Role of lipids in chemical modification of proteins and development of complications in diabetes


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Abstract

Hyperglycaemia is the major risk factor for the development of complications in both Type I and Type II diabetes; however, there is growing evidence from several clinical trials that dyslipidaemia, including hypertriglyceridaemia, is a significant and independent risk factor for diabetic complications. In this paper, we propose that chemical modification of proteins by lipids may be a underlying pathogenic mechanism linking dyslipidaemia to diabetic complications. Thus the major AGEs (advanced glycation end-products) in tissues, such as carboxymethyl-lysine, carboxyethyl-lysine and hydroimidazolones, may, in fact, be ALEs (advanced lipoxidation end-products), derived from lipids. Increased lipid peroxidation and accelerated ALE formation, possibly catalysed by hyperglycaemia and oxidative stress, may be the mechanistic link between dyslipidaemia and diabetic complications. If correct, this proposal would suggest that inhibition or reversal of glycation, which is a central theme of this symposium, may not be sufficient for protection against diabetic complications.

Introduction: chemical modifications of protein in diabetes – AGEs (advanced glycation end-products) or ALEs (advanced lipoxidation end-products)

Hyperglycaemia has been identified as the major risk factor for the development of complications in both Type I and Type II diabetes [1,2]. Yet it is widely recognized that patients with similar age of onset and duration of diabetes and a similar history of glycaemic control differ significantly in the severity of diabetic complications. From the viewpoint of the Maillard or glycation hypothesis, this difference in susceptibility to complications may be attributed to individual differences in rates of formation or accumulation of AGEs in tissue proteins. Individuals may vary in the kinetics of synthesis of AGE-proteins, turnover of AGE-proteins or pro-inflammatory reactions to AGE-proteins involving receptors such as RAGE (the receptor for AGEs) [3] or scavenger receptors [4]. Differences in antioxidant defences [5], detoxification systems [6] and renal function [7] may also affect levels of AGE precursors, indirectly affecting the rate of formation of AGEs and the risk for complications. Thus numerous factors, in addition to levels of glucose, may modulate the effects of glycaemia on AGE formation and diabetic complications.

In addition to glucose, metabolic intermediates, such as triose phosphates, glyoxal and methylglyoxal, are recognized as important precursors of AGEs [8], and changes in the concentrations of these compounds, both inside and outside the cell, will affect the rate of AGE formation. There is also growing evidence from several clinical trials that dyslipidaemia, including hypertriglyceridaemia, is a significant and independent risk factor for diabetic complications [9–13]. In this paper, we propose that chemical modification of proteins by lipids may be a underlying pathogenic mechanism linking dyslipidaemia to diabetic complications. This proposal is based on the fact that lipid peroxidation produces several dicarbonyl intermediates that are identical to those formed during glycoxidation reactions, including glyoxal and methylglyoxal [14], which are precursors of the major AGEs, including CML [Nε-(carboxymethyl)lysine], CEL [Nε-(carboxyethyl)lysine], imidazolone derivatives of arginine, and argpyrimidine [15]. Recent work in animal models suggests that these dicarbonyl precursors may, in fact, be derived primarily from lipid peroxidation reactions, and that the major ‘AGEs’ in tissues may, in fact, be ALEs, derived from lipids. Increased lipid peroxidation and accelerated ALE formation, possibly catalysed by hyperglycaemia and oxidative stress, may be the mechanistic link between dyslipidaemia and diabetic complications. If correct, this proposal would suggest that inhibition or reversal of glycation, which is a central theme of this symposium, may not be sufficient for protection against diabetic complications.

