

The extended postprandial phase in diabetes

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Abstract

Atherosclerosis is a major complication of diabetes, yet the reason for this remains obscure. Mechanisms of plaque formation are discussed and, in particular, metabolic alterations in the postprandial phase in diabetes are examined. A major metabolic effect of insulin deficiency is a failure to suppress non-esterified fatty acids. The importance of non-esterified fatty acids in the formation of the lipoproteins is discussed, as well as the effects of non-esterified fatty acids on insulin secretion and glucose transport, since the hallmark of Type II diabetes is insulin resistance. The genesis of large triacylglycerol-rich lipoproteins is examined and, in particular, the formation of the intestinally derived chylomicron particle is discussed in some depth with reference to microsomal triacylglycerol transfer protein and apolipoprotein B48, the structural protein for the intestinally derived lipoproteins. The role of microsomal triacylglycerol transfer protein polymorphisms is mentioned. The final section of this review examines alterations to the low-density lipoprotein particle that are found in patients with diabetes and the mechanisms that create an atherogenic low-density lipoprotein particle in diabetes. In conclusion, the lipoprotein cascade is severely disrupted in diabetes, with a major abnormality being found in the metabolism of non-esterified fatty acids. It appears that, at each level of disruption of the normal pathway, the alterations that have been described have the potential to accelerate cholesterol deposition in the plaque and to cause plaque disruption, explaining in part the increased cardiovascular disease found in diabetes.

Diabetes and vascular disease

Diabetes is predicted to become an epidemic by the year 2025. The major complications of diabetes relate to damage to large and small arteries. On average these reduce the life span by around 8 years, with patients with diabetes having the same risk of MI (myocardial infarction) as non-diabetic patients who have already survived a heart attack [1]. Major risk factors for MI include hypercholesterolaemia, hypertriglyceridaemia, insulin resistance and hypertension. These factors are often found in people with diabetes, and increase the risk of MI in an incremental manner.

The atherosclerotic plaque is formed by the accumulation of cholesterol in the subendothelial compartment of the artery wall. Atherosclerosis is partly an inflammatory process [2]. The steps involved in plaque formation include monocyte and platelet adhesion to the artery wall, macrophage cholesterol accumulation as the monocyte enters the subendothelial space and transforms into the scavenging macrophage, and smooth muscle cell migration and proliferation. The stable plaque, which contains only a small amount of lipid and is protected by a layer of healthy smooth muscle cells, is of little danger, but destabilization of a plaque by metalloproteinase activity exposes the thrombolytic collagen and is an important step in artery occlusion [3]. The cardioprotective HMG-CoA (3-

hydroxy-3-methylglutaryl-CoA) reductase inhibitors, which have a powerful cholesterol-lowering effect, may also reduce inflammation [4].

The postprandial phase

Blood sugar is the biochemical parameter used to diagnose and control diabetes, but there is good evidence to suggest that the metabolic defect in the pathway to clinical diabetes is the dysregulation of fatty acid metabolism, with a rise in the levels of NEFA [non-esterified ('free') fatty acids]. As the late Denis McGarry [5] wrote some years ago "What if Minkofsky were ageusic?" In that paper Professor McGarry suggested that it was the sweet taste of urine that first focused attention on blood sugar as being the important abnormality in diabetes, and for many years glucose metabolism continued to be considered as the major metabolic defect. It is clear that, even in the non-fasting state, NEFA represent an energy source for body tissues that is equivalent to or greater than glucose [6]. Although NEFA concentrations are suppressed following a meal in subjects with diabetes and insulin resistance, the suppression is less than in healthy controls, with both fasting and 2 h postprandial levels being raised considerably.

Normally, binding of insulin to its receptor induces tyrosine phosphorylation of insulin receptor substrate-1 and activation of phosphoinositide-3-kinase. This induces translocation of the Glut4 glucose transporters to the cell surface and promotes glucose uptake. In a series of experiments, Shulman's group [7,8] showed that NEFA inhibit muscle insulin receptor substrate-1 tyrosine phosphorylation and phosphoinositide-3 kinase activation, thus reducing muscle glucose uptake. Many years ago, Randle et al. [9] had

Key words: cardiovascular disease, chylomicron, diabetes, low-density lipoprotein (LDL), microsomal triglyceride transfer protein (MTP), non-esterified fatty acids (NEFA), postprandial phase.

Abbreviations used: apo B, apolipoprotein B; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; LDL, low-density lipoprotein; MI, myocardial infarction; MTP, microsomal triacylglycerol transfer protein; NEFA, non-esterified fatty acids; VLDL, very-low-density lipoprotein.

