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The effects of ageing on glycation and the interpretation of glycaemic control in Type 2 diabetes

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Summary

To investigate the discrepancy in the assessment of glycaemic control using glycated haemoglobin (HbA_{1c}) and glycated proteins (fructosamine), the effect of age on these variables was measured in non-diabetic individuals. In 232 non-diabetics, there was a linear relationship between HbA_{1c} and age ($r=0.49$, $p<0.0001$). Mean HbA_{1c} rose from 3.82% to 4.44% between the ages of 20 and 70. Consequently, when Type 2 diabetic patient samples ($n=128$, median age 63 years) were classified according to European guidelines into good or poor glycaemic control using both an age-matched ($n=101$) and a younger ($n=108$, median age 37 years) non-diabetic reference population, fewer patients were in good control (14% vs. 25%) and more in poor control (73% vs. 53%) when the younger reference population was used (both $p<0.05$). In a subgroup of 126 non-diabetic subjects, HbA_{1c} rose with age ($r=0.48$), but serum fructosamine and fasting glucose did not ($r=0.07$, $r=0.009$, respectively, p = NS). Age-associated differences in non-diabetic HbA_{1c} values may affect the assessment of glycaemic control in diabetic patients. It may also partly explain discrepancies found when comparing fructosamine with HbA_{1c} as a measure of glucose control. Age-related HbA_{1c} reference intervals may therefore be required for the treatment of patients and the accurate auditing of clinic performance.

Introduction

The non-enzymic and irreversible binding of glucose to the N-terminal amino acid of proteins has long been known to food chemists as the 'browning reaction'.[1] While this glycation process may alter the structure and function of proteins and thus be relevant to the small-vessel complications of patients with diabetes mellitus,[2–4] glycation of haemoglobin and serum proteins (in the form of fructosamine) has become an important means of objectively assessing glycaemic control in diabetic patients. Since blood glucose levels can vary markedly within a few hours or from day to day in some patients with diabetes, the introduction of long-term indicators of glycaemic control such as HbA_{1c} or fructosamine has led to target guidelines for diabetic control now widely used by both patients and clinicians. Indeed, glycated haemoglobin measurement was the cornerstone of treatment assessment in the Diabetes Control and Complications Trial (DCCT) which showed conclusively that improved glucose control can lead to a major reduction in the development of long-term diabetic complications.[5]

When routine serum fructosamine measurement was introduced, it was seen as a cheaper, analytically simpler and more standardized alternative to glycated haemoglobin assays. Nevertheless, its general acceptance has been limited by the fact that the classification of glycaemic control obtained by this measure has often been discrepant with that of HbA_{1c}.[6–8] However, in contrast to fructosamine measurement, results obtained using differing glycated haemoglobin assay methods can vary markedly from one another. In an attempt to take account of this, European guidelines have suggested that a diabetic patient's

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glucose control be classified according to the number of standard deviations (SDs) between the patient's results and the chosen assay's non-diabetic mean value.[9] This approach puts great emphasis on the establishment of an accurate assay reference range or interval. Since locally-derived reference intervals are traditionally determined using young, healthy hospital staff, any age variation in non-diabetic glycated haemoglobin may render comparison to older diabetic patients inappropriate. Therefore, this study tested whether non-diabetic glycated haemoglobin and fructosamine values varied with the age of the subjects chosen.

Methods

Haemoglobin A_{1C} measurements were performed on 232 non-diabetic individuals (95 male, 137 female, median age 47, range 16–74, fasting plasma glucose <6.4 mmol/l[10,11]) using an HPLC instrument (Hi-AutoA_{1C}, Model 8121, Kyoto Daiichi Kagakiu). These subjects comprised hospital staff and individuals attending a clinic for lipid assessment. None were on drug treatment known to affect blood glucose.

In a subgroup of 126 of these individuals (54 male, 72 female, median age 57 years, range 23–74), serum fructosamine (Fructosamine Plus, Roche Diagnostics), serum albumin and body mass index (BMI) were also measured.

One hundred and twenty-eight Type 2 diabetic patient samples (median age 63, range 50–75, median HbA_{1C} 6.4%) were classified according to European guidelines into good (HbA_{1C} value <3 SD from non-diabetic mean value), borderline (3–5 SD) and poor (>5 SD) glycaemic control using reference intervals derived from both an age-matched ($n=101$) and a younger population ($n=108$, median age 37, range 25–50).

