Evaluation of N^{ϵ} -(3-formyl-3,4-dehydropiperidino)lysine as a novel biomarker for the severity of diabetic retinopathy

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Abstract

Aims/hypothesis Recent studies suggest that oxidative stress should be monitored alongside HbA_{1c} to identify subgroups of diabetic patients at high risk of initiation or progression of retinopathy. The acrolein-derived advanced lipoxidation end-product (ALE), N^{ϵ} -(3-formyl-3,4-dehydropiperidino)lysine (FDP-lysine), is a useful biomarker that reflects the cumulative burden of oxidative stress over long periods of time. The purpose of the present study was to investigate whether serum and haemoglobin levels of FDP-lysine are associated with the severity of diabetic retinopathy in type 1 and type 2 diabetic patients.

Methods Serum and haemoglobin levels of FDP-lysine were measured by competitive ELISA in 59 type 1 and 76 type 2 diabetic patients with no retinopathy, non-proliferative retinopathy or proliferative retinopathy (mean age [$\pm$ SEM] 54.3 $\pm$ 1.3 years), and in 47 non-diabetic control individuals (mean age 51.9 $\pm$ 2.1 years).

Results Serum and haemoglobin levels of FDP-lysine were significantly increased in diabetic patients compared with control individuals ($p=0.04$ and $p=0.002$, respectively). However, no significant association was found between levels of serum FDP-lysine and the severity of diabetic retinopathy ($p=0.97$). In contrast, increased haemoglobin FDP-lysine levels were observed in patients with proliferative retinopathy compared with patients without retinopathy and with non-proliferative retinopathy ($p=0.04$). The relationship of FDP-lysine with proliferative retinopathy was unaltered after adjustment for HbA_{1c}, or other clinical parameters.

Conclusions/interpretation Our data suggest that haemoglobin FDP-lysine may provide a useful risk marker for the development of proliferative diabetic retinopathy independently of HbA_{1c}, and that elevated intracellular ALE formation may be involved in the pathogenesis of this sight-threatening complication of diabetes.

Keywords Acrolein · Advanced lipoxidation end-products · Diabetic retinopathy · FDP-lysine · Haemoglobin · Oxidative stress · Serum

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Abbreviations

ALE	advanced lipoxidation end-product
AU	arbitrary units
FDP-lysine	N^{ϵ} -(3-formyl-3,4-dehydropiperidino)lysine
HSA	human serum albumin
ROS	reactive oxygen species
SOD	superoxide dismutase

Introduction

Retinopathy is one of the most common microvascular complication of diabetes and the leading cause of acquired

blindness in the working population of developed countries [1]. After 20 years of diabetes, nearly all patients with type 1 diabetes will have at least some retinopathy. Overall, diabetic retinopathy is slightly less common in type 2 diabetic patients, but is still the most frequent microvascular complication suffered by this group [2]. Although the pathogenic basis of diabetic retinopathy is not wholly understood at the cellular and molecular level, large prospective clinical trials have demonstrated a strong relationship between time-averaged mean levels of glycaemia (measured as HbA_{1c}) and the rate of development of retinopathy in both type 1 and type 2 diabetes [3, 4].

Hyperglycaemia is believed to contribute to retinal microangiopathy through the induction of oxidative stress [5]. Tissue oxidative stress can be evoked in diabetes through several mechanisms such as overproduction of oxygen radicals by the mitochondrial electron transport chain [6] and impairment of antioxidant defences [7, 8]. Reactive oxygen species (ROS) may directly cause oxidative damage of lipids, proteins and nucleic acids, impairing cell functions and leading to cell death by apoptosis [9]. Moreover, ROS are considered to be a causal link between elevated glucose and activation of other known pathways of hyperglycaemic damage, i.e. the polyol, hexosamine, diacylglycerol–protein kinase C and AGE pathways [6]. Investigation of appropriate animal models strongly supports the premise that oxidative stress plays an important role in the pathogenesis of diabetic retinopathy. Superoxide levels are elevated in the diabetic retina [10, 11] and inhibition of superoxide accumulation with antioxidants [12, 13] or overproduction of mitochondrial superoxide dismutase (SOD) [14, 15] can prevent lesions of retinopathy in experimentally induced diabetes in rodents.

