Role of insulin and counter-regulatory hormones in the control of hepatic glycerolipid synthesis and low-density-lipoprotein catabolism in diabetes

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Diabetes mellitus exists in two forms: the first is characterized by insulin deficiency (insulin-dependent diabetes), and the second results from tissue resistance to insulin (non-insulin-dependent diabetes). The consequence of this relative lack of insulin action is that metabolism is controlled to a greater extent by the counter-regulatory, or 'stress hormones'. The latter include the glucocorticoids, the catecholamines, glucagon and growth hormone [1].

The purpose of this article is to review the role of insulin and the counter-regulatory hormones in controlling the synthesis of glycerolipids and their secretion from the liver in very-low-density lipoprotein (VLDL). The subsequent metabolism of VLDL and the loss of triacylglycerol eventually leads to the production of low-density lipoprotein (LDL). These lipoproteins are mainly removed from the circulation by a process of receptor-mediated endocytosis [2]. The cholesterol that constitutes the major lipid of LDL can then be secreted as such, or as bile salts in the bile. This route also represents a means of excretion, since there is incomplete reabsorption of these compounds in the intestine. Alternatively, it can be re-secreted in lipoproteins or stored as cholesterol ester.

The processes of hepatic lipid synthesis, VLDL secretion and LDL catabolism are major metabolic areas that help to regulate the concentration of triacylglycerol and cholesterol in the circulation. Hypertriglyceridaemia and hypercholesterolaemia are commonly observed in uncontrolled diabetes [1, 3–5], and these are believed to contribute to the increased atherosclerotic risk associated with diabetes.

Effects of insulin and the counter-regulatory hormones on the synthesis of glycerolipids in the liver

The rate at which the liver is able to synthesize glycerolipids depends upon the supply of substrates for this synthesis and upon the activity of the enzymes that are involved. Fatty acids can be supplied by three separate routes. They are formed from hepatic synthesis de novo, from the uptake of chylomicron remnants and from the mobilization of adipose tissue stores.

Synthesis de novo is, of course, depressed in uncontrolled diabetes because of the lack of insulin and the increased effect of glucagon which causes cyclic AMP concentrations to increase in the liver. The supply of chylomicron remnants may increase, since diabetics often consume diets that are relatively rich in fat. Finally, the supply of fatty acids from adipose tissue increases dramatically in uncontrolled diabetes which is characterized by high circulating concentrations of unesterified fatty acid. Thus, the availability of fatty acids should not be equated to the control of fatty acid synthesis, nor should one expect that the control of triacylglycerol synthesis in liver should be necessarily similar to that of lipogenesis.

The net availability of fatty acids for glycerolipid synthesis in the liver is governed by the rate of fatty acid supply relative to the rate of $\beta$-oxidation. This latter process increases strikingly in diabetes so that energy production depends to a large extent on this process. The liver also secretes ketones as an alternative fuel for muscle tissue and the brain (Fig. 1). These events are also promoted by the lack of insulin and the increased effectiveness of glucagon. Although the proportion of fatty acids that are oxidized increases relative to those that are esterified, the absolute rate of triacylglycerol synthesis can be sustained or even increased as a result of the large increase in fatty acid supply from adipose tissue. The liver is the main organ responsible for controlling the rise in un-

![Fig. 1. Some effects of the increased effectiveness of glucocorticoids, glucagon and catecholamines on metabolism](image-url)

The bold lines represent major routes of metabolism. Reprinted from [47] with permission.
esterified fatty acids and it does this by sequestering excess fatty acids as triacylglycerol [6, 7].

The other precursor necessary for triacylglycerol synthesis is glycerolphosphate. This concentration is maintained or increased in starvation and in diabetes [8, 9] because of the increased availability of glycerol from the breakdown of triacylglycerol in adipose tissue, and from gluconeogenic amino acids which are released by protein degradation (Fig. 1). Although glycolysis will be decreased in the liver in diabetes, it should be remembered that glucose is not normally a preferred substrate in hepatic metabolism [1, 10, 11]. There is now increasing evidence that glycerol, lactate and amino acids are normally used preferentially for glycogen and fatty acid synthesis. In diabetes, these processes will be depressed and the precursors will be directed to triacylglycerol synthesis in what would have been unfavourable circumstances for maintain-