Between batch coefficient of variation (CV) for the HbA_{1C} method was 4.5% at 3.9% HbA_{1C}. Fructosamine CV was 1.9% at 304 µmol/l and glucose CV 2.5% at 5.2 mmol/l.

Statistical analysis used the McNemar test for paired samples. Correlation coefficients were calculated by the least squares method. Regression slopes and intercepts were compared using t-tests. Statgraphics software was used throughout.

Results

There was a linear relationship between HbA_{1C} and age in the 232 non-diabetic subjects ($r=0.49$, $p<0.0001$). There was no significant difference between males and females in the regression line slope (0.0108 ± 0.00262 SEM vs. 0.0132 ± 0.00178, respectively) or intercept (3.631 ± 0.122 vs. 3.538 ± 0.090, respectively). Mean HbA_{1C} rose from 3.82% to 4.44% between the ages of 20 and 70 (Figure 1).

As a consequence, the reference interval derived from non-diabetic subjects age-matched to the Type 2 patients was higher (mean HbA_{1C} 4.31%, SD 0.37%) than that of the younger age group (mean 4.03%, SD 0.30%). Therefore, according to European guidelines, fewer diabetic patients were in good control (14% vs. 25%) and more in poor control (73% vs. 53%) when the younger reference interval was used (both $p<0.05$ by McNemar tests, Table 1).

In the subgroup of 126 non-diabetic subjects, the rise in HbA_{1C} with age ($r=0.49$) was not reflected by similar increases in serum fructosamine concentrations (Figure 2) or fasting glucose measurements (Figure 3) ($r=0.07$, $r=0.009$ respectively, $p=$NS). This was despite serum albumin concentrations (median 44 g/l, range 35–52) and body mass indexes (median 25.5 kg/m^2, range 18.2–35.2) showing no linear relationship to age in this sample ($p>0.05$). The fructosamine/albumin ratio was also unrelated to age ($p>0.05$).

In these non-diabetic subjects, HbA_{1C} showed no relationship to either fasting glucose ($r=0.109$, $p=0.23$) or fructosamine ($r=0.105$, $p=0.24$) and neither did fructosamine with fasting glucose ($r=0.073$, $p=0.42$).

Discussion

This study has shown that non-diabetic HbA_{1C} values increase with subject age, which in turn may affect the assessment of glycaemic control in diabetic

Table 1 Classification of glycaemic control in 128 type 2 diabetic patients using age-matched and younger controls

	Good control (HbA_{1C} <3 SDs from non-diabetic mean value)	Borderline control (3–5 SDs)	Poor control (>5 SDs)
Age-matched controls	32 (25%)	28 (22%)	68 (53%)
Younger controls	18* (14%)	17 (13%)	93** (73%)

* $p<0.05$ vs. age-matched controls; ** $p<0.01$ vs. age-matched controls.