While there is no dispute that hyperglycaemia is a critical factor in the aetiology of diabetic retinopathy, there are subgroups of patients where time-averaged mean HbA_{1c} values do not predict clinical prognoses [16]. This might be explained by the fact that HbA_{1c} fails to reflect the magnitude of acute glycaemic fluctuations, which may foster a pro-oxidative state [17]. In addition, genetically determined factors appear to influence susceptibility to diabetic retinopathy [18], which possibly involves polymorphic variations in genes that govern the generation of, or protection from, oxidative damage [19, 20]. These findings have important clinical implications and suggest that it may be beneficial to monitor oxidative stress in concert with HbA_{1c} to identify subgroups of diabetic patients at high risk of initiation and progression of retinopathy. Unfortunately, currently used markers of oxidative stress *in vivo*, such as thiobarbituric acid-reacting substances [21] and F2-isoprostanes [17], are relatively limited in their clinical usefulness as they only provide a ‘snapshot’ of ROS formation at the time of sampling and do not report integrated oxidative stress over a prolonged period.

Lipid peroxidation caused by ROS yields lipid hydroperoxides that decompose to reactive aldehydes such as 4-hydroxynonenal, malondialdehyde and acrolein. These compounds subsequently react with the nucleophilic sites of proteins generating relatively stable chemical adducts known as advanced lipoxidation end-products (ALEs) [22]. Acrolein is one of the most reactive products of lipid peroxidation, and this aldehyde has been shown to preferentially modify lysine residues of proteins [23]. Reaction of acrolein with the ϵ -amino group of lysine leads to the formation of *N*^ε-(3-formyl-3,4-dehydropiperidino) lysine (FDP-lysine) [23]. The presence of FDP-lysine has been demonstrated in human serum and urine [24–26] and represents a marker of cumulative oxidative stress [27].

As a chronic marker of oxidative stress, FDP-lysine might represent a useful prognostic biomarker for the development of diabetic retinopathy independently of HbA_{1c}. Therefore, in the present study, serum and haemoglobin levels of FDP-lysine have been quantified in well-characterised diabetic patients with differing stages of retinopathy and compared with age-matched non-diabetic controls.

Methods

Patients and healthy volunteers The patients consisted of 59 type 1 and 76 type 2 diabetic patients of white ethnic background. All were outpatients in the Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Belfast. Forty-seven healthy volunteers who were matched for sex, age and ethnicity served as controls. To rule out any confounding effects of renal impairment in causing an elevation of FDP-lysine levels, diabetic patients with clinical evidence of proteinuria were excluded from the study. Blood specimens were analysed for HbA_{1c}, cholesterol, HDL-cholesterol, LDL-cholesterol and triacylglycerol using National Health Service approved methods at the Clinical Chemistry, Regional Endocrine, Haematology and Coagulation Laboratories, Royal Victoria Hospital, Belfast. BP was measured at the right brachial artery using an Omron HEM-705CP automated sphygmomanometer (Omron Healthcare, Henfield, UK). Mean systolic and diastolic BPs were averages of the last two of three measurements. Hypertension was defined as a mean systolic BP ≥ 160 mmHg, and/or a mean diastolic BP of ≥ 95 mmHg or a history of antihypertensive medication [28]. Retinopathy was assessed by fundus photography and was graded according to the UK National Screening Committee protocol [29]. Diabetic retinopathy in each patient was classified as ‘none’ (level R0), ‘non-proliferative’ (levels R1/R2) or ‘proliferative’ (level R3). The study was performed in accordance with the declaration of Helsinki and was approved by the Research Ethics Committee of

Queen's University Belfast. All patients gave written informed consent to participate.

