckle. At the end of this period rats were anaesthetized with pentobarbital (60 mg/100 g body wt. in 0.9% NaCl, intraperitoneally), and 5 min later an arterial blood sample was taken from the abdominal aorta. Tissues were dissected out and weighed. The whole of the intestine was removed and homogenized in 150 ml of 3% (w/v) $HClO_4$ in a Waring blender. The rat carcasses (minus the intestine and mammary gland) and whole pups were autoclaved and homogenized with 350 ml of water.

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Treatment of tissues and carcasses

Samples (0.2 g) of tissues (liver, white adipose tissue, brown adipose tissue and mammary gland), together with samples of homogenized intestine and carcass (5 g), were saponified and extracted with light petroleum (b.p. 40–60 °C; Stansbie *et al.*, 1976) to determine the lipid content and the accumulation of [^{14}C]lipid.

Analysis of plasma

Arterial blood samples were centrifuged for 10 min at 2000 *g* at 4 °C, and plasma was then collected for measurement of radioactivity to assess the accumulation of [^{14}C]lipid. The triacylglycerol content of plasma was determined by the method of Eggstein & Kreutz (1966). Plasma Triton WR1339 was measured as described by Schurr *et al.* (1972).

Analysis of data

Subtraction of the amount of radioactive lipid remaining in the intestine from the amount of [1-^{14}C]triolein administered allowed calculation of the amount absorbed by the rat. The specific radioactivity of the administered [1-^{14}C]triolein and the radioactivity measured in the CO_2 and in each tissue and the plasma allowed determination of the accumulation of the μmol equivalents of [1-^{14}C]triolein. Total tissue weights were determined, for each rat, by dissection of the liver and mammary gland and by extraction of the eviscerated-carcass lipid for adipose tissue. These values then allowed calculation of the total tissue and plasma (taking plasma volume to equal 4.1 % of total body weight: Otway & Robinson, 1967; Lovo & Hustvedt, 1975) accumulation of [1-^{14}C]triolein. The results are mean values ± S.E.M., expressed as μmol equivalents of [1-^{14}C]triolein, and were compared by Student's unpaired *t* test.

RESULTS AND DISCUSSION

In view of the hyperphagia associated with lactation (Fell *et al.*, 1963), a surprising feature of previous measurements in our laboratory of the rate of triacylglycerol entry into the plasma with Triton WR1339 was that, at peak lactation (10–14 days), the rate of entry expressed per 100 g body wt. was significantly lower than in virgin or 24 h-weaned rats (Agius *et al.*, 1981). One might expect not only increased entry of chylomicrons derived from the diet in lactating rats, but also increased hepatic secretion of very-low-density lipoproteins (Whitelaw & Williamson, 1977; Zammit, 1981). To ensure that rats in different physiological states have received similar amounts of fat before administration of Triton WR1339, we have used conscious rats and an intragastric load of [1-^{14}C]triolein in the present experiments so that the absorption and disposal of the dietary lipid could be measured. It has been shown that 90 % of absorbed [^{3}H]triolein appears in the lymph as triacylglycerol; the remainder of the radioactivity is in phospholipid and cholesterol (Tso *et al.*, 1980).

The total plasma triacylglycerols after administration of Triton WR1339 were not significantly different in virgin and lactating rats, but were significantly higher in 48 h-weaned rats ($P < 0.01$; Table 1). The calculated entry rates were 38 ± 4, 21 ± 2 and 41 ± 2 (μmol/h per 100 g body wt.) for virgin, lactating and 48 h-weaned rats respectively. The decreased entry rate ($P < 0.01$) in lactating rats is similar to that reported previously (Agius *et al.*, 1981). Measurements of the radioactivity accumulated in the plasma in these experiments showed a large decrease (at least 50 %) in lactating rats compared with the other groups (Table 1). Separation of the plasma lipids by t.l.c. indicated that at least 85 % of the accumulated radioactivity was associated with triacylglycerols (results not shown). Although there was a tendency for Triton WR1339 to decrease absorption of [1-^{14}C]triolein in virgin and lactating rats compared with untreated rats, this was only significant in the last group (Table 1). However, as there was no difference in absorption between the three groups treated with Triton WR1339, the large decrease in the plasma ^{14}C-labelled triacylglycerols in the lactating group cannot be due to impaired absorption (Table 1).

