

Abnormalities of cholesterol metabolism in diabetes

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The basic lesion leading to the high morbidity and mortality in diabetes is atherosclerosis. The histological picture of atherosclerosis in the diabetic is similar to that found in the non-diabetic, yet atherosclerosis is more frequent and more severe in diabetes (West, 1978; Krolewski *et al.* 1987*a,b*). Gangrene of the foot is five times more common and myocardial infarction and strokes are also more frequent than in non-diabetic subjects (Fuller *et al.* 1983; Brand *et al.* 1989). The relationship between cholesterol and atherosclerosis is well known in the non-diabetic population. For example, Castelli *et al.* (1986) reporting on the famous Framingham study, in which about 5000 men and women between 30 and 60 years of age on entry to the study were followed and reviewed at regular intervals, found that there was a direct and independent relationship between elevated serum cholesterol levels and symptoms suggestive of atherosclerosis. In the diabetic population Brand *et al.* (1989) examined the incidence of diabetes in intermittent claudication in 1813 men and 2004 women during a 34-year follow-up. For both sexes diabetes was associated with a two–threefold excess risk of intermittent claudication compared with its absence. Reckless *et al.* (1978) have shown that the connection between lipoprotein-cholesterol and the prevalence of vascular disease in the diabetic patient is the same as the non-diabetic, but it is not known why the diabetic with similar lipid profile to his non-diabetic counterpart is much more at risk of developing atherosclerosis. For this reason, there has been a proliferation of research work on more complex alterations in lipoprotein-cholesterol in the diabetic in an effort to understand the way in which diabetes speeds up the atherogenic process.

THE PHYSIOLOGY OF CHOLESTEROL METABOLISM

To understand some of the complex alterations in cholesterol metabolism in the diabetic patient, it is necessary to discuss the physiology of cholesterol metabolism in broader outlines. Cholesterol is either absorbed by the gut from the food or synthesized from acetate in the cell. Absorption of cholesterol is dependent on the small intestinal mucosa being intact. The cholesterol molecule is absorbed by pinocytosis and transferred to the lacteal in the villus where it is assembled, together with triacylglycerols, phospholipids and protein to make very-low-density chylomicrons. These are transported via the thoracic duct and eventually to the liver. Diabetes in humans and experimentally induced in animals may be associated with intestinal hypertrophy. Increased intestinal cholesterol absorption or synthesis, or both, has been shown to accompany poorly-controlled diabetes (Devery *et al.* 1987; O'Meara *et al.* 1990).

The acceptance of cholesterol by the liver is dependent to a large part on receptors which recognize the protein moiety of the chylomicron, namely, Apo E. The chylo-

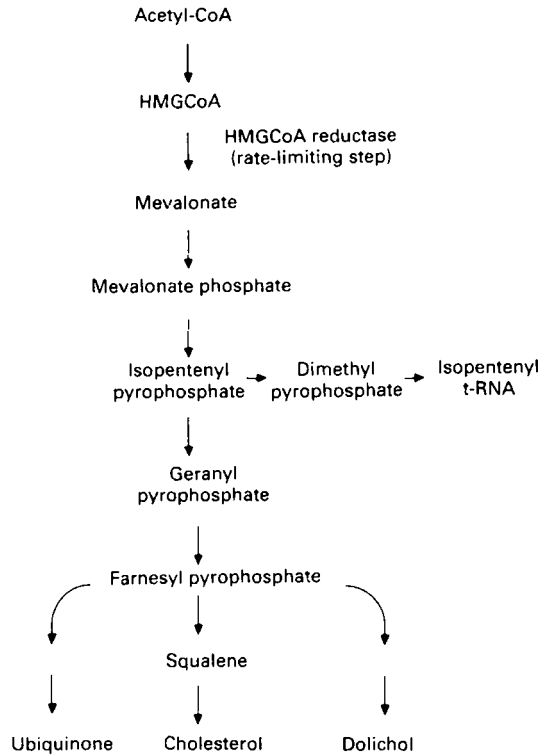


Fig. 1. Pathway for cholesterol synthesis. HMGCoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase (*EC* 1.1.1.88).

