Insulin, diabetes and hepatic very-low-density lipoprotein metabolism

GEOFFREY F. GIBBONS
Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE, U.K.

Triacylglycerol synthesized in the liver from dietary carbohydrate is secreted into the plasma and delivered to peripheral tissues as very-low-density lipoprotein (VLDL). Fatty acids are then derived from the VLDL triacylglycerol by the action of lipoprotein lipase (EC 3.1.1.34) in these peripheral tissues, particularly muscle and adipose tissue sites. In muscle, the fatty acids may be utilized as a source of energy, whereas in adipose tissue they are re-esterified and stored as triacylglycerol. Insulin plays a key role in the co-ordination of these events, the efficiency of which breaks down under conditions of insulin deficiency or insulin resistance. Some consequences of this breakdown include abnormalities of lipoprotein metabolism which are responsible for the hyperlipidaemia characteristic of the diabetic states. This paper has two objectives. First, to review what is known of the role of insulin in controlling VLDL secretion from the liver. Secondly, to discuss how recent findings in this area could account, in part, for the abnormalities of VLDL metabolism observed in the insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) forms of diabetes. Because these diabetic states differ from each other in their effects on lipoprotein metabolism, each will be dealt with separately below.

Hepatic VLDL secretion in NIDDM

This form of diabetes is often referred to as type II or maturity-onset diabetes and is characterized partly by tissue resistance to insulin. There is general agreement that this form of diabetes is associated with an increase in the rate of VLDL production (for a review, see [1]). However, controversy exists as to the exact cause of this defect, particularly in regard to the role of dietary carbohydrates in the diseased state. This paper has two objectives. First, to review what is known of the role of insulin in controlling VLDL secretion from the liver. Secondly, to discuss how recent findings in this area could account, in part, for the abnormalities of VLDL metabolism observed in the insulin-dependent (IDDM) and non-insulin-dependent (NIDDM) forms of diabetes. Because these diabetic states differ from each other in their effects on lipoprotein metabolism, each will be dealt with separately below.

Abbreviations used: VLDL, very-low-density lipoprotein; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; apo, apolipoprotein.

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Short- and longer-term effects of insulin on hepatic VLDL secretion

NIDDM is often associated with an absolute increase in the level of plasma insulin, particularly in the postprandial state [1, 14]. The direct effect of insulin, however, on hepatic VLDL secretion is a matter of some controversy. First, it has been proposed that the hyperinsulinaemia which occurs in insulin-resistant states is directly responsible for the enhanced secretion of hepatic VLDL [4, 15]. Several more recent studies, however, have shown that when insulin was added to isolated hepatocytes for periods up to 24 h, the secretion of VLDL triacylglycerol was diminished [3, 12, 16–20]. There was also a decline in the secretion rate of VLDL-associated phospholipid [16, 18], cholesterol ester [20], apolipoprotein (apo) B and apo E [16, 17, 19]. The inhibitory effect on the secretion of non-esterified cholesterol was slight compared with that on esterified cholesterol secretion [20] and this gave rise to changes in the ratio of these lipids in the newly secreted VLDL.

It might be argued that the inhibitory effect of insulin on hepatic VLDL secretion is merely an artefact of the in vitro model. However, several studies in vivo have also provided evidence that insulin administration results in a decline in hepatic VLDL production [21–23]. Although a decreased hepatic fatty acid flux resulting from the anti-lipolytic effect of insulin cannot be ruled out, it has been shown, in at least one instance, that the inhibitory effect on VLDL secretion was independent of fatty acid uptake by the liver [22].

Two mechanisms for the inhibitory effect of insulin have been proposed. First, during a 20 h incubation with cultured hepatocytes, insulin, whilst inhibiting the total secretion of apo B and apo E into the medium, also reduced the proportion of these apoproteins which were associated with the VLDL. Concomitantly, the proportion of secreted apoprotein associated with the other fractions of the medium such as low-density lipoprotein and albumin increased. It was thus proposed that insulin inhibited hepatic VLDL secretion by interfering with the normal intracellular association of apoprotein and lipid during the assembly of the nascent particles [16]. Second, insulin inhibited the incorporation of S-methionine into both the large- and small-molecular-mass variants of apo B [19]. Whether this was a secondary effect of the reduced association of lipid and apoprotein (see above) or whether insulin directly inhibited the synthesis of the apo B variants de novo is not yet known.