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demonstrated that excessive oxidation of fatty acids interfered with insulin-mediated glucose uptake by muscle cells. The concept as they described it, however, probably only holds in severe NEFA oversupply, such as occurs in Type I diabetes [6].

Most obese people have raised NEFA levels [10,11], and both acute and chronic elevation of NEFA produce insulin resistance. Acute elevation of NEFA was shown to inhibit glucose uptake, glycogen synthesis and endogenous glucose production in men [12,13]. There was a suggestion that the effect might be sex specific, but recently Homko et al. [14] showed that women are susceptible to the same NEFA-induced insulin resistance as men. An interesting new observation has been that the effect of physiological elevation of plasma NEFA in hyperglycaemia may not be the same as in normoglycaemia. Staehr et al. [15] demonstrated that physiological elevation of plasma NEFA in Type I diabetic patients stimulates gluconeogenesis as well as glycogenolysis and causes mild fasting hyperglycaemia. In the same experiment but using less insulin, so that the patients had moderate hyperglycaemia, these changes were attenuated. That study suggests that, once diabetes has developed, NEFA may lose their importance. Another feature of insulin resistance is intracellular triacylglycerol deposition in muscle, liver and other tissues [16]. However, although this triacylglycerol deposition is associated with insulin resistance, it is probably not the causative agent, but rather a marker for some other fatty acid-derived entity [17].

Although raised NEFA levels are a feature of diabetes and have been shown to impair insulin action, a direct relationship between an increase in NEFA, atherosclerosis and MI has been difficult to establish [18]. On the other hand, NEFA have been implicated in sudden death in a population, which included diabetic patients [19]. It has also been shown that increased NEFA stimulate the cardiac autonomic nervous system in normal subjects [20]. In Type II diabetes, increased postprandial NEFA have been associated with increased oxidative stress and dysregulation of the autonomic nervous system, again implicating postprandial NEFAs in sudden death in diabetes [21].

MTP (microsomal triacylglycerol transfer protein) and lipoprotein synthesis

The chylomicron is the largest of the lipoproteins and is synthesized in the intestine, mostly in the postprandial period, although there is a low level of chylomicrons present even in fasting plasma [22–24]. Chylomicron cholesterol is derived from both dietary cholesterol and cholesterol synthesized in the enterocyte. We have shown increased intestinal cholesterol synthesis in diabetes due to an increase in HMG-CoA reductase [25,26]. The triacylglycerol component of the particle is entirely dietary, while much of the phospholipid comes from bile. MTP is essential for the assembly of the chylomicron in the endoplasmic reticulum of the enterocyte and the VLDL (very-low-density lipoprotein) in the liver [27]. MTP mediates lipidation of

apo B (apolipoprotein B), allowing correct folding of the lipoprotein to proceed [28]. In the absence of MTP or lipid, apo B is quickly degraded. MTP interacts with apo B through ionic binding, and the lysine and arginine residues are particularly important for this association. MTP is thought to bind first to apo B, after which the addition of lipid allows the hydrophilic surface of the apo B to orient towards the aqueous milieu of the endoplasmic reticulum, while the lipid forms the core of the particle [29]. MTP probably facilitates the transfer of further triacylglycerol into the endoplasmic reticulum, where it fuses with the nascent particle to form the large lipid-rich mature chylomicron or VLDL particle.

It is not entirely clear how MTP is regulated *in vivo*. The promoter region of the MTP gene contains a positive sterol response element for cholesterol and a negative insulin response element [30], and studies in isolated hepatocytes have shown up-regulation by cholesterol and down-regulation by insulin [31,32]. Fatty acids and triacylglycerols appear to be the most important physiological regulators of MTP, and Lin et al. [33] have shown up-regulation of intestinal MTP mRNA in hamsters within 24 h of initiating a high-fat diet; the level remained elevated as long as the diet was continued. They found a slower response by the liver, but a high-fat diet significantly increased levels in both organs after 31 days. Bennett et al. [34] showed that the increase in MTP on fat feeding depended on the type of fatty acid, with saturated fat up-regulating MTP mRNA to a greater extent than polyunsaturated fat. We have shown recently that intestinal MTP mRNA expression was lower in statin-treated non-diabetic subjects compared with untreated subjects [35]. The statin-treated subjects had significantly lower plasma cholesterol levels, but there was no correlation between plasma cholesterol and MTP mRNA [35]. We have also examined the effect of diabetes on intestinal MTP in animal models. We found a 4-fold increase in MTP mRNA expression in streptozotocin-diabetic rats compared with control rats [36]. We cannulated the lymph duct of these animals in order to ensure that we could relate intestinal MTP expression to chylomicron production without the confounding effect of hepatic clearance [37]. We found that the increase in MTP in the diabetic rats resulted in a lipid-rich particle rather than in an increased number of particles. In the alloxan-diabetic rabbit model we also found an increase in intestinal MTP, but this time it was associated with an increased number of chylomicron particles, showing a species difference [38]. Because apo B is produced in excess and degraded rapidly if not associated with lipid, we had expected that the increase in MTP would produce more particles rather than larger particles, and this is what we found in the rabbit.