micron is broken down before it reaches the liver by the action of lipoprotein lipase (*EC* 3.1.1.34) which hydrolyses the triacylglycerol portion to give chylomicron remnants (Taskinen, 1987). These are rapidly taken up by the Apo E receptor in the liver. The cholesterol released enters the pool of hepatic cholesterol together with endogenously synthesized hepatic cholesterol. Cholesterol synthesis occurs mainly in the liver but also in the intestine and in most of the body's cells. Synthesis of cholesterol is via the acetate pathway (Fig. 1). Therefore, an estimate of cholesterol synthesis may be made by measuring 3-hydroxy-3-methylglutaryl-CoA reductase (*EC* 1.1.1.88); or by measuring radiolabelled acetate incorporation into cholesterol in the cell. *De novo* synthesis is controlled by a feedback mechanism through the low-density-lipoprotein (LDL) receptor (Brown *et al.* 1974). Cholesterol, once synthesized, may be stored in the cell following conversion to cholesteryl ester by the enzyme acyl-CoA:cholesterol-0-acyltransferase (*EC* 2.3.1.26; ACAT). In the liver cholesterol becomes associated with triacylglycerols, apolipoprotein B100 and phospholipids. This complex particle has a very low density and is termed very-low-density lipoprotein (VLDL). Triacylglycerol secretion by the liver is controlled by insulin, insulin deficiency leading to increased triacylglycerol release (Streja *et al.* 1977).

Lipoprotein lipase again is responsible for hydrolysis of the VLDL-triacylglycerol and the remnant particle is converted to intermediate-density lipoprotein (IDL), so named

because of its higher density. IDL is rapidly converted into LDL, having shed Apo C and Apo E proteins. Lipoprotein lipase is insulin sensitive, hence the deficiency of insulin leads to increase in triacylglycerol levels in both the chylomicrons and VLDL (for review, see Gibbons, 1990).

High-density lipoprotein (HDL) is assembled by the combination of the shed proteins and cholesterol from the VLDL following hydrolysis by lipoprotein lipase. Because of the structure of HDL it is thought to act as an acceptor of cholesterol from cells. HDL first attaches itself to the cell membrane by means of the HDL receptor (Reichl & Miller, 1989) and then accepts cellular free cholesterol. The cholesterol in the HDL is esterified by the enzyme lecithin-cholesterol acyltransferase (*EC* 2.3.1.43) (Glomset, 1968) and transferred to the lower-density lipoproteins by the action of cholesteryl ester transfer protein (CETP). This cholesterol is then cleared by the liver following the uptake of LDL by the LDL receptor. CETP has been shown to have an increased activity in insulin-dependent diabetic patients (Bagdade *et al.* 1991). The significance of this finding is still unclear. However, a defect of this type is potentially atherogenic because it may lead to an increase in cholesteryl ester-rich lipoprotein particles with altered LDL-receptor binding properties.