The physiological role of the inhibitory effect of insulin on hepatic VLDL secretion has been the subject of some debate. During the postprandial period, elevated levels of plasma insulin are coincident with a high rate of hepatic lipid synthesis and an increased entry into the plasma of intestinal...
triacylglycerol in the form of chylomicrons. It has been proposed that the inhibition of hepatic VLDL secretion by the increased circulating insulin serves to prevent an excessive postprandial hyperlipidaemia [3, 19]. Alternatively, or, in addition, the insulin-mediated delay in the secretion of newly synthesized hepatic triacylglycerol means that the liver is able to maintain or increase triacylglycerol output during the preprandial period when insulin levels are low [24]. This arrangement would ensure an adequate supply of triacylglycerol for use as an energy source by muscle tissue when dietary carbohydrate is not immediately available [25]. In any event, it appears that insulin may allow the liver to act as a temporary lipid-storage organ in which triacylglycerol is stored or secreted in a rhythmic fashion depending upon the animal’s nutritional status.

The inhibitory effect of insulin on hepatic VLDL secretion is consistent with an alternative explanation for the increased secretion rates observed in NIDDM, a state which is characterized by insulin resistance. Thus hepatic insensitivity to insulin would result in a deficiency of insulin at the intracellular level. Thus the normal insulin-mediated constraints would be removed and VLDL secretion would increase. This explanation is supported by recent work which showed a positive correlation between plasma VLDL triacylglycerol and the extent of insulin resistance. This relationship was independent of the plasma insulin concentration [26].

Although these short-term effects of insulin on VLDL secretion are relatively clear-cut, changes which occur during longer periods of insulin treatment are more complex and difficult to explain from a physiological viewpoint. For instance, a study of the time course of insulin action in cultured hepatocytes showed that inhibition was maximal between 6 and 12 h after addition of the hormone (Fig. 1). During the next 12 h period, there was a mitigation of the inhibitory effect and continued treatment for a further 24 h led to an actual increase in VLDL triacylglycerol secretion (Fig. 1) [20]. A similar diminution of the inhibitory effect of insulin after long periods of treatment has also been observed previously [17].

**Hepatic VLDL secretion in IDDM**

Over the last several years, many studies using experimental animals have shown that induction of diabetes by injection of anti-insulin serum, alloxan or streptozotocin, results in a decrease in the secretion of VLDL. As measured in perfused livers in vitro [27–31]. Longer-term regulation of hepatic VLDL secretion in diabetes has also been studied by culturing hepatocytes from rats made diabetic 2–3 days earlier using streptozotocin (S. Bartlett, J. Duerden & G. F. Gibbons, unpublished work). Over a 24 h period there was a 50% decrease in the secretion of VLDL triacylglycerol compared with that observed in normal cells. Moreover, however, there was no change in the rate of secretion of non-esterified cholesterol, and the secretion rate of VLDL-cholesterol ester increased about three-fold. Addition of insulin to the diabetic hepatocytes decreased VLDL triacylglycerol output by a further 65% over the 24 h culture period. Thus treatment with insulin, at least for a relatively short period (24 h), was unable to restore the normal rate of hepatic VLDL secretion in chronically insulin-deficient animals.

