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Mechanisms, Significance and Treatment of Vascular Dysfunction in Type 2 Diabetes Mellitus

Focus on Lipid-Regulating Therapy

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Contents

Abstract

Endothelial dysfunction and increased arterial stiffness occur early in the pathogenesis of diabetic vasculopathy. They are both powerful independent predictors of cardiovascular risk. Advances in non-invasive methodologies have led to widespread clinical investigation of these abnormalities in diabetes mellitus, generating a wealth of new knowledge concerning the mechanisms of vascular dysfunction, risk factor associations and potential treatment targets.

Endothelial dysfunction primarily reflects decreased availability of nitric oxide (NO), a critical endothelium-derived vasoactive factor with vasodilatory and anti-atherosclerotic properties. Techniques for assessing endothelial dysfunction include ultrasonographic measurement of flow-mediated vasodilatation of the brachial artery and plethysmography measurement of forearm blood flow responses to vasoactive agents. Arterial stiffness may be assessed using pulse wave analysis to generate measures of pulse wave velocity, arterial compliance and wave reflection.

The pathogenesis of endothelial dysfunction in type 2 diabetes is multifactorial, with principal contributors being oxidative stress, dyslipidaemia and hyperglycaemia. Elevated blood glucose levels drive production of reactive oxidant species (ROS) via multiple pathways, resulting in uncoupling of mitochondrial oxidative phosphorylation and endothelial NO synthase (eNOS) activity, reducing NO availability and generating further ROS. Hyperglycaemia also contributes to accelerated arterial stiffening by increasing formation of advanced glycation end-products (AGEs), which alter vessel wall structure and function. Diabetic dyslipidaemia is characterised by accumulation of triglyceride-rich lipoproteins, small dense low-density lipoprotein (LDL) particles, reduced high-density lipoprotein (HDL)-cholesterol and increased postprandial free fatty acid flux. These lipid abnormalities contribute to increasing oxidative stress and may directly inhibit eNOS activity.

Although lipid-regulating agents such as HMG-CoA reductase inhibitors (statins), fibric acid derivatives (fibrates) and fish oils are used to treat diabetic dyslipidaemia, their impact on vascular function is less clear. Studies in type 2 diabetes have yielded inconsistent results, but this may reflect sampling variation and the potential over-riding influence of oxidative stress, dysglycaemia and insulin resistance on endothelial dysfunction. Results of positive intervention trials suggest that improvement in vascular function is mediated by both lipid and

non-lipid mechanisms, including anti-inflammatory, anti-oxidative and direct effects on the arterial wall. Other treatments, such as renin-angiotensin-aldosterone system antagonists, insulin sensitisers and lifestyle-based interventions, have shown beneficial effects on vascular function in type 2 diabetes. Novel approaches, targeting eNOS and AGEs, are under development, as are new lipid-regulating therapies that more effectively lower LDL-cholesterol and raise HDL-cholesterol. Combination therapy may potentially increase therapeutic efficacy and permit use of lower doses, thereby reducing the risk of adverse drug effects and interactions. Concomitant treatments that specifically target oxidative stress may also improve endothelial dysfunction in diabetes. Vascular function studies can be used to explore the therapeutic potential and mechanisms of action of new and established interventions, and provide useful surrogate measures for cardiovascular endpoints in clinical trials.

Type 2 diabetes mellitus is associated with marked increased risk of cardiovascular disease.[1] Impaired endothelial function and increased arterial stiffness are early signs of diabetic vasculopathy.[2,3] These early vascular changes may be causally related to the microvascular and macrovascular complications of diabetes.[2-5] Recent prospective studies demonstrate that endothelial function[6-10] and arterial stiffness[11-18] are both independent predictors of cardiovascular events, including cardiovascular mortality, and could, therefore, be useful surrogate endpoints in clinical trials. Advances in noninvasive methodologies have led to widespread clinical investigation of vascular function in diabetic patients.[19] This has generated a wealth of new knowledge concerning risk factor associations, mechanisms and therapy of diabetic vasculopathy.

Diabetic dyslipidaemia reflects diverse abnormalities in lipoprotein metabolism including, chiefly, hypertriglyceridaemia, low high-density lipoprotein (HDL)-cholesterol and increased small dense low-density lipoprotein (LDL) particles.[20] These risk factors contribute significantly to abnormalities in vascular function in type 2 diabetic patients, consistent with their ability to predict cardiovascular events in diabetes and the metabolic syndrome.[21]

Strategies for lipid regulation in diabetic dyslipidaemia include lifestyle changes, aimed at weight reduction and physical exercise,[22,23] and the use of pharmacotherapeutic agents such as HMG-CoA reductase inhibitors (statins), fibric acid derivatives (fibrates), fish oils and nicotinic acid (niacin).[24] This review focuses on the mechanisms of vascular dysfunction in type 2 diabetes, with specific reference to dyslipidaemia and oxidative stress, and on the effectiveness of lipid-regulating drugs and other therapies in correcting these abnormalities.

To fully understand and appreciate the importance of the trials reviewed requires an initial presentation of the concepts and techniques for the measurement of endothelial function and arterial stiffness, as well as the clinical significance of these tests.

1. Vascular Dysfunction: Concepts and Definitions

1.1 Endothelial Dysfunction

Endothelial dysfunction occurs early in the process of atherogenesis[4] (figure 1) and results from endothelial cell activation and/or injury. The endothelium has important physiological functions, which are mediated by the release of vasoactive factors that regulate vessel wall tone, cellular growth, haemostasis and inflammation.[25] Arguably, the most critical of these endothelium-derived molecules is nitric oxide (NO).[26]

In response to shear stress or stimulation of endothelial surface receptors by agents such as acetyl-

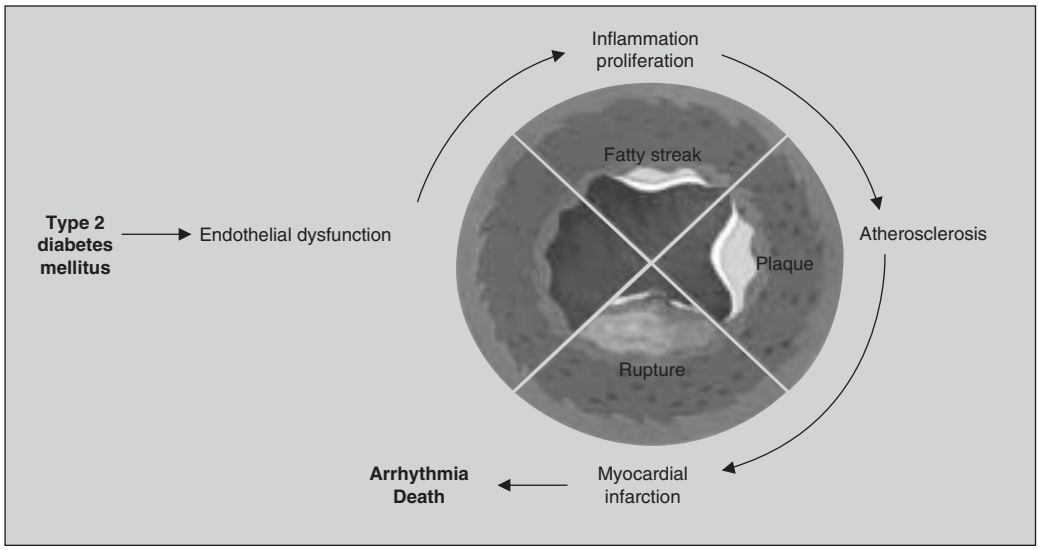

Fig. 1. Endothelial dysfunction is an early phase of atherosclerosis in type 2 diabetes mellitus.

choline or bradykinin, a G-protein signal transduction pathway is activated that, by increasing cytosolic calcium levels, stimulates endothelial NO synthase (eNOS) [figure 2]. Calcium enters the cell via membrane channels or is released from intracellular stores, and forms a complex with calmodulin, which subsequently binds to eNOS. This increases the activity of eNOS, forming NO and citrulline from arginine, molecular oxygen and reduced nicotinamide adenine dinucleotide phosphate (NADPH). NO is then released into the subendothelial space

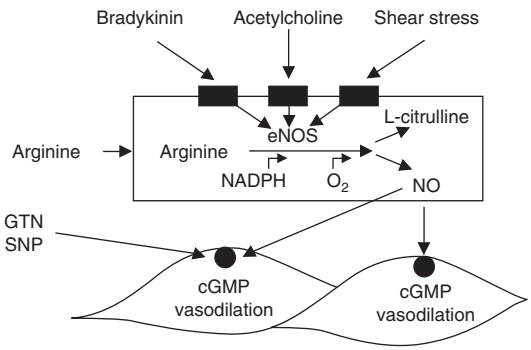

Fig. 2. The arginine/nitric oxide (NO) pathway. In response to a variety of stimuli, oxidation of arginine by endothelial NO synthase (eNOS) produces NO. **cGMP** = cyclic guanosine monophosphate; **GTN** = glyceryl trinitrate, **NADPH** = reduced nicotinamide adenine dinucleotide phosphate; **SNP** = sodium nitroprusside.

and vascular lumen, where it exerts potent vasodilatory, antimitotic, anti-inflammatory, antithrombotic and antioxidant effects.

Endothelial dysfunction also reflects altered release of vasoconstrictors such as endothelin-1, and other vasodilators such as endothelial-derived hyperpolarising factor (EDHF) and prostacyclin.[27]

Endothelial dysfunction due to impaired release and/or action of NO has been demonstrated in asymptomatic patients who have conventional cardiovascular risk factors such as hypercholesterolaemia,[28,29] cigarette smoking,[30] hypertension,[31] diabetes,[32,33] male sex,[34] postmenopausal status,[35] advanced age,[36] a family history of premature coronary disease,[37] renal failure[38] and hyperhomocysteinaemia.[39] These, and other less traditional risk factors, combine with genetic influences to promote vascular contraction, remodelling, inflammation, thrombosis and plaque rupture, through the common pathway of endothelial dysfunction (figure 3).

1.2 Arterial Stiffness

Arterial stiffness is influenced by both structural and functional properties of the vessel wall, which are determined by its content of smooth muscle,

elastin and collagen. The fracturing of elastin fibres and qualitative changes in collagen that occur with aging, lead to a gradual increase in aortic arch diameter, with a consequent decrease in aortic distensibility and arterial buffering.[40] Arterial stiffness is also related to endothelial function:[41,42] reduced endothelial production of NO and increased release of endothelin-1 or angiotensin II can affect arterial stiffness via a vasotonic effect on vascular smooth muscle.[43-45]

2. *In Vivo* Tests of Vascular Function

2.1 Endothelial Function

Endothelial function has been most widely studied *in vivo* in humans by measuring the NO-mediated vasodilatory responses of peripheral conduit[46,47] and resistance arteries[48] to various stimuli. In the brachial artery, shear stress is generated by hyperaemia following a period of induced local ischaemia, and flow-mediated dilatation (FMD) is measured using high-resolution ultrasonography.[47] The use of edge-detection software reduces within-subject variability of this technique to approximately 15%.[49] Blood flow changes in the forearm microcirculation following intra-arterial infusion of muscarinic receptor agonists such as acetylcholine can be measured using venous occlusion strain-gauge plethysmography.[48]

These responses in the different arterial beds of the peripheral circulation are generally associated with each other,[50] and provide a reasonable surrogate measure for the coronary circulation.[51,52] Indeed, the risk factors associated with endothelial dysfunction are similar for both peripheral and coronary arteries.[53] Endothelial function in coronary vessels may also be studied in response to pharmacological agonist or shear stress stimuli, using quantitative angiography to measure vessel diameter changes.[54] Laser Doppler fluximetry may be used to measure endothelial function of the skin microcirculation,[55] but experience is limited.

Elevated plasma levels of endothelial markers (e.g. von Willebrand factor [vWF]) and microalbuminuria may also provide indirect estimates of endothelial dysfunction in diabetes.[3,56-58] Similarly, the soluble adhesion molecules E-selectin, vascular cellular adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 may be measured

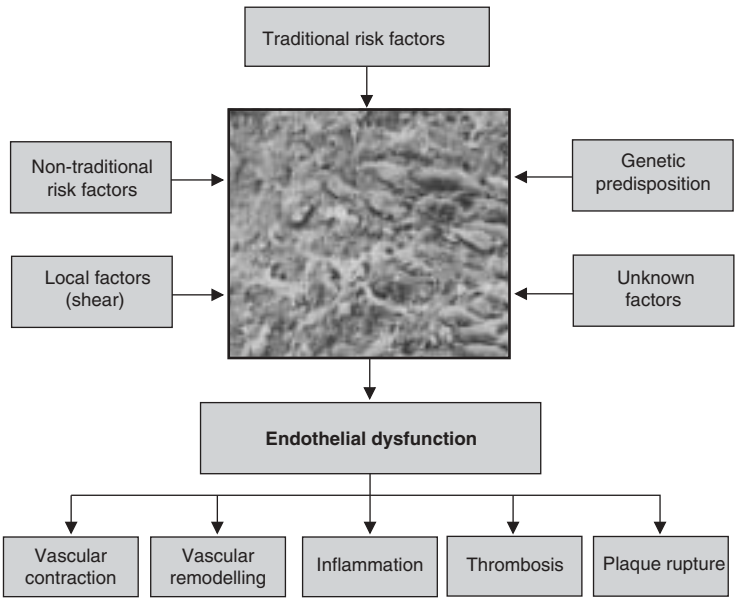

Fig. 3. The endothelium as a transducer of cardiovascular disease risk factors.

in plasma as indirect indices of endothelial activation.[59-61]

Impaired mobilisation or depletion of endothelial progenitor cells formed from bone marrow may be involved in the pathogenesis of endothelial dysfunction, and their circulating levels could also potentially be used as a surrogate biological marker of endothelial dysfunction. A recent study showed that the number of endothelial progenitor cells sampled from peripheral blood was closely associated with the Framingham Risk Score and a better predictor of brachial artery reactivity than traditional cardiovascular risk factors.[62]

2.2 Arterial Stiffness

As predicted by the Bramwell-Hill equations, pulse wave velocity (PWV) is directly related to arterial elasticity[63] and is the most common method of assessing arterial stiffness. Central PWV is a measure of aortic arterial stiffness, and is calculated using carotid and femoral arterial pressure/flow waveforms obtained via applanation tonometry, acoustic transducers, Doppler ultrasound or magnetic resonance imaging.[64] Arterial compliance and/or distensibility (i.e. compliance relative to initial volume) of the superficial arteries (brachial, femoral and carotid) may be assessed by ultrasound and phase-locked wall-tracking techniques,[65] which measure displacement diameter relative to change in pressure within the cardiac cycle.

