

Review

The link between hyperglycaemia and diabetic nephropathy

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Summary. A large number of experimental studies in animals and retrospective or non-randomised prospective studies in humans provide support for the concept that the microvascular complications of diabetes mellitus are dependent on hyperglycaemia. This review focuses on four potential biochemical pathways linking hyperglycaemia to changes within the kidney which can plausibly be linked to the functional and structural changes characterising diabetic nephropathy. These four pathways are the polyol pathway, non-enzymatic glycation, glucose autooxidation and de novo synthesis of diacylglycerol leading to protein kinase C and phospholipase A₂

activation. Rather than being independent, there are several potential interactions between these four pathways which may explain confusing and overlapping effects observed in studies examining inhibitors of individual pathways. As many of the steps which follow on glucose metabolism are subject to modification by dietary and pharmacological means, the further delineation of the pathogenetic sequence leading to tissue damage in diabetes should allow a logical and effective approach to the prevention or treatment of the complications of diabetes.

Despite an increased ability to control the metabolic disturbances of diabetes mellitus, diabetic nephropathy remains a major cause of morbidity and mortality. It is now the most common underlying problem in patients presenting for management of end-stage renal failure in many Western countries, accounting for over 25 % of the cases. Whilst controversial, most data indicate that the liability to develop diabetic nephropathy is linked to the overall control of the blood glucose level. It should be remembered that the strongest evidence for this in human subjects comes from non-randomised prospective studies [1] or from retrospective studies [2] and that some similar retrospective studies have shown no clear association between the degree of glycaemic control and the risk of diabetic nephropathy [3]. Animal studies have provided fairly direct support for an aetiological link between hyperglycaemia and histological changes in the kidney [4], but until results from the Diabetes Control and Complications Trial are available [5], there is no absolute certainty of the link in humans between hyperglycaemia and diabetic nephropathy. However, the most reasonable position to take at this stage, and the departure point for the discussion in the rest of this review, is that hyperglycaemia sets in motion a series of biochemical disturbances in critical tissues (including the kidney) leading to functional changes, followed by irreversible structural changes and

finally the features that we recognise as clinical disease. Whatever role we attribute to hyperglycaemia, it is apparent that this sequence is very much affected by genetic and environmental (such as dietary) factors that lead to a variable expression of disease even in those with a similar duration and severity of metabolic disturbance.

Having accepted the surmise that hyperglycaemia is directly linked to the development of diabetic nephropathy, there is no shortage of plausible biochemical mechanisms which could lead to functional changes and/or tissue damage. Because of available space, this review will focus on four biochemical pathways which have recently been the subject of extensive investigation. Where possible, potential links with functional and structural changes in the kidney will be described. It will be emphasised that the mechanisms are not mutually exclusive and that there is the likelihood of important interactions between them.

The polyol pathway (Fig. 1)

A potential link between the formation of sorbitol from glucose catalysed by aldose reductase in tissues of diabetic subjects and the development of diabetic complications was first recognised more than 20 years ago [6–8]. Evidence for participation of the polyol pathway in the pa-