In untreated rats the amount of [1-^{14}C]triolein oxidized to $^{14}CO_2$ followed the pattern reported in previous work from this laboratory (Oller do Nascimento & Williamson, 1986) and confirmed the conservation of dietary fat in lactation and on cessation of lactation (Table 1). Triton WR1339 significantly inhibited the production of $^{14}CO_2$ by about 75 % in virgin and 48 h-weaned rats, but caused no decrease in the low rate of production in lactating rats (Table 1). The recovery of absorbed [1-^{14}C]triolein in $^{14}CO_2$ plus [^{14}C]lipid in eviscerated carcass plus plasma was 58 %, 30 % and 70 % in untreated virgin, lactating and 48 h-weaned rats respectively. Treatment with Triton WR1339 increased these percentages to 96 %, 58 % and 103 % respectively. It is noteworthy that the increase in the percentage recovered in the three groups treated with Triton WR1339 was similar (about 30 %), suggesting that there was no major difference between the experimental groups in the conversion of the lipid load into water-soluble non-lipid products (carbohydrate, amino acids, bicarbonate) (Leyton *et al.*, 1987). However, in the Triton-WR1339-treated lactating rats some 30–40 % of the absorbed [1-^{14}C]triolein was unaccounted for.

In the present experiments the eviscerated carcass did not

Table 1. Effects of Triton WR1339 treatment on the absorption, oxidation and carcass accumulation of [1-^{14}C]triolein in virgin, lactating and weaned rats

For experimental and other details see the text. The results are mean values ± S.E.M., with the numbers of observations in parentheses, and are expressed as μmol of [1-^{14}C]triolein equivalents. Values for Triton WR1339-treated rats that are significantly different by Student's *t* test from the appropriate untreated group of rats are shown by $*P < 0.05$, $***P < 0.001$; differences for Triton WR1339-treated rats compared with treated virgin rats are shown by $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$.

State of rats	Triton WR1339 treatment	Body wt. (g)	[1-^{14}C]Triolein disposal (μmol/rat)				Plasma triacylglycerol (μmol/rat)
			Absorbed	$^{14}CO_2$	Carcass	Plasma	
Virgin (5)	–	174 ± 9	470 ± 28	123 ± 19	145 ± 10	8 ± 2	5 ± 1
Virgin (6)	+	181 ± 5	436 ± 37	26 ± 3***	155 ± 19	236 ± 25***	347 ± 35***
Lactating (5)	–	249 ± 13	530 ± 12	38 ± 7	117 ± 22	6 ± 1	7 ± 2
Lactating (5)	+	260 ± 5	415 ± 38*	27 ± 5	106 ± 6	106 ± 17***,†††	275 ± 26***
48 h-weaned (4)	–	281 ± 15	459 ± 8	74 ± 9	242 ± 31†	6 ± 1	12 ± 3
48 h-weaned (4)	+	283 ± 17	507 ± 27	21 ± 1***	207 ± 16	294 ± 34***	575 ± 31***,††

Table 2. Effects of Triton WR1339 on tissue accumulation of [1-^{14}C]triolein in virgin, lactating and weaned rats

For experimental and other details see the text. The results are mean values ± S.E.M., and are expressed as μmol of [1-^{14}C]triolein equivalents per total mass of tissue, except for brown adipose tissue, which is per g. Values for Triton-WR1339-treated rats that are statistically different by the Student's *t* test from the appropriate untreated group are shown: $*P < 0.05$, $**P < 0.01$.

State of rats	Triton WR1339 treatment	Accumulation of [1-^{14}C]triolein (μmol/tissue mass)					
		Liver	White adipose tissue	Brown adipose tissue	Mammary gland	Litter	Mammary gland + litter
Virgin (5)	−	23.3 ± 1.89	25.8 ± 5.90	27.7 ± 6.0	−	−	−
Virgin (6)	+	34.3 ± 4.54*	19.6 ± 2.70	9.87 ± 2.35*	−	−	−
Lactating (6)	−	10.6 ± 1.33	5.21 ± 2.85	1.97 ± 1.22	147.2 ± 7.77	102 ± 21	249 ± 17
Lactating (5)	+	23.9 ± 2.82**	6.75 ± 1.43	0.91 ± 0.13	162.0 ± 38.5	18 ± 6**	179 ± 39
24 h-weaned (4)	−	37.3 ± 6.87	106.5 ± 17.7	10.9 ± 4.43	10.5 ± 1.72	−	−
24 h-weaned (4)	+	45.7 ± 5.99	45.7 ± 17.7*	1.42 ± 0.15	9.43 ± 1.38	−	−