HYPERTRIACYLGLYCEROLAEMIA IN DIABETES

The commonest abnormality in the diabetic patient is the finding of a raised serum triacylglycerol level, the cause being most importantly the decrease in the inhibitory effect of insulin on triacylglycerol release from the liver and, secondly, the decreased activity of lipoprotein lipase leading to slowing down of the hydrolysis of triacylglycerol in the circulation. The hypertriacylglycerolaemia in uncontrolled diabetes is rapidly correctable by control of diabetes. Whether hypertriacylglycerolaemia is a risk factor in the development of atherosclerosis is perhaps still an open question, but the Paris Prospective Study is the most compelling evidence for triacylglycerol as an independent risk factor (Fontbonne *et al.* 1989). In this study, during a mean follow-up of 11 years, twenty-six of 943 subjects with abnormal glucose tolerance died from coronary heart disease. Univariate analysis showed that plasma triacylglycerol levels were significantly higher in subjects who died from coronary heart disease compared with those who did not. A multivariate regression analysis showed that the plasma triacylglycerol level was the only factor positively and significantly associated with coronary heart disease. I have reservations, however, about the power of statistics in such a small sample (twenty-eight subjects) to answer definitively whether triacylglycerols really are an independent risk factor. Of greater interest is the relationship between a raised serum triacylglycerol and HDL and LDL. It is accepted that a low HDL level is an independent risk factor for atherosclerosis in both the non-diabetic (Gordon *et al.* 1977) and diabetic patient (Reckless *et al.* 1978). The deficiency in lipoprotein lipase activity diminishes the formation of HDL from VLDL. HDL is often low in type 2 diabetic patients, although it may be normal or raised in type 1 diabetes (Nikkila & Hormila, 1978). Hence, the relationship between triacylglycerol and HDL is of extreme importance in the clearance of cellular cholesterol from the tissues to the liver. The disturbances, as stated, above all lead to impairment in the reverse transport of cholesterol from the tissue to liver for excretion. Support for these findings comes from recent results in our laboratory. We have shown that cellular cholesterol in diabetic patients with poor control was signifi-

cantly higher than that in control subjects. Normocholesterolaemic diabetic patients shared the same elevated cellular cholesterol as hypercholesterolaemic non-diabetic patients (McBrinn *et al.* 1991).

The enzyme ACAT, which esterifies cholesterol in the cell, has been shown to be low in diabetic animals in both liver and intestine. Enzyme levels have been related to glycaemic control, the lowest levels being found in the most poorly-controlled animals (O'Meara *et al.* 1990). We have also shown that ACAT is lower in diabetic patients than in controlled subjects (Owens *et al.* 1990b). The significance of the lower ACAT levels suggests that more free cholesterol is available for transport out of storage leading to a higher pool of circulating cholesterol.

LDL

The major carrier of cholesterol to the cell is the LDL particle. Elevated serum LDL is a major risk factor of atherosclerosis and explains the association between atheroma and cholesterol. LDL is often raised in type 1 diabetics in poor control and even in young diabetic patients the LDL may be higher than in their non-diabetic siblings (Sosenko *et al.* 1980). LDL composition is often abnormal (James & Pometta, 1990). We and others have demonstrated a raised esterified cholesterol content in LDL (Owens *et al.* 1990a; Bagdade *et al.* 1991). The significance of the abnormality is related to the inability of this modified LDL to down-regulate cholesterol synthesis in the cell. This appears to be due to the poorer binding of esterified LDL to the LDL receptor (D. Owens, unpublished results). A normal serum LDL cholesterol level does not reveal these very important differences in the metabolic effect of the particle and again the abnormal particle may play a very important role in the pathogenesis of atherosclerosis. Insulin has been shown to stimulate LDL receptors and insulin deficiency is another mechanism which would result in poor clearance of LDL from the blood and could lead to an increase in *de novo* synthesis in the cell (Mazzone *et al.* 1984).

Lipoprotein(a) (Lp(a)) is an unusual serum lipoprotein characterized by the presence of a unique glycoprotein(a) linked to apoprotein B100 by disulphide bridges. Lp(a) has been shown to be an important marker of coronary heart disease (Kostner *et al.* 1981; Armstrong *et al.* 1986). Lp(a) may be elevated in poorly-controlled non-insulin-dependent diabetic patients (Levitsky *et al.* 1991) and may be decreased with improved glycaemic control (Haffner *et al.* 1991).

Diabetes affects not only the large vessels, due to the early and aggressive onset of atheroma, but also the small vessels, leading to retinopathy and blindness, neuropathy with loss of sensation in the feet, postural hypotension diarrhoea, impotence and proteinuria leading to renal failure. Recent studies on the effect of proteinuria on lipoproteins have shown an association with lower HDL levels (Winocour *et al.* 1987).

