

## Lipids, thrombosis and cardiovascular disease in diabetes

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Diabetes is associated with a two- to five-fold increase in mortality from CHD (Fuller *et al.* 1980), an excess risk which does not appear to be explained by the well-recognized abnormalities of the other cardiovascular risk factors in diabetic patients (Rosengren *et al.* 1989). Lipoprotein abnormalities are common in subjects with non-insulin-dependent diabetes mellitus (NIDDM). The major changes seen in this condition are an increase in concentrations of the triacylglycerol-rich lipoproteins, in particular VLDL, a particle which itself is probably not atherogenic. Nevertheless, increased concentrations of VLDL are associated with other lipoprotein abnormalities in NIDDM, including triacylglycerol enrichment of LDL and a consequent increase in oxidizability, as well as with a reduction in concentration of HDL. Both of these are potentially atherogenic. Moreover, the increase in VLDL concentration found in NIDDM induces abnormalities of coagulation and fibrinolysis, which, by producing a procoagulant state, may further contribute to the increased incidence of CHD. The role of these changes in explaining the excess risk, along with the possible therapeutic implications, will be explored in the present article.

### LIPOPROTEIN ABNORMALITIES IN NON-INSULIN-DEPENDENT DIABETES MELLITUS

Although levels of total cholesterol are similar in diabetic and non-diabetic subjects, disturbances in the subclasses of cholesterol are common in NIDDM. Concentrations of HDL-cholesterol are reduced (Laakso *et al.* 1993), and there is an increase in the proportion of small dense LDL particles, which may be particularly related to cardiovascular risk (Austin *et al.* 1995). These abnormalities may both be the consequence of impaired removal of non-esterified fatty acids from triacylglycerol-rich lipoproteins through subnormal activity of lipoprotein lipase (EC 3.1.1.34), and resulting triacylglycerol enrichment of IDL and LDL (Feingold *et al.* 1992). The role of reduced activity of lipoprotein lipase is also evident in the other major abnormality of lipoproteins in NIDDM, i.e. hypertriacylglycerolaemia, in particular after a fatty meal (Tan *et al.* 1995). All these lipid abnormalities are related to insulin resistance (Stewart *et al.* 1993), are commoner in obese subjects, and antedate the development of the diabetic state (Haffner *et al.* 1990).

### DYSLIPIDAEMIA AND CHD IN NON-INSULIN-DEPENDENT DIABETES MELLITUS: EPIDEMIOLOGICAL OBSERVATIONS

The relationships between CHD and high concentrations of total and LDL-cholesterol have been well defined, with an increase of about 3% in CHD incidence and mortality with every 1% increase in LDL-cholesterol (Law *et al.* 1994). The relationship between cholesterol concentrations and CHD has been compared for more than 5000 diabetic and 348 000 non-diabetic men screened for the Multiple Risk Factor Intervention Trial (MRFIT; Stamler *et al.* 1993). Although the proportional increase in CHD risk per unit increase in cholesterol concentration was smaller in the diabetic subjects, the higher background risk implied that the absolute increase per unit change in cholesterol was