It is known that the capacity of the liver to synthesize triacylglycerols in starvation is maintained or increased provided that glycerolphosphate concentrations are optimum [8, 14]. This can be demonstrated by inhibiting \( \beta \)-oxidation with octanoyl-\( \text{(-)}\)carnitine which then diverts fatty acids into esterification. This capacity is partly provided by the activity of phosphatidate phosphohydrolase (phosphatidate phosphatase; EC 3.1.3.4) which increases in starvation, diabetes and several conditions of metabolic stress [6, 7]. These increases are caused by the actions of glucocorticoids, glucagon and growth hormone which appear to stimulate the rate of synthesis of the phosphohydrolase [14, 15]. Glucagon also increases the stability of the phosphohydrolase [17]. The action of insulin is antagonistic to the counter-regulatory hormones in that it prevents the rise in phosphohydrolase activity [15, 16] and it decreases the enzyme's stability [17]. It is also significant that the increases in the activities of phosphatidate phosphohydrolase [18-21] and diacylglycerol acyltransferase (EC 2.3.1.2.) [18, 22] that are seen in diabetes together with a fatty liver, are reversed by the injection of insulin [19, 21]. The rise in phosphohydrolase activity enables the liver to anticipate an increased fatty acid supply by providing it with an enhanced capacity for triacylglycerol synthesis in what otherwise may be unfavourable circumstances for maintain-

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**Fig. 2. Effects of insulin and dexamethasone on the degradation of \( ^{125}\text{I-LDL} \) by hepatocytes**

Cells were prepared and maintained in culture for the first 20 h. After a further hour of incubation with serum-free medium containing albumin, cells were transferred to medium containing the indicated concentrations of hormones [45, 46]. After 6 h at \( 37^\circ\text{C} \) the medium was again changed, the hormone concentrations were maintained and 10 \( \mu\text{g} \) of \( ^{125}\text{I-LDL} \) protein/ml and 1 mM-iodotyrosine were added. The latter was necessary to inhibit further degradation of the liberated \( ^{125}\text{I-tyrosine} \) to free iodine [48]. Degradation was then measured after a further 18 h of incubation. (a) shows the effects of dexamethasone and (b) the effects of insulin alone (●), or in the presence of 10 nm-\( \triangle \) or 100 nm-\( \blacktriangle \) dexamethasone. Results are means ± S.E.M. for the number of independent experiments indicated in parentheses. The results that are significantly different from the corresponding incubation in the absence of dexamethasone (a) or insulin (b) are indicated by asterisks.

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ing the rate of esterification. However, the first physiological requirement may be to produce energy and ketones from the fatty acids. If this is sufficient to control the rise in the accumulation of fatty acids and acyl-CoA esters, then the high phosphohydrolase activity need not be expressed.

It has been proposed that the phosphohydrolase exists in the cytosol of the hepatocyte in a metabolically inactive form [6, 7]. The accumulation of fatty acids, acyl-CoA esters or phosphatidate (caused by increased esterification) causes the cytosolic enzyme to attach to the endoplasmic reticulum and to catalyse diacylglycerol synthesis. Thus, the effective activity of the phosphohydrolase increases in proportion to the fatty acid accumulation [6, 7]. It should also be noted that fatty acids have also been reported to increase the activity of diacylglycerol acyltransferase [23]. Thus, the enzymic control of triacylglycerol synthesis should not be considered in terms of rate-limiting enzymes, but rather the provision of a protective pathway where capacity is not normally exceeded under physiological conditions.

The increased triacylglycerol synthesis in diabetes can be observed in terms of a fatty liver [19] and also through the increased secretion of VLDL that can occur [1, 3-5]. Triacylglycerol secretion in VLDL requires the simultaneous production of phosphatidylcholine [24]. The enzyme that normally regulates this process is phosphocholine:cytidylyltransferase (EC 2.7.8.2) [25]. This enzyme is also activated by a fatty-acid-induced translocation of the cytosolic form of the enzyme to the endoplasmic reticulum [25]. Such a coordinated control of the cytidylyltransferase and the phosphohydrolase helps to provide the required lipids for VLDL secretion [6, 7].

**Effects of insulin and counter-regulatory hormones on the secretion of VLDL**

Glucocorticoids are known to stimulate the secretion of VLDL in vivo [26-28], in perfused liver [29, 30], and in monolayer cultures of rat hepatocytes [30]. Insulin antagonizes this action of glucocorticoids [31]. Furthermore, there is increasing evidence that insulin, especially in the long term, can decrease the secretion of triacylglycerol and apolipoprotein B [31-37]. This conclusion runs contrary to previous views. However, the inhibition of VLDL secretion by insulin has been rationalized by postulating that it normally prevents lipidaemia during periods of enhanced hepatic lipogenesis [4]. Good insulin control is normally associated with normolipidaemia. The lack of insulin action during postprandial periods should facilitate VLDL secretion in order to supply muscle tissues with an alternative source of fuel to glucose [4].

