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Review article

DIABETES AND MACROVASCULAR DISEASE: RISK FACTORS FOR ATHEROGENESIS AND NON-INVASIVE INVESTIGATION OF ARTERIAL DISEASE

M. L. WAHLQVIST, I. R. N. RELF, K. A. MYERS and C. S. LO

Vascular Unit and Clinical Nutrition and Metabolism Unit, Prince Henry's Hospital, St Kilda Road, Melbourne, Victoria, Australia: Department of human Nutrition, Deakin University, Geelong, Victoria, Australia.

This paper reviews the risk factors that may contribute to atherosclerotic vascular disease in diabetics. Many of the risk factors have been identified on the basis of cross-sectional clinical studies or from mortality data, and as a result various aetiological hypotheses about the mechanism of atherogenesis have been formed.

Because of the importance of identifying changes in the arterial wall well in advance of the clinical events that lead to morbidity or mortality, emphasis is given also to the various techniques of non-invasive assessment of vascular disease that can be applied to diabetic as well as non-diabetic patients. One particular form of non-invasive investigation of arterial function, namely the Doppler pulse rate velocity and wave-form analysis, is comparatively simple.

We present some preliminary data to suggest that this technique may be useful in the evaluation of pre-symptomatic stages of atherosclerosis in diabetes.

Introduction: the problem of macrovascular disease in diabetes

About three quarters of all deaths of diabetics are due to macrovascular disease (West, 1978). Diabetics not only die at an earlier age than non-diabetics from macrovascular disease affecting coronary and cerebrovascular systems, but also suffer considerable morbidity from disease affecting peripheral arteries, renal arteries and to a lesser extent the mesenteric arteries (Bryfogle & Bradley, 1957; Strandness, Priest & Gibbons, 1964; Kessler, 1971; Kannel & McGee, 1979*b*; Keen & Jarrett, 1979; Fuller, Shipley & Rose, 1981).

The impact of diabetic macrovascular disease in women is greater than it is in men. Coronary heart disease is 1.7 times as common in diabetic men as in non-diabetic, but 2.7 times more common in diabetic women as in non-diabetic (Garcia *et al.*, 1974; Kannel & McGee, 1979*a,b*). However, not all cardiac disease in diabetics is attributable to macrovascular coronary heart disease. Whereas congestive cardiac failure is 2.2 times as common in diabetic men as in non-diabetic, it is 5.4 times as common in diabetic women as in non-diabetic (Kannel & McGee, 1979*b*). The presumed diabetic cardiomyopathy may be on the basis of microvascular disease, changes in myocardial metabolism, or in myocardial membrane properties (Jarrett, 1977; Kannel & McGee, 1979*b*; Ledet *et al.*, 1979; Chobanian *et al.*, 1982).

Risk factors for macrovascular disease in diabetics

Diabetes appears to be a risk factor in its own right, but also may enhance the influence of the conventional risk factors that operate in non-diabetic subjects. It must be borne in mind that diabetics may be affected to differing degrees by any given risk factor (Pell & D'Alnonzo, 1970; Beach *et al.*, 1979; Beach & Strandness, 1980). Risk for atherosclerotic vascular disease may not be the same as for other arterial wall changes in diabetics (Ferrier, 1964; Neubauer, 1971; Lundbaek, 1973; Ledet, 1981). Various putative risk factors are presented in Table 1 and discussed below. For some, the evidence is clear and, for others, is in an interesting state of evolution.

Table 1. *Risk factors for atherosclerotic vascular disease in diabetes*

Genetic predisposition	Low LDL concentrations
Sex differences	Elevated FFA concentrations
Duration of disease	Altered FA patterns
Abnormal glucose tolerance	Enhanced platelet aggregation
Hyperinsulinaemia	Cigarette smoking
Hypercholesterolaemia	Hypertension
Hypertriglyceridaemia	Obesity
	Lack of exercise

Genetic predisposition

The striking geographic differences in the frequency of macrovascular disease among diabetics appear to reflect the frequency of macrovascular disease in the non-diabetic population, rather than there being a specific genetic basis for the complication. Where low rates of macrovascular disease are found in diabetic patients, the food intake pattern both in diabetic and non-diabetic groups is characterized by a greater contribution to energy intake from carbohydrate and a lesser contribution from fat (Rudnick & Anderson, 1962; West & Kalbfleisch, 1971).

Sex differences

Diabetes eliminates the protection of females from vascular disease, as has been discussed previously.

Age and duration of disease

Age itself is an acknowledged and important risk factor for atherosclerotic vascular disease in natural populations, hence it is assumed that the older diabetic will show more macrovascular disease. Such an age relationship does not imply that atherosclerosis is part of the ageing process. It seems likely also that duration of diabetes will affect the prevalence and severity of atherosclerotic vascular disease (Deckert, Poulsen & Larsen, 1978). Non-invasive methods of assessing arterial disease may be of value in comparing diabetic and non-diabetic populations in relation to the influence of age.