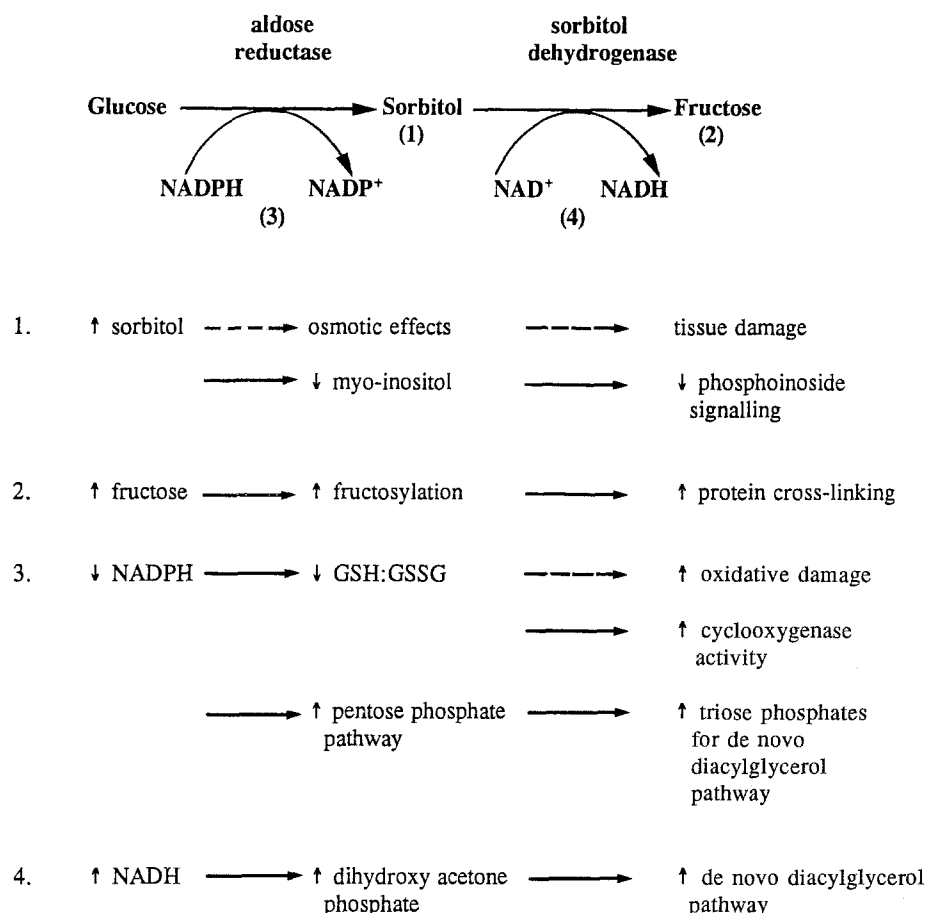


Fig. 1. The aldose reductase pathway with demonstrated or postulated biochemical consequences of potential relevance to the pathogenesis of diabetic nephropathy (and other complications of diabetes). GSH, reduced glutathione; GSSG, oxidized glutathione

thogenesis of diabetic nephropathy comes mainly from animal data using aldose reductase inhibitors. Although the results from different laboratories have not been uniform, most groups have been able to demonstrate that if administered from the time of induction of diabetes by streptozotocin in rats, a variety of aldose reductase inhibitors will reduce the subsequent increase in urinary albumin excretion [9–12]. The increased glomerular filtration rate of early diabetes may also be normalized [11, 13] although this is not required for the effect on albumin excretion to be observed [12]. Human data relating to the effects of aldose reductase inhibitors in diabetic nephropathy are relatively sparse. It has recently been shown that the aldose reductase inhibitor ponalrestat decreases the hyperfiltration observed in some diabetic subjects [14].

Further evidence that the polyol pathway may play a role in diabetic complications has come from using galactose feeding of non-diabetic animals to achieve increased polyol accumulation without the other metabolic disturbances of diabetes. Galactose is converted to galactitol by aldose reductase, and accumulates in tissues. It has been clearly demonstrated that galactose-feeding leads to the development of retinal lesions resembling those of diabetes in rats and dogs [15–17]. It also leads to changes in kidney physiology and structure resembling the effects of diabetes [18, 19], although the effectiveness of aldose reductase inhibitors in preventing the changes has differed between studies.

The mechanisms by which excessive activity of the polyol pathway could lead to complications of diabetes are complex and are summarised schematically in Figure 1. It was initially considered that osmotic effects of sorbitol accumulation might damage tissues [20], but even in the lens of the eye where an osmotic effect is potentially the greatest sorbitol levels do not exceed 2 mmol/l, even after severe hyperglycaemia [21]. Moreover, although galactose feeding leads to an increase in nerve water content, similar changes are not seen in experimental diabetes [22]. These and other observations make osmotic effects unlikely to be the major mechanisms of tissue damage associated with the polyol pathway.