INSULIN RESISTANCE AND CHOLESTEROL

Epidemiological studies have shown a relationship between raised insulin levels and atherosclerosis in non-diabetic subjects. The raised insulin in the presence of normal blood sugars suggests insulin resistance. In type 2 diabetes, in obesity and in conditions such as cortisol excess (Cushing's Syndrome), raised insulin levels are frequently found. Insulin resistance is often associated, as previously explained, with hypertriacyl-

glycerolaemia and, therefore, low HDL levels, both of which are strongly and independently related to coronary heart disease (for review, see Stout, 1990). Stout & Vallance-Owen (1969) did the original animal studies showing that insulin in experimental conditions could cause atheroma (see also Stout, 1970). They suggested a mechanism whereby insulin rather than glucose might be the important factor in the development of atheroma. The hypothesis was backed up or supported by many epidemiological studies showing the relationship between hyperinsulinaemia and coronary artery disease (Zavaroni *et al.* 1989; DeFronzo & Ferrannini, 1991). A recent study, for example, has shown that patients with angina and normal coronary arteries have insulin resistance with elevated serum insulin levels but without hyperglycaemia (Dean *et al.* 1991). Reaven (1988) has publicized the correlation between hyperinsulinaemia, hypertension and hyperlipidaemia. To evaluate the role of insulin on cholesterol synthesis, we have recently completed a series of experiments in type 1 insulin-dependent diabetic patients using the biostator (artificial pancreas). Clamping blood sugar for 4 h at either high or low level and infusing insulin at either a high or low rate, we have shown that hyperglycaemia does not stimulate cholesterol synthesis in the mononuclear cell. However, hyperinsulinaemia as derived from high insulin infusion rate causes a significant rise in cholesterol synthesis as measured by acetate incorporation. Hyperglycaemia, only in the presence of hyperinsulinaemia, caused a further rise in the rate of cholesterol synthesis in the cell (Stinson *et al.* 1991). In another set of experiments on type 2 non-insulin-dependent diabetic patients and obese hyperinsulinaemic non-diabetic patients, these findings were confirmed after a high-energy meal. In these experiments we have shown that there was a relationship between hyperinsulinaemia and cholesterol synthesis, whereas there was no relationship between blood sugar and cholesterol synthesis after the meal.

GLYCOSYLATION OF LDL

Finally, what effect does hyperglycaemia have on cholesterol metabolism? Glucose is known to form chemically reversible early glycosylation products with protein. LDL, because of its protein moiety, is, therefore, subject to glycosylation. Glycosylated LDL binds poorly to the LDL receptor (Steinbrecher & Witztum, 1984) and is one explanation for the increased cellular cholesterol and raised LDL-cholesterol found in poorly-controlled diabetic subjects. Furthermore, hyperglycaemia may retard the efflux of cholesterol from the cell through covalent cross-linking of plasma lipoprotein to cellular protein. At high levels of glycosylated collagen, LDL binding increases with increased LDL concentration (Brownlee *et al.* 1985). Lyons *et al.* (1987) have shown in a series of experiments that non-enzymic glycosylation of LDL may lead to increased accumulation of cholesteryl esters in macrophages, suggesting another possible mechanism by which hyperglycaemia may contribute to the acceleration of atherosclerosis in diabetes.

CONCLUSION

The present review has focused on cholesterol metabolism and particularly on LDL-cholesterol alterations in diabetes. It must be accepted that there are many alterations in the diabetic state that may promote atherosclerosis, for example altered platelet function

and abnormal growth factors, including perhaps the altered LDL found in the diabetic which may also act as a growth factor (Owens *et al.* 1990a). Glycosylation of collagen may play a part in the development of atherosclerosis. The purpose of the present paper was not to give an overall review of the pathogenesis of diabetes but rather to present recent evidence suggesting that the measurement of serum lipoproteins does not reveal major disturbances which occur in lipoprotein metabolism in the diabetic patient. Understanding these abnormalities may lead to a rational theory which could prevent the onset of severe atherosclerosis in the diabetic patient.

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