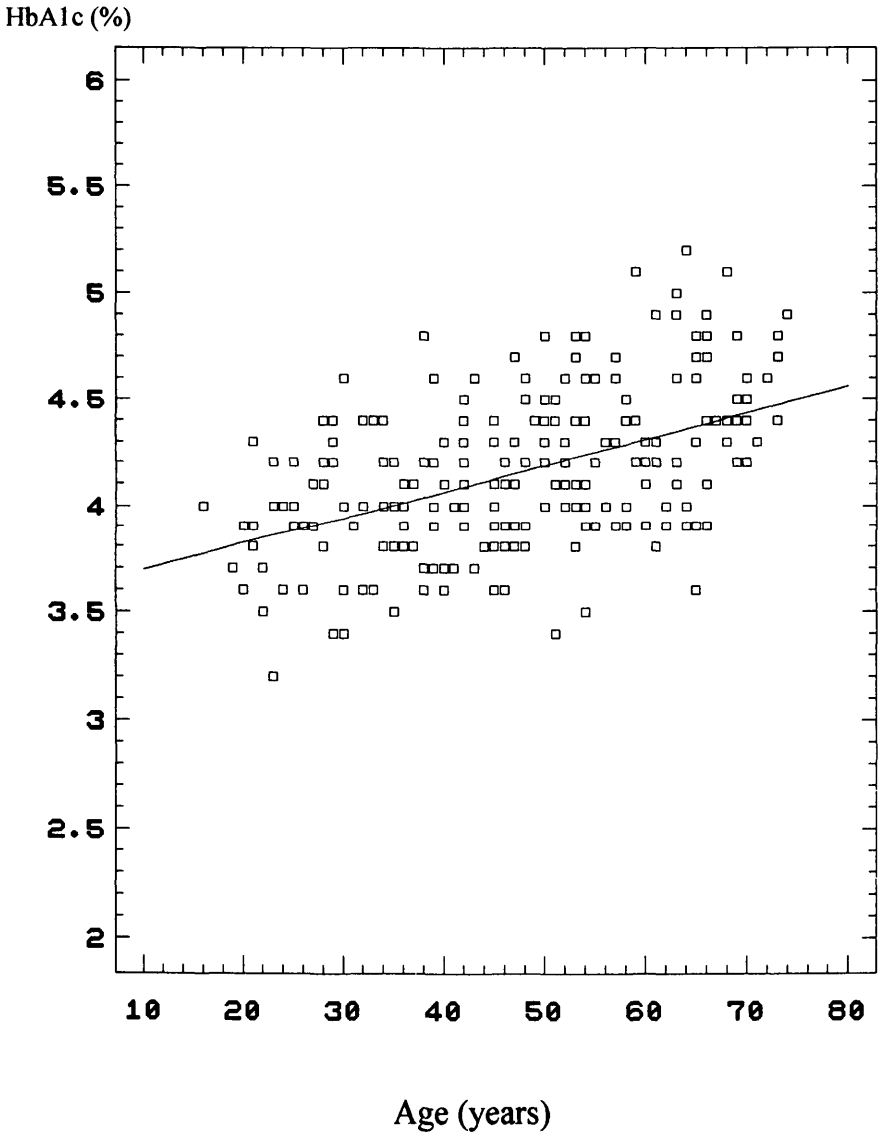

Figure 1. HbA_{1c} vs. age in 232 non-diabetic subjects ($y=0.0124x+3.57$, $r=0.490$, $p<0.00001$).

patients. By using a reference interval derived from young individuals, more Type 2 diabetic patients appeared in poorer glycaemic control when using European guidelines than if an age-matched non-diabetic population was used (73% vs. 53%). Therefore, to take account of this, age-related reference ranges may need to be used when interpreting HbA_{1c} results.

This report has also shown clear differences when comparing HbA_{1c} with fructosamine in non-diabetic subjects of varying ages. Some of this inconsistency can be explained by the fact that HbA_{1c} reflects glycaemic control over the preceding 6–8 weeks compared with 1–3 weeks for fructosamine.[12,13] Fructosamine measurements can also be influenced by other factors such as the serum albumin concentration[14] and body mass index (BMI) of the patient.[15] However, despite the subjects in this study having similar serum albumin concentrations/BMIs and presumably being in stable glucose control, HbA_{1c} values increased with subject age, whereas fructosamine did not.

The reasons for this discrepancy remain speculative. Like other studies,[16,17] this investigation showed little change in fasting plasma glucose with increasing age which, at first glance, would appear to indicate that serum fructosamine is the more representative marker of glucose control. However, this deduction does not take account of the larger glycaemic excursions which are likely to occur post-prandially in elderly individuals.[16] It thus remains difficult to establish whether it is HbA_{1c} or fructosamine that is most accurately reflecting glycaemia in these subjects.

In addition, it has been suggested that degrees of glucose intolerance may explain only one-third of the variance of glycated haemoglobin levels in non-diabetic subjects.[18,19] Thus, another possible