Newer measures include large and small artery compliance (termed C_1 and C_2, respectively), derived from the 'Windkessel' modelling of the circulation,[66] and the 'augmentation index' (AIx), which reflects the augmentation of the forward travelling pressure wave resulting from wave reflection.[67] Brachial pulse pressure may also be used as a surrogate measure of systemic arterial stiffness,[68] particularly in diabetic patients where it is often linearly associated with central pulse pressure. Central and peripheral pulse pressure, aortic compliance, and C_1 and C_2 are significantly associated with conduit vessel endothelial function,[69,70] suggesting that large and small vessel stiffness may be either a cause or consequence of endothelial dysfunction.

3. Prognostic Significance of Arterial Dysfunction

3.1 Endothelial Function

Endothelial dysfunction has been shown to be independently predictive of cardiovascular events in patients with known coronary artery disease (CAD). In one study, impaired coronary endothelial vasoreactivity, angiographically visible coronary atherosclerosis and arterial hypertension were the only independent predictors of cardiovascular events over a mean follow-up period of 6.7 years.[6] There was a 40% reduction in cardiovascular events for those patients in the upper versus lower tertile of FMD, and a 20% reduction for those with normal versus abnormal coronary vasoreactivity. This was consistent with coronary vasoreactivity data from a long-term follow-up (mean 28 months) of patients with mild CAD.[7]

Impaired FMD of the brachial artery has also been shown to be of prognostic value over 5 years in patients with chest pain (for FMD <10%: negative predictive value 85%, positive predictive value 52%).[8] An absolute 1% decrease in FMD increased the risk of a cardiac event by 33%. Similarly, in CAD patients, there was a 19% increased risk of cardiovascular events per 1 mL/min per 100mL decrease in acetylcholine-induced forearm blood flow.[9]

Importantly, endothelial dysfunction of the peripheral circulation has been shown to have prognostic value in hypertensive patients without clinical evidence of angina pectoris or heart disease.[10] In this study, patients in the upper tertile of increase in acetylcholine-stimulated forearm blood flow had 43% fewer cardiovascular events than those in the lowest tertile, with 24-hour mean blood pressure being the only other independent predictor of events. However, no prognostic studies have been performed in individuals with type 2 diabetes.

3.2 Arterial Stiffness

Several indices of arterial stiffness have each been shown to be strong prognostic indicators in

nondiabetic individuals. Brachial artery pulse pressure, central PWV, AIx,[16] small artery compliance (C_2)[18] and elastic modulus (the reciprocal of distensibility)[17] were independent predictors of all-cause and cardiovascular mortality in healthy[11,12] and elderly[15] subjects, patients with hypertension[13,14] and end-stage renal failure,[16,17] and in individuals with high cardiovascular risk.[18] An absolute 10% increase in AIx was associated with a 51% increase in all-cause mortality and a 48% increase in cardiovascular mortality amongst patients with end-stage renal failure.[16] Similarly, in a study of 419 individuals, 41% of whom reported one or more cardiovascular events during up to 7 years of follow-up, a 2-unit decrease in C_2 was associated with a 50% increase in risk of cardiovascular events, independent of age.[18]

Arterial stiffness may be an even stronger predictor of cardiovascular events in patients with type 2 diabetes. Both aortic PWV and glucose tolerance status, but not systolic blood pressure, were shown to be independent predictors of total mortality amongst subjects, some of whom had diabetes: a 1 m/sec increase in PWV was associated with an 8% increase in all-cause and cardiovascular mortality.[71] In the Hoorn Study,[5] pulse pressure predicted 9-year cardiovascular mortality independently of age, gender and mean arterial pressure, in diabetic, but not nondiabetic, individuals. Type 2 diabetic patients with end-stage renal disease had increased overall and cardiovascular 5-year mortality compared with nondiabetic patients,[72] and aortic PWV was an independent predictor of both cardiovascular and overall mortality in all patients combined.

In summary, *in vivo* measures of both endothelial function and arterial stiffness have been demonstrated to have significant prognostic value in individuals with or at risk of CAD. However, the specificity and positive predictive value of these tests are still relatively poor. Studies in individuals with type 2 diabetes have not yet been performed. *In vitro* measurement of the number of circulating endothelial progenitor cells may provide a more useful marker of endothelial dysfunction, although further work is required. In addition, no formal comparison of the relative ability of endothelial function and arterial stiffness to predict cardiovascular events has yet been performed in either diabetic or nondiabetic individuals. Given the influence of endothelial function on arterial stiffness,[69,70] it is expected that the predictive value of these measures would be comparable.

4. Vascular Dysfunction in Type 2 Diabetes Mellitus

4.1 Endothelial Function

Endothelial dysfunction in individuals with type 2 diabetes is present in resistance and conduit vessels of the peripheral circulation,[33,73-75] as well as in the coronary circulation.[76] Plasma levels of vWF, a marker of endothelial cell damage and activation, are increased,[77] as are VCAMs, ICAMs and selectins.[78-80] The prevalence of microalbuminuria and the transcapillary escape rate of albumin are also increased, indicating widespread vascular dysfunction.[81]

Since insulin resistance has been shown to be associated with impaired endothelial function[82,83] and they both share associations with common metabolic abnormalities, it has been suggested that endothelial dysfunction is an integral aspect of the insulin resistance syndrome. Accordingly, insulin receptor substrate-1-deficient mice develop features of both insulin resistance and endothelial dysfunction.[84] Several features of the insulin resistance syndrome which are common to type 2 diabetes, including low HDL-cholesterol,[85] hypertension[86] and visceral obesity,[87] have also been associated with endothelial dysfunction in nondiabetic individuals. However, endothelial dysfunction in patients with type 2 diabetes occurs independently of obesity[88] and hypertension,[89] both of which can further impair endothelial function. Other factors that are associated with endothelial dysfunction in type 2 diabetes include diabetic dyslipidaemia,[20] postprandial hyperlipidaemia,[90-92] fasting and postprandial hyperglycaemia,[93-95] increased oxidative stress[20] and inflammation.[96,97] Increased oxidative

stress results from these and other risk factors, and may be central to the aetiology of diabetic endothelial dysfunction and its sequelae (figure 4).

4.2 Arterial Stiffness

Increased arterial stiffness has been demonstrated consistently in type 2 diabetes using a variety of techniques. Brachial and central pulse pressure,[98] central PWV[98-100] and AIx[101] are increased, while both large (C_1)[102,103] and small (C_2)[104,105] artery compliance are reduced. This may partly be a consequence of endothelial dysfunction, which is known to affect both large and small vessel stiffness.[70,106,107] In nondiabetic individuals, increased arterial stiffness with aging is related to advanced glycation end-product (AGE)-induced collagen cross-linking in connective tissue and matrix components,[108-110] as well as calcium deposition in the extracellular matrix leading to calcification of elastin fibres.[111] Diabetic arteries age at an accelerated rate[109,110] under the influence of dysglycaemia,[102,105,112] insulin resistance,[113] duration of diabetes[100] and in the presence of risk factors known to be associated with endothelial dysfunction, such as dyslipidaemia,[102] hypertension[114-116] and oxidative stress.[20] Increased arterial stiffness in diabetes may be due to vascular insulin resistance[117] and either the direct or indirect consequences of hyperglycaemia, including the formation of AGEs.[109,118,119] AGEs potentially increase type IV collagen synthesis in the arterial wall[120] and increase its resistance to collagen matrix degradation.[121] Increased arterial stiffness in diabetes may also reflect arterial wall accumulation of laminin, fibronectin and hyaluronic acid.[122]

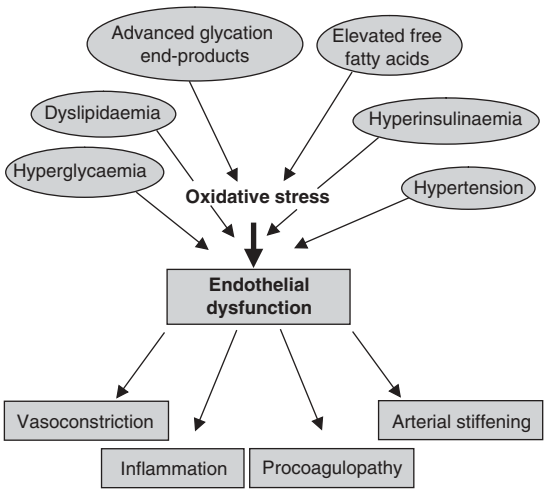

Fig. 4. Aetiology and consequences of endothelial dysfunction in type 2 diabetes mellitus.

5. Diabetic Dyslipidaemia

The dyslipidaemia of type 2 diabetes is characterised by hypertriglyceridaemia, low HDL-cholesterol, an increase in chylomicron remnants and accumulation of small dense LDL and HDL particles. This so-called 'atherogenic lipid profile' is a major risk factor for coronary disease in diabetic patients.[123] The aetiology of diabetic dyslipoproteinaemia is complex,[124] relating collectively to poor glycaemic control,[125] hyperinsulinaemia and insulin resistance,[126,127] and dysregulated fatty acid and lipoprotein metabolism[128,129] (figure 5). Insulin resistance stimulates hepatic output of triglyceride-rich very low-density lipoprotein (VLDL), specifically the large $VLDL_1$ species, partly due to increased delivery of free fatty acids from adipose tissue to the liver,[130,131] expansion in liver fat content[132,133] and reduction in the direct inhibitory effect of insulin on hepatic apolipoprotein (apo) B secretion.[126,134] In diabetes, increased fatty acid release by adipose tissue induces hepatic and skeletal muscle insulin resistance.[135] Fatty acids may also be lipotoxic to pancreatic β cells, thereby contributing to β-cell failure and hyperglycaemia.[136] Clearance of chylomicrons is impaired in diabetes as a result of reduced synthesis of endothelial-bound lipoprotein lipase (LPL) in adipose tissue[137,138] and decreased receptor-mediated endocytosis in the liver.[139] The increased hepatic production of $VLDL_1$ particles in diabetes delays chylomicron clearance by competing for and saturating the lipolytic capacity of LPL, and the activity of VLDL and remnant receptors within the liver. These mechanisms collectively account for postprandial lipaemia,[140] which may be a particularly important causal mechanism of vascular dysfunction in diabetes.

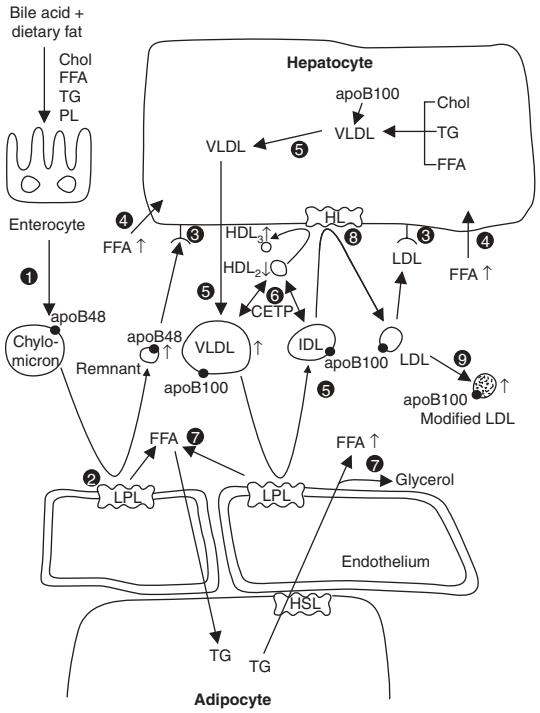

Fig. 5. Schematic representation of abnormalities in lipoprotein metabolism in type 2 diabetes mellitus. (**1**) increased apolipoprotein (apo)B48 synthesis and chylomicron triglyceride (TG); (**2**) decreased lipoprotein lipase (LPL) activity; (**3**) decreased chylomicron remnant and low-density lipoprotein (LDL) clearance via decreased receptor activity; (**4**) increased free fatty acid (FFA) delivery to liver; (**5**) increased production and secretion of very low-density lipoprotein (VLDL)-TG and VLDL-apoB100; (**6**) increased cholesteryl ester transfer protein (CETP) transfer of TG to high-density lipoprotein (HDL) and LDL; (**7**) increased FFA generation from TG lipolysis in adipose tissue; (**8**) increased activity of hepatic lipase (HL) leading to LDL formation, especially small, dense LDL (see text); (**9**) increased formation of modified (glycosylated, oxidised) LDL. Chylomicron remnants, VLDL and HDL are also modified (reproduced from Watts and Playford,[20] with permission from Elsevier). **Chol** = cholesterol; **HSL** = hormone sensitive lipase; **IDL** = intermediate-density lipoprotein; **PL** = phospholipid; ↓ indicates decrease; ↑ indicates increase.