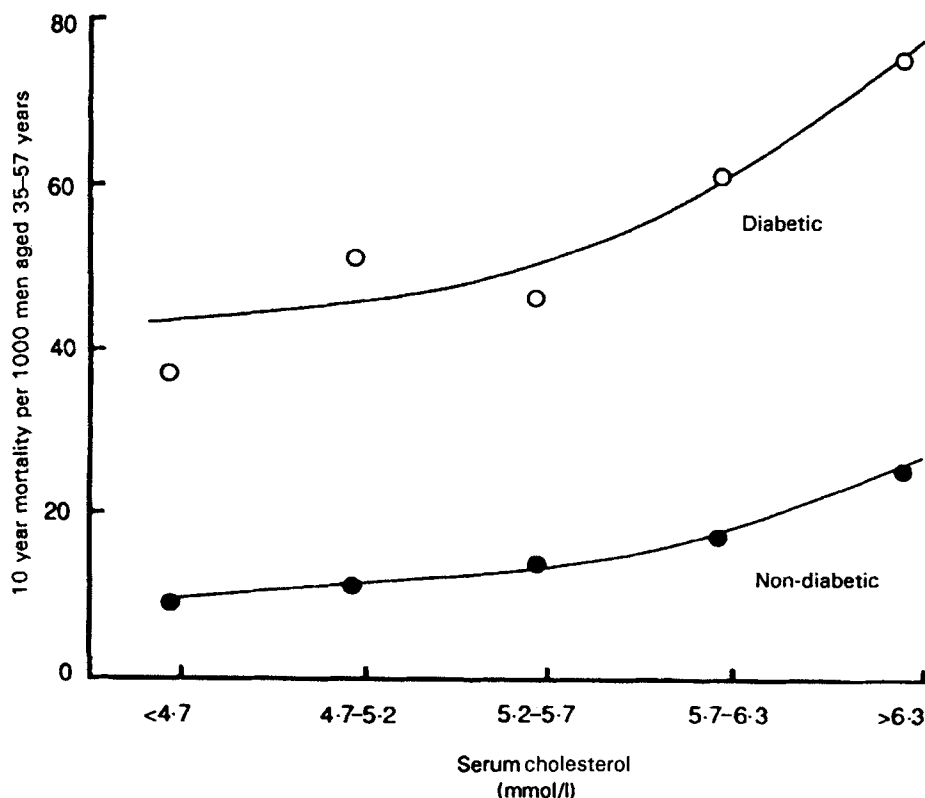


Fig. 1. Serum cholesterol and 10-year coronary heart disease mortality in 342 815 non-diabetic and 5163 diabetic men aged 35-57 years. (From J. D. Neaton, personal communication.)

greater in diabetes (Fig. 1). The therapeutic implications of this observation will be considered later (p. 278).

Several studies support the importance of triacylglycerol-rich lipoproteins in predicting CHD in patients with NIDDM. Thus, a Finnish study found that in multiple-regression analysis, LDL-triacylglycerols, but not LDL- or HDL-cholesterol, predicted 5-year incidence of CHD (Uusitupa *et al.* 1990), while a parallel study from the same group found that high triacylglycerol concentration (> 2.3 mmol/l) but not a high LDL-cholesterol (> 4.1 mmol/l) predicted incident CHD over 7 years (Laakso *et al.* 1993). In the Paris Prospective Study of 943 subjects with abnormal glucose tolerance, a triacylglycerol level above the median (> 1.39 mmol/l), but not an elevated cholesterol concentration (> 5.7 mmol/l), was significantly predictive of 11-year CHD mortality (Fontbonne *et al.* 1989).

#### MECHANISMS OF ASSOCIATION: ATHEROGENESIS

It is generally accepted that LDL, and not VLDL, is an atherogenic particle. Furthermore, the use of multiple-regression techniques will tend to reduce the predictive associations of variables such as triacylglycerols, which have a high biological variability (Austin, 1989). Thus, the finding that concentrations of triacylglycerol-rich lipoproteins predict CHD in

three separate longitudinal studies of NIDDM and glucose intolerance are at first difficult to understand.

The mechanism of vessel-wall damage by LDL-cholesterol is incompletely understood. Certain subfractions, and in particular small dense LDL, appear to be most harmful (Austin *et al.* 1990), perhaps because these particles are substantially more vulnerable to lipid oxidation (Steinberg *et al.* 1989). Whether this, in turn, is because of increased content of oxidizable fatty acids, or due to lower content of antioxidants, is unclear. The elevated triacylglycerol concentrations found in NIDDM patients are associated with increased proportions of small dense LDL (Austin *et al.* 1995; Lahdenperä *et al.* 1995), with increased lipid oxidation and reduced concentrations of antioxidants in these particles (Chait *et al.* 1993), these perhaps representing the mechanisms of association of CHD with hypertriacylglycerolaemia in these patients.