Since insulin suppresses MTP in the cell, we wondered whether insulin resistance would be associated with an increase in MTP, helping to explain the increase in triacylglycerol levels in the insulin resistance syndrome. We examined the insulin-resistant Zucker obese fatty rat, and found that MTP mRNA was increased compared with controls, and chylomicrons were also increased [39]. We have now examined MTP mRNA in human diabetic patients, and

found increased MTP levels in diabetes, which is associated with an increase in small chylomicron particles [40].

Because MTP plays such a crucial role in the production of postprandial lipoproteins, polymorphisms of the MTP gene have been examined. Karpe et al. [41] have demonstrated in normal men that the rare homozygous MTP-493 T/T polymorphism was associated with reduced cholesterol, while in familial hypercholesterolaemia it was associated with reduced triacylglycerol [42]. In Type II diabetic patients we have very recently found that the common heterozygous MTP-493 G/T variant is associated with reduced LDL (low-density lipoprotein) cholesterol and smaller chylomicron particles [43]. In diabetic patients it has also been shown that carriers of the T allele are protected from non-alcoholic steatosis [44]. MTP inhibitors have been shown to be very effective lipid-lowering drugs, but unfortunately they cause hepatic steatosis [44].

Triacylglycerol-rich lipoproteins

It must be remembered that chylomicrons cannot be identified entirely by ultracentrifugation, and that the usual classification of chylomicrons is by size, based on their ultracentrifugation density [45]. Apo B48 defines the intestinally derived particle, whereas apo B100 defines the hepatic VLDL particle with intermediate-density lipoprotein and LDL containing virtually no apo B48. The atherosclerotic importance of the intestinally derived apo B48-containing small particle may be considerable, since it has been shown that these particles can indeed migrate through the endothelial wall [46], and also since a receptor that binds apo B48 has been demonstrated on the macrophage [47]. We have examined intestinally derived apo B48-containing particles in Type II diabetes and demonstrated a significant increase in the postprandial level [24,48,49]. Not only are the levels increased, but the duration of the elevation is greater in the diabetic patient, showing that the particles are cleared more slowly. Although chylomicrons are cholesterol-poor compared with LDL, being made up mostly of triacylglycerols, it has to be remembered that whereas clearance of LDL takes days, chylomicrons are cleared in minutes. Hence the cholesterol load that could be delivered to the atherosclerotic plaque by chylomicrons is potentially very great [50]. There have been no studies directly examining this concept, but Karpe et al. [51] have related apo B48 to the progression of coronary atherosclerosis in normal men, and in Type II diabetic patients, Mero et al. [52] have demonstrated a correlation between the severity of atherosclerosis and postprandial apo B48 levels. Fasting serum triacylglycerols are usually taken to be a surrogate marker for postprandial lipoproteins, and in some studies we have shown that fasting VLDL triacylglycerol reflects the postprandial excursions in VLDL triacylglycerol [53,54]. However, fasting chylomicron apo B48 and apo B100 do not reflect the postprandial chylomicron apoB48 and apo B100 levels [49,53,54]; hence it may be important to measure the postprandial phase in future studies.

The VLDL particle is defined as the hepatically derived apo B100-containing particle. Around half of the chylomicron density fraction is composed of hepatically derived particles on ultracentrifugation [50]. We have demonstrated a delay in the clearance of apo B100 in diabetic patients, and have shown that improved diabetic control [54] or an oleic acid-rich diet, such as is found in Mediterranean countries, helps to normalize postprandial VLDL excursions [49]. It has been suggested that delayed clearance might be due to apo E deficiency on the particle [55], so we carried out experiments in animals to examine whether the particle in diabetes had some abnormality or whether delayed clearance was due entirely to deficiency of lipoprotein lipase, an insulin-sensitive enzyme. We cannulated the lymph ducts of diabetic and control rabbits to harvest pure chylomicron particles [56]. Having labelled both chylomicron triacylglycerol and cholesterol, we re-injected the particles into the diabetic and control rabbits, and found that the particles from diabetic animals were apo E deficient; these particles were cleared more slowly even by control animals. The importance of the apo E deficiency seems equal to that of the dysfunction of lipoprotein lipase in diabetic animals, since there was no significant difference in clearance when particles from diabetic animals when injected into controls or particles from control animals were injected into diabetic animals. We have since demonstrated a significant decrease in apo E in triacylglycerol-rich particles from diabetic patients [71].

