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Rates of triacylglycerol entry into the circulation in the lactating rat

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The rate of entry of triacylglycerols into the circulation in lactating rats decreased after parturition and increased in mid-lactation. The decrease in entry rate after parturition may contribute to the disappearance of the hyperlipaemia of pregnancy. A method is described for the determination of the rate of entry *in vivo* of both triacylglycerols and lipid synthesized *de novo*.

The plasma triacylglycerol concentration increases during the last few days of pregnancy and decreases at parturition; it increases slightly or shows little change during lactation (Scow *et al.*, 1964; Bosch & Carnejo, 1967; Zinder *et al.*, 1974). The decrease in concentration at parturition has been attributed to the removal of triacylglycerol by the mammary gland (Otway & Robinson, 1968). The rate of entry of triacylglycerol into the circulation has been studied during pregnancy (Otway & Robinson, 1968), but not during lactation, and the possibility that changes in the plasma triacylglycerol concentration during lactation may be related to changes in the rate of entry of triacylglycerol into the circulation needs also to be considered. Evidence is presented in this paper that the rate of entry of triacylglycerol into the circulation decreases in early lactation relative to the virgin rat, but increases again in mid-lactation. We have shown previously that the rate of hepatic lipogenesis in lactating rats increases after premature removal of the pups (Agius *et al.*, 1979). In the present paper we report the effects of premature removal of the pups on the rate of entry into the circulation of triacylglycerols and lipid synthesized *de novo*.

Experimental

Albino Wistar rats fed *ad libitum* on Oxoid breeding diet (Oxoid, London S.E.1, U.K.) were maintained on a 12h-light/12h-dark cycle), the light period running from 08:00 to 20:00h. Virgin rats weighed 200–240g. At birth all litters were culled to 10 pups. The early-lactating rats (less than 24h *post partum*) weighed 260–320g and the mid-lactating rats (10–14 days *post partum*) weighed 320–350 g. Weaned rats (340–390g) refers to mid-lactating rats which had their litters removed 24h before the experiment. All experiments were started between 08:00 and 09:00h.

The rate of entry of triacylglycerol into the circulation was measured by a modification of the method of Otway & Robinson (1967). The rats were anaesthetized with ether for 4min, and the detergent Triton WR1339, which prevents the removal of triacylglycerol from the plasma (Scanu, 1965), was injected [1 ml of 10% (w/v) in 0.15M-NaCl] into the femoral vein. Triton WR1339 was a gift from Dr. R. B. Fears, Beecham Pharmaceutical Research Division, Tadworth, Surrey, U.K. Blood (0.4ml) was collected from the tip of the tail (under ether anaesthesia; 2min) at 1h intervals for 4h. Plasma triacylglycerol was determined by a modification of the method of Eggstein & Kreutz (1966). Plasma (0.1ml) was saponified with 0.5ml of alcoholic KOH [2.8% (w/v) in 90% (v/v) ethanol] at 70°C for 90min. The protein was then precipitated with 0.1M-$MgSO_4$ (1.5ml) and the glycerol was determined by an enzymic method (Eggstein & Kreutz, 1966). The concentration of free glycerol in the non-saponified plasma was negligible relative to that after saponification of the plasma lipid.

The increase in plasma triacylglycerol concentration was linear with time during the first 4h after Triton injection. From the rate of increase of plasma triacylglycerol concentration, and assuming a plasma volume of 4% of the body weight (Otway & Robinson, 1967; Lovo & Hustvedt, 1975), the increase in total plasma triacylglycerol was determined and hence the rate of triacylglycerol entry into the circulation. The results were expressed as μmol of triacylglycerol entering the circulation/h per 100g body wt. or per rat. On a fat-free diet the liver is

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responsible for approx. 80% of the triacylglycerol entering the circulation (Byers & Friedman, 1960; Reaven *et al.*, 1979), and the results were also expressed on a unit-liver-weight basis.

The rate of increase of lipid synthesized *de novo* into the circulation was measured by combining ^3H_2O incorporation into lipid *in vivo* with Triton inhibition of triacylglycerol removal from the circulation and measuring the rate of increase of ^3H-labelled lipid in the circulation after injection of Triton and ^3H_2O. The rats were anaesthetized with pentobarbital (50mg/kg body wt.) and polythene cannulae (no. 1619R Bardic 1-Catheter, C. R. Bard International Ltd., Clacton-on-Sea, Essex, U.K.; no. 2FG intravenous cannula, Portex Ltd., Hythe, Kent, U.K.) were inserted into the aorta and inferior vena cava through the left femoral artery and vein respectively. They were tied into place, the incision was closed and the experiment started after 30min. Triton WR1339 was injected through the venous cannula followed by 5mCi of ^3H_2O (0.5ml). ^3H_2O was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Blood samples (0.8ml) were withdrawn at 1h intervals for 5h from the arterial cannula, followed by the injection of an equal volume of saline into the venous cannula. The cannulae were left filled with saline (0.15M-NaCl), as it was found relatively easy to dislodge the small clots. The plasma ^3H_2O specific radioactivity, which was determined at 1h intervals, remained constant throughout the experiment. From each blood sample 0.1ml of plasma was used for determination of triacylglycerol and 0.5ml of whole blood for determination of tritiated lipid as described previously (Robinson *et al.*, 1978). The radioactivity incorporated into blood lipid increased linearly between 2 and 5h after ^3H_2O injection (results not shown). The rate of secretion of fatty acid synthesized *de novo* was determined from the total ^3H in saponified lipid of blood, assuming that 13.3 μmol of ^3H are incorporated into 1 μmol of fatty acid (Jungas, 1968).