Abnormal glucose tolerance

Abnormal glucose tolerance without evidence of clinical diabetes is associated

with risk of macrovascular disease intermediate between that of normal glucose tolerance and frank diabetes. This applies both to coronary heart disease and peripheral vascular disease (Heinle *et al.*, 1969; Fuller *et al.*, 1981; Keen *et al.*, 1981; Efendic *et al.*, 1982; Keen *et al.*, 1982). In the Whitehall prospective study of cardiovascular mortality, and the Paris population study, the risk of hyperglycaemia is not the usual stepwise increase of mortality with increasing degrees of glucose intolerance, but rather a 'threshold' effect which operates with a sharp doubling of mortality at the 95th percentile of the 2-h blood glucose concentrations (Fuller *et al.*, 1980; Eschwege *et al.*, 1980).

Hyperinsulinaemia

Various experimental studies have supported the hypothesis that elevated levels of circulating insulin *per se* might predispose to atherosclerotic vascular disease (Ganda, 1980; Stout, 1981). Hyperinsulinaemia is recognized in non-insulin dependent diabetes and is augmented on sulphonylurea therapy, and may be inferred to occur in insulin dependent diabetes where relatively high amounts of injected insulin are given to maintain blood sugar control. The effects of insulin antibodies may promote high levels of circulating insulin also.

There is evidence that insulin can stimulate smooth muscle cell proliferation in the arterial wall and alter various aspects of arterial wall metabolism, both lipid and mucopolysaccharide (Sirek, Sirek & Cukerman, 1981; Stout, 1981).

Population studies in Australia, Scotland, and Finland indicate that elevated serum insulin levels are associated with coronary heart disease mortality as an independent risk factor (Logan *et al.*, 1978; Pyorala, 1979; Welborn & Wearne, 1979). In a prospective study of 7000 non-diabetic men aged 43-54 years and followed for an average of 63 months, Ducimetiere and colleagues (1980) found that the fasting serum insulin was predictive of subsequent coronary events as an independent variable, together with systolic blood pressure and cigarette smoking, but not obesity nor triglyceride levels.

Hyperlipidaemia

Hypercholesterolaemia has long been recognized as one of the cardinal risk factors for atherosclerotic vascular disease in population studies (Kannel *et al.*, 1964). Diabetics are assumed to be at least as likely to show the prevailing hypercholesterolaemia as the non-diabetic population, being susceptible to similar genetic and environmental influences. The metabolic aberration of diabetes, however, increases triglyceride levels rather than cholesterol levels (Bagdade *et al.*, 1967; Goldberg, 1981). Hypertriglyceridaemia is a common metabolic abnormality in diabetes but is not shown to be an independent risk factor (Ducimetiere *et al.*, 1980; Fielding, 1981; Steiner, 1981).

Irrespective of actual lipid levels, lipoprotein metabolism is altered in diabetes (Ganda, 1980) and it has been suggested that diabetes might increase the sensitivity of arteries to risk factors such as LDL-cholesterol levels (Steiner, 1981) without marked differences in the actual LDL-cholesterol levels. HDL-cholesterol concentrations can be low in some diabetic populations and the disproportionately low HDL-cholesterol concentration in diabetic women is cited as

an explanation for the loss of protection of females from macrovascular disease (Gordon *et al.*, 1977; Reckless *et al.*, 1978).

Free fatty acid concentrations in plasma tend to be higher where there is lack of insulin or insulin action, and this phenomenon is well recognized in diabetics. Experimental data indicate a relationship between plasma free fatty acid concentrations and morphological changes in arteries in animal studies (Reinila, 1981). The effects of free fatty acids may be mediated through accelerated prostacyclin degradation in plasma and thus the increased tendency to platelet aggregation (Mikhailidis, Mikhailidis & Dandona, 1981). Fatty acid patterns may also be different in diabetics, with possible implications for prostaglandin metabolism (Jones *et al.*, 1983).

Enhanced thrombogenesis

Various aspects of haemostasis are altered in diabetes, including elevated fibrinogen levels and decreased fibrinolysis (Ganda, 1980), increased platelt aggregation (Kazmir *et al.*, 1981), and an adverse influence on the ratio of thromboxane, which tends to increase platelet aggregation, to prostacyclin, which is protective (Halushka *et al.*, 1981; Wall, Rubinstein & Cooper, 1981).

Cigarette smoking

In the Framingham study, diabetic men smoked fewer cigarettes each day than did non-diabetic men. Smoking added to risk of cardiovascular disease in diabetes without appearing to be synergistic with the diabetic state (Kannel & McGee, 1979*c*).