Important compositional changes in lipoproteins are present in type 2 diabetes.[141] Cholesteryl ester enrichment of VLDL and triglyceride enrichment of both LDL and HDL probably arise from expansion of the VLDL-triglyceride pool, and from the associated increase in neutral lipid exchange among lipoproteins, via the action of cholesteryl ester transfer protein (CETP).[142,143] Increased phospholipid transfer protein activity in type 2 diabetes may also contribute to hypertriglyceridaemia and compositional changes in HDL.[144-146] Increased lipolysis of triglyceride-rich HDL and LDL, as a consequence of an effect of insulin resistance that increases the activity of hepatic lipase,[147,148] produces lipoprotein particles that are smaller, denser and potentially more atherogenic.[149-153] Hence, the effect of diabetes on LDL subtypes is to increase the proportion of LDL_3 particles. These small dense LDL particles have an increased sensitivity to oxidative modification and are more able to penetrate the arterial intima and bind to proteoglycans than the more buoyant large LDL particles.[154-157] The effect of diabetes on HDL size is to decrease the levels of both HDL_2 and HDL_3, with a greater reduction in HDL_2. These reductions in HDL particles are paralleled by a decrease in plasma levels of apoA-I and HDL lipoproteins containing both apoA-I and apoA-II (LpA-I **:** A-II) particles.[158] The lowering of HDL is important in two respects as it implies a significant reduction in the rate of reverse cholesterol transport and a decrease in the direct anti-atherogenic effects of HDL (e.g. its antioxidant and anti-inflammatory properties).[159] These various lipoprotein abnormalities and the associated risk of cardiovascular disease in type 2 diabetes are likely determined by various genes that regulate lipid and lipoprotein metabolism, for example LPL,[160] apoE,[161] apoC-III[162,163] and CETP.[164] This illustrates an important node for the genetic control of endothelial dysfunction in type 2 diabetes.

6. Pharmacotherapy for Dyslipidaemia in Type 2 Diabetes

The American Diabetes Association (ADA) has made recommendations regarding nutrition therapy,[22] weight loss and physical exercise[23] for the modification of lipids and lipoproteins in type 2 diabetes. However, effective management of diabetic dyslipidaemia generally requires additional lipid-regulating pharmacotherapy. Subgroup analyses of primary and secondary prevention trials indicate that lipid modification with fibric acid derivatives and statins in diabetic patients is associated with significant reduction in cardiovascular events.[165]

Table I. Plasma lipid and lipoprotein targets: current recommendations for the management of diabetic dyslipidaemia

Study (year)	Lipid target priorities (mmol/L)		
	first	second	third
EAS[171] (1998)	LDL-C <3.0	HDL-C >1.0	TG <2.0
NCEP[172] (2001)	LDL-C <2.6	Non-HDL-C <3.4 if TG >2.3	
ADA[24] (2002)	LDL-C <2.6	HDL-C >1.2	TG <2.3
NHFA and CSANZ[170] (2001)	LDL-C <2.5	HDL-C >1.0	TG <2.0

ADA = American Diabetes Association; **CSANZ** = Cardiac Society of Australia and New Zealand; **EAS** = European Atherosclerosis Society; **HDL-C** = high-density lipoprotein-cholesterol; **LDL-C** = low-density lipoprotein-cholesterol; **NCEP** = National Cholesterol Education Program; **NHFA** = National Heart Foundation of Australia; **TG** = triglycerides.

The ADA recommends aggressive lowering of LDL-cholesterol using statins in the first instance to reduce the risk of CHD in patients with diabetes[24] (table I). Intensive LDL-cholesterol lowering, together with reduction of other cardiovascular risk factors, is now recommended even for type 2 diabetic patients whose LDL-cholesterol is not substantially elevated.[166-169] Detailed recommendations for target lipid levels, lifestyle modifications and lipid pharmacotherapy have also been provided for Australian physicians[170] (table I).

6.1 HMG-CoA Reductase Inhibitors (Statins)

Statins are potent LDL-cholesterol-lowering agents with significant, but lesser, effectiveness in lowering triglycerides. Reductions of up to 55% in LDL-cholesterol and 30% in triglycerides are possible, as well as increases in HDL-cholesterol of up to 15%.[172] The principal mechanism by which statins reduce cholesterol relies upon the inhibition of cholesterol synthesis at the hydroxymethylglutaryl coenzyme A reductase step, with reciprocal regulation of LDL and other liver receptors, and increased catabolism of LDL and remnant lipoproteins.

In the Heart Protection Study,[173] the first primary prevention trial to specifically study the effect of statin therapy in men and women with diabetes, allocation to treatment with simvastatin 40 mg/day lowered LDL-cholesterol by 1.0 mmol/L and reduced the rate of first major vascular events by approximately one-quarter. Subgroup analysis of secondary prevention trials has also shown statins to be at least as effective in reducing major coronary events in diabetic versus nondiabetic subjects.[174-176] The benefits of statin therapy occurred despite average baseline LDL-cholesterol levels that were only moderately elevated (3.2–3.7 mmol/L),[173,175,176] suggesting that they may reduce cardiovascular risk via additional non-lipid mechanisms, and particularly in diabetic patients. These non-lipid effects include improvement in endothelial function[177] as well as inhibition of monocyte adhesion and platelet aggregation, thrombus formation[178] and inflammation.[178,179]

Rosuvastatin, the most recently developed of these medications, has been shown to be more efficacious than pravastatin, atorvastatin or simvastatin in achieving therapeutic targets for plasma LDL-cholesterol, triglycerides and HDL-cholesterol.[180-182] Its non-lipid lowering effects have not yet been reported, but its pleiotropic properties are likely to be similar to other drugs of this class.[183] As with all statins, the frequency of myopathy increases with the dose of rosuvastatin. Myopathy[184] is most likely with doses of 80 mg/day and in the presence of impaired renal function. In doses of up to and including 40 mg/day, the adverse effects of rosuvastatin are similar to other statins.[181]

6.2 Fibric Acid Derivatives

Fibric acid derivatives are agonists of peroxisome proliferator-activated receptor (PPAR)-α. PPARs are ligand-activated nuclear hormone receptors that regulate several genes involved in lipid metabolism and atherogenesis.[185] They regulate diabetic dyslipidaemia by increasing the clearance of VLDL, intermediate-density lipoprotein (IDL) and LDL-cholesterol, and increasing the production of HDL-cholesterol.[186] These metabolic changes are a consequence of transcriptional effects in the

liver that increase LPL, acyl-coenzyme A synthase, fatty acid transfer protein, and apoA-I and apoA-II, while decreasing the expression of apoC-III, an inhibitor of LPL activity.[187,188] Fibric acid derivative therapy plays an important role in controlling diabetic dyslipidaemia as it significantly decreases fasting and postprandial hypertriglyceridaemia, and may increase HDL-cholesterol by as much as 20%.[172] The reduction in LDL-cholesterol is much less with fibric acid derivatives than with statins, and LDL-cholesterol can actually increase in individuals with marked hypertriglyceridaemia.[189] However, fibric acid derivatives increase the formation of large, less dense LDL particles,[172] which may be of major clinical importance.[190] Evidence in humans that fenofibrate can potentially enhance reverse cholesterol transport in type 2 diabetes has recently been provided by stable isotope kinetic studies,[186] and experimental studies in circulating mononuclear cells which show increases in liver X receptor (LXR)-α and adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1) messenger RNA (mRNA) levels.[191]

In the Helsinki Heart Study,[192] gemfibrozil was associated with a nonsignificant reduction in coronary heart disease (CHD) in type 2 diabetic patients without previous heart disease. In VA-HIT (Veterans Affairs High-Density Lipoprotein Intervention Trial),[193] a secondary prevention study of men with previous myocardial infarction, gemfibrozil decreased coronary events by 22%. This effect was most pronounced in individuals with diabetes, insulin resistance and the metabolic syndrome.[194] About 23% of the reduction in cardiovascular events was explained by lipid changes, specifically increases in HDL_3-cholesterol.[195] That a large proportion of the cardiovascular benefit of fibric acid derivatives was not explicable by lipid changes in VA-HIT suggests that these agents have direct beneficial effects on reducing atherogenesis by, for example, increasing NOS expression, and decreasing expression of endothelin-1, activator protein-1 and nuclear factor-kappa B (NF-κB).[185,187,188]

In the DAIS (Diabetes Atherosclerosis Intervention Study),[196] fenofibrate significantly reduced the progression of coronary atherosclerosis over a 39-month follow-up period. Fenofibrate is a more specific and potent PPAR-α agonist than gemfibrozil,[197] and accordingly has a greater effect in increasing the expression of apoA-I and apoA-II. Its benefit in protecting diabetic patients against CAD may, however, principally relate to increasing LDL particle size[190] and to direct pleiotropic effects on the artery wall.[185]

6.3 Fish Oils

Secondary prevention trials in CHD demonstrate increased survival with n-3 fatty acid supplementation,[198,199] thereby supporting numerous prospective cohort studies describing an inverse association between fish intake and CHD mortality.[200-205] In the GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico)-Prevenzione trial, cardiovascular death in patients with previous myocardial infarction was reduced by approximately 30%, and was similar amongst individuals with and without diabetes.[198] The cardioprotective properties of n-3 fatty acids include a favourable effect on the typical dyslipidaemic profile of insulin resistance and type 2 diabetes.[206-208] Fish oils consistently reduce plasma triglycerides in both diabetic[209] and nondiabetic[210] individuals by as much as 40%.[211] They act as weak PPAR-α agonists, with free fatty acids being directed towards oxidation and away from lipid storage.[212] While fish oil normally has little effect on total HDL-cholesterol, both purified docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), increased HDL_2-cholesterol by 16% and 12%, respectively, in treated hypertensive type 2 diabetic individuals.[213] Fish oil may also increase LDL particle size,[214] an effect which may be predominantly mediated by DHA, rather than EPA.[215,216]

6.4 Nicotinic Acid (Niacin)

Nicotinic acid-based agents, which are available as intermediate release or extended release formulations, can decrease plasma triglyceride levels by up to 30%, decrease LDL-cholesterol by up to 15% and also increase HDL-cholesterol by up to 25%.[172]

The Coronary Drug Project[217] showed that in patients with established CHD, treatment with nicotinic acid reduced recurrent non-fatal myocardial infarction by 27% over 5 years, and reduced total mortality by 11% after 15 years of follow-up.

The cardiovascular benefits of nicotinic acid therapy need to be further demonstrated in clinical endpoint trials in patients with insulin resistance and diabetes. However, nicotinic acid may impair insulin action, decrease glucose tolerance, and increase plasma glucose and uric acid levels;[218,219] therefore, current guidelines do not recommend the use of nicotinic acid in patients with diabetes.[24] The mechanism for these adverse effects appears to be related to a decrease in insulin sensitivity caused by an elevation in free fatty acids.[220,221] However, extended release forms of nicotinic acid appear to lack these adverse effects.[222]

6.5 Ezetimibe

Ezetimibe belongs to a novel class of lipid-regulating drugs that potently impair the net intestinal absorption of biliary and dietary cholesterol.[223] It is well tolerated both as monotherapy and combination therapy.[224] In pooled analysis of phase II trials, administration of ezetimibe 10 mg/day to patients with primary hypercholesterolaemia significantly decreased LDL-cholesterol by 18%, significantly increased HDL-cholesterol by 3.5% and resulted in a nonsignificant 5% reduction in triglycerides,[223] with similar results found in phase III trials.[225,226] The role of ezetimibe monotherapy in patients with type 2 diabetes is probably limited, since it chiefly lowers LDL-cholesterol alone. Its potential as combination therapy is more promising for diabetic dyslipidaemia.

6.6 Combination Therapy

In many patients with dyslipidaemia, lipid-regulating monotherapy (e.g. statins or fibric acid derivatives) may not provide adequate improvement in their lipid profiles. Several possible treatment combinations include statin/fibric acid derivative, fibric acid derivative/ezetimibe, statin/nicotinic acid and statin/fish oil regimens. Fibric acid derivatives, nicotinic acid and fish oils are generally more effective than statins at lowering VLDL-triglyceride levels. Since their mechanisms of action on lipoprotein metabolism are complementary, the addition of either a fibric acid derivative, nicotinic acid or fish oils to a statin should theoretically provide desirable correction of plasma triglycerides, LDL-cholesterol and HDL-cholesterol, and will, in particular, reduce the formation of atherogenic small dense LDL particles. In 120 patients with diabetic dyslipidaemia, treatment with both atorvastatin and fenofibrate decreased plasma triglycerides by 50% and LDL-cholesterol by 46%, as well as increased HDL-cholesterol by 22%.[227] A clinical trial assessing the benefits of adding fenofibrate to simvastatin in individuals with type 2 diabetes is currently in progress.[228]

Ezetimibe does not inhibit or induce cytochrome P450 (CYP)[229] [e.g. CYP3A] or *N*-acetyltransferase enzymes and is, hence, ideally suited to combination therapy with statins, fibric acid derivatives and nicotinic acids. Ezetimibe could also be used in combination therapy to produce further decreases in LDL-cholesterol and triglycerides and increases in HDL-cholesterol. In nondiabetic patients, addition of ezetimibe to a statin results in significant additional reductions in LDL-cholesterol and triglycerides of approximately 12–14% and 10%, respectively, and increases in HDL-cholesterol of approximately 2%.[230,231] Such combination therapy will permit use of a lower dosage of statins, avoiding potential statin-related complications.

Combination lipid-regulating therapy, specifically use of statins with fibric acid derivatives or nicotinic acids, may increase the risk of adverse drug interactions such as myositis and hepatotoxicity.[232] Care should be taken when using combination therapy in patients with significant liver, muscle or renal dysfunction, in those with a history of liver or muscle enzyme elevation on treatment with statin or fibric acid derivative alone, where there is concomitant use of drugs known to impair CYP3A4 mixed function oxidase or polypharmacy, and where there is lack of physician and/or patient commitment to close clinical and laboratory monitoring.

