Acute meal-induced changes in hepatic glycerolipid metabolism are unimpaired in severely diabetic rats: implications for the role of insulin

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Received 13 July 1995

Abstract The effect of food intake on the partitioning of diacylglycerol between phospholipid and triacylglycerol synthesis, and on the fractional rate of secretion of triacylglycerol was studied in starved-refed diabetic rats by using the technique of selective labelling of hepatic fatty acids in vivo. Acute and phasic responses in these parameters similar to those observed previously in normal animals were obtained, in spite of the absence of any insulin response to refeeding. Labelling of the major phospholipids (phosphatidylcholine and phosphatidylethanolamine) increased markedly at the expense of triacylglycerol labelling. In addition, the fractional rate of secretion of newly-labelled triacylglycerol was decreased. The data suggest that insulin is not obligatorily involved in any decrease in hepatic triacylglycerol secretion in the prandial period, but that it may act synergistically with other meal-induced signals to mediate this effect in normal animals.

Key words: Insulin; Liver; ApoB; Cell volume; Secretion

1. Introduction

The rate of secretion of triacylglycerol (TAG) by the liver is the resultant of at least three main processes: (i) the rate of de novo synthesis of diacylglycerol (DAG) (determined by the availability of acyl-CoA and its partitioning between oxidation and esterification); (ii) the partitioning of DAG between phospholipid (PL) and TAG synthesis; and (iii) the partitioning of the synthesised TAG between the cytosolic and secretory pools. In previous studies, we have observed that when hepatic fatty acids are selectively pulse-labelled in vivo with 1-[^{14}C]oleate, both the partitioning of label into phospholipids and into the intracellular (presumably predominantly cytosolic) pool of triacylglycerol are actually increased during the prandial period (in 24 h-starved-refed [1] and metal-fed [2] rats). Consequently, the partitioning of [^{14}C]-fatty acid label towards secretion is greatly decreased, due to the cumulative (multiplicative) effects of these changes [3]. Such a diversion of flux away from TAG secretion would complement the effects of the decrease in the rate of non-esterified fatty acid (NEFA) delivery to the liver that occurs during a meal, such that the absolute rate of TAG secretion by the liver would be expected to be efficiently curtailed. This scenario would appear to fit in with the observations (see e.g. [4–7]) that insulin actually inhibits TAG and apoB secretion by cultured hepatocytes. Consequently, it has been suggested that the prandial increase in portal insulin concentration is sufficient to inhibit VLDL secretion by the liver [8–10]. However, the role of insulin in the control of TAG secretion has proved controversial, partly because some workers have found that the hormone stimulates TAG secretion in the perfused liver [11] and in prolonged cultures of rat hepatocytes [12] and partly because conditions characterised by hyperinsulinaemia in vivo are associated with an increased rate of TAG secretion by the liver (see e.g. [13]).

Therefore, in the present study we have employed the technique of selective labelling of hepatic fatty acids in vivo to address the question as to whether a normal insulin secretory response is required for meal-induced changes in the partitioning of DAG and TAG to occur. The results suggest that insulin does not play an obligatory role in these responses, as they are unimpaired in severely diabetic rats given a meal after a period of starvation.

2. Materials and methods

2.1. Animals

Female Wistar rats (180–200 g) were made severely diabetic by a single intraperitoneal injection of streptozotocin (80 mg/kg). Two sets of rats were used. The first set were fitted with a jugular cannula under halothane anaesthesia 4 days after streptozotocin injection. They were allowed to recover for a further 5 days, after which time their food intake had been restored to normal. The second set were made diabetic and used 10 days later without further treatment. In both groups blood glucose concentrations were >35 mM and plasma insulin <3 μU/ml. Male rats (400–500 g) were used as donors of post-heparin plasma from which remnant lipoproteins were prepared. They were given sucrose in their drinking water (10%) for 48 h prior to being anaesthetised. After functional hepatotectomy they were injected with heparin (100 U/kg body weight, in 0.5 ml saline) and, after 10 min, exsanguinated through the aorta. The plasma was used for the preparation of cholesteryl 1-[^{14}C]oleate- and [^{3}H]cholesterololeoyl ether-labelled VLDL- and chylomicron remnants (d < 1.016) [14].