Hypertension

Hypertension and particularly elevated systolic blood pressure are risk factors by multivariate analysis for diabetic macrovascular disease in population studies with the end point of coronary heart disease mortality or cardiovascular mortality (Kannel & McGee, 1979*b*; Fuller *et al.*, 1980; Jarrett, McCartney & Keen, 1982). For the juvenile-onset insulin dependent diabetic, the Joslin Clinic experience is that hypertension is the major identifiable risk factor for both coronary heart disease and renal disease (Christlieb *et al.*, 1981).

Obesity

Obesity will not only increase the risk and severity of non-insulin dependent diabetes but also the likelihood of associated risk factors such as hypertension and hyperlipidaemia and hyperinsulinaemia. Obese women diabetics who sustain a myocardial infarction have a high mortality rate (Tansey, Opie & Connelly, 1977).

Lack of exercise

Physical training in non-insulin dependent diabetic patients improves glucose, insulin, and lipid profiles (Saltin *et al.*, 1979; Ruderman, Ganda & Johansen, 1979). However, the long-term beneficial effect in terms of preventing the incidence of macrovascular disease, or favourably influencing mortality, has yet to be studied prospectively.

Concepts of atherogenesis in diabetes

Many factors are likely to play a role in the acceleration of macrovascular disease in diabetes. The endothelial cell lining of large and medium arteries is more susceptible to damage from increased blood viscosity, excessive shear stresses, and arterial stiffening (McMillan, 1981). The intimal lining of arteries responds to various forms of injury in a uniform manner characterized by the development of lipid-rich atherosclerotic-like lesions (Moore, 1981). The mechanical effects of hypertension will further increase the risks of endothelial damage, and cigarette smoking will add to the problem with impaired oxygen transport.

The arterial wall cannot synthesize any significant quantity of cholesterol which therefore must be deposited from the circulating blood. But even without alterations in plasma cholesterol concentrations, alteration in lipoprotein metabolism in diabetes may enhance the likelihood of acquisition of cholesterol in the arterial wall. LDL-cholesterol is more likely to be deposited, especially if the HDL-cholesterol levels are low as in untreated or inadequately treated diabetes.

Of the many mechanisms whereby arterial wall metabolism may be adversely affected in diabetes (St Clair, 1976) the presentation of high concentrations of free fatty acids from the circulation, as well as the increased local synthesis of fatty acids from acetate, as stimulated by hyperinsulinaemia, could initiate a chain of events leading to formation of cholesterol esters. The removal of cholesterol esters may be adversely affected: glycosoaminoglycan metabolism and connective tissue metabolism can be altered in diabetes such that cholesterol is retained in the arterial wall (Iverius, 1972; Berenson, Srinivasan & Radhakrishnamurthy, 1974). Hyperinsulinaemia may also stimulate smooth muscle cell proliferation in the arterial wall, possibly stimulating the uptake of LDL by these cells.

Finally, through alterations in fibrinogen turnover and enhanced platelet aggregation, arterial occlusive events are more likely.

Monitoring macrovascular disease in diabetes

In clinical and epidemiological studies, mortality data and the ascertainment of morbidity such as myocardial infarction, stroke, or clinically evident peripheral vascular disease, have been the time-honoured end points taken as monitoring the frequency and severity of macrovascular disease. Clearly the capacity to evaluate changes in the arterial wall at much earlier stages in the development of the disease process would be highly desirable.

The invasive arteriographic approach will provide useful anatomical demonstrations of early disease. For example serial femoral arteriography in high risk individuals has been used to demonstrate reversibility of femoral atherosclerosis (Blankenhorn, 1978). Relationships between abnormal glucose tolerance and coronary artery atherosclerosis have been demonstrated in patients submitted to coronary angiograms (Falsetti *et al.*, 1970).

The non-invasive techniques developed for macrovascular disease may well be applied to diabetics (Baskett, 1982; Bernstein, 1981, 1982) – these are summarized in Table 2. Pulse wave velocity and wave form analysis using Doppler

Table 2. *Non-invasive monitoring of atherosclerotic vascular disease (NIMA VD)*

Blood pressure ratios (with or without exercise)
Plethysmography
Doppler pulse wave velocity and wave-form analysis
Doppler imaging (of blood flow)
B mode imaging (of vessel wall)
Combination of Doppler and B mode techniques
Computerized enhancement of venous injection arteriography
Nuclear magnetic resonance

scanning techniques can reveal early evidence for stiffening of the central and peripheral arterial system in diabetes (McMillan, 1981; Relf, 1983), which may well indicate the severity of pre-symptomatic atherosclerosis. We report some preliminary results of this form of assessment in the next section. It is also possible to represent visually the anatomical location of, or image blood flow using a Doppler Ultrasound and to image the vessel wall with B mode techniques.