Greene and co-workers have suggested that tissue damage secondary to polyol pathway activity is caused by the impairment of myo-inositol uptake consequent to sorbitol accumulation [23, 24]. This causes depletion of tissue phosphoinositides, and would have the potential to result in decreased phosphatidylinositol bisphosphate hydrolysis and decreased diacylglycerol formation [25]. Diacylglycerol is the regulatory activator of protein kinase C [26] which in turn activates sodium-potassium ATPase. Although there is evidence supporting parts of this sequence especially in nerve [27] the situation in the kidney appears to be different. Diacylglycerol is produced in increased amounts by a de novo synthetic pathway in glomeruli [28] and cultured mesangial cells [29], and this is associated with increased, not decreased, protein ki-

nase C activity. Decreased myo-inositol uptake and content, and polyphosphoinositide turnover can be demonstrated in glomeruli from diabetic rats [28], and this could contribute to impaired inositol trisphosphate release, which in turn could account for poor Ca^{2+} mobilisation and mesangial cell contractile activity in mesangial cells cultured in conditions designed to simulate the diabetic environment [29, 30].

Even by 1971 it was recognised that important alterations in the ratio of pyridine nucleotides would occur as a result of flux through the polyol pathway [7]. Reduction of glucose to sorbitol utilises NADPH and oxidation of sorbitol to fructose catalysed by sorbitol dehydrogenase increases NADH.

Increased NADPH utilization by activity of the aldose reductase pathway could have several consequences in the cell. In particular, it could be linked to increased prostaglandin synthesis in two possible ways. It would be expected to lead to decreased levels of reduced glutathione (GSH), which in turn might be associated with increased endogenous hydrogen peroxide and cyclooxygenase activity [32]. Alternatively, the NADPH utilization would be expected to increase activity of the pentose phosphate pathway, providing triose phosphate intermediates for the *de novo* synthetic pathway for diacylglycerol (and subsequently prostaglandin synthesis). The *de novo* diacylglycerol synthetic pathway would be enhanced by the increased NADH:NAD⁺ ratio, which would favour the formation of dihydroxyacetone at the triose isomerase step. The *de novo* pathway of diacylglycerol formation and its relation to prostaglandin synthesis is described more fully in a later section of this review.

The final consequence of increased flux through the aldose reductase pathway which could be important in diabetic complications is increased formation of fructose. Non-enzymatic fructosylation of tissues occurs at a considerably increased rate compared with glycation [33] and fructose levels are increased up to 23-fold in tissues such as the lens where the aldose reductase pathway is active [34]. These observations provide an explanation for the observed effects of aldose reductase inhibitors in decreasing collagen fluorescence in diabetic rats [33, 35], an effect shared by aminoguanidine, an inhibitor of formation of advanced glycation end products. This provides a link between the aldose reductase pathway and non-enzymatic glycation.

It can be seen that there are several consequences of increased flux of glucose through the aldose reductase pathway which could have relevance to diabetic complications. There are abundant data from animal studies supporting a role for some of these effects in the early stages of diabetic nephropathy, but it should be emphasised that data in humans are very limited, and no studies have convincingly demonstrated reversal of established pathological changes following inhibition of aldose reductase.

Non-enzymatic glycation

It has been known for many years that glucose can form glycation products with protein (Schiff bases), by a non-enzymatic process dependent on the glucose concentra-

tion. The Schiff bases rearrange to form the more stable Amadori products. These early glycation reactions are reversible and therefore reach a steady state, even in long-lived proteins [36]. Particular interest has focused in recent years on the subsequent steps which can occur. The early glycation products may undergo a complex series of chemical rearrangements to form advanced glycation end products (AGEs), which accumulate over a life-time. By interacting with other proteins, they may form cross-links with significant structural effects. Aging in non-diabetic subjects is associated with progressive accumulation of AGEs, but the process is greatly accelerated by diabetes. As it has been shown that a highly significant correlation exists between accumulated levels of AGEs on collagen and severity of diabetic retinopathy [37], a pathogenetic role for them has been suggested. This is supported by experiments showing that an inhibitor of the advanced glycation product formation, aminoguanidine, prevented basement membrane thickening in diabetic rats [36]. Many other consequences of non-enzymatic glycation have been postulated which might be of relevance to diabetic complications, but they are beyond the scope of this review. It should, however, be emphasised that there is an important potential link between the polyol pathway and non-enzymatic glycation, with aldose reductase inhibitors having the ability to simulate the action of aminoguanidine in inhibiting diabetes-induced fluorescence in rat collagen [33, 35]. The description of specific macrophage receptors for advanced glycation end products and their down-regulation by insulin [38] provides an additional level of complexity. Moreover, non-enzymatic glycation of reactive amino groups in model proteins increases the rate of free radical production by nearly 50-fold [39], providing a link with the oxidative damage described in the next section.