Results and discussion

The plasma triacylglycerol concentration was lower in early-lactating rats (less than 24h *post partum*) than in mid-lactating rats (10–14 days *post partum*) (Table 1). Removal of the pups for 24h from mid-lactating rats resulted in a 3-fold increase ($P < 0.005$) in the plasma triacylglycerol concentration (Table 1). The rate of triacylglycerol entry into the circulation for virgin rats, measured in this study (Table 1), agrees with the results of Otway & Robinson (1967, 1968). After parturition the rate of triacylglycerol entry, expressed on a unit-body-weight basis, decreased by about 57% relative to the rate for virgin rats (Table 1; Otway & Robinson, 1968) or pregnant rats (Otway & Robinson, 1968). The rate of triacylglycerol entry for mid-lactating rats, expressed on a whole-rat basis, was higher (79%; $P < 0.005$) than that of early-lactating rats, but similar to that for virgin rats. However, on a unit-body-weight basis the rate for mid-lactating rats was lower (30%; $P < 0.0025$) than that for virgin rats. Removal of the pups (24h) at mid-lactation resulted in a 50% increase ($P < 0.01$) in the entry rate. On a whole-rat basis the rate of triacylglycerol entry was higher in lactating-weaned rats than in virgin rats (Table 1). On a unit-liver-weight basis the rate of triacylglycerol secretion was highest in virgin rats and lowest in early-lactating rats.

The decrease in triacylglycerol entry in early lactation and the increase in mid-lactation when the pups were removed for 24h (Table 1) parallel the changes in the rates of fatty acid synthesis *de novo* in the liver, which decreases in early lactation (Agius & Williamson, 1980) and increases when the pups are removed for 24h in mid-lactation (Agius *et al.*, 1979). A correlation between hepatic fatty acid

Table 1. *Rates of entry of triacylglycerol into the circulation in vivo in virgin, early-lactating, mid-lactating and 24h-weaned rats*

For experimental details see the text. The results are means ± S.D. for the numbers of rats shown in parentheses. Values that are significantly different by the Student's *t* test from the corresponding values for the virgin rat are shown: *$P < 0.05$; **$P < 0.005$.

Parameter	Virgin (9)	Early-lactating, 24h *post partum* (5)	Mid-lactating, 10–14 days *post partum* (5)	24h-weaned (mid-lactating) (5)
Body wt. (g)	224 ± 17	301 ± 29**	336 ± 16**	357 ± 32**
Liver wt. (g)	8.5 ± 0.7	12.8 ± 1.9**	16.4 ± 1.4**	17.0 ± 2.0**
Plasma triacylglycerol (mM)	1.08 ± 0.33	0.66 ± 0.20*	1.06 ± 0.22	3.47 ± 0.90**
Triacylglycerol secretion				
μmol/h per 100g body wt.	29.2 ± 4.2	12.6 ± 2.3**	20.3 ± 2.9**	29.0 ± 8.2
μmol/h per rat	67.1 ± 11.2	37.7 ± 6.0**	67.3 ± 8.2	102.5 ± 23.0**
μmol/h per g of liver	7.9 ± 1.4	3.0 ± 0.8**	4.2 ± 0.7**	6.2 ± 1.9*

synthesis *de novo* and triacylglycerol secretion was reported for perfused liver from fed and starved–re-fed rats (Windmueller & Spaeth, 1967), and it was suggested that the rate of fatty acid synthesis may be an important determinant of lipid secretion. Insulin increases the rate of secretion of triacylglycerol in the perfused liver (Topping & Mayes, 1972), and glucagon depresses the synthesis of triacylglycerol from exogenous fatty acids in hepatocytes (Geelen *et al.*, 1978). The increase in the rate of triacylglycerol secretion in mid-lactating rats after removal of the pups for 24 h (Table 1) may be related to the increase in the plasma insulin concentration (Agius *et al.*, 1979). There is no evidence for a lower plasma insulin concentration in early-lactating rats relative to mid-lactating rats; however, the plasma glucagon concentration rises on the last day of pregnancy (Girard *et al.*, 1973; Saudek *et al.*, 1975), and it is possible that the plasma insulin/glucagon ratio may be lower in early lactation than in mid-lactation. This may explain the lower rate of triacylglycerol entry in early-lactating rats. It is suggested that the low rate of triacylglycerol entry into the circulation in early-lactating rats observed in this study may be an important contributory factor to the disappearance of the hyperlipaemia of pregnancy.