LDL

There is now overwhelming evidence that inhibition of HMG-CoA reductase by statin therapy reduces cardiovascular disease in both diabetic and non-diabetic subjects [57,58]. A major effect of the statins is up-regulation of the B/E receptor, which clears chylomicrons and VLDL as well as LDL. There is little information about the effect of HMG-CoA reductase inhibitors on postprandial lipoproteins in diabetes, but we have shown a significant decrease in chylomicrons and VLDL on statin therapy [53]. The LDL particle is derived mostly from VLDL, and it is therefore interesting both that little attention has been paid to the postprandial lipoproteins in defining the composition of LDL and that VLDL has not been targeted in an effort to reduce the atherogenicity of LDL. The composition of VLDL determines LDL composition, and it has been suggested that there is a two-compartment VLDL system with the large, triacylglycerol-rich VLDL being the precursor of small, dense LDL [59]. Garvey et al. [60], using NMR of whole sera to analyse lipoprotein size and composition, have recently shown, in 46 insulin-resistant subjects and 46 untreated Type II diabetic patients, that there was a 2–3-fold increase in large VLDL, producing an increase in serum triacylglycerols and a decrease in LDL size, together with an increase in LDL particle number. These changes were associated with a decrease in the large, cardioprotective high-density lipoprotein. The size and density of LDL increases its ability to become oxidized [61], an atherogenic modification.

Small, dense LDL is associated with cardiovascular disease in the general population [62], but the relationship has not been confirmed in diabetes.

The LDL particle is the major cholesterol-carrying particle, although it must be remembered that the half-life of LDL is 3–4 days and that the amount of cholesterol that it carries may not entirely reflect the importance ascribed to it in atherosclerosis. Perhaps more importantly, the length of time that it spends in the circulation allows for LDL modification, and it is modification of lipoprotein and cholesterol that may explain the relationship between LDL and atheroma [63]. In diabetes, LDL is modified by both glycation and oxidation, and modified LDL is taken up by the scavenger receptor, which does not have the ability to regulate cellular cholesterol synthesis and clearance in the way that the LDL receptor does [64]. The degree of glycaemia governs the glycation of the particle, and glycated LDL is more susceptible to oxidation [65]. Hence an improvement in diabetic control will decrease the amount of modified LDL in the circulation. We have demonstrated that, in diabetic patients, the susceptibility of LDL to oxidation is dependent on the amount of polyunsaturated fatty acid in the LDL particle, and that an alteration in diet from oleic acid to linoleic acid significantly reduced the the susceptibility of LDL to oxidation [66,67]. We have re-examined the composition of LDL in diabetes, and found that there is a 10-fold increase in fatty acids that are tightly attached to the LDL particle, resisting a 48 h ultracentrifugation [68]. This discovery arose from our observation that a 2-fold increase in fatty acids in the LDL particle could not be accounted for by an increase in LDL triacylglycerol, cholesteryl ester or phospholipids [69]. It is generally accepted that NEFA are carried in the circulation attached to albumin, and if in excess by other proteins, including lipoproteins. Cistola and Small [70] demonstrated that the affinity of albumin for NEFA had been largely overemphasized when they showed that the threshold NEFA/albumin molar ratio after which NEFA started to bind to other proteins was 1 rather than 3, as held previously. Since oxidation of LDL is dependent on the amount of polyunsaturated fatty acids in the particle [66], some of the increase in the susceptibility of diabetic LDL to oxidation is due to attachment of NEFA to the particle. These studies suggest that there would be a benefit in lowering NEFA in diabetic patients through improved postprandial metabolic control.

Conclusion

In conclusion, the dyslipidaemia of diabetes is found in the postprandial phase and is a major determinant of the atherogenicity of LDL, which may account, to a large extent, for the up to 5-fold increase in atherosclerosis in the diabetic patient. There is, however, increasing evidence that postprandial lipoproteins themselves are atherogenic, and future studies may show that normalization of the postprandial phase in diabetes will yield important benefits in cardiovascular prevention.

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