6.7 Insulin Sensitisers

Thiazolidinediones (e.g. rosiglitazone, pioglitazone), also known as 'glitazones', are synthetic PPAR-γ agonists, which are currently used in the treatment of type 2 diabetes to correct insulin resistance.[233] Despite the drugs not being specifically used for the treatment of dyslipidaemia, pioglitazone and rosiglitazone both have significant antiatherogenic effects.[234,235]

Pioglitazone increases HDL-cholesterol by as much as 15%, reduces triglycerides (by 10–20%)[236,237] and increases LDL particle size,[238] but has little effect on LDL-cholesterol.[236,239,240] Although rosiglitazone appears to increase LDL-cholesterol by approximately 10% it also significantly increases HDL-cholesterol by similar amounts,[241,242] and increases LDL particle size,[242] an effect also observed with troglitazone.[243,244] When combined with atorvastatin, LDL-cholesterol and triglycerides decreased, while an increase in LDL particle size remained.[242] The incremental beneficial lipid effects may be related to the ability of thiazolidinediones to reduce insulin resistance.[233]

To date, few direct head-to-head comparisons of rosiglitazone and pioglitazone regarding lipid profiles including LDL particle size have been performed. However, in a comparison of type 2 diabetic patients with similar characteristics, pioglitazone was associated with greater beneficial effects than rosiglitazone on triglycerides and HDL-cholesterol in both monotherapy and combination treatment:[245] there was a greater decrease in serum triglycerides and HDL-cholesterol increased rather than decreased. Similar effects between the two drugs were observed after patients were assigned to either pioglitazone or rosiglitazone for either 3[246] or 4 months[247] after previous treatment with troglitazone.

Although thiazolidinediones may therefore be useful in the management of type 2 diabetes, of some concern are the results from a recent case-control study that suggests they may also cause a small but significant increased risk of heart failure as a result of sodium and water retention.[248] In particular, combining rosiglitazone with insulin is contraindicated. It is clear that long-term prospective clinical trials aimed at determining the effect of these insulin sensitisers on cardiovascular events in patients with type 2 diabetes are required. Metformin, which reduces gluconeogenesis and improves insulin sensitivity, also marginally improves lipid profiles,[249] possibly by activating adenosine monophosphate (AMP)-activated kinase.[250]

6.8 Summary

Modification of lipids and lipoproteins in patients with diabetic dyslipidaemia usually requires pharmacotherapy including statins and/or fibric acid derivatives in addition to lifestyle changes such as dietary modification, weight loss and physical exercise. Statin therapy either alone or in combination therapy is now recommended even for those diabetic individuals with normal LDL-cholesterol since statins have beneficial pleiotropic effects on the vasculature beyond those caused by lipid lowering alone. Increasing HDL-cholesterol is important in the context of endothelial function, which is positively associated with HDL-cholesterol in both nondiabetic[251,252] and diabetic[74] individuals. However, increasing HDL-cholesterol in diabetic patients can often prove to be problematic: although HDL consistently increases with the use of fibric acid derivatives and nicotinic acid,[253] the latter may impair insulin sensitivity and decrease glucose tolerance. Novel agents for regulating HDL metabolism[253] include inhibitors of CETP,[254] and LXR agonists that stimulate the expression of the newly identified ABCA1.[255] The latter also show promise in animal models for improving both hepatic and peripheral insulin sensitivity.[256] Ezetimibe is a novel lipid-regulating drug that potently impairs cholesterol absorption and can also be used in combination therapy to produce further reductions in LDL-cholesterol, triglycerides and, possibly, increases in HDL-cholesterol. Insulin sensitisers, such as thiazolidinediones, can raise HDL and LDL particle size and metformin also marginally improves lipid profiles. Further primary and secondary prevention trials in men and women with type 2 diabetes are

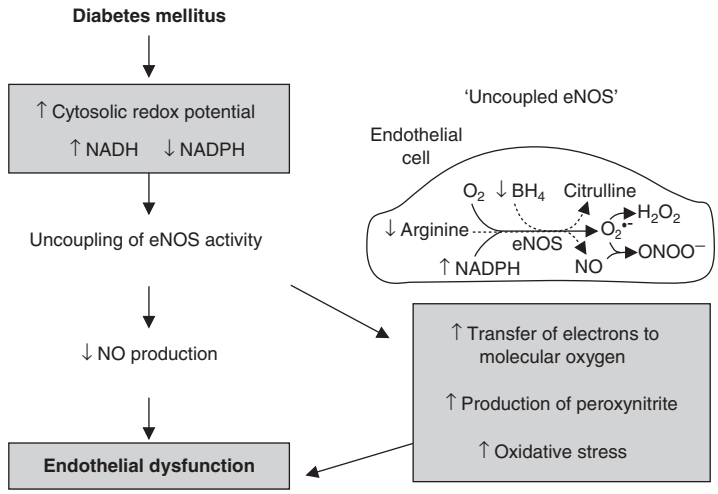

Fig. 6. Uncoupling of endothelial nitric oxide (NO) synthase (eNOS) activity. Effect on endothelial function and oxidative stress in type 2 diabetes mellitus. **BH_4** = tetrahydrobiopterin; **H_2O_2** = hydrogen peroxide; **NADH** = reduced nicotinamide adenine dinucleotide; **NADPH** = reduced nicotinamide adenine dinucleotide phosphate; **O_2** = molecular oxygen; **$O_2^{\bullet-}$** = superoxide; **$ONOO^-$** = peroxynitrite.

required to assess whether the beneficial effects on lipids and lipoproteins with these pharmacotherapies translate into reduced cardiovascular events. A key question relevant to the present review is whether reduction in cardiovascular risk due to improved plasma lipid and lipoproteins is predictable on the basis of improvements in endothelial function.

7. Mechanisms of Endothelial Dysfunction in Type 2 Diabetes

To understand how lipid-regulating agents and other therapies influence endothelial dysfunction in diabetes requires knowledge of the underlying pathobiochemical mechanisms involved in endothelial cell injury. These are discussed in this section, with specific reference to the roles of oxidative stress and dyslipidaemia.

The precise pathogenetic mechanisms underlying the development of endothelial dysfunction in type 2 diabetes remain unclear, but they probably involve uncoupling of both eNOS activity (leading to reduced NO production) [figure 6] and mitochondrial oxidative phosphorylation (figure 7), as well as the activation of vascular NAD(P)H oxidase. These three mechanisms essentially result in increased generation of superoxide ($O_2^{\bullet-}$) radicals and, in the case of eNOS uncoupling, in the overproduction of peroxynitrite (figure 8). We have previously hypothesised that the main factors that combine to cause these biochemical disturbances are dyslipoproteinaemia and oxidative stress.[20] Additional factors that may contribute, either individually or synergistically, to endothelial cell dysfunction in diabetes include hypertension,[89] visceral obesity,[87] inflammation,[96,97] insulin resistance,[82,83] hyperglycaemia,[93-95] postprandial hyperlipidaemia[90,91] and elevated plasma levels of asymmetrical dimethylarginine (ADMA),[257] an endogenous competitive inhibitor of eNOS.[20] It is likely that these are causally related to an increase in vascular oxidative stress (figure 4).[258]

7.1 Oxidative Stress

Oxidative stress essentially refers to an imbalance between reactive oxygen species (ROS) and antioxidant defences, which results in net overproduction of ROS. The genesis of ROS involves the production of superoxide by the coupling of electrons to molecular oxygen, and its subsequent reduction to yield hydrogen peroxide (H_2O_2) and, finally, hydroxyl radicals ($OH^{\bullet}$). There are several specific biochemical sources of ROS in vascular cells, including mitochondrial electron transport, xanthine

oxidase, cyclo-oxygenase (COX), NOS and NAD(P)H oxidase. Superoxide reacts with NO to form peroxynitrite, an amplification pathway for superoxide-mediated oxidative stress. NO and peroxynitrite are also referred to as reactive nitrogen species (RNS). Accumulation of ROS and/or RNS directly impairs several cellular functions by oxidising DNA, proteins and lipids. These highly reactive biomolecules also activate proinflammatory and pro-atherogenic intracellular signalling pathways involving NF-κB, protein kinase C (PKC) and mitogen-activated protein (MAP) kinase,[260] and impair insulin sensitivity by inactivating phosphoinositide 3 (PI3)-kinase and Akt (protein kinase B) signalling.

Increased oxidative stress in diabetes has been consistently demonstrated in experimental studies.[261] Its primary causal factor is hyperglycaemia, although the dose-response relationship between hyperglycaemia and oxidative stress remains undefined. Hyperglycaemia uncouples oxidative phosphorylation and eNOS, and has other pro-oxidant effects, including increased glycosylation of functional proteins, glucose auto-oxidation and activation of the polyol pathway.[262] Increased cellular uptake of glucose stimulates PKC, which activates pro-oxidative enzymes such as peroxidase and COX, and induces the production of proinflammatory cytokines. Long-term hyperglycaemia also increases the formation of AGE-modified plasma proteins, which in turn induce receptor-mediated production of ROS. Glyco-oxidation of glucose generates a series of ROS, including superoxide, hydrogen peroxide and hydroxyl radicals. Glucose shunting through the polyol pathway depletes cellular NADPH, which in turn decreases glutathione-redox cycling, an important mechanism for scavenging free radicals. Increased polyol pathway activity also stimulates the COX pathway, and increases the cytosolic level of NADH and the cellular redox potential, thereby indirectly contributing to uncoupling of oxidative phosphorylation. Activation of PKC, increased formation of AGEs and increased polyol pathway activity are all, therefore, hyperglycaemia-induced mechanisms and are implicated in vascular damage via increases in superoxide production in the mitochondrial electron-transport chain.[262] In addition, hyperglycaemia contributes to uncoupling of eNOS by promoting the oxidation of tetrahydrobiopterin (BH_4), which is required for

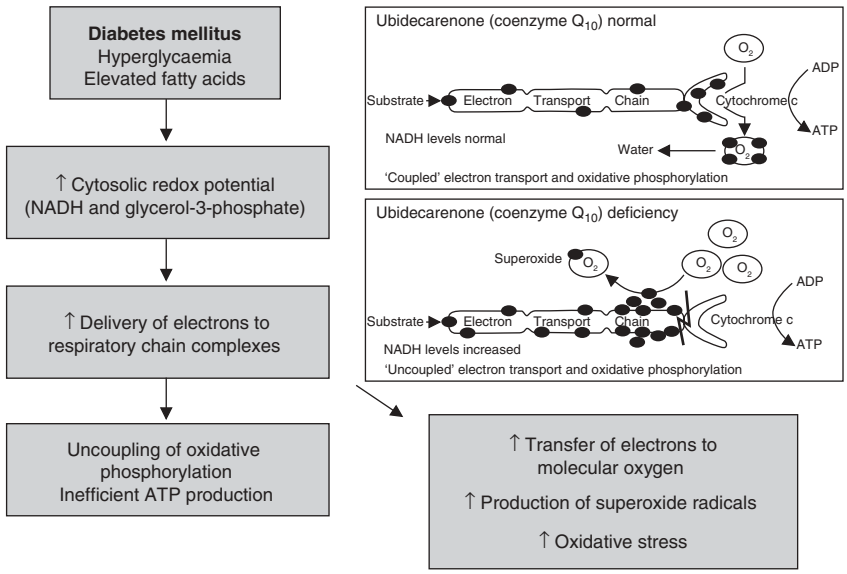

Fig. 7. Uncoupling of oxidative phosphorylation. Effect on oxidative stress in type 2 diabetes mellitus. **ADP** = adenosine diphosphate; **ATP** = adenosine triphosphate; **NADH** = reduced nicotinamide adenine dinucleotide.

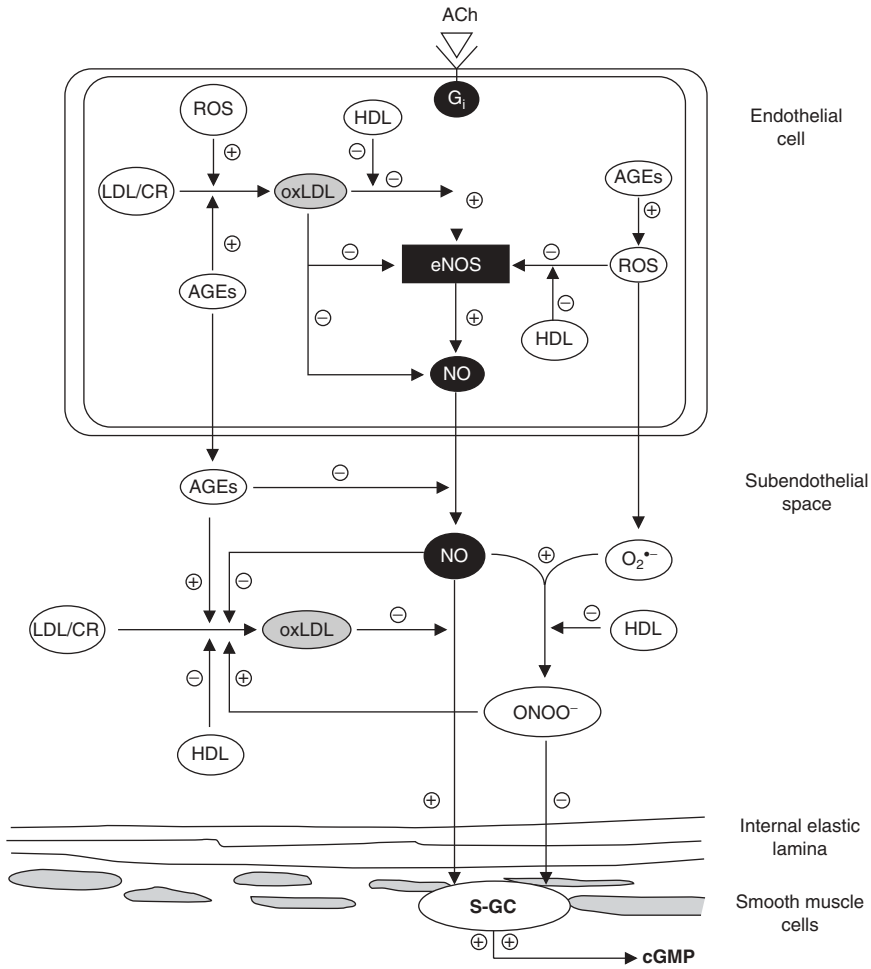

Fig. 8. Potential mechanisms leading to vascular dysfunction in type 2 diabetes mellitus. (+) indicates stimulatory and (−) indicates inhibitory regulations. In the endothelial cell nitric oxide (NO) is synthesised following stimulation of endothelial NO synthase (eNOS) by activation of a G_i protein signal transduction pathway in response to, for example, a muscarinic receptor agonist such as acetylcholine (ACh); this process is probably regulated by the expression of caveolin-I from plasmalemmal caveolae, which is associated with eNOS. Synthesis and release of NO is inhibited by reactive oxygen species (ROS), advanced glycation end-products (AGEs) and oxidised low-density lipoprotein (oxLDL), and this manifests as endothelium-dependent vascular dysfunction. In the subendothelial space, NO is inactivated by conversion by superoxide ($O_2^{\bullet-}$) to peroxynitrite ($ONOO^-$), and by direct quenching by oxLDL and AGEs; this manifests as endothelium-independent vascular dysfunction. High-density lipoprotein (HDL) may exert a protective effect on vascular function by neutralising the effects of oxLDL, ROS, AGEs on the formation and function of NO. The effect of oxLDL may be mimicked by modified chylomicron remnants (CR). Oxidative modification of HDL is counteracted by platelet activating factor acetylhydrolase and paraoxonase associated with the particle, modified HDL being rapidly cleared from plasma compared with modified LDL.[259] In type 2 diabetes the balance of effects in the figure is in favour of decreased synthesis and bioavailability of NO (reproduced from Watts and Playford,[20] with permission from Elsevier). **cGMP** = cyclic guanosine monophosphate; **S-GC** = soluble guanylyl cyclase.

tight regulation of NO production from arginine and molecular oxygen. As a consequence of eNOS uncoupling, the production of NO is decreased, and electrons are transferred to molecular oxygen to form further superoxide and peroxynitrite, a highly potent oxidant. The impact of diabetes on the uncoupling of eNOS may also involve PKC-dependent activation of NAD(P)H oxidase in the artery

wall,[263] an effect that may be under significant genetic control.[264]

As well as increased generation of ROS and RNS, reductions in tissue level of antioxidants, in particular vitamin E, superoxide dismutase, glutathione peroxidase and catalase, have also been demonstrated in diabetes.[265-268] Decreased antioxidant defences evidently compound overall oxidative stress.