Preparation of serum and haemoglobin samples Venous blood was drawn into two polypropylene tubes (5 ml per tube) containing clot activator or heparin for the isolation of serum and haemoglobin, respectively, and stored on ice. For the serum, blood was allowed to clot for ~30 min, before being centrifuged at 1,000 *g* for 10 min at 4°C. Serum was immediately frozen at –80°C for later use. For haemoglobin, isotonic buffer (0.9% NaCl with 5 mmol/l sodium phosphate, pH 8) was added to whole blood. After trituration, the suspension was centrifuged at 600 *g* for 10 min. Supernatant was discarded and the pellet resuspended in isotonic buffer by trituration, then re-centrifuged. The erythrocyte pellet was resuspended in hypotonic buffer (5 mmol/l sodium phosphate, pH 8) to lyse the cells and the samples were centrifuged at 12,000 *g* for 10 min. Protein concentrations of the haemoglobin samples were measured with a BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Haemoglobin samples were immediately frozen at –80°C for later use.

FDP-lysine assay Serum and haemoglobin levels of FDP-lysine were determined with a competitive ELISA assay using a well-characterised monoclonal antibody specific for acrolein-modified FDP-lysine residue (mAb5F6) prepared as previously described [27]. FDP-lysine-human serum albumin (HSA) was prepared by incubating 10 mg/ml HSA with 10 mmol/l acrolein (stabilised; Sigma, Poole, UK) in 50 mmol/l PBS (pH 7.2) at 37°C for 24 h in the dark. After dialysis against PBS, FDP-lysine-HSA was stored at –20°C. Microtitre plates (96 wells) were coated with 0.1 µg/ml FDP-lysine-HSA in coating buffer (0.05 mol/l carbonate buffer, pH 9.6, 200 µl per well) overnight at 4°C. ELISA plates were washed extensively three times with washing buffer (0.2 mmol/l KH₂PO₄, 1.4 mmol/l K₂HPO₄, 3 mmol/l NaCl, 0.005 mmol/l sorbic acid potassium, 0.01% Tween-20) and wells were blocked for 2 h with 3% skim milk. After rinsing with washing buffer, 50 µl competing antigen (FDP-lysine-HSA standard [0.1–3 µg/ml]), serum (1:4 vol./vol.) or haemoglobin (30 mg/ml) in dilution buffer (0.05% Tween-20 and 0.2% BSA in 75 mmol/l PBS pH 7.4) and 50 µl of mAb5F6 (50 ng/ml) were added for 2 h at room temperature. After washing, peroxidase conjugated anti-mouse IgG (diluted 1:5,000 vol./vol.) was added and incubated for 1 h. Following three subsequent washing steps, the colour reaction was induced with 150 µl per well 3,3',5,5'-tetramethyl-benzidine (TMB) diluted in substrate buffer (340 µl TMB solution [52 mmol/l 3,3',5,5'-TMB (Sigma), 1 ml DMSO, 5 ml methanol] plus 10 µl 30% H₂O₂ [wt/wt] per 20 ml substrate buffer [0.2 mol/l potassium dihydrogen citrate, 0.7 mmol/l sorbic acid

potassium]) and stopped after 15 min by the addition of 50 µl per well of 1 mol/l H₂SO₄. Absorbance was read using a microtitre plate reader (Multifunction Microplate Reader, Safire; TECAN, Reading, UK) at 450 nm. Results were first determined as % B/B_0 , where B = the absorbance in the presence of standard or sample and B_0 = absorbance in the absence of standard or sample. The FDP-lysine content of the samples was then quantified by using a standard calibration curve. Final results are given as: serum FDP-lysine concentrations (µg/ml) referring to the 1 µg/ml of synthesised FDP-lysine-HSA standard; haemoglobin FDP-lysine concentrations (arbitrary units [AU]/mg protein) is equal to 1 µg/ml of synthesised FDP-lysine-HSA standard per mg haemoglobin. All samples were run in triplicate. Intra-assay CV values of our ELISA system were 2.7% and inter-assay values were 6.6%.