#### MECHANISMS OF ASSOCIATION: THROMBOGENESIS AND IMPAIRED FIBRINOLYSIS

Hypertriacylglycerolaemia is associated with a number of abnormalities of coagulation and fibrinolysis. Thus, Factor VII coagulant activity is increased in proportion to triacylglycerol concentration, perhaps consequent on activation of the serine protease, which cleaves the Factor VII precursor to the active molecular species, by binding to the surface of the VLDL particle (Miller *et al.* 1986). Plasminogen activator inhibitor-1 (PAI-1) is a fast-acting inhibitor of fibrinolysis, which alters the thrombotic balance in favour of occlusion. Levels of PAI-1 are elevated in young men surviving a myocardial infarction and also predict recurrent events (Hamsten *et al.* 1987). We have found that increased PAI-1 activity in patients admitted to hospital with acute myocardial infarction predicts a poor response to thrombolytic therapy, and that elevated levels on the third day after the initial event predict in-hospital re-infarction (Gray *et al.* 1993b). Levels of PAI-1 are increased in NIDDM patients (Gray *et al.* 1993c), and further increased in diabetic patients with CHD (Gray *et al.* 1993a). We have found that diabetic patients show enzymic evidence of poorer response to thrombolysis than do non-diabetic subjects, which is largely related to their higher admission levels of PAI-1 (Gray *et al.* 1993b).

PAI-1 is expressed in, and synthesized by, both liver and endothelium. In hepatocytes, PAI-1 synthesis is increased by inducing insulin resistance (Anfossa *et al.* 1993), a relationship which has also been noted in clinical studies (Juhan-Vague *et al.* 1993; Potter van Loon *et al.* 1993). However, the interpretation of such clinical studies is made complex by the powerful interrelationships between various features of the insulin-resistance syndrome, any of which may explain the increased levels of PAI-1 in insulin resistance and in NIDDM. Thus, PAI-1 activity relates to elevated concentrations of triacylglycerol (Vague *et al.* 1986; Asplund-Carlson *et al.* 1993), to central and global obesity (Folsom *et al.* 1993) and to hyperproinsulinaemia (Nagi *et al.* 1990). Expression studies and investigation of tissue culture have been able to resolve some of these issues, demonstrating, for example, that both VLDL particles from hypertriacylglycerolaemic subjects (Stiko-Rahm *et al.* 1990) and oxidized LDL particles (Latron *et al.* 1991) are able to induce secretion of PAI-1 from cultured human umbilical-vein endothelial cells. There appear to be important gene-environment interactions in this mechanism, in that NIDDM patients homozygous for a common allele (4G) located 675 base pairs upstream from the start of transcription of the PAI-1 gene showed higher levels of PAI-1, and greater impact of hypertriacylglycerolaemia, than those homozygous for the 5G allele (Panahloo *et al.* 1995). It appears, then, that hypertriacylglycerolaemia, and the parallel increase in the proportion of oxidized small dense LDL, may help directly to explain the elevated levels of

PAI-1 activity and the impaired fibrinolysis seen in NIDDM (Panahloo & Yudkin, 1996), this perhaps being especially relevant in those with the 4G4G PAI-1 genotype.

#### MECHANISMS OF ASSOCIATION: ENDOTHELIAL DYSFUNCTION AS A COMMON ANTECEDENT?

Associations are not proof of causation, and it may be hypothesized that the relationship between hypertriacylglycerolaemia, insulin resistance, elevated levels of PAI-1 and cardiovascular risk could represent consequences of a common antecedent. Skeletal-muscle blood flow makes an important contribution to insulin action, with the effects of insulin including an increase in muscle blood flow, thereby increasing supply of both substrate and hormone to the insulin-sensitive tissues (Laakso *et al.* 1992). Insulin needs to be translocated to the abluminal side of the capillary to access the cell receptor, and part of this transport is via an energy-dependent receptor-mediated transpinoctosis (King & Johnson, 1985). Insulin transport across endothelium may be rate-limiting for its action during changes in insulin concentration (Ader & Bergman, 1994). Damage to capillary or resistance vessel endothelium might thereby impair insulin action. Parallel changes in adipose tissue might adversely affect the proteoglycan binding of the exoenzyme lipoprotein lipase, which would impair the clearance of triacylglycerol-rich lipoproteins (Eckel, 1989). Furthermore, the activation of endothelial expression of PAI-1 by cytokines or by other participants in endothelial damage or acute-phase reaction (Sawdey & Loskutoff, 1991) could partly underlie the relationships between insulin resistance, hypertriacylglycerolaemia and impaired fibrinolysis. In thirty-three subjects with NIDDM, we have found significant relationships between concentrations of von Willebrand factor, a marker of endothelial dysfunction, and the degree of insulin resistance as measured by the modified Harano method (Fig. 2), as well as between levels of von Willebrand factor and those of triacylglycerol ( $r_s = 0.36$ ,  $P = 0.04$ ), providing support for the hypothesis of an endothelial aetiology of both insulin resistance and the metabolic syndrome.