Inhibition of AGE/ALE formation and of diabetic complications by PM (pyridoxamine)

In recent studies on the Maillard or AGE hypothesis, we have observed that the AGE inhibitor PM [16] protects...
against the development of early nephropathy, retinopathy and neuropathy in the STZ (streptozotocin)-induced diabetic rat [17–19]. In the studies on nephropathy, we found that urinary albumin and plasma creatinine concentrations in diabetic rats were correlated with the increases in CML, CEL, AGE-like fluorescence (excitation 370 nm, emission 400 nm) and cross-linking of skin collagen [17]. Treatment with the AGE inhibitors PM and aminoguanidine decreased levels of CML, CEL, fluorescence and cross-linking of collagen and preserved renal function, consistent with the proposed mechanism of action of these drugs as AGE inhibitors and with the role of the Maillard reaction and AGEs in the development of diabetic complications. Unexpectedly, however, neither PM nor aminoguanidine affected the increase in pentosidine in skin collagen of the diabetic rats. Since CML and CEL may be formed from lipids, as well as carbohydrates, while pentosidine is derived exclusively from carbohydrates [14], we considered the possibility that the increases in CML and CEL in the diabetic rat might result from reactions of lipids with proteins. This interpretation of the experiments was supported by the observations that the diabetic animals were severely hyperlipidaemic, with mean triacylglycerols increasing 6–10 times and total cholesterol by up to 2-fold, compared with control rats, and that the ALE MDA (malondialdehyde)–lysine was also increased significantly in the skin collagen of diabetic rats [17]. PM and aminoguanidine caused a substantial (>50%) decrease in triacylglycerols and normalization of cholesterol in the diabetic rats, comparable with the decreases in CML, CEL, fluorescence and MDA–lysine. There were also significant correlations among CML, plasma triacylglycerols and albuminuria in these animals (Figure 1). PM and aminoguanidine appeared to decrease all of these biomarkers in a comparable manner, while they had no effect on glycaemia, glycation of haemoglobin or collagen, or pentosidine formation. These results suggested that lipids, rather than carbohydrates, might be a major source of chemical modification of proteins in the STZ-diabetic rat, and that lipoxidative chemical modification of protein might be important in the development of diabetic complications.

### Role of lipids and lipoxidation in renal disease – studies in the Zucker rat

We had shown previously [20] that PM was a potent inhibitor of lipoxidation reactions in vitro, inhibiting the formation of CML and CEL, as well as MDA and hydroxynonenal adducts to proteins. The inhibitory effect of PM was observed during oxidation of arachidonate in the presence of RNase or during the metal-catalysed oxidation of low density lipoprotein. To address the role of lipids in the chemical modification of proteins and the development of nephropathy in animal models, we studied the effects of PM on the development of renal disease in the Zucker obese (fa/fa) rat [19]. This animal model, which has a homozygous defect in the leptin receptor gene, has many of the characteristics of the pre-diabetic state, known as the metabolic syndrome (Syndrome X), including obesity, hyperlipidaemia, insulin resistance and hypertension; however, the blood glucose concentration is normal, the same as that of lean (heterozygous) littermates. The Zucker

![Figure 1](image-url)
obese rat develops albuminuria and creatininaemia at a rate similar to that of the poorly controlled STZ-diabetic rat, and also develops severe hypertriglyceridaemia and hypercholesterolaemia.

As in the diabetic rats, renal function in the Zucker rat was markedly protected by PM, and preservation of renal function was accompanied by significant decreases in both triacylglycerols and cholesterol [19]. Consistent with a role for lipids and lipoxidation in the chemical modification of proteins, levels of both CML and CEL were increased by about 2-fold in the skin collagen of the Zucker rat, an extent similar to that observed in the STZ-diabetic rat, which had a similar degree of hyperlipidaemia. Unexpectedly, pentosidine was also increased in the Zucker rats, without a corresponding increase in glycaemia. Consistent with its effects on lipoaemia, PM also decreased the levels of CML and CEL by about 50%, arguing that these compounds were derived from lipids in both the STZ-diabetic and Zucker obese rat. Surprisingly, PM also marginally (P = 0.07) inhibited the increase in pentosidine in the Zucker rat, suggesting that this might have been formed from ascorbate or another carbohydrate precursor, rather than glucose. In summary, these experiments suggest that lipids may be a major source of chemical modifications of proteins and contribute to development of chronic renal disease in diabetes and obesity.