In summary, increased vascular oxidative stress is a feature of diabetes, resulting from increased local production of oxygen (and nitrogen) free radicals, induced by hyperglycaemia and the increased supply of reducing equivalents to mitochondria and eNOS. Uncoupling of oxidative phosphorylation may be the central mechanism for the activation of inflammatory signalling and impaired insulin action that accelerates diabetic vasculopathy. As well as hyperglycaemia, altered lipid metabolism, in particular the increased turnover of free fatty acids, contributes to mitochondrial uncoupling and impaired vascular insulin signalling. Hence, elevated free fatty acids are not only causally related to insulin resistance and dyslipidaemia, but also to endothelial dysfunction. Significantly, the increased flux of fatty acids to liver and skeletal muscle contributes to diabetic dyslipidaemia, which compounds the effect of oxidative stress on endothelial function. Of relevance to this review, all the aforementioned cellular events that lead to increased oxidative stress could potentially be regulated therapeutically.

7.2 Dyslipidaemia

The important role of dyslipidaemia in the development of endothelial dysfunction in diabetes is underscored by demonstrations in well controlled type 2 diabetic patients of a significant correlation between abnormal acetylcholine-mediated vasodilatation of forearm resistance arteries and plasma lipid and lipoprotein changes. In these studies,[269,270] endothelial dysfunction was significantly correlated with elevated triglycerides, low HDL-cholesterol[74] and small dense LDL particle size.[271] As reviewed in this section, dyslipidaemia can contribute to the pathogenesis of endothelial dysfunction in diabetes via several mechanisms involving the accumulation in plasma of small dense LDL particles and chylomicron remnants, as well as reduced HDL particle levels and elevated non-esterified fatty acid (NEFA) levels.[20,272]

7.2.1 Low-Density Lipoproteins

Small dense LDL particles are highly susceptible to oxidative modification,[155,156] particularly when glycated and subjected to dysglycaemia and oxidative stress in type 2 diabetes.[153,273-276] Oxidised LDL can potentially impair endothelium-mediated vasodilator function by several mechanisms including decreased expression of eNOS mRNA,[277] stimulation of PKC[278] and disruption of a G_i protein signal transduction pathway[279,280] that activates eNOS in response to physiological stimuli. These adverse mechanisms have the effect of inhibiting the formation and action of NO.[281] Both oxidised LDL and native LDL may potentially impair eNOS activity and, hence, the formation of NO, by increasing the expression of caveolin-I within endothelial plasmalemmal caveolae,[282-285] which binds eNOS and prevents its activation by the calcium-calmodulin complex. Oxidised LDL may also enhance the synthesis of endothelin-1[286] and the expression of angiotensin II receptors on endothelial cells, thereby impairing vasodilator function and stimulating NAD(P)H oxidase activity; this effect may be regulated by the NAD(P)H oxidase p22 *phox* gene.[264] Native LDL in itself has pro-oxidant properties that compound the adverse vascular effect of modified LDL,[287] and it may also impair endothelial function by increasing the plasma levels of ADMA.[288] Although the evidence for these effects is derived from *in vitro* studies, oxidatively modified LDL found in patients with type 2 diabetes stimulates the expression of atherogenic cytokines in endothelial cells,[289] possibly involving an effect on NOS activity.

7.2.2 Triglyceride-Rich Lipoproteins

A cardinal feature of disordered lipid metabolism in diabetes is postprandial dyslipidaemia. This has been consistently shown to impair endothelial function *in vivo*,[90,91] and is considered to involve two principal mechanisms: (i) accumulation of remnant

lipoproteins; and (ii) increased oxidative stress. There is evidence that remnant lipoproteins may induce endothelial dysfunction,[290-292] but the precise contribution of these lipoprotein particles to vascular dysfunction has not yet been explored in diabetes. Both VLDL and chylomicron remnants equivalent to those found in the plasma of patients with CAD, have been shown to specifically impair endothelium-dependent vasodilatation in aortic ring preparations.[290,293] This may be related to the lipid moiety of the remnants, as with the effects of LDLs, and involves an alteration in G protein-coupled receptor-mediated activation of the arginine/NO pathway.[293] Accordingly, a significant and independent association has been reported between lipoprotein remnant levels and impaired endothelium-dependent vasodilatation of the coronary circulation in humans.[294]

Ultrasonographic studies of post-ischaemic FMD of the brachial artery have yielded informative results: in healthy individuals, a single high-fat meal (50g), compared with an isocaloric low-fat meal (0g), significantly and maximally suppressed brachial artery FMD after 4 hours, with the greatest suppression seen in those with the highest postprandial triglyceridaemia.[291] Similar postprandial impairments in arterial stiffness were observed in nondiabetic individuals.[295] Interestingly, the impairment in FMD could be prevented by acute coconsumption of a mixture of antioxidant vitamins.[296] Consistent with this, 4 weeks of dietary supplementation with tocopherol (vitamin E) was reported to significantly improve FMD of the brachial artery in patients with high plasma remnant lipoprotein levels.[297]

Hence, it appears that pathological accumulation of lipoprotein remnants may impair endothelial function by inducing a state of oxidative stress, either in the short term after a meal or in the long term after repeated postprandial challenges. Direct evidence for this has recently been produced in humans by a study showing significant inverse association between meal-induced increase in superoxide production by leucocytes and brachial artery FMD.[298] Support for this concept is also derived from studies involving diabetic patients, in which reversal of postprandial oxidative stress was associated with improvement in endothelial function of the brachial artery.[299,300]

7.2.3 High-Density Lipoproteins

Both *in vitro*[301-303] and *in vivo*[304,305] studies provide evidence that HDL inhibits the oxidation of LDL. The mechanism for this antioxidant effect probably involves platelet-activating factor acetylhydrolase and paraoxonase (specifically the PON-1 isoenzyme), enzymes associated with HDL that can hydrolyse lipid hydroperoxide within the LDL particle.[301,306] Accordingly, in type 2 diabetes, a low pool size or altered chemical composition of HDL is associated with low paraoxonase-specific activity,[307] and this will enhance the oxidative modification of LDL. Hence, allelic variations in paraoxonase activity may also contribute to the pathogenesis of diabetic vasculopathy.[308,309] The important role of HDL is supported by experimental demonstrations that it directly improves endothelial function,[310-315] as well as evidence that show it decreases the expression of endothelial cell adhesion molecules stimulated by oxidised LDL.[304,314] Intravenous infusion of recombinant HDL in humans was recently shown to improve acetylcholine-mediated vasodilation of the forearm resistance arteries in hypercholesterolaemic men,[315] but this remains to be confirmed in diabetes. Nevertheless, it supports a direct effect of HDL on the synthesis, release and action of NO. Whether impaired endothelial function in type 2 diabetes is more closely related to low plasma levels of apoA-I or LpA-I **:** A-II particles has not been reported, although cross-sectional data suggest that CAD in type 2 diabetes is more closely associated with low levels of apoA-I and LpA-I **:** A-II particles and, hence, low HDL_3-cholesterol.[158] This may be related to the greater potential of LpA-I **:** A-II particles to increase cellular cholesterol efflux[316] and to possibly improve endothelial function. The endothelial protective effects conferred by HDLs have recently been reviewed in detail.[317]

7.2.4 Non-Esterified Fatty Acids

Finally, in type 2 diabetes, the lipolysis of expanded triglyceride-rich lipoprotein pools and increased release of NEFAs from adipocytes, as a consequence of increased hormone-sensitive lipase activity, may also partially contribute to the development of endothelial dysfunction.[318] The mechanisms involved are probably similar to those for oxidised LDL and chylomicron remnants, that is, either via a direct inhibitory effect on eNOS activity[319] or through the generation of ROS.[320] An increased plasma flux of NEFAs following a fatty meal could also contribute directly to postprandial endothelial dysfunction in diabetes.[318,321,322]

7.2.5 Summary

All components of dyslipidaemia in both postabsorptive and postprandial states contribute to endothelial dysfunction in type 2 diabetes. This includes the accumulation of small dense LDL and remnant lipoproteins, as well as low HDL-cholesterol and increased plasma NEFA levels. The modification of lipoprotein particles via glycation and/or oxidation increases their propensity to induce endothelial dysfunction. We propose that the presence of oxidative stress compounds the atherogenicity of diabetic dyslipidaemia at the stage of subclinical endotheliopathy. Importantly, the genesis of both dyslipidaemia and oxidative stress in diabetic and insulin-resistant states may be through the dysregulation of adipocyte fatty acid metabolism that results in increased flux of NEFAs to the liver, skeletal muscle and endothelial cells. The clinical importance of the aforementioned mechanisms is that the efficacy of the therapeutic correction of dyslipidaemia in improving endothelial dysfunction in diabetes may rely on the ability of the treatments to correct oxidative stress, and that the efficacy of these therapeutic agents could potentially be enhanced by coadministration of antioxidant supplements.

8. Effect of Lipid-Regulating Therapies on Vascular Function in Type 2 Diabetes

The mechanisms for the potential improvement in vascular function in type 2 diabetes with statins, fibric acid derivatives and fish oils are summarised in table II.

8.1 Statins

Improvement in endothelial function[338-340] and decrease in arterial stiffness[341-345] have been demonstrated following statin therapy in nondiabetic individuals with dyslipidaemia. The beneficial effect of statins appears to be mediated by a variety of mechanisms, including a reduction in LDL-cholesterol and triglycerides, increased expression and activity of eNOS and decreases in inflammation and oxidative stress (table II). Whether statins improve endothelial function in individuals with type 2 diabetes is, surprisingly, still controversial (table III).

Rapid improvement in brachial artery FMD has been demonstrated in placebo-controlled trials with type 2 diabetic patients receiving simvastatin 40 mg/day[300] or cerivastatin 0.15 mg/day:[353] in both studies, significant improvements occurred within 3 days, despite a lack of significant changes in lipids or lipoproteins. A direct antioxidant effect of statin therapy on improving FMD is supported by the demonstration that simvastatin reduced basal levels of nitrotyrosine (NT), a marker of oxidative stress, and also attenuated the postprandial rise in NT fol-

Table II. Possible mechanisms of action of three lipid-regulating agents that improve vascular function

Parameter	Statin	Fibric acid derivative	Fish oil
↓ LDL-cholesterol	++[174]	+/–[172]	+/–[323,324]
↑ LDL particle size	+[325]	++[187]	+[215,216]
↓ Triglycerides	+[172]	++[172]	++[323]
↓ Chylomicron remnants	++[326]	+/– [326]	+/–[327]
↑ HDL-cholesterol	+[174]	++[172,187]	+/–[323]
↑ PPAR-α activation/ expression	+[179]	+++[187,328]	+[329]
↓ Vascular inflammation	+[179]	+[328]	+[330]
↑ NO production	+[179]	+[331]	+/–[332,333]
↓ Endothelin-1 synthesis/ expression	+[179]	+[185]	+/–[334,335]
↓ Oxidative stress	+[179]	+[336]	+[337]

LDL = low-density lipoprotein; **NO** = nitric oxide; **PPAR** = peroxisome proliferator-activated receptor; **statin** = HMG-CoA reductase inhibitor; ↓ indicates decreased; ↑ indicates increased; **+** indicates minor effect; **++** indicates moderate effect; **+++** indicates major effect; **+/–** indicates equivocal effect.