Statistical analysis Data are presented as means ± SEM. Statistical analyses were performed using Prism V4.02 (Graphpad Software, San Diego, CA, USA). Normal distribution was assessed by the D'Agostino and Pearson omnibus normality test. The Mann–Whitney U test was used for statistical analysis of serum and haemoglobin FDP-lysine between diabetic patients and control individuals. Multiple comparisons were performed using one-way ANOVA with Newman–Keuls test for normally distributed data and Kruskal–Wallis analysis followed by Dunn's test for non-normally distributed data. The χ^2 test was used to compare differences in proportions between groups. Bivariate logistic regression and receiver operating characteristics curve analyses were performed using SPSS statistical software V15 (SPSS, Chicago, IL, USA).

Results

The serum level of FDP-lysine in diabetic patients was significantly greater than in non-diabetic volunteers (22.4 ± 0.51 and 19.61 ± 0.7 µg/ml, respectively; $p=0.04$; Fig. 1a). Likewise, the haemoglobin level of FDP-lysine was elevated in diabetic patients compared with non-diabetic individuals (0.38 ± 0.02 AU/mg protein and 0.22 ± 0.03 AU/mg protein, respectively; $p=0.002$; Fig. 1b).

Patients were subdivided into three groups according to retinopathy status, and FDP-lysine levels were investigated. Eighty-one patients had no retinopathy and 30 patients were classified as having non-proliferative retinopathy, whilst 24 patients were categorised as having proliferative retinopathy (Table 1). Because of the criteria used for recruitment, no significant differences in age, sex or renal function were evident among the groups. Fasting blood glucose and HbA_{1c} were significantly higher in diabetic patients compared with non-diabetic controls, and the severity of

Fig. 1 Comparisons of FDP-lysine in serum of control individuals and diabetic patients (**a**), and from haemoglobin (Hb) from controls vs diabetic patients (**b**). The horizontal bars indicate mean values. * $p < 0.05$, ** $p < 0.01$ vs control individuals

retinopathy was associated with a longer duration of diabetes. There was no significant difference in serum blood lipid profiles or diastolic BP among the groups; however, compared with controls, systolic BP and BMI were higher in diabetic patients with non-proliferative retinopathy and no retinopathy, respectively (Table 1). No association was found between levels of serum FDP-lysine and the severity of diabetic retinopathy ($p = 0.97$; Fig. 2a). In contrast, haemoglobin FDP-lysine levels were significantly different between the retinopathy groups ($p = 0.04$; Fig. 2b). Increased haemoglobin levels of FDP-lysine were seen when patients with proliferative retinopathy (0.47 ± 0.05 AU/mg protein) were compared with diabetic patients without retinopathy (0.36 ± 0.02 AU/mg protein; $p < 0.05$) and with non-proliferative retinopathy (0.32 ± 0.04 AU/mg protein; $p < 0.05$). A significant elevation in haemoglobin FDP-lysine levels was also evident in patients with no retinopathy ($p < 0.01$), non-proliferative retinopathy ($p < 0.05$) and proliferative retinopathy ($p < 0.001$) when compared with control individuals.

To determine whether haemoglobin FDP-lysine was independently associated with retinopathy status, we divided diabetic patients into two groups according to whether or not they had proliferative disease. Bivariate logistic regression analysis was then performed to examine the relationship between haemoglobin FDP-lysine and retinopathy before and after adjustment for potential confounding variables. Confounding variables chosen for inclusion in the logistic regression analysis were those known from

Table 1 Clinical characteristics of controls and diabetic patients grouped according to retinopathy status