Fatty acids utilized for glycerolipid synthesis in the liver may originate from plasma non-esterified fatty acids, hepatic synthesis *de novo* or degradation of hepatic or plasma lipoproteins (Nikkila, 1969). In most metabolic situations, plasma non-esterified fatty acids are the predominant precursors (Schonfield & Pfleger, 1971; Topping & Mayes, 1972). Direct measurements of the rate of secretion of lipid synthesized *de novo* have been performed in the perfused liver (Windmueller & Spaeth, 1966). In the present study the rate of secretion of lipid synthesized *de novo* was measured *in vivo* by combining ^3H_2O incorporation into lipid with Triton inhibition of triacylglycerol removal from the circulation. The rate of entry of lipid synthesized *de novo* in the mid-lactating rats that were left with their litters was about 10% of the rate of entry of triacylglycerol (Table 2). After removal of the pups for 24 h the rate of secretion of lipid synthesized *de novo* increased by 240% (Table 2) and the contribution to the rate of entry of triacylglycerol was about 19%. This increase, however, does not account for the difference in the rate of triacylglycerol entry between lactating and weaned rats. This method of combining measurements of triacylglycerol (non-radioactive) entry with entry of newly synthesized lipid (^{3}H-labelled) should also be applicable to situations where short-term changes occur in lipid metabolism.

Table 2. *Rates of entry of triacylglycerol and lipid synthesized de novo into the circulation in mid-lactating and 24 h-weaned rats*

For experimental details see the text. Rates of lipid synthesized *de novo* are expressed as μmol of fatty acid, and the rates of triacylglycerol secretion are expressed as μmol of triacylglycerol. Values are means ± S.D. for the numbers of rats shown in parentheses. Values that are significantly different by the Student's *t* test from the corresponding values for the mid-lactating rat are shown: *$P<0.05$; **$P<0.005$.

Rate of secretion	Mid-lactating (7)	24 h weaned (4)
Triacylglycerol		
μmol/h per 100 g body wt.	20.2 ± 7.5	32.1 ± 4.3*
μmol/h per rat	59.8 ± 24.0	104.7 ± 17.3**
Fatty acid synthesized *de novo*		
μmol/h per 100 g body wt.	5.8 ± 0.9	18.7 ± 10.2**
μmol/h per rat	17.8 ± 7.8	60.6 ± 31.8**

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References

Agius, L. & Williamson, D. H. (1980) *Biochem. J.* **190**, 477–480

Agius, L., Robinson, A. M., Girard, J. R. & Williamson, D. H. (1979) *Biochem. J.* **180**, 689–692

Bosch, V. & Carnejo, G. (1967) *J. Lipid Res.* **8**, 138–141

Byers, S. O. & Friedman, M. (1960) *Am. J. Physiol.* **198**, 629–631

Eggstein, M. & Kreutz, F. H. (1966) *Klin. Wochenschr.* **44**, 262–267

Geelen, M. J. H., Groener, J. E. M., DeHaas, C. G. M., Wisserhof, T. A. & Van Golde, L. M. G. (1978) *FEBS Lett.* **90**, 57–60

Girard, J. R., Cuendet, G. S., Marliss, E. B., Kervan, A., Rieutort, M. & Assan, R. (1973) *J. Clin. Invest.* **52**, 3190–3200

Jungas, R. L. (1968) *Biochemistry* **7**, 3708–3717

Lovo, A. & Hustvedt, B.-E. (1975) *Biochim. Biophys. Acta* **409**, 51–58

Nikkila, E. A. (1969) *Adv. Lipid Res.* **7**, 63–134

Otway, S. & Robinson, D. S. (1967) *J. Physiol. (London)* **190**, 321–332

Otway, S. & Robinson, D. S. (1968) *Biochem. J.* **106**, 677–682

Reaven, G. M., Risser, T. R., Ida Chen, Y.-D. & Reaven, E. P. (1979) *J. Lipid Res.* **20**, 371–378

Robinson, A. M., Girard, J. R. & Williamson, D. H. (1978) *Biochem. J.* **176**, 343–346

Saudek, C. D., Finkowski, M. & Knopp, R. H. (1975) *J. Clin Invest.* **55**, 180–187

Scanu, A. M. (1965) *Adv. Lipid Res.* **3**, 63–138

Schonfield, G. & Pfleger, B. (1971) *J. Lipid Res.* **12**, 614–621

Scow, R. O., Chernick, S. S. & Brinley, M. S. (1964) *Am. J. Physiol.* **206**, 796–804

Topping, D. L. & Mayes, P. A. (1972) *Biochem. J.* **126**, 295–311

Windmueller, H. G. & Spaeth, A. E. (1966) *J. Biol. Chem.* **241**, 2891–2899

Windmueller, H. G. & Spaeth, A. E. (1967) *Arch. Biochem. Biophys.* **122**, 362–369

Zinder, O., Hamosh, M., Fleck, T. R. C. & Scow, R. O. (1974) *Am. J. Physiol.* **226**, 744–748