Table III. Clinical trials investigating the effects of HMG-CoA reductase inhibitor (statin) therapy on vascular function in patients with type 2 diabetes mellitus

Study (year)	Study design	n	Intervention dose (mg/day) [duration]	Baseline lipid profile (mmol/L)	Glycaemic control (diabetes duration [y])	Method(s)	Baseline measurement (intervention group)	Absolute effect
Economides et al.[346] (2004)	r, db, pc, pg	40[a]	Atorvastatin 20 [12wk]	TC 5.3; TG 1.2; HDL-C 1.55; LDL-C 3.2	HbA_{1c} 8.0% (8)	FMD Laser Doppler (ACh)	5.0% 149 ± 77% flux increase	No significant change No significant change
Ichihara et al.[347] (2002)	r, db, pc, pg	22	Fluvastatin 20 [6mo]	TC 4.1; TG 1.1; HDL-C 1.23; LDL-C 2.2	HbA_{1c} 6.0%	Central PWV	19.9 ± 1.6 m/sec	↓ 14%
Ceriello et al.[300] (2002)	r, db, pc, co	30	Simvastatin 40 [3d] Simvastatin 40 [3mo]	TC 7.5; TG 3.7; HDL-C 0.9; LDL-C 3.6	HbA_{1c} 7.8%	FMD	4.8 ± 0.8% 4.9 ± 0.7%	↑ 2.5% ↑ 4.3%
van Venrooij et al.[348] (2002)	r, db, pc, pg	133	Atorvastatin 10 vs 80 [30wk]	TC 6.0; TG 2.7; HDL-C 1.05; LDL-C 3.7	HbA_{1c} 8.4% (11.5)	FMD	3.4 ± 0.6% 3.2 ± 0.4%	No significant change
van Etten et al.[349] (2002)	uc	23	Atorvastatin 80 [4wk]	TC 5.8; TG 2.2; HDL-C 1.2; LDL-C 4.1	HbA_{1c} 8.6% (8)	FABF (5-HT) FABF (SNP)	53 ± 30%	No significant change
Tan et al.[350] (2002)	r, db, pc, pg	80	Atorvastatin 10 [3mo] + 20 [3mo]	TC 6.2; TG 1.4; HDL-C 1.16; LDL-C 4.4	HbA_{1c} 7.9% (10)	FMD	5.3 ± 2.6%	↑ 1.2%
Sheu et al.[351] (2001)	uc	12	Simvastatin 20–40 [12wk]	TC 5.9; TG 2.6; HDL-C 1.03; LDL-C 3.7	HbA_{1c} 8.1%	FMD	5.6 ± 1.7%	↑ 8.0% (LDL <80 mg/dL)
van de Ree et al.[352] (2001)	uc	17	Simvastatin 40 [6wk]	TC 5.7; TG 2.9; HDL-C 1.04; LDL-C 3.4	HbA_{1c} 7.2% (8.4)	FABF (5-HT)	56 ± 7%	No significant change
Tsunekawa et al.[353] (2001)	r, pc, pg	27 8	Cerivastatin 0.15 [3d] Cerivastatin 0.15 [3mo]	TC 6.1; TG 1.4; HDL-C 1.49	HbA_{1c} 7.3% (11.5)	FMD	4.3 ± 0.6% 4.4 ± 0.6%	↑ 4.4% ↑ 4.4%
Mansourati et al.[354] (2001)	r, db, pc, pg	18	Simvastatin 40 [3mo]	LDL-C 4.4	HbA_{1c} 9.2% (9)	Laser Doppler (ACh)	0.7 ± 0.6 mL/min/100g	No significant change
Sheu et al.[355] (1999)	uc	21	Simvastatin 10 [24wk]	TC 6.3; TG 2.1; LDL-C 4.2	HbA_{1c} not provided (>5)	FMD	6.1 ± 0.8%	No significant change

a 20 type 2 diabetes; 20 type 1 diabetes.

5-HT = serotonin; **ACh** = acetylcholine; **co** = crossover; **db** = double-blind; **FABF** = forearm blood flow using venous occlusion plethysmography; **FMD** = brachial artery flow-mediated dilatation; **HbA_{1c}** = glycosylated haemoglobin; **HDL-C** = high-density lipoprotein-cholesterol; **LDL-C** = low-density lipoprotein-cholesterol; **pc** = placebo-controlled; **pg** = parallel group; **PWV** = pulse wave velocity; **r** = randomised; **SNP** = sodium nitroprusside; **TC** = total cholesterol; **TG** = triglycerides; **uc** = uncontrolled; ↑ indicates significant increase ($p < 0.05$); ↓ indicates significant decrease ($p < 0.05$).

lowing oral fat and glucose load.[300] Similarly, short-term cerivastatin therapy also decreased plasma levels of 8-isoprostane, another marker of oxidative stress, but increased plasma nitrite/nitrate, an index of NO function.[353] In longer-term trials with a greater number of diabetic patients, treatment with atorvastatin 10–20 mg/day for 6 months improved FMD,[350] but this was not confirmed with dosages of either 10 or 80 mg/day over 30 weeks[348] or with 20 mg/day over 12 weeks.[346] Reductions in LDL-cholesterol of 40–50% were achieved in each of these trials, but there was no correlation between FMD and LDL-cholesterol at baseline,[348] nor between the changes in FMD and changes in LDL-cholesterol.[346,350] The trials did not assess changes in oxidative stress, but improvement in FMD with atorvastatin 10–20 mg/day was significantly and inversely associated with changes in C-reactive protein (CRP), a marker of systemic inflammation.[350] This supports the observations of an inverse association between endothelial function and CRP levels in CAD patients,[356] and the ability of statins to reduce CRP in hyperlipidaemic patients.[357] CRP may have a direct role in promoting inflammation.[358] Endothelial cells from the coronary artery incubated with recombinant CRP for between 6 and 24 hours have been shown to induce large increases in the expression of VCAM-1, ICAM-1 and E-selectin.[359]

In uncontrolled trials, lower doses of simvastatin 10 mg/day[355] or 20–40 mg/day[351] did not improve FMD over periods of 3–6 months. However, improvements did occur in the latter study in subjects whose LDL-cholesterol was reduced to <2.1 mmol/L from 3.7 ± 0.2 mmol/L.[351] Neither simvastatin 40 mg/day[352] nor atorvastatin 80 mg/day[349] improved endothelial function of the forearm microcirculation in trials lasting between 4 and 6 weeks, despite reductions in LDL-cholesterol of between 41%[352] and 56%.[349]

Discrepancies in the effects of statins on endothelial function in the various trials may be related to differences in the level of glycaemic control in the subjects, the duration of the intervention, study design (in particular, whether or not they were placebo-controlled) and the vascular bed studied (conduit vessel versus forearm microcirculation) [table III]. Improvements in FMD have tended to occur in those subjects with good-to-moderate glycaemic control, and also in larger studies that used a placebo control group for comparison with the intervention group. The absolute improvements in FMD of between 1% and 4% that occurred with statin therapy in these trials (table III) could have the potential to reduce the risk of cardiovascular events by at least 25%.[8]

In haemodialysis patients with type 2 diabetes, central PWV (aorto-iliac) was significantly decreased following treatment with fluvastatin 20 mg/day for 6 months, compared with placebo.[347] Although these patients had lower LDL-cholesterol at baseline (2.38 ± 0.21 mmol/L), there was still a 14% decrease in PWV observed. The decreases in serum LDL-cholesterol observed in the fluvastatin group were not different from the nonsignificant changes in the placebo group. However, there was a significantly greater decrease in serum oxidised LDL-cholesterol observed in the fluvastatin group. The decrease in PWV was independent of blood pressure, and the reduction in oxidised LDL-cholesterol was more significant than the reduction in LDL-cholesterol alone. Inhibition of oxidised LDL-cholesterol might, therefore, have contributed more to the decrease in arterial stiffness than the lipid-lowering effects of fluvastatin.

8.2 Fibric Acid Derivatives

Improvement in coronary vasomotor function following fibric acid derivative therapy has been demonstrated in dyslipidaemic CAD patients without diabetes.[360] There have been relatively few studies assessing the effects of fibric acid derivatives on vascular function in type 2 diabetes, but the results are consistent (table IV). Three placebo-controlled studies using different fibric acid derivatives all reported improvements in brachial artery FMD amongst patients with type 2 diabetes (table IV). Ciprofibrate 100 mg/day,[299] gemfibrozil 600mg twice daily[361] and fenofibrate 200 mg/day[362] were each administered for 3 months. There was improvement in both fasting and 4-hour postprandial FMD with ciprofibrate,[299] with postpran-

Table IV. Clinical trials investigating the effects of fibric acid derivative and fish oil therapy on endothelial function in patients with type 2 diabetes mellitus

Study (year)	Study design	n	Intervention dose (duration)	Baseline lipid profile (mmol/L)	Glycaemic control (diabetes duration [y])	Method(s)	Baseline measurement (intervention group)	Absolute effect
Fibric acid derivatives								
Evans et al.[299] (2000)	r, db, pc, pg	20	Ciprofibrate 100 mg/day (3mo)	TC 5.8; TG 2.8; HDL-C 1.06; LDL-C 3.5	HbA_{1c} 8.2%	FMD Post-prandial FMD	3.8 ± 1.8% 1.8 ± 1.3%	↑ 1.0% ↑ 1.6%
Avogaro et al.[361] (2001)	r, db, pc, co	10	Gemfibrozil 600mg twice daily (3mo)	TC 5.8; TG 3.9; HDL-C 1.1; LDL-C 2.9	HbA_{1c} 9.6%	FMD	7 ± 3%	↑ 9%
Playford et al.[362] (2002)	r, db, pc, pg	40	Fenofibrate 200 mg/day (3mo)	TC 5.4; TG 2.3; HDL-C 0.98; LCL-C 3.1	HbA_{1c} 6.6%	FMD	3.3 ± 0.8%	↑ 1.5%
Fish oils								
Woodman et al.[363] (2003)	r, db, pc, pg	51	EPA 4 g/day (6wk) DHA 4 g/day (6wk)	TC 4.5; TG 1.5; LDL-C 2.6; HDL-C 1.1	HbA_{1c} 7.2% (5.2)	FMD	3.1 ± 0.6% 5.3 ± 1.3%	No significant change No significant change
McVeigh et al.[364] (1994)	r, db, pc, co	20	EPA/DHA 3 g/day (6wk)	TC 5.3; TG 1.8	HbA_{1c} 9.7% (5.2)	C_1 C_2	1.50 ± 0.19 mL/mm Hg 0.015 ± 0.004 mL/mm Hg	↑ 0.18 mL/mm Hg ↑ 0.07 mL/mm Hg
McVeigh et al.[332] (1993)	r, db, pc, co	23	EPA/DHA 3 g/day (6wk)	TC 5.3; TG 1.8	HbA_{1c} 9.6%	FABF (ACh)	2.5 ± 1.2 mL/100mL/min	↑ 2.8 mL/100mL/min
Wahlqvist et al.[365] (1989)	cs	22	Fish consumption ≥100 g/wk			Arterial compliance ($66.7/PWV^2$)	$0.59 \pm 0.03 s^4/m^2$	↑ $0.13 s^4/m^2$

ACh = acetylcholine; **C_1** = large artery compliance; **C_2** = small artery compliance; **co** = crossover; **cs** = cross-sectional; **db** = double-blind; **DHA** = docosahexaenoic acid; **EPA** = eicosapentaenoic acid; **FABF** = forearm blood flow using venous occlusion plethysmography; **FMD** = brachail artery flow-mediated dilatation; **HbA_{1c}** = glycosylated haemoglobin; **HDL-C** = high-density lipoprotein-cholesterol; **LDL-C** = low-density lipoprotein-cholesterol; **pc** = placebo-controlled; **pg** = parallel group; **PWV** = pulse wave velocity; **r** = randomised; **TC** = total cholesterol; **TG** = triglycerides; **uc** = uncontrolled; ↑ indicates significant increase ($p < 0.05$).

dial reduction in FMD being positively associated with postprandial triglyceride enrichment of VLDL- and LDL-cholesterol, and inversely correlated with HDL-cholesterol. The postprandial increase in oxidative stress, which was positively correlated with triglyceride enrichment of VLDL, was also significantly reduced with ciprofibrate. The improvement in FMD with gemfibrozil treatment occurred with a reduction in fasting plasma triglycerides and an increase in HDL-cholesterol and insulin sensitivity.[361] However, associations between the improvement in endothelial function and either insulin sensitivity or lipid parameters were not examined statistically. The improvement in FMD with fenofibrate occurred with a reduction in plasma triglycerides, and an increase in HDL-cholesterol and LDL particle size. Changes in percentage FMD were positively associated with in-trial LDL particle size and inversely associated with total cholesterol, fasting plasma triglycerides and apoB. In multivariate analysis, apoB was the only significant predictor of an increase in FMD. Although significant, the magnitude of the improvements in FMD with fibric acid derivative therapy have mostly been relatively small (table IV). However, as indicated in section 3.1, even small increases in FMD of the order of 1% could translate into a 25% reduction in cardiovascular events, similar to the clinical cardiovascular effects of gemfibrozil observed in the VA-HIT trial.[195]

Improvement in endothelial function of the forearm resistance vessels was also observed in subjects treated with a combination of fenofibrate and the antioxidant ubidecarenone (coenzyme Q_{10}), but not in those treated with fenofibrate or ubidecarenone alone.[336] Changes in forearm blood flow response to intra-arterial infusions of acetylcholine were negatively associated with changes in glycosylated haemoglobin (HbA_{1c}), but not with changes in plasma lipids or lipoproteins. Differences in the effects of fenofibrate on conduit[336] and microcirculatory[362] function in diabetes in our studies might relate to several factors, including a greater contribution of NO to vasodilator function in the brachial artery, the greater precision in the measurement of FMD using a computerised method and use of background aspirin (acetylsalicylic acid) therapy in the forearm studies. There have been no reports on the effects of fibric acid derivatives on arterial stiffness in type 2 diabetic patients to date.

Recent data from nondiabetic hypertriglyceridaemic subjects showed that improvements in endothelial function following fibric acid derivative intervention were not only associated with lowering of plasma triglycerides, but also with a reduction in NEFAs[366] and CRP.[367,368] The possible mechanisms of the beneficial effect of fibric acid derivatives on vascular function are summarised in table II. We propose that the favourable effects of these agents on vascular function are mediated by lipid and nonlipid mechanisms,[369] in particular the consequences of PPAR-α activation in the arterial wall.