Characteristic	Control	No retinopathy	NPDR	PDR	<i>p</i> value
<i>n</i>	47	81	30	24	
Sex (M/F)	23/24	40/41	16/14	14/10	NS ^a
Age (years)	51.9 ± 2.1	53.3 ± 1.7	55.5 ± 2.9	56.3 ± 3.1	NS
Diabetes (type 1/type 2)	na	25/56	16/14	18/6	<0.001 ^a
Diabetes duration (years)	na	10.5 ± 1.2	22.0 ± 2.4 ^d	29.0 ± 2.6 ^d	<0.001
Fasting blood glucose (mmol/l)	4.8 ± 0.1	9.2 ± 0.3 ^c	10.31 ± 0.6 ^c	9.6 ± 0.6 ^c	<0.001
HbA _{1c} (%)	5.5 ± 0.1	8.2 ± 0.1 ^c	8.8 ± 0.4 ^c	9.3 ± 0.4 ^c	<0.001
Total cholesterol (mmol/l)	na	4.6 ± 0.1	4.7 ± 0.2	4.4 ± 0.2	NS
Triacylglycerol (mmol/l)	na	1.9 ± 0.1	1.7 ± 0.2	2.1 ± 0.4	NS
HDL-cholesterol (mmol/l)	na	1.4 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	NS
LDL-cholesterol (mmol/l)	na	2.4 ± 0.1	2.3 ± 0.2	2.1 ± 0.2	NS
Systolic BP (mmHg)	128.3 ± 2.8	133.9 ± 1.9	140.2 ± 2.2 ^c	136.8 ± 3.2	0.002
Diastolic BP (mmHg)	77.7 ± 1.2	77.9 ± 0.9	77.5 ± 1.4	73.4 ± 1.6	NS
Hypertension (absent/present)	na	65/16	22/8	19/5	NS ^a
BMI (kg/m ²)	27.1 ± 0.7	30.0 ± 0.6 ^b	30.0 ± 1.2	28.8 ± 1.2	0.007
Smokers (current or former/never)	20/27	26/55	8/22	9/15	NS ^a
ACR (mg/mmol)	na	3.05 ± 0.8	3.4 ± 1.1	5.5 ± 1.4	NS
Macular oedema (absent/present)	na	81/0	27/3	24/0	0.005 ^a
Treated with insulin (yes/no)	na	41/40	24/6	22/2	<0.001 ^a

Data are means ± SEM

^a p value of χ^2 test

Post hoc analysis: ^b $p < 0.01$ and ^c $p < 0.001$ vs control individuals; ^d $p < 0.001$ vs diabetic individuals with no retinopathy

ACR, albumin to creatinine ratio; na, not applicable; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy

Fig. 2 Association of serum (a) and haemoglobin (Hb) (b) concentrations of FDP-lysine with the severity of diabetic retinopathy. The horizontal bars indicate mean values. Post hoc analysis: * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs control individuals; † $p<0.001$ vs NPDR and no retinopathy. NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy

previous studies to be risk factors for the progression of diabetic retinopathy, including HbA_{1c}, diabetes duration, sex, BMI, diastolic BP and hypertension [28, 30, 31]. Type of diabetes and treatment with insulin were also included as possible confounding factors since these parameters varied between the two groups ($p<0.001$ in both cases; χ^2 test). In an unadjusted analysis, a significant association was found between the haemoglobin level of FDP-lysine and retinopathy (Table 2; OR 1.38; $p=0.024$). After adjustment for confounders, ORs remained essentially unaltered (Table 2), suggesting that FDP-lysine residues on haemoglobin are independently associated with the development of proliferative retinopathy.

We constructed a receiver operating characteristics curve plot to compare haemoglobin FDP-lysine levels of patients with non-proliferative retinopathy with those of diabetic patients with proliferative retinopathy (Fig. 3). This facilitated selection of the optimal cut-point (threshold) associated with proliferative diabetic retinopathy. The optimal cut-point for balancing sensitivity and specificity of the haemoglobin FDP-lysine test was defined as the point

on the curve closest to the upper left hand corner (dotted line, Fig. 3). This point corresponded to an FDP-lysine level of 0.3753 AU/mg protein, giving a sensitivity of 72% and a specificity of 71% for proliferative retinopathy.