2.2. The partitioning of hepatic fatty acids into glycerolipids in vivo

These measurements were performed on the set of rats fitted with the jugular cannulae. The animals were starved for 24 h and injected with a known dose of the labelled lipoproteins (approximately 100,000 dpm, 3 μg protein) before, or during the course of refeeding. Control, fed animals were injected 2 h into the light phase. The animals were placed into metabolic chambers and, after 15 min, they were injected with 1 ml of 10% Triton WR 1339 solution in filtered saline to inhibit peripheral utilisation of labelled secreted TAG and allow the [^{14}C]TAG accumulation during the last 45 min prior to sampling, at which time they were anaesthetised, their abdominal cavity was opened, a sample of aortic blood was taken and a lobe of the liver was freeze-clamped between metal blocks cooled in liquid N_2. The rest of the liver was excised and frozen in liquid N_2. The total liver weight was recorded. The labelled lipid fractions from the liver and plasma of animals or from the cells and incubation media of hepatocyte incubations were analysed as described previously after separation of the various lipid fractions by thin layer chromatography [14]. It is to be emphasised that Triton was only injected during the last 45 min of any refeeding period (see [1,2] for full details).

The second group of animals (non-cannulated) were subjected to the same starvation-refeeding protocol and used to obtain either aortic or

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SSDI 0014-5793(95)00838-1

portal blood samples, as indicated. These animals did not receive Triton. Plasma triacylglycerol was quantitated using a commercial kit (Sigma, Poole, UK, cat. no. 337-10A) and glucose was measured spectrophotometrically in perchloric acid extracts of whole blood [15]. Portal serum insulin concentrations were measured by Dr. L. Ashworth, Department of Medicine, University of Newcastle-upon-Tyne as described previously [16].

3. Results and discussion

The rats used in this study were severely diabetic and had a mean blood glucose concentration value of 38.0 ± 2.0 mM and aortic serum insulin of 1.7 ± 1.2 μU/ml in the fed state. After 24 h starvation their blood glucose concentration fell to 19.5 ± 2.1 mM, indicating that the rate of gluconeogenesis could not be increased sufficiently to compensate for the loss of dietary carbohydrate intake. Immediately upon refeeding, however, the blood glucose level was restored (Fig. 1). There was a much more gradual increase in plasma triacylglycerol concentration during refeeding; although the concentration increased 3-fold after 4 h of refeeding it was still less than half that observed in fed diabetic animals even after 7 h of refeeding. This may have been due to a combination of the following: (i) a refeeding-induced enhancement in the rate clearance of TAG-rich lipoproteins, (ii) a slow rate of secretion of chylomicrons from the gut, or (iii) an inhibition of hepatic VLDL-TAG secretion upon refeeding. The data described below suggest that the last-mentioned possibility may play an important role.

3.1. Partitioning of DAG and TAG

In our previous studies [1,2] we observed that refeeding after a period of starvation results in an acute and transient diversion of the labelled [^{14}C]oleate away from incorporation into triacylglycerol and into the labelling of the major phospholipids, phosphatidylethanolamine (PE) and phosphatidylcholine (PC). There was a concomittant inhibition in the fractional rate of secretion of newly-labelled TAG. The consequences of such concerted effects have been discussed elsewhere [3]. One plausible explanation for these coordinated changes (the time-courses of which are very similar) is that they are both mediated by the meal- induced increase in insulin secretion that would be expected to occur during the prandial period in normal animals. The results obtained in the present study (Fig. 2) suggest that any role of insulin is likely not to be obligatory. Thus, although there was no increase in portal serum insulin concentration upon refeeding of the starved diabetic animals (see Table 1), we observed changes in both DAG- and TAG-partitioning similar to those observed in non-diabetic rats. Indeed, the changes occurred more rapidly than in normal animals and were both more pronounced and longer lasting than in non-diabetic animals (see [1,2]). These results may suggest that (a) changes in DAG and TAG partitioning occur in the absence of any meal-induced insulin secretion, and (b) that if insulin plays a role in the response elicited in normal, non-diabetic animals, this can be mimicked and/or compensated for effectively in diabetic animals through some other physiological effect associated with food intake and/or absorption.

Table 1
Absence of insulin secretory response to food intake in streptozotocin-diabetic rats

Animals	Portal serum insulin concentration (μU/ml)	
	Starved	Starved-refed
Diabetic	4.3 ± 0.8(4)	5.6 ± 3.2(4)
Normal	8.1 ± 5.8(3)	38.4 ± 4.1(3)

Rats were made diabetic by injection of 80 mg streptozotocin per kg body weight. After 10 days they were either starved for 24 h and refed for 1 h, or used in the starved state. The average food intake during 1 h of refeeding was 4.5 ± 0.2 g for diabetic, and 2.3 ± 0.1 for normal rats. Portal blood was sampled after anaesthesia with pentobarbital (60 mg per kg). Values are means (± S.E.M.) for the number of animals shown in parentheses.