8.3 Fish Oils

The considerable evidence supporting a beneficial effect of n-3 fatty acids on vascular function[333,364,370,371] extends to those with type 2 diabetes.[332,372] Acetylcholine-induced, endothelium-dependent relaxation of the forearm microcirculation was significantly increased in type 2 diabetic individuals following treatment with 3 g/day of EPA/DHA fish oil for 6 weeks.[332] The same patients demonstrated improved oscillatory but not large vessel compliance,[364] without changes in blood pressure, cardiac output or systemic vascular resistance. However, there was no association in either study between the improvements in vascular function and changes in plasma lipids or lipoproteins. In a study examining the effects of purified fish oils, DHA, but not EPA, improved endothelial function of the forearm resistance vessels in overweight dyslipidaemic men, most of whom probably had the metabolic syndrome.[373] However, in a similar study of type 2 diabetic individuals, neither EPA nor DHA improved FMD of the brachial artery.[363] Concurrent hypertension, concomitant medications,[68,374-376] specific characteristics of the different vessels studied (coronary,[377] forearm[332,373] and brachial[363]) and possible unidentified atherosclero-

sis might have limited the potential improvements in endothelial function with these fish oils in this trial.

While release of NO is the main factor affecting FMD in conduit vessels,[378] fish oils also exert effects on vascular function by a number of other mechanisms: they may decrease vasoreactivity by suppressing the formation of thromboxane A_2 or cyclic endoperoxides, increasing COX-derived prostanoids, and by affecting the release of ADP and endothelium-derived hyperpolarising factor.[333] Selective incorporation of DHA into endothelial membranes could increase membrane fluidity, calcium influx and the endogenous synthesis and release of NO. Other potential mechanisms include improvements in lipid profile (decreased VLDL-cholesterol and triglycerides, and increase in LDL particle size[216]), and a reduction in postprandial lipaemia,[327] inflammation[379] and oxidative stress[380] (table II).

8.4 Nicotinic Acid

Nicotinic acid has been consistently shown to decrease the incidence of cardiovascular disease in a limited number of clinical trials.[381] This may not only relate to increases in HDL-cholesterol, but also to improvement in endothelial function, as suggested in CAD patients with low HDL-cholesterol and normal LDL-cholesterol.[382] In nondiabetic subjects with angiographic coronary disease and low plasma HDL-cholesterol, treatment for 30 months with gemfibrozil and (if necessary) nicotinic acid and/or colestyramine (cholestyramine) to raise HDL-cholesterol by 25% and lower LDL-cholesterol to <2.8 mmol/L, failed to restore FMD to normal.[383] However, this result might have been due to normal endothelial function in those subjects with angiographic disease but without hypertension.

Caution should be observed in using nicotinic acid to treat diabetic patients, as it is known to impair glycaemic control.[218] No studies so far have assessed the effect of nicotinic acid-based agents on endothelial function in type 2 diabetes.

8.5 Summary

Controversy still exists as to whether or not statins improve endothelial function in individuals with type 2 diabetes. Discrepancies may be related to wide differences amongst subjects in glycaemic control, study design, study duration and vascular bed. If beneficial effects of statins on endothelial function do exist, they appear to be related more to reductions in oxidative stress and inflammation than to changes in LDL-cholesterol. Improvement in endothelial function with fibric acid derivative therapy has been consistently demonstrated reductions in fasting and postprandial triglycerides and increases in HDL-cholesterol. However, the favourable effects of fibric acid derivatives are probably a consequence of PPAR-α activation in vascular wall cells in addition to favourable effects on circulating plasma lipids and lipoproteins. Fish oils can also consistently improve vascular dysfunction in diabetes via a variety of mechanisms, including improvements in dyslipidaemia and direct effects on the arterial wall. Nicotinic acid preparations that raise HDL-cholesterol may also assist in restoring endothelial function. However, their use is not recommended for diabetic patients owing to potential impairment in glycaemic control.

9. Effect of Other Therapies on Vascular Function in Type 2 Diabetes

Improvements in vascular function in subjects with type 2 diabetes have recently been demonstrated using a wide variety of agents other than specific lipid-regulating therapies (table V).

9.1 Antioxidants

Oxidative stress is believed to play a major role in the development of atherosclerosis. Observational studies have shown associations between tocopherol and betacarotene supplementation with a reduction in cardiovascular events. However, these effects have not been supported in large-scale randomised clinical trials of both primary and secondary prevention.[198,399-402] Similarly, since oxidative stress is increased in diabetes and contributes to

Table V. Intervention trials investigating the effects of non lipid-regulating therapies on endothelial function in patients with type 2 diabetes mellitus (DM2)

Study (year)	Design	n	Intervention [duration]	Method(s)	Effect (relative change)
Evans et al.[384] (2003)	pc, pg (ascorbic acid), uc (insulin)	20	Insulin lispro 0.2 IU/kg; ascorbic acid 1 g/day [6wk]	FMD	282% ↑ vs baseline (insulin lispro) 577% ↑ vs baseline (ascorbic acid + lispro)
Caballero et al.[385] (2003)	r, db, pc, pg	87	Troglitazone 600 mg/day [12wk]	FMD	34% ↑ vs placebo (recently diagnosed DM2: n = 25)
van Etten et al.[386] (2002)	uc, op	23	Intra-arterial methyltetrahydrofolic acid 1 μg/100mL FAV/min [30 min]	FABF (5-HT)	66% ↑ vs baseline
Watts et al.[387] (2002)	r, pc, pg	40	Ubidecarenone (coenzyme Q_{10}) 200 mg/day [12wk]	FMD	66% ↑ vs placebo
Gaenzer et al.[388] (2002)	uc	21	Insulin therapy (physician discretion) [3mo]	FMD	85% ↑ vs baseline
Vehkavaara et al.[389] (2000)	uc	18	Isophane insulin nocte (self-adjusted) [6mo]	FABF (ACh)	44% ↑ vs baseline
Cheetham et al.[390] (2001)	r, db, pc, co	12	Losartan 50 mg/day [4wk]	FMD	42% ↑ vs baseline
Cheetham et al.[391] (2000)	r, db, pc, co	9	Losartan 50 mg/day [4wk]	FABF (ACh)	79% ↑ vs placebo
Maiorana et al.[392] (2001)	r, co	16	Aerobic plus resistance exercise training [8wk]	FABF (ACh) FMD	83% ↑ vs no exercise 194% ↑ vs no exercise
Butler et al.[393] (2000)	r, db, pc, co	11	Allopurinol 300 mg/day [1mo]	FABF (ACh)	65% ↑ vs placebo
Chowienczyk et al.[394] (2000)	uc	10	Raxofelast 600mg bid [1wk]	FABF (ACh)	53% ↑ vs baseline
Mather et al.[376] (2001)	db, pc, pg	44	Metformin 500mg bid [12wk]	FABF (ACh)	85% ↑ vs placebo
Heitzer et al.[395] (2000)	uc	23	Intra-arterial sapropterin (tetrahydrobiopterin) 500 μg/min	FABF (ACh)	85% ↑ vs baseline
Heitzer et al.[396]	uc	39	Intra-arterial thioctic acid (0.7 mg/min)	FABF (ACh)	39% ↑ vs baseline
(2001)		21	Intra-arterial ascorbic acid (24 mg/min)		31% ↑ vs baseline
O'Driscoll et al.[397] (1999)	r, db, pc, co	10	Enalapril 10mg bid [4wk]	FABF (ACh)	105% ↑ vs placebo
Ting et al.[398] (1996)	uc	10	Intra-arterial ascorbic acid (24 mg/min)	FABF (MCh)	36% ↑ vs baseline

5-HT = serotonin; **ACh** = acetylcholine; **bid** = twice daily; **co** = crossover; **DB** = double-blind; **FABF** = forearm blood flow using venous occlusion plethysmography; **FAV** = forearm volume; **FMD** = brachial artery flow-mediated dilatation; **MCh** = methacholine; **op** = open-label; **pc** = placebo-controlled; **pg** = parallel group; **r** = randomised; **uc** = uncontrolled; ↑ indicates increase.

endothelial dysfunction, antioxidants and other regulators of oxidative stress may protect against and/or reverse diabetic vasculopathy. However, at present there is evidence both for and against improvement in vascular function with antioxidant therapy in patients with type 2 diabetes.

Oral supplementation with the tocopherol analogue raxofelast improved forearm microcirculatory endothelial function in type 2 diabetic men,[394] but neither tocopherol supplementation (1600 IU/day for 8 weeks)[403] nor ascorbic acid (vitamin C) [1.5 g/day for 3 weeks] was effective.[404] In contrast, improvement in microcirculatory endothelial function has been reported in patients with type 2 diabetes following intra-arterial administration of ascorbic acid[398] or folic acid.[386] Infusion of the powerful antioxidant thioctic acid (lipoic acid) was also beneficial, particularly in diabetic patients with low plasma levels of ubidecarenone.[396] This supports an important role for ubidecarenone in endothelial dysfunction in type 2 diabetes, and we have recent data in dyslipidaemic diabetic patients demonstrating improvement in endothelial function of the brachial artery following oral ubidecarenone

supplementation.[387] Also, ubidecarenone and fenofibrate synergistically improved endothelium-dependent and -independent function of forearm resistance arteries in the same patients.[336] In hypercholesterolaemic patients, ascorbic acid augmented the improvement in acetylcholine-stimulated forearm blood flow following atorvastatin therapy,[405] but there have been no reports on the effects of combined statin and antioxidant therapy in diabetic patients. We postulate that the synergistic effect of fenofibrate and ubidecarenone involves co-activation of PPAR-α in endothelial and smooth muscle cells that improves release and action of NO and depresses the synthesis of endothelin-1, but this requires verification in cell biological studies. This is an important objective for future studies, since the full benefit of statins on vascular function could potentially be blunted by inhibition in the cellular synthesis of ubidecarenone, a by-product of the cholesterol biosynthetic pathway.[406]

One month of treatment with allopurinol, a xanthine oxidase inhibitor, reduced oxidative stress and restored endothelial function in type 2 diabetic individuals,[393] but had no effect on the same measures in healthy controls. The specific impact of antioxidant therapy on arterial stiffness in type 2 diabetes is difficult to elucidate, since antioxidants may also improve blood pressure,[407,408] although this effect may be modest. Oral supplementation with ascorbic acid 500 mg/day for 4 weeks in type 2 diabetic patients reduced both AIx and PWV,[408] with a parallel decrease in blood pressure.

9.2 ACE Inhibitors and Angiotensin II Receptor Antagonists

In type 2 diabetic patients without evidence of vascular disease, the ACE inhibitor enalapril improved stimulated and basal NO-dependent endothelial function of the forearm microcirculation.[397] These effects may be mediated by improvement in insulin sensitivity,[409] reduction in the direct effects of angiotensin II on the arterial wall or an increase in the local effects of bradykinin. Angiotensin II can increase free radical formation, impair NO generation and cause smooth muscle contraction,[410] especially in high-risk patients with CAD. Accordingly, angiotensin receptor antagonists have been demonstrated to improve conduit vessel endothelial function,[390] central pulse pressure and PWV[411] in type 2 diabetes. The absolute 2% improvement in FMD[390] could translate into a relative risk reduction in cardiovascular events of between 25% and 40%,[8] especially in high-risk coronary patients. The mechanism of the benefit seen with angiotensin receptor antagonists, and possibly ACE inhibitors, may relate to reduction in the local generation of superoxide radicals because of decreased activity of vascular NAD(P)H oxidase.[412] Studies on the effects of hypertensive therapies on arterial stiffness are confounded by changes in blood pressure and heart rate, both of which affect arterial stiffness measures.[68] However, ACE inhibitors have consistently been associated with blood pressure-independent decreases in arterial stiffness.[68]

9.3 Advanced Glycation End-Product Cross-Link Breakers

Treatments that directly target structural components of the arterial wall, such as AGEs, might be expected to have significant effects on arterial stiffness. The newly developed AGE cross-link breaking compound alagebrium chloride (ALT-711) has been shown to quickly reverse the increases in large artery stiffness seen in streptozotocin-induced diabetic rats, as assessed by several *in vivo* and *in vitro* measures.[413] The same compound improved aortic PWV and systemic arterial compliance after 8 weeks of treatment in elderly hypertensive patients.[414] Such treatments are, therefore, likely to offer much promise in reducing the accelerated arterial stiffening seen in type 2 diabetes. The effects of new therapeutic approaches that control the accumulation of AGEs, including inhibitors of glycation and of the RAGE receptor, on arterial function in diabetes are promising lines of investigation for the future.