A most promising non-invasive approach, the use of computerized enhancement of venous injection arteriography, also known as digital subtraction arteriography, is being evaluated (Mistretta *et al.*, 1982). But perhaps the most exciting technique of all is nuclear magnetic resonance (NMR) imaging using an NMR body scanner which will allow recognition of the soft tissue of the arterial wall. Potentially, this method could be used to study metabolic events in the arterial wall *in situ* (Budinger *et al.*, 1982; Pykett, 1982; Shulman, 1983), although such techniques will take years to define adequately before coming into general clinical use.

Preliminary results using the Doppler Spectroscan Mark II

We have assessed relative arterial compliance or elasticity using the Doppler Spectroscan Mark II, as calculated from the reciprocal of the square of the pulse wave velocity. Abnormal values obtained from this method indicate macroangiopathy but the specificity for the atherosclerotic arterial vascular disease remains to be established.

In non-diabetic subjects, a progressive decline in arterial compliance with advancing years is evident. The value for left subclavian to external iliac arterial compliance drops from an index of 1.5 in the third decade, 1.2 in the fourth decade to 1.0 in the fifth decade and 0.7 in the sixth decade and beyond (Relf, Myers and Wahlqvist – unpublished data). It is of interest therefore that the mean index for a group of adult diabetics without clinical evidence of macrovascular disease is about 0.5 (range 0.3–1.0). Arterial wall compliance in healthy control subjects was significantly and negatively associated with the area under the glucose tolerance curve (36 subjects, $r = 0.32$, $P<0.05$). The range of compliance for healthy subjects was from 0.5 to 2.2.

Significant associations with compliance and the conventional cardiovascular risk factors have been shown in healthy subjects in the left subclavian to external iliac arterial segment. HDL-cholesterols are positively related to compliance at a borderline level of significance, but plasma total cholesterol, systolic blood pres-

sure and diastolic blood pressure levels showed highly significant negative correlations with the compliance index (see Table 3).

Statistically significant associations have been demonstrated *in vivo* between conventional risk factors commonly related to end points of vascular disease and disturbances of arterial function measured non-invasively. This suggests that the technique of Doppler Ultrasound scanning would be useful in identifying pre-clinical macrovascular disease in diabetics as well as in assessing the response to therapies such as dietary regimes and exercise.

Table 3. *Relationships between arterial compliance assessed by Doppler Ultrasound, and potential risk factors for macrovascular disease, in healthy subjects*

Factor	*All subjects*			*Men*		
	No. of subjects[a] *n*	*Correlation coefficient r*	*P value*	*No. of subjects*[a] *n*	*Correlation coefficient r*	*P value*
Total cholesterol	43	−0.44	< 0.005	35	−0.43	< 0.01
HDL cholesterol	40	0.38	< 0.05	33	0.33	n.s.
Triglyceride	43	−0.24	n.s.	35	−0.29	n.s.
Oral glucose tolerance (area under curve)	36	−0.32	< 0.05	29	−0.21	n.s.
Systolic BP	44	−0.57	< 0.001	36	−0.52	< 0.01
Diastolic BP	44	−0.62	< 0.001	36	−0.55	< 0.001

n.s., not significant, $P > 0.05$.

[a]Of the 44 healthy subjects, 38 men (m) and 6 women (f). Age groups were 20 to 29 (m 10, f 3), 30 to 39 (m 11), 40 to 49 (m 8, f 2), 50+ (m 9, f 1).

The arterial segment examined for pulse wave velocity, from which arterial compliance was calculated, was left subclavian to external iliac.

Nutritional intervention

Several risk factors have the potential for nutritional modification (Table 4). The extent to which this is possible or worthwhile will depend on studies which link dietary intervention with risk factor profiles and measurement of arterial disease, preferably non-invasively.

Table 4. *Atherogenesis in diabetes and possibilities for nutritional intervention*

Endothelial damage	Due to:	*impaired oxygen transport
		*hypertension
		cigarette smoking
*Altered lipoprotein metabolism		
*Altered FFA metabolism		
*Altered arterial wall metabolism	Especially:	fatty acid synthesis
		cholesterol ester formation and removal
		glycosaminoglycan metabolism
		connective tissue metabolism
*Hyperinsulinaemia	Affecting:	arterial wall metabolism
		arterial wall smooth muscle cell proliferation
Increased platelet aggregation	Due to:	*altered fatty acid patterns
		*hyperlipidaemia

*Potentially nutritionally related

In conclusion, the more clear definition of risk factors for macrovascular disease in diabetics, together with the improved delineation of the atherogenic process in diabetics, paves the way for various forms of intervention, not the least of these being nutritional at several points (Editorial, 1981).

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