include the mammary gland: the [^{14}C]lipid accumulation in this and other tissues are given in Table 2. As expected from previous studies, in the lactating rat the major portion of the absorbed [^{14}C]lipid accumulated in mammary gland and the suckling pups, whereas in the 48 h-weaned rats it was in white adipose tissue (Table 1; Oller do Nascimento & Williamson, 1986, 1988; Oller do Nascimento *et al.*, 1989). Unexpectedly, administration of Triton WR1339 did not decrease the accumulation of [^{14}C]lipid (less that 10% was in the form of [^{14}C]sterols) in mammary gland of lactating rats, but there was a significant decrease in the pups (Table 1). There was no indication that pups of mothers treated with the detergent suckled less, and the weight of milk clot in their stomachs was not different from that in control pups. It is noteworthy that when lipoprotein lipase activity in mammary gland is decreased by bromocryptine treatment there is also no decrease in [^{14}C]lipid accumulation in mammary gland, but the transfer to the pups is decreased (Oller de Nascimento *et al.*, 1989).

In 48 h-weaned rats treated with Triton WR1339 the [^{14}C]lipid accumulation in white adipose tissue was decreased by 58% (Table 1). At first sight it is surprising that Triton WR1339 did not decrease the accumulation of [^{14}C]lipid in white adipose tissue of virgin and lactating rats or in liver of all three groups, but a likely explanation is the residual plasma content of these tissues, which after Triton WR1339 treatment has a high concentration of [^{14}C]lipid (Table 1). If it is assumed that the plasma content of the white-adipose-tissue mass is 2.5%, then it can be calculated that some 65–85% of the apparent [^{14}C]lipid after Triton WR1339 is due to plasma contamination. Similar calculations, assuming a 5% plasma content, would only account for 6% of the observed [^{14}C]lipid accumulation in mammary gland (Table 2). Inclusion of the [^{14}C]lipid accumulated in mammary gland and pups after Triton WR1339 gives a value for the percentage recovery of absorbed [1-^{14}C]triolein of 98% for lactating rats. Thus Triton WR1339 appears to be relatively ineffective in inhibiting triacylglycerol uptake into mammary tissue. This is not due to differences in availability of the detergent, because the plasma concentrations were 4.8 ± 0.13, 6.0 ± 0.35 and 6.5 ± 0.13 mg/ml in virgin, lactating and 48 h-weaned rats respectively.

The apparent failure of Triton WR1339 to prevent accumulation of [^{14}C]lipid into lactating mammary gland suggests that the lipoprotein lipase in this tissue is different, in terms of sensitivity or accessibility of the Triton-WR1339-coated lipoproteins, from that of adipose tissue or muscle. Lipoprotein lipase activity extracted from acetone-dried powders of mammary gland was completely inhibited by 12.5 μg of Triton WR1339/ml (results not shown). There is evidence that lipoprotein lipase of mammary gland has different properties, in that there is a lack of heparin-releasable enzyme in intact cells and membrane fragments (Clegg, 1981*a*,*b*), although release of the enzyme has been shown in perfused glands (Scow *et al.*, 1973). Unlike the enzyme of adipose tissue, that of mammary tissue appears more resistant to a decrease in activity on starvation (Oller do Nascimento & Williamson, 1988; Clegg, 1988).

An alternative explanation for the present findings is that the [^{14}C]lipid is trapped in the capillary bed via association with lipoprotein lipase on the surface of endothelial cells. It is unlikely this is the case, because injection of heparin (500 units/kg body wt.) at the end of the experiment to release lipoprotein did not increase plasma radioactivity or plasma triacylglycerols (results not shown).

This study has two important implications for work already published from our laboratory. Firstly, it can be calculated that continued uptake of triacylglycerol into the mammary gland in the presence of Triton WR1339 leads to an underestimate of the rate of entry into plasma during lactation (Agius *et al.*, 1981) by at least 100%. Similarly, Nagata & Zilversmit (1987) have reported that Triton WR1339 does not completely inhibit the utilization of intestinal lipoproteins in rabbits, but the validity of their findings is in doubt, because the dose of Triton WR1339 administered was only 20 mg/100 g body wt., or 20% of that used in the present study.

Secondly, the inhibitory effects of an intragastric triolein load on mammary-gland lipogenesis are partially reversed by simultaneous administration of Triton WR1339, and this was interpreted as indicating a direct effect of absorbed lipid on the gland which was prevented by the inhibition of lipoprotein lipase activity by the detergent (Mercer & Williamson, 1988). In view of the present results, this interpretation is no longer tenable, although they do not exclude the possibility that the detergent acts by decreasing the formation of ketone bodies in liver, owing to the inhibition of chylomicron-remnant production in muscle.

Finally, these results underline the value of the [1-^{14}C]triolein load as a test for functional lipoprotein lipase activity *in vivo*.

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