9.4 Insulin and Sulphonylureas

The regular use of insulin therapy in the treatment of type 2 diabetes remains controversial. Al-

though it may be important in achieving glycaemic control, elevated levels of insulin may cause alterations in the vasculature involving smooth muscle cell proliferation[415] and lipid accumulation.[416] Long-term insulin therapy may have detrimental effects via weight gain, and chronic hyperinsulinaemia could also potentially increase hepatic secretion of VLDL-triglyceride, blood pressure, and plasma fibrinogen[417] and plasminogen activator inhibitor (PAI-1).[418,419] Similarly, controversy also surrounds the effects of insulin on vascular function in states of insulin resistance, with positive[389,420] and negative[421] effects reported. More recently, a study reported that 6 weeks of insulin therapy improved both fasting and postprandial endothelial function, an effect that was augmented by 1 g/day of ascorbic acid.[384] Improvement in fasting FMD correlated with the increase in HDL-cholesterol levels, while improvement in postprandial FMD was associated with a reduction in postprandial VLDL-triglyceride content. In patients with poorly controlled type 2 diabetes treated with diet alone, markers of endothelial damage and activation (including vWF, E-selectin and ICAM-1), and urinary albumin excretion rate were not significantly altered with either insulin or sulphonylureas.[422] There is also concern that the action of sulphonylureas on vascular ATP-sensitive potassium channels may contribute to elevated blood pressure and impairment in vascular reactivity.[423]

9.5 Insulin Sensitisers

Insulin resistance itself may be central to the pathogenesis of endothelial dysfunction.[424] Thiazolidinediones (glitazones) directly reduce insulin resistance by enhancing insulin action via the activation of PPAR-γ receptors in peripheral tissues, thereby potentially correcting endothelial dysfunction. Troglitazone treatment for 12 weeks improved FMD in recently diagnosed type 2 diabetic patients, an effect that was closely related to improvements in fasting insulin.[385] However, these effects did not apply to patients with chronic diabetes, nor those with evidence of macrovascular disease. Troglitazone also had no additional effect on endothelial function in insulin-resistant obese individuals with impaired insulin sensitivity but normal endothelial function, despite improvements in insulin sensitivity.[425] In patients with type 2 diabetes, acetylcholine stimulated increases in forearm blood flow were improved with rosiglitazone but not with metformin.[426] This beneficial effect of rosiglitazone was not related to the improved insulin sensitivity, as measured by the euglycaemic clamp, suggesting a direct effect on the arterial wall. The favourable effect of glitazones on vascular function is likely to be mediated by direct activation of PPAR-γ receptors in endothelial and smooth muscle cells that potentially decrease inflammatory signalling pathways and oxidative stress. Troglitazone[427] and pioglitazone[428] have potent inhibitory effects on the progression of early atherosclerotic lesions and cause rapid regression of intima-media thickness of the carotid artery, probably through decreasing insulin resistance. Both HbA_{1c} and postprandial serum triglycerides were decreased after troglitazone, but were not associated with the decrease in intima-media thickness.[427] Metformin improved both insulin sensitivity and endothelial function in one study,[376] with insulin sensitivity being the only independent predictor of endothelial function following treatment ($r = -0.66$, $p < 0.001$). Metformin may also exert its effects by lowering ADMA levels following improvements in metabolic control.[429]

9.6 Sapropterin (Tetrahydrobiopterin (BH_4)), Methyltetrahydrofolic Acid and Arginine

A few studies have reported on the effect of certain substrates and co-factors that could potentially recouple eNOS activity in diabetes. Tetrahydrobiopterin (BH_4) is an essential cofactor for eNOS that is required for the conversion of arginine to NO and, thus, deficiency of BH_4 decreases NOS bioactivity. In diabetic patients, but not controls, endothelial dysfunction of the microcirculation was considerably improved by concomitant treatment with sapropterin, the proprietary form of BH_4.[395]

Positive effects on endothelial function have also been observed with oral arginine supplementation in healthy individuals, and in patients with hypercho-

lesterolaemia and CAD,[430] but mixed results were seen in hypertensive patients with CAD.[431] ADMA, an endogenous inhibitor of NOS, is elevated in patients with type 2 diabetes,[432] possibly as a result of a glucose-induced impairment of dimethylarginine dimethylaminohydrolase, which results in ADMA accumulation and endothelial dysfunction.[257] Response to arginine supplementation in type 2 diabetic individuals may, therefore, depend on the level of glycaemic control. However, only one recent study on the effect of arginine supplementation has so far been reported in type 2 diabetes, showing a positive effect on brachial artery FMD and forearm blood flow response.[433]

Several studies in nondiabetic individuals have demonstrated an improvement in endothelial function following folic acid supplementation, an effect that may be mediated via a reduction in plasma homocysteine and/or oxidative stress.[434] In patients with type 2 diabetes, intra-arterial administration of methyltetrahydrofolic acid, the active form of folic acid, has also been shown to restore endothelial dysfunction.[386] Studies investigating whether oral folic acid supplements can improve vascular function and reduce cardiovascular events in these individuals are required.

9.7 Hormone Replacement Therapy, Aspirin and Anti-inflammatory Agents

Short-term estrogen supplementation for 7 days has been shown to improve FMD in both type 2 diabetic and nondiabetic postmenopausal women.[435] Similarly, hormone replacement therapy for 6 months improved endothelium-dependent vascular relaxation of gluteal arteries in similar subjects.[436] The beneficial effects of estrogen in these studies are likely to be mediated by either direct upregulation of eNOS or, possibly, by an antioxidant effect.[437]

Aspirin is now recommended to individuals with diabetes because of their increased cardiovascular risk.[438] Type 2 diabetes and insulin resistance are associated with an increase in proinflammatory cytokines,[439] and impaired vascular function has been associated with markers of inflammation.[440] In addition to reducing platelet aggregation, aspirin reduces inflammation and prevents the impairment in endothelial function following an inflammatory stimulus.[441] Both aspirin and celecoxib, an inhibitor of COX-2, improved endothelial function in patients with CAD.[442,443]

9.8 Nutritional and Lifestyle Changes

Three recent lifestyle intervention programmes have highlighted the importance of including lifestyle changes, that is, weight loss, exercise and diet, in preventing the development of diabetes in individuals with impaired glucose tolerance. The Da Qing trial,[444] DPS (Finnish Diabetes Prevention Study)[445] and the DPP (Diabetes Prevention Program)[446] have each demonstrated reductions of approximately 30–60% in the development of type 2 diabetes in middle-aged men and women with impaired glucose tolerance over periods of 2.8–6 years. Similar lifestyle modifications recommended by the Australian National Heart Foundation[170] and the ADA[22,23] aimed at improving dyslipidaemia might also, therefore, be expected to have beneficial effects on vascular function and/or reductions in cardiovascular events.

Weight loss improves endothelial function in men with the metabolic syndrome, whether it is achieved through diet, use of serotonergic agonists[447] or physical activity.[448] Several adipocytokines (adipocyte-derived plasma proteins) may influence the development of atherosclerosis and vascular dysfunction. The proinflammatory cytokines tumour necrosis factor (TNF)-α and interleukin (IL)-6 have been demonstrated to increase insulin resistance *in vitro*, and IL-6 has numerous adverse effects on adipose tissue including inhibition of LPL activity and induction of lipolysis.[449] In premenopausal obese women, weight reduction of at least 10% for 1 year improved endothelial function, and was associated with a reduction in proinflammatory cytokines.[450] A further mechanism by which weight loss might improve vascular function is an increase in the adipocytokine, adiponectin. Hypoadiponectaemia is a feature of CAD,[451] insulin resistance and diabetes,[452] and is negatively

correlated with body mass index. Adiponectin enhances hepatic insulin sensitivity and reduces triglycerides in skeletal muscle,[449] and is inversely associated with dyslipidaemia.[453] Adiponectin is also inversely associated with CRP in both plasma and adipose tissue.[454] Its anti-inflammatory effects include suppression of macrophage-to-foam cell transformation and downregulation of adhesion molecule expression,[455,456] and it is also involved in the repair process of damaged vasculature.[457] Adiponectin has recently been associated with impaired FMD in hypertensive patients.[458] The favourable metabolic and vascular effects of decreasing TNFα and IL-6, may be mediated via an increase in plasma adiponectin levels.

Combining aerobic exercise with weight training leads to improvements in vascular function in diabetic patients,[392] while aerobic training alone decreases arterial stiffness.[459] The ADA recommends at least 3 days per week of moderate intensity exercise (50–70% maximal oxygen consumption [VO_{2max}]) for at least 20–45 minutes,[460] consistent with improvements in vascular function.[392,459] The relative benefits of weight-reducing regimens based on the Atkins® [1] diet (high protein, high fat, low carbohydrate) versus a traditional low fat, low energy diet on vascular function in diabetes requires examination, given their recently reported differential effects on weight loss and metabolic risk factors.[461,462] Mediterranean-style diets containing n-3 fatty acids from either fish or canola oil prevent postprandial impairments in FMD in healthy normolipidaemic individuals.[463] Although observational evidence suggests a cardioprotective effect for alcohol (ethanol),[464] there is insufficient evidence at present to suggest that alcohol consumption enhances endothelial function in the short-term,[465] especially in diabetic patients. Other nutritional factors that warrant examination in future studies include dietary consumption of isoflavones [466,467] and a low AGEs diet.[468]

9.9 Summary

The central role of oxidative stress in regulating the bioavailability of NO would suggest that antioxidants and other agents that reduce oxidative stress should aid in restoring endothelial function in diabetic patients. Although several antioxidants, including ascorbic acid, tocopherol, folic acid and ubidecarenone, have improved vascular function in diabetic patients, other studies have been negative, perhaps reflecting the wide variation in oxidative stress and/or individual antioxidant requirements of subjects between studies. A reduction in the generation of superoxide radicals may account for some of the beneficial effects of many other agents via, for example, their effects on NAD(P)H oxidase, inflammatory signalling pathways or their direct antioxidant effects. Lifestyle and nutritional interventions such as exercise, weight loss and n-3 fatty acids, which are each associated with decreased cardiovascular events, have each also been demonstrated to improve vascular function in type 2 diabetic patients.

10. Conclusions and Future Perspectives

Endothelial dysfunction occurs as a consequence of the impaired release and/or action of NO and other vasoactive factors (including endothelin-1 and EDHF) from endothelial cells. It is present in individuals at risk of cardiovascular disease, including those with type 2 diabetes and/or dyslipidaemia, and has been demonstrated clinically using a variety of classical techniques. The importance of endothelial dysfunction and increased arterial stiffness relates to their early role in the pathogenesis of atherosclerosis and their ability to independently predict cardiovascular events.

Prospective trials and intervention studies have typically employed several different techniques for measurement of vascular function. Plethysmography, measuring blood flow in the forearm resistance vessels in response to a variety of vasoactive agents, and brachial artery ultrasound, measuring FMD following an ischaemic stimulus, are the

1 The use of trade names is for product identification purposes only and does not imply endorsement.

two most commonly used methods. Both these techniques provide surrogate measures of endothelial function of the coronary circulation. Each technique has its own specific advantages: although plethysmography, with the intra-arterial infusion of pharmacological agents, allows a mechanistic examination of the pathways responsible for endothelial dysfunction, brachial ultrasound FMD is less invasive, less costly and less time-consuming. The contribution of different endothelial-derived vasoactive molecules to blood flow changes also varies along the arterial tree, for example, NO may be the principal mediator of brachial artery FMD, with a greater relative contribution from EDHF in forearm resistance arteries. Arterial stiffness is most commonly assessed by measurement of PWV, although there is now available a wide range of commercial instruments, which provide measures that also have independent prognostic value.

The mechanisms that underlie the pathogenesis of endothelial dysfunction in type 2 diabetes are multifactorial. Two principal contributors, which appear to operate synergistically, are the presence of diabetic dyslipidaemia and increased oxidative stress. Both may be the consequence of an increased flux of free fatty acids to the liver, skeletal muscle and endothelial cells due to dysregulated adipocyte fatty acid metabolism. Hyperglycaemia contributes to the dyslipidaemia and oxidative stress and may also be a consequence of altered fatty acid metabolism. Dyslipidaemia, oxidative stress and hyperglycaemia also play a central role in the accelerated rate of arterial stiffening in patients with type 2 diabetes. The efficacy of the therapeutic correction of dyslipidaemia in improving vascular function may partly be due to reduction in oxidative stress.

Together with lifestyle modifications, statins and fibric acid derivatives (or a combination of the two) are the principal therapies recommended by expert bodies for individuals with diabetic dyslipidaemia. Although statin therapy may be successful in reaching the required targets for total and/or LDL-cholesterol, the results of the trials we have reviewed indicate that they are not always effective in improving endothelial dysfunction. Moreover, in patients with type 2 diabetes, improvements in the degree of dyslipidaemia following statin therapy have not been paralleled by changes in endothelial function, but have in some studies been shown to be associated with reduction in oxidative stress. Similarly, although fibric acid derivatives improve endothelial function in diabetic subjects when administered alone, treatment may be most effective when combined with an antioxidant such as ubidecarenone. On the basis of prospective data, the size of the treatment effects with statins and fibric acid derivatives reported in various trials reviewed here predict at least a 25% reduction in the incidence of cardiovascular events, consistent with the findings in the corresponding clinical end-point trials.

In addition to lipid-modifying agents and antioxidants, numerous other interventions have demonstrated significant treatment effects on endothelial function, including angiotensin II receptor antagonists, insulin and AGE cross-link breakers. In aiming to achieve optimal improvement in endothelial function, the treatment of dyslipidaemia in diabetic patients should therefore not be considered in isolation. Whether the addition of lipid-regulating therapy to other agents such as ACE inhibitors or angiotensin II receptor antagonists improves endothelial dysfunction has not been formally tested. However, data from clinical endpoint trials suggest that, in patients with established CHD, statins and fibric acid derivatives have incremental effects to these treatments in reducing cardiovascular events.

While this review has focused on patients with type 2 diabetes, our conclusions may also apply to individuals without diabetes, but with features of the metabolic syndrome. Similarly, we have not considered the effects of lipid-regulating pharmacotherapy in patients with type 1 diabetes, or in particular subsets of type 2 diabetic individuals, such as those with nephropathy or autonomic neuropathy, who may be more resistant to therapy. The effects on vascular function of newer treatments for dyslipidaemia such as thiazolidinediones, combined PPAR-α and PPAR-δ agonists, cholesteryl ester transfer protein antagonists and ezetimibe need further examination. Since they operate via compli-

mentary pathways to standard lipid therapies, combinations of these drugs may offer the potential for even more effective improvement in vascular function amongst patients with type 2 diabetes or the metabolic syndrome.

Investigating the effects of pharmacotherapy on endothelial function and arterial stiffness affords a good paradigm for exploring the clinical potential of new therapies, including pipeline lipid-regulating agents. This is supported by the demonstrations that these tests provide surrogate measures that predict cardiovascular events. These surrogate endpoint trials may be employed in two ways in cardiovascular medicine: (i) to identify agents that should be tested in clinical endpoint trials; and (ii) to provide mechanisms for the benefit of these treatments observed in large clinical trials. Given that the favourable effects of agents such as the statins and fibric acid derivatives on endothelial dysfunction could be predominantly mediated by non-lipid mechanisms, a novel prospect for the future is whether measures of arterial function, such as those reviewed here, could *per se* be employed to guide the intensity of treatment. We predict that this could be the next paradigm shift in the management of cardiovascular disease of high-risk patients, such as those with diabetes and the metabolic syndrome.

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