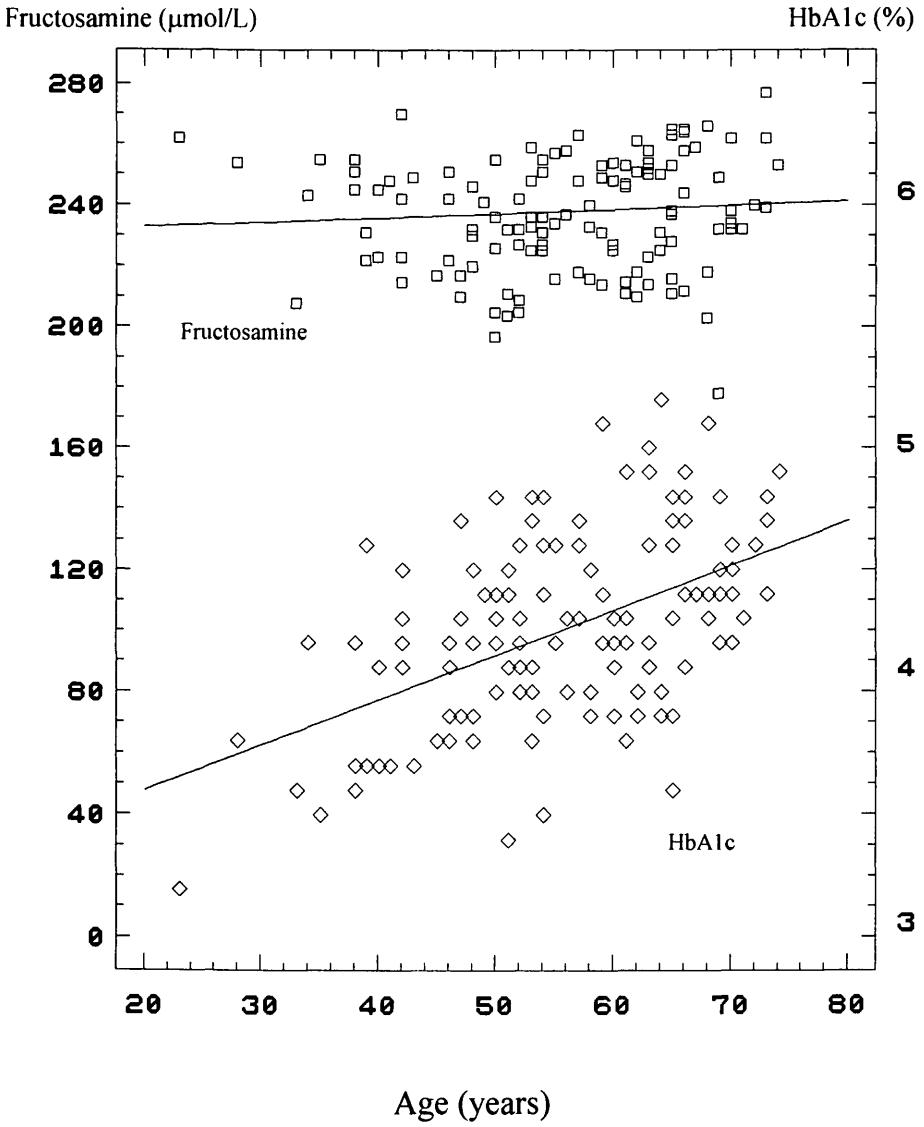

Figure 2. HbA_{1c} (diamonds) vs. age ($y=0.018x+3.23$, $r=0.493$, $p<0.00001$), and Fructosamine (squares) vs. age ($y=0.140x+230$, $r=0.073$, $p=0.42$) in 126 non-diabetic subjects.

explanation for our HbA_{1C} findings would be if a factor which changes with increasing subject age also affected HbA_{1C} values. For example, if red blood cell lifespan was longer in elderly subjects, this would allow greater glycation of their haemoglobin. In fact, it would seem that erythrocyte lifespan paradoxically shortens with increasing subject age.[20]

Our HbA_{1C} results are consistent with those of a previous more limited study of 48 non-diabetic individuals where values were found to be higher in elderly patients[21] although, curiously, this was not the case in another study where total glycated haemoglobin was measured instead of specifically HbA_{1C}.[17] Our fructosamine data are also in agreement with a European fructosamine workshop report which concluded that, above 16 years, age had little effect on serum fructosamine concentrations.[22] However, no study as yet has found both these findings in the same subjects. These differences, if applicable to diabetic patients, may be an additional reason for the discrepancy found when comparing HbA_{1C} with fructosamine.