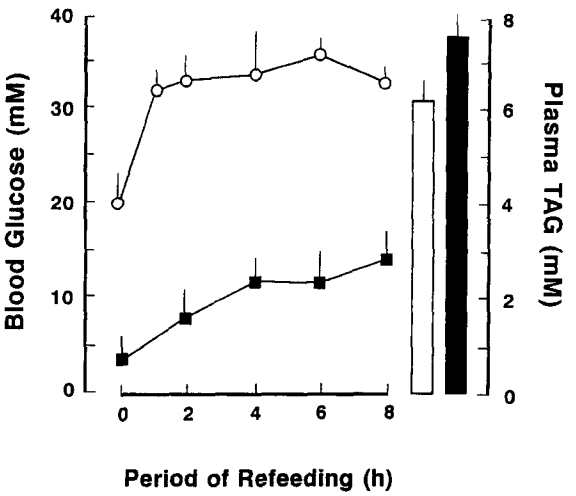

Fig. 1. Time course of changes in blood glucose (○) and plasma triacylglycerol (■) concentrations in 24 h-starved diabetic rat refed ad libitum for the number of hours indicated. Vertical bars indicate the values in fed diabetic animals for glucose (empty bar) and triacylglycerol (solid bar). Values are means (± S.E.M.) for four animals.

The mechanism through which the shift from TAG to phospholipid labelling (mostly PC and PE, see below) occurs is of obvious interest, but outside the scope of the present study. Nevertheless, it is worth noting that the present data suggest that it is unlikely that this is achieved through a decrease in membrane diacylglycerol concentration in response to a decrease in fatty acid availibility for esterification. Such a mechanism would be expected to operate under conditions of decreased fatty acid supply to the liver if the affinity of diacylglycerol acyltransferase (DGAT) in the membrane for DAG is lower than that of the ethanolamine- and choline phosphotransferases, as suggested in [17]. However, the fact that we observe these changes both in diabetic animals (in which NEFA concentration increases upon refeeding; see [16]) and in normal animals (in which NEFA decrease upon refeeding [1,2]), suggests that this is unlikely to be the explanation.

3.2. Relative labelling of PC and PE

Previous studies [15] have suggested that the synthesis of PC and PE may be differentially affected by starvation and refeeding. Therefore, in the present study we wanted to find out whether the large increase in the relative proportion of [^{14}C]oleate label diverted to phospholipid at the expense of triacylglycerol labelling during refeeding was accompanied by any changes in the relative proportions of PC and PE labelled. (As in our previous studies [1,2,16] we found that only a very minor proportion of newly-labelled phospholipids were secreted into the plasma. Therefore, only the intra-hepatic

labelled phospholipids were analysed.) As shown in Fig. 3., there was a progressive increase in the proportion of total phospholipid-associated label that could be accounted for in PE at the expense of labelling of PC. These changes occurred much more slowly than that observed for the increased partitioning of DAG to phospholipid labelling and peaked only after 5 h of refeeding of the diabetic rats, by which time the partitioning of DAG had almost fully reverted to its basal pattern (Fig. 2). Several possibilities could account for the observed change in PC/PE labelling, including changes in the effective activity of CTP: phosphocholine cytidyltransferase (CT) and in the rate of methylation of PE to PC. The activity of CT is known to be regulated acutely through changes in translocation to a particulate fraction within the cell and possibly through reversible phosphorylation (see [18] for review). The respective transferases may have different affinities for different species of diacylglycerol [15] and the prevalence of these may change in the membrane pool(s) concerned. It is also possible that differences in PC and PE labelling may arise from different rates of turnover and/or remodelling [19]. In view of the fact that the label is effectively provided as 1-[^{14}C]oleate within the liver cell in our experiments, any change in the preferential use of the monoenoic fatty acid for PC synthesis [20] may affect the labelling pattern.