It could be postulated that the process of endothelial damage could both initiate and compound the atherothrombotic process. Thus, a loss of endothelially-bound lipoprotein lipase might be responsible for an accumulation of triacylglycerol-rich lipoprotein, with consequent enhanced oxidation of small dense LDL and reduced levels of HDL increasing the damage to endothelium and deposition of fatty plaques. There is accumulating evidence that markers of inflammation indicate an increased risk of CHD events (Mendall *et al.* 1996), maybe in part by damage to endothelium or matrix by activated macrophages (Casscells *et al.* 1996), but perhaps also as a consequence of the compounding effects of the acute-phase protein, fibrinogen, on the atherothrombotic process (Casscells *et al.* 1996), and those of the inflammatory cytokines on expression of another pro-coagulant molecule, PAI-1. In Table 1 are shown the complex interrelationships between markers of the acute-phase response (C-reactive protein and fibrinogen) and of endothelial damage (von Willebrand factor, fibronectin, thrombomodulin) with insulin resistance (fasting insulin concentrations) and the cardiovascular risk markers under consideration (triacylglycerol, PAI-1) in 125 Caucasian non-diabetic subjects recruited from general practice. From these data it appears that elevated levels of acute-phase proteins correlate with markers of endothelial damage, and that both are correlated with hyperinsulinaemia, an indicator of insulin resistance. Furthermore, elevated levels of triacylglycerol and of PAI-1 appear to relate both to hyperinsulinaemia and to endothelial dysfunction and/or inflammation.