By contrast to these conclusions, other investigators found no significant short-term effects of insulin [31, 38, 39], whereas others reported increases in VLDL secretion [40, 41]. Furthermore, glucagon acting through cyclic AMP [37, 42] and adrenaline through α-adrenoceptors [43] are able to decrease VLDL secretion. Consequently, these acute-acting stress hormones are opposite in their effects to those of the glucocorticoids which normally operate through longer term control.

Despite the controversy that surrounds the action of insulin on VLDL secretion, it is likely that the increased supply of fatty acids in diabetes and the increased effectiveness of the glucocorticoids would help to facilitate the observed increases in hepatic triacylglycerol secretion [6, 7].

**Effects of insulin and the counter-regulatory hormones on hepatic LDL receptors and the catabolism of LDL**

The triacylglycerol of VLDL is hydrolysed preferentially in muscle tissue in uncontrolled diabetes, since the activity of lipoprotein lipase (EC 3.1.1.34) in adipose tissue is diminished because of the lack of insulin action [44]. This degradation first produces intermediate-density lipoproteins and then LDL. Between 70-80% of the LDL are removed from the circulation by the liver by receptor-mediated endocytosis [2].

The binding of LDL at 4°C to the ‘classic’ LDL receptor on rat hepatocytes was measured at 37°C as described by Salter et al. [48] and this is expressed relative to the rate of degradation of the LDL to [125I]iodotyrosine in the same cells. These latter results are taken from one of the experiments described in Fig. 2. $r=0.86$; similar correlations were obtained in six other experiments. The values for incubations containing insulin alone (●), dexamethasone alone (○), insulin plus dexamethasone (▲) or no hormones (△) are shown.

**Fig. 3. Relationship between the binding of 125I-LDL to the ‘classic’ LDL receptor on rat hepatocytes at 37°C and the rate of LDL degradation**

Binding of 125I-LDL to the ‘classic’ LDL receptor of rat hepatocytes was measured at 37°C as described by Salter et al. [48] and this is expressed relative to the rate of degradation of the LDL to [125I]iodotyrosine in the same cells. These latter results are taken from one of the experiments described in Fig. 2. $r=0.86$; similar correlations were obtained in six other experiments. The values for incubations containing insulin alone (●), dexamethasone alone (○), insulin plus dexamethasone (▲) or no hormones (△) are shown.
hypercholesterolaemia. However, it should be mentioned that glaucon and adrenaline appear to have the opposite effects on LDL binding and degradation to that of dexamethasone (D. N. Brindley, N. F. Brown, A. M. Salter, S. C. Fisher & R. Fears, unpublished work). This difference is reminiscent of their effects mentioned above concerning VLDL secretion.

Conclusions

In uncontrolled diabetes the liver supplies other organs with sources of energy in the form of glucose, ketones and triacylglycerols. Fatty acids are released from adipose tissue and are either used directly by muscle tissue or by the liver. The potential of the liver for β-oxidation is increased and this facilitates ketogenesis. However, the increased fatty acid supply can cause an accumulation of fatty acids and acyl-CoA esters and this activates triacylglycerol synthesis. This is facilitated by the movement of phosphatidate phosphohydrolase to the endoplasmic reticulum and it can result in a fatty liver. The long-term control of the phosphohydrolase is similar to that of regulatory enzymes in gluconeogenesis, amino acid breakdown and of the urea cycle [6, 7, 15]. Namely, the activities are increased by glucocorticoids and cyclic AMP with insulin acting antagonistically. This change in endocrine regulation appears to be designed to co-ordinate the secretion of glucose and triacylglycerol (Fig. 1).

The secretion of triacylglycerols in VLDL is also stimulated by glucocorticoids and insulin antagonizes this effect. However, the re-uptake of the LDL particles that result from the metabolism of the VLDL is retarded by glucocorticoids and increased by insulin. Consequently, the lack of the effectiveness of insulin and the increased action of glucocorticoids on these aspects of lipoprotein metabolism can contribute to hypertriglyceridaemia and hypercholesterolaemia. The consequence of this is likely to be an increased risk of premature atherosclerosis in poorly controlled diabetes.

We thank the British Heart Foundation, the Alberta Heart and Stroke Foundation, the Alberta Heritage Foundation for Medical Research, the Humane Research Trust and the Science and Engineering Research Council for financial support.

References