At first sight, the inhibitory effect of insulin deficiency on hepatic VLDL secretion in the diabetic animals appears at odds with the low rate of VLDL secretion in hepatocytes from normal animals which had been treated with insulin for 24 h. However, the two experimental situations are not directly comparable for at least two reasons. First, the diabetic animals have been insulin-deficient for at least 2 days, whereas, in the cells from normal animals, insulin inhibited VLDL secretion only when the experimental period was confined to 24 h. Longer-term treatment with insulin reversed this effect (see above). Secondly, insulin deficiency is almost invariably associated with high levels of plasma glucagon [32–35] and it is known that glucagon inhibits hepatic VLDL secretion [11, 12]. It is possible, therefore, that, in the whole animal, hyperglucagonaemia reinforces the effects of chronic insulin deficiency in reducing hepatic VLDL output.

**Non-hepatic production of VLDL in IDDM**

There have been some reports that the entry rate of VLDL into the plasma is increased in patients with IDDM [36, 37]. Also, in well-fed experimental animals in which VLDL clearance was blocked, the entry of VLDL into the plasma from all sources was significantly increased in 3-day streptozotocin-diabetic animals (J. Duerden, unpublished work). On the basis of the experiments in vitro described above, hepatic VLDL secretion is decreased under these circumstances. This obviously raises the question as to the source of the increase in whole plasma VLDL which occurs in type I diabetes and in experimental diabetes. In this respect, direct measurement of the appearance of VLDL in mesenteric lymph in rats showed that this was significantly increased in fasted diabetic rats compared with their fasted controls [38, 39]. The involvement of lipoproteins of intestinal origin in diabetes has been the subject of considerable attention (e.g. [40–42]). In support of a significant role for this organ in VLDL production in this state, intestinal triacylglycerol and cholesterol synthesis are known to increase in diabetes [39, 43].
Effect of diabetes on the metabolism of triacylglycerol-rich lipoproteins

CYNTHIA M. ARBEENEY and HOWARD A. EDER
Department of Medicine, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York, NY 10461, U.S.A.

In diabetic humans and in experimental animals the concentrations of triacylglycerol-rich lipoproteins are often markedly increased. The increased levels of these lipoproteins have been shown to be due to increased production by the liver and intestine and/or decreased removal of the triacylglycerol-rich lipoproteins. We have utilized the streptozotocin-diabetic rat model to elucidate the underlying mechanisms responsible for the hyperlipidaemia of diabetes (Bar-On et al., 1976a). Our studies of the pathogenesis of the diabetic hyperlipidaemia have been focused on alterations in removal rates of triacylglycerol-rich lipoproteins in diabetic animals (Arbeeney et al., 1987).

The removal of these lipoproteins involves at least two steps. One is the reduction of the triacylglycerol content of the lipoprotein by the action of lipoprotein lipase (EC 3.1.1.34) that results in the formation of remnant particles which are depleted of triacylglycerol, enriched in cholesterol esters and have alterations in apolipoprotein (apo) composition. The second step is the uptake by the liver of these remnant lipoproteins. Decreased tissue lipoprotein lipase activity in diabetic animals and patients has been found in many studies (Wilson et al., 1987). In some instances, this may be due to reduced amounts of apo C-II present in the triacylglycerol-rich lipoproteins (Bar-On et al., 1976b; O'Loneyn et al., 1985).

We have characterized the lipid and apoprotein composition of the lipoproteins from diabetic rats and have determined the effects of insulin treatment on these parameters. In addition, we have measured the effects of other interventions including the administration of heparin and feeding of fish oil on lipoprotein concentrations and composition.

Rats made diabetic with streptozotocin exhibited hyperglycaemia and hypertriglyceridaemia. Serum triacylglycerol concentrations were elevated more than 6-fold, while serum cholesterol levels were elevated by 67%. In the diabetic rats, very-low-density lipoprotein (VLDL) triacylglycerol, cholesterol and protein concentrations were elevated 7.7-, 9.3- and 4-fold, respectively (Table 1). Low-density lipoprotein (LDL) cholesterol and protein were slightly increased as was high-density lipoprotein (HDL) cholesterol and protein, but

Abbreviations used: apo, apolipoprotein; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LCAT, lecithin:cholesterol acyltransferase (phosphatidylcholine:sterol acyltransferase; EC 2.3.1.43).

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