Discussion

To the best of our knowledge, this is the first clinical study examining serum and haemoglobin levels of FDP-lysine in diabetes. We found that both serum and haemoglobin levels of FDP-lysine were significantly elevated in patients with diabetes compared with control individuals. Our results are consistent with previous reports showing that urinary levels of acrolein-lysine adduct are elevated in type 1 and type 2 diabetic patients [25, 26]. In addition, they further confirm the widely accepted view that increased oxidative stress and lipid peroxidation are characteristic of the diabetic milieu.

A primary goal of the present study was to evaluate whether protein-bound FDP-lysine adduct was associated with diabetic retinopathy progression and to determine if it could provide a robust biomarker for oxidative stress over prolonged time-frames. We have shown that serum FDP-lysine levels were not significantly associated with retinopathy status. In contrast, haemoglobin FDP-lysine adducts were associated with the severity of diabetic retinopathy, with elevated levels being detected in patients with proliferative disease. The disparity between these findings may lie in the different half-lives of serum proteins and haemoglobin. Albumin, the most abundant serum protein, has a relatively short half-life of ~20 days [32] meaning that serum FDP-lysine levels may only reflect integrated oxidative stress over ~2–3 weeks. In contrast, erythrocytes have a lifespan of ~120 days [32] and thus haemoglobin FDP-lysine adducts may represent the accumulation of ROS-induced damage over several months.

Table 2 Unadjusted and adjusted ORs and 95% CIs for haemoglobin FDP-lysine and retinopathy

Haemoglobin FDP-lysine	OR	95% CI	<i>p</i> value
Unadjusted	1.38	1.04–1.82	0.02
Adjusted for:			
HbA _{1c}	1.39	1.04–1.84	0.02
Diabetes duration	1.37	1.02–1.84	0.04
Sex	1.38	1.04–1.82	0.02
BMI	1.33	1.00–1.76	0.05
Diastolic BP	1.37	1.03–1.82	0.03
Hypertension	1.38	1.04–1.83	0.02
Type of diabetes	1.36	1.02–1.81	0.04
Treatment with insulin	1.35	1.02–1.79	0.04

ORs represent the increased odds of proliferative retinopathy associated with a 0.1 AU/mg protein increase in haemoglobin FDP-lysine concentration

Fig. 3 Receiver operating characteristics curve plot comparing haemoglobin FDP-lysine levels of patients with non-proliferative retinopathy with those of diabetic patients with proliferative retinopathy. The diagonal broken line is the line of no discrimination. For the presence of proliferative retinopathy a ‘decision threshold’ haemoglobin FDP-lysine level of 0.3753 AU/mg protein was defined as the far left point in the curve, giving 72% sensitivity and 71% specificity. AUC=0.743

In the ELISA assay, haemoglobin FDP-lysine was normalised to haemoglobin content using the BCA protein assay kit (haemoglobin constitutes ~95% of intracellular protein in erythrocytes [33]). In future studies, the precision of the assay may be improved by normalising to the exact haemoglobin concentration of the samples. This may be particularly important in studies where patients and controls are not matched for age, sex or ethnicity, since all of these factors may affect the haemoglobin concentration of erythrocytes [34–36].