Glucose autooxidation

The metabolism of glucose can lead to the formation of reactive oxygen species [40]. This process is catalysed by metal ions. Oxidative modification of lipids and proteins can follow. It has been postulated that oxidative processes are major mechanisms for tissue damage in diabetes [41]. With respect to the kidney, the analogy between diabetic nephropathy and atherosclerosis has been drawn [42]. Oxidative modification of low density lipoproteins (LDL) is a key step in the formation of foam cells in atherosclerosis, as such modification is required to allow macrophage receptors to recognise LDL [43]. It has been demonstrated that high fat diets exacerbate certain types of experimental nephropathy [44], perhaps by a similar mechanism to that occurring in atherosclerosis. Glucose-induced oxidative modification of lipids, especially LDL, would be expected to exacerbate this process. Controlled studies of the effect of diets of varying fat content or of pharmacological modification of hyperlipidaemia on the progress of diabetic nephropathy in humans are not yet available, but an uncontrolled study indicated a relationship between serum lipid levels and rate of progression of diabetic nephropathy in humans [45].

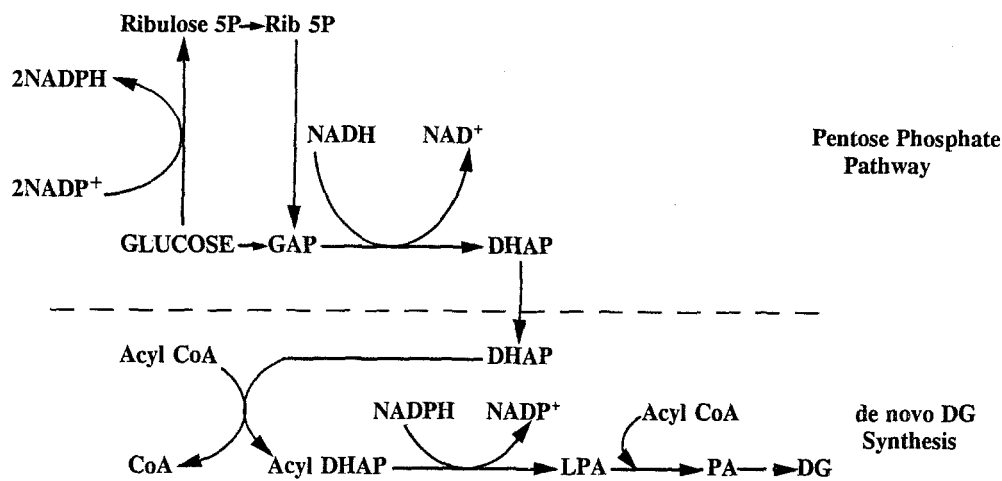


Fig. 2. The de novo synthetic pathway of diacylglycerol (DG) formation from glucose. It is favoured by the altered ratios of pyridine nucleotides consequent upon increased aldose reductase pathway activity demonstrated in Figure 1. DG is the physiological regulator of protein kinase C activity, which in turn can regulate phospholipase A₂ activity and prostaglandin production. Ribulose 5P, ribulose 5-phosphate; Rib 5P, ribose 5-phosphate; GAP, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; LPA, lysophosphatidic acid; PA, phosphatidic acid

Although human data are lacking, oxidative damage to proteins dependent on oxygen radical generation from glucose metabolism might also be important in the genesis of diabetic nephropathy.