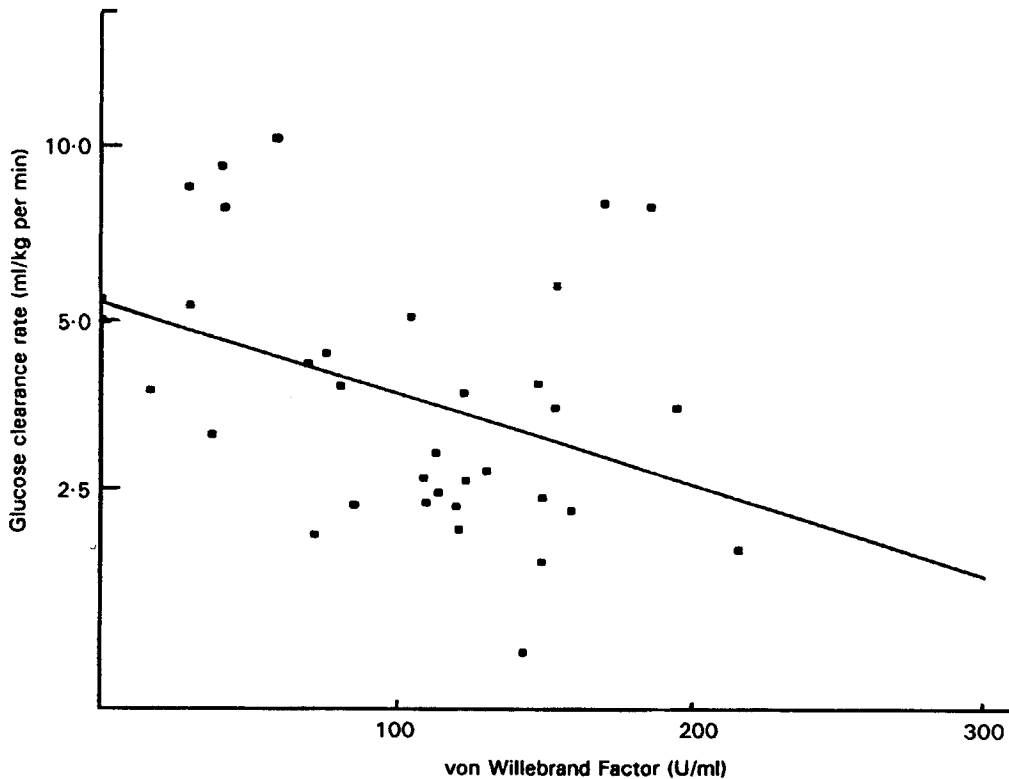


Fig. 2. The relationship between von Willebrand factor (marker of endothelial dysfunction) levels and metabolic clearance rate of glucose in thirty-three non-insulin-dependent diabetic subjects.  $r = -0.35$ ,  $P = 0.048$ .

Table 1. Relationship between markers of cardiovascular risk, endothelial damage, and inflammation in 123 non-diabetic subjects

	Insulin	Triacylglycerol	Fibronectin	vWF	CRP	Fibrinogen
Fasting insulin†	—	0.41***	0.23*	0.12	0.22*	0.20*
Triacylglycerol†	0.41***	—	0.32***	0.14	0.27**	0.20*
PAI-1†	0.42***	0.34***	0.03	0.03	0.21*	0.15*
Fibronectin†			—	0.30**	0.29**	0.12
vWF			0.30**	—	0.31***	0.16
CRP†					—	0.22*
Fibrinogen					0.22*	—

PAI-1, plasminogen activator inhibitor-1; vWF, von Willebrand factor (marker of endothelial dysfunction); CRP, C-reactive protein.

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

†Variable logarithmically transformed.

#### DYSLIPIDAEMIA IN NON-INSULIN-DEPENDENT DIABETES MELLITUS: IMPLICATIONS FOR THERAPY

While LDL-cholesterol concentrations are similar in diabetic and non-diabetic subjects, as pointed out previously, the findings of two new studies have provided clear indications for cholesterol lowering in high-risk subjects (Scandinavian Simvastatin Survival Study

Group, 1994; Shepherd *et al.* 1995). Thus, the 4S Study (Scandinavian Simvastatin Survival Study Group, 1994) used simvastatin for secondary prevention in 4444 men, demonstrating a 32% reduction in cardiovascular mortality over 5 years. The West of Scotland Pravastatin Study (Shepherd *et al.* 1995) showed similar proportional benefits in a primary prevention setting. A subgroup analysis of the 4S Study, assessing the benefits of these drugs in about 200 diabetic patients with CHD and a total cholesterol concentration of  $> 5.5$  mmol/l showed similar benefits to those in the non-diabetic patients in the study (Pyörälä *et al.* 1995). The advent of these new drugs has now made effective cholesterol lowering possible and, as a consequence, the 2 year loss of life expectancy that hypercholesterolaemia can be calculated to produce in a 45-year-old non-diabetic man, and the 16 month loss for a 45-year-old diabetic (Yudkin, 1993) might in theory be reversed by more than half by these hydroxymethylglutaryl-CoA inhibitors.

The interpretation of the relationships between hypertriacylglycerolaemia and CHD in diabetic patients clearly has a major impact on therapy. If lowering of elevated levels of triacylglycerol were capable of producing elevation of HDL-cholesterol, reduced proportions or oxidation of small dense LDL, and reductions in levels of PAI-1 and Factor VII, these more proximal markers of CHD risk could provide more meaningful surrogate risk markers than levels of triacylglycerol itself. If, however, the elevated concentrations of triacylglycerol provided an indication of pre-existing endothelial damage, then their reduction may be without benefit. It is only through the establishing of intervention trials with hard endpoints that such conclusions could be reached.

#### CONCLUSION

The complexities of the atherothrombotic process continue to be unravelled in directions not predicted by the cholesterol–heart hypothesis. Fibrinolysis, oxidation, inflammation and endothelial dysfunction are all major players in the game, and the data are not yet available to decide whether some of the lipid and fibrinolytic markers, such as triacylglycerol and PAI-1, are true risk factors, defined on the basis of reversibility and experimentation (Bradford Hill, 1965).

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