**PM inhibits lipoxidation chemistry in vivo**

Because of the lipid-lowering effects of PM, it is possible that the decrease in ALEs could be explained by the decrease in lipoaemia alone, without direct inhibition of AGE/ALE formation. In order to confirm that PM functioned as an ALE inhibitor in vivo, we analysed the urine of PM-treated control, diabetic and hyperlipidaemic rats for products formed during the PM-mediated inhibition of lipoxidation reactions in vitro. Several lipid-derived PM adducts (Figure 2), detected originally in in vitro reactions of PM with linolate or arachidonate, were readily identified and quantified in the plasma and urine of PM-treated control, diabetic and obese rats [21]. All were formed by modification of the primary amino group of PM, which appears to be acting as a sacrificial nucleophile for trapping intermediates in lipoxidation reactions. Among the PM adducts, N-formyl-PM is formed during reactions of both carbohydrates and lipids with protein (T.O. Metz and J.W. Baynes, unpublished work) and is considered, like CML, to be a non-specific indicator of AGE/ALE formation. N-Hexanoyl-PM is formed during inhibition of chemical modification of proteins by ω-6 fatty acids, including both linolate and arachidonate. The analogous product, N⁵-(hexanoyl)lysine, has been detected by immunohistochemistry in atherosclerotic plaque [22], suggesting that PM may inhibit N-hexanoylation of protein in vivo. N-Pentanedioloyl-PM and N-nonanedioloyl-PM monoamides, derived from arachidonate and linolate respectively, were also detected in urine, along with the pyrrole and formylpyrrole derivatives of PM (results not shown), which are formed from arachidonate. The high ratio of N-pentanedioloyl-PM to N-nonanedioloyl-PM in urine (typically >100) suggested that, despite the higher concentration of linolate in blood and tissues, arachidonate or related ω-6-polyunsaturated fatty acids, possibly prostanoid intermediates, are the primary substrates for lipoxidation reactions in vivo. All of the PM adducts were detectable in both plasma and urine of PM-treated non-diabetic control and lean rats, but at only 5–10% of the concentration in the urine of diabetic and hyperlipidaemic rats. We therefore conclude that PM does function, at least in part, as an inhibitor of advanced lipoxidation reactions in vivo, and that the inhibition of ALE formation contributes to its efficacy in protecting against renal disease in diabetes and obesity.

**Role of hyperglycaemia**

The concentration of PM adducts in urine is not solely a function of the degree of lipoaemia, but appears to be increased by hyperglycaemia. The concentrations of PM adducts, which may be used as an indicator of the rate of lipid peroxidation, were up to 10-fold higher in urine from the Zucker diabetic fatty (ZDF) rat, a sub-strain of the Zucker obese rat that proceeds to develop Type II diabetes, compared with the Zucker obese rat itself, despite the fact plasma triacylglycerols were 2–3-fold higher in the non-diabetic rat [21]. Based on this admittedly limited evidence, it appears that hyperglycaemia or other aspects of the diabetic state may enhance the production of lipid-derived PM adducts. These observations with PM are consistent with clinical experience with diabetes, that hyperglycaemia exacerbates the cardiovascular effects of dyslipidaemia and that more rigorous attention to dyslipidaemia is essential for effective, long-term management of the diabetic patient.
Summary
In concluding this article, we wish to emphasize that we are not challenging the identification of hyperglycaemia and protein glycation as risk factors for diabetic complications, or the pathogenic role of glucose, hyperglycaemia, glycation and AGEs in diabetic complications. However, we do propose a variation on the AGE hypothesis, i.e. that hyperglycaemia exacerbates the chemical modification of proteins by lipids, and that lipids and ALEs, rather than carbohydrates and AGEs, may be the immediate and major source of chemical modifications leading to tissue damage, pro-inflammatory processes and chronic complications in diabetes. In all of the hyperlipidaemic animals, including the non-diabetic fa/fa rat, there was a substantial increase in the urinary excretion of PM adducts derived from lipids, indicating an increase in lipid peroxidation. Thus, while severe hyperlipidaemia may be sufficient to induce lipoxidative damage, hyperlipidaemia combined with hyperglycaemia and possibly an increase in oxidative stress in diabetes and obesity [4,21] appears to exacerbate the chemical modification of proteins by lipids in diabetes. These observations suggest a central role for lipid peroxidation and the lipoxidative modification of proteins in the development of cardiovascular and renal disease in diabetes.

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References
9 UK Prospective Diabetes Study (UKPDS) (1993) Diabetes 36, 1021–1029

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