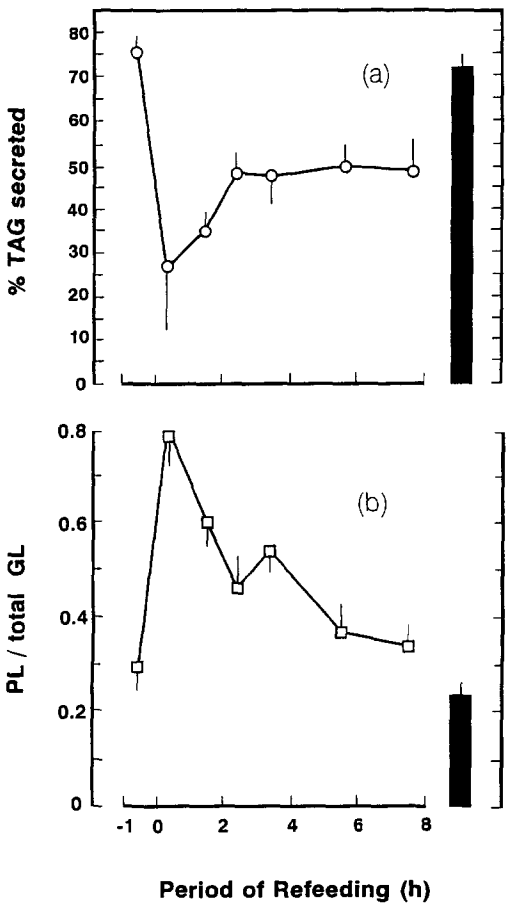

Fig. 2. Acute changes in (a) the fraction of newly-labelled TAG secreted into the plasma and (b) the partitioning of 1-[^{14}C]oleate label between phospholipid and triacylglycerol. Vertical bars indicate the values obtained in fed diabetic animals. PL, phospholipid; total GL, total glycerolipid. Values are means (±S.E.M.) for four animals for each time point.

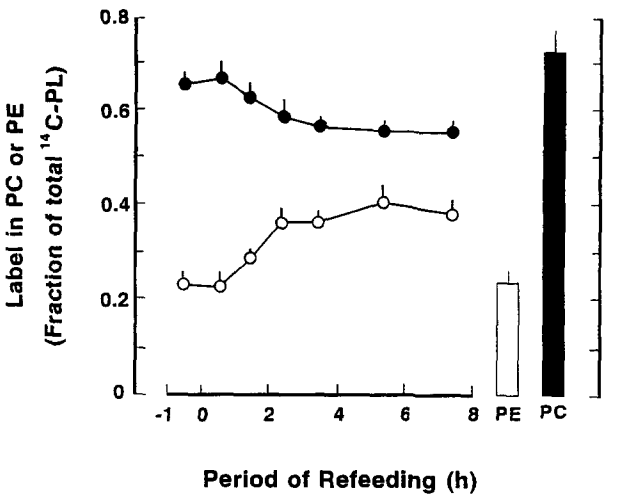

Fig. 3. Changes in the relative incorporation of 1-[^{14}C]oleate into phosphatidylcholine (●) and phosphatidylethanolamine (○) in the livers of 24 h-starved diabetic rats refed for the periods indicated. The vertical bars indicate the values obtained for fed diabetic rats. Values (means ±S.E.M.) are expressed as a percentage of the label incorporated with the total phospholipid fraction.

In conclusion, these studies show that the diversion of flux of acyl moieties away from the secretory pool of TAG [21] that occurs in normal animals upon (re)feeding also occurs in several diabetic rats. Indeed, the increase in PL/TAG labelling ratio, and the decrease in the fractional rate of secretion of labelled TAG were of a larger magnitude and occurred more rapidly than in normal animals (compare with data in [1,2]). These observations suggest that, if insulin is involved in the inhibition of very-low-density-lipoprotein-TAG secretion during the prandial/early absorptive phase, its secretion is not obligatory for the response to occur. It is possible that, in the normal rat, other meal-induced changes e.g. increases in the portal concentrations of osmotically active substrates and ions (such as Na^+-cotransported amino acids and K^+ ions), which would be anticipated to alter liver metabolism through hepatocyte swelling [22], could also be involved synergistically with the effects of insulin (which can increase cell volume in its own right [23]). In diabetic animals or in insulin-resistant states, the effects of these other factors would compensate for the absence of insulin action on the liver. For example, it is known that amino acid transport across the hepatocyte plasma membrane is increased several-fold in diabetic animals [24] and this may account for the more rapid and extensive changes in glycerolipid metabolism observed in the present study.

Acknowledgements: We thank Ms A.M. Caldwell for excellent assistance. This work was supported by the Leverhume Trust and the Scottish Office Agriculture and Fisheries Department.

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