Mounting evidence from animal studies suggests that oxidative stress is a major pathogenic factor for diabetic retinopathy. In particular, exogenous antioxidants [12, 13] or overproduction of endogenous defence enzymes such as mitochondrial SOD protect against the development of retinal microvascular lesions in animal models of diabetes [14, 15]. Despite this, results from clinical studies have failed to unambiguously establish if oxidative stress plays any role in the development of retinopathy in diabetic patients. The outcomes of trials of antioxidants on the progression of retinopathy in diabetic patients have been contradictory, although in some cases the concentrations of antioxidants in the retina may not have been sufficiently elevated to produce beneficial effects [5]. Other studies investigating the association between serum markers of oxidative stress and the progression of diabetic retinopathy have also been inconclusive. For example, Hartnett et al. (2000) demonstrated increased levels of thiobarbituric acid-reacting substances and decreased levels of SOD and glutathione peroxidase in serum of diabetic patients when

compared with non-diabetic controls, but no significant associations were found with the severity of retinopathy [21]. Most commonly used markers only reflect short-term levels of oxidative stress and this has greatly contributed to the ambiguity in this area. Despite the obvious limitations on the cross-sectional design of the current investigation, we believe that the haemoglobin FDP-lysine data presented herein provide some of the strongest evidence to date that oxidative stress is associated with the development of human diabetic retinopathy.

Although hyperglycaemia-induced ROS production is widely considered to be a causal factor in the initiation and progression of diabetic retinopathy, the exact mechanism by which oxidative stress contributes to the development of this condition remains to be elucidated. Mitochondria are believed to be the main source of superoxide in the diabetic retina [10] and this can lead to further biochemical imbalance such as activation of the polyol, hexosamine, diacylglycerol–protein kinase C pathways and accelerated accumulation of AGEs [6]. Furthermore, ROS may directly contribute to progressive vasodegeneration in the diabetic retina by causing mitochondrial cytochrome *c* release and activation of caspase-3, thereby initiating capillary cell apoptosis [5, 11, 37]. Findings from the present study also support a possible role for ROS-mediated lipid peroxidation in the development of diabetic retinopathy. Lipid aldehydes (e.g. acrolein, 4-hydroxynonenal, malondialdehyde), produced as a consequence of lipid peroxidation reactions, are believed to be important mediators of oxidative stress and can induce cell death by activating both the mitochondrial and death receptor pathways of apoptosis [38, 39]. ALEs and their precursors have been implicated in macrovascular endothelium dysfunction and atheromatous lesion formation [27], but their precise role in the development of diabetic microvascular complications remains unresolved. Currently, no drugs have been designed that specifically inhibit ALE formation [40], but future development of such compounds should allow for a clearer understanding of the contribution of lipid-derived aldehydes and ALEs in the aetiology of retinopathy and other diabetic complications.

There is no question that time-averaged mean HbA_{1c} levels are closely linked to the development and progression of diabetic eye disease [3, 4]. However, as demonstrated from the Diabetes Control and Complications Trial, patients with identical HbA_{1c} levels may in fact have significantly different risk profiles for diabetic retinopathy [41]. Recent work has suggested that monitoring oxidative stress in parallel with hyperglycaemia may help to identify subsets of patients at high risk of advancement of diabetic retinopathy [19–21]. Carboxymethyl-lysine (CML), a product of glycooxidation and lipoxidation reactions, is often regarded as a general marker of oxidative stress [42] and a number of studies have shown cross-sectional associations

between serum CML levels and the severity of diabetic retinopathy [43–45]. In most cases, however, serum CML levels strongly correlate with HbA_{1c} [46], suggesting that serum CML may not provide any additional risk information beyond that provided by monitoring mean levels of glycaemia. In contrast, other studies have reported that CML concentrations in protein from memory T cells [16] and in skin collagen [47] are of predictive value for the risk of future progression of retinopathy and appear to be independent of HbA_{1c}. However, the complexity of sample preparation may preclude the widespread use of these biomarkers. In the present study we found that FDP-lysine residues on haemoglobin, which reflect cumulative oxidative stress, are also independent predictors of retinopathy in type 1 and type 2 diabetic patients. Thus haemoglobin FDP-lysine, which is relatively straightforward to measure, may prove to be a very useful tool for identifying patients at high risk of diabetic retinopathy not evident from their HbA_{1c} profiles. To specifically address this issue, prospective studies are now warranted.

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