De novo synthesis of diacylglycerol, protein kinase C activation, phospholipase A₂ activation and increased prostanoid synthesis (Fig. 2)

Following some earlier observations on glucose conversion to lipids and phospholipids in mouse pancreatic islets [46] it was shown that glucose can be metabolised directly to diacylglycerol, by a process involving conversion of dihydroxyacetone phosphate to lysophosphatidic acid, phosphatidic acid and diacylglycerol in neonatal rat pancreatic islets [47]. This pathway has now been shown to operate in many other tissues, including retinal capillary endothelial cells [48, 49] heart from diabetic rats [50], granulation tissue in a skin chamber exposed to high glucose concentrations [51], glomeruli from diabetic rats [28] and mesangial cells isolated from diabetic rats or cultured under conditions designed to simulate diabetes [29].

Diacylglycerol is the regulatory stimulator of protein kinase C [26], and its elevation is the presumed mechanism for the elevated protein kinase C activity observed in several tissues obtained from diabetic animals or exposed in vitro to high glucose concentrations, including rat retinal capillary endothelial cells [49], rat granulation tissue [52], rat renal glomeruli [28, 53–55] and rat mesangial cells [29]. Activation of protein kinase C in turn could have several consequences of potential importance in the pathogenesis of diabetic complications [48] including enhanced DNA synthesis and growth rate in vascular cells [56], increased hormone and growth factor receptor turnover [57], increased smooth muscle contraction [58] and increased cyclic AMP responses to different hormones in vascular cells [59].

Another consequence of increased protein kinase C activity may be increased prostaglandin synthesis. Increased glomerular and mesangial cell prostaglandin production in experimental diabetes was demonstrated several years ago [60, 61] and has been confirmed by many

investigators. Part of the mechanism for increased prostaglandin synthesis is presumably increased conversion of arachidonic acid to prostaglandins dependent on cyclooxygenase activation [62, 63] perhaps related to altered redox state of the cell due to aldose reductase activity [12, 31]. In addition, however, and probably playing a larger part in the increased glomerular prostaglandin production, increased phospholipase A₂ activity has been demonstrated in glomeruli and mesangial cells from diabetic rats [12, 29, 64]. Phospholipase A₂ activation has been attributed to activation of protein kinase C [53–55] directly or via phosphorylation of lipocortin [65]. There are of course many ways in which elevated production of vasodilatory prostaglandins and/or thromboxane A₂ could lead to the early functional and later structural changes in the kidney in diabetes, and much research has been devoted to delineating their role. Suffice it to say that it seems likely that overproduction of vasodilatory prostaglandins by renal glomeruli plays a role in the early renal hyperfusion and hyperfiltration [62, 63, 66–68] although their exact contribution is controversial [69, 70]. The reduction in mesangial cell contractility induced by prostaglandin E₂ and prostacyclin in vitro [30, 60] suggests that increased production of vasodilatory prostaglandins could also affect glomerular permeability. A recent report also suggests an important role for overproduction of thromboxane A₂. Treatment of diabetic rats with a thromboxane synthetase inhibitor greatly reduced the degree of albuminuria and the degree of renal type IV collagen gene expression [71]. The latter effect may well have resulted from decreased pressure within the glomerular loops, suggesting that thromboxane A₂ might contribute to increased glomerular efferent arteriolar tone in diabetes.

Conclusion

From this brief review, it can be seen that there are several potential mechanisms by which hyperglycaemia could lead to a sequence of biochemical events culminating in the physiological changes characterising early diabetes. The later structural changes can be understood as long-term consequences of the biochemical and physiological changes. The exact role played by these processes, and the

contribution of many other physiological and biochemical changes observed in diabetes which have not been dealt with in this review remain to be clarified. However, as many of the processes can be modified by dietary or pharmacological means, it is essential that the detailed sequence of events in the pathogenesis of diabetic nephropathy be defined by continuing and extending the combination of cell biology, animal and human experimentation that has led to such dramatic advances in our knowledge in the last few years.

Acknowledgements. Experimental work of the authors referred to in this manuscript was supported by grants from National Health and Medical Research Council of Australia, Hoechst Diabetes Research and Development Fund, the Kellion Diabetes Foundation and the Diabetes Australia Research Trust.

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