The findings are of clinical relevance because, in contrast to fructosamine, HbA_{1C} reference intervals are likely to be influenced by the age of the subjects chosen and so may partly explain the diversity of locally-derived reference intervals quoted when using the same glycated haemoglobin instrument.[23] It would seem appropriate that clinicians should aim for their diabetic patients to have comparisons in glycated haemoglobin values made to those of their non-diabetic chronological peers. To this end, age-related reference intervals may be required for glycated haemoglobin measurements to facilitate more accurate glycaemic control targets for patients and for better auditing of clinic performance.

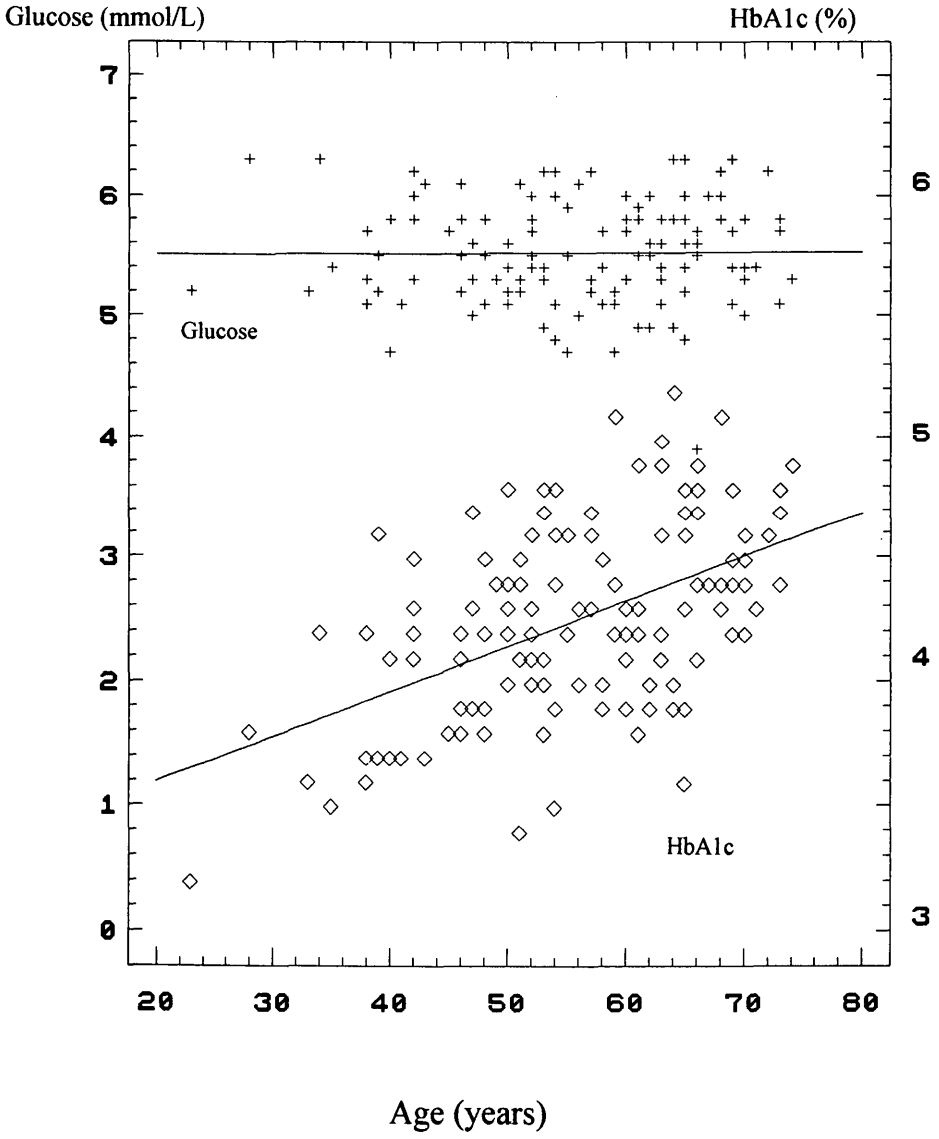

Figure 3. HbA_{1c} (diamonds) vs. age ($y=0.018x+3.23$, $r=0.493$, $p<0.00001$), and fasting plasma glucose (crosses) vs. age ($y=0.0004x+5.50$, $r=0.009$, $p=0.92$) in 126 non-diabetic subjects.

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