Age does not influence levels of HbA₁c in normal subject

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Summary

To resolve whether haemoglobin A₁c (HbA₁c) levels in normal subjects increase with age, we measured HbA₁c in 399 patients undergoing routine oral glucose tolerance test (OGTT). The OGTT results categorized the patients into 127 normal, 94 impaired glucose tolerance (IGT) and 178 diabetic. None of these groups showed a significant correlation between HbA₁c and age and we cannot, therefore, see a need for age-specific reference ranges for HbA₁c. Some of the confusion in the literature may have arisen from less rigorous categorization of subjects than we used, resulting in the inclusion of some individuals with IGT or diabetes in the ‘normal’ groups of other studies. The prevalence of such abnormality would be expected to be greater amongst older subjects, falsely suggesting a correlation between HbA₁c and age, and we were able to demonstrate this with our own data when insufficiently rigorous criteria were applied for the selection of normal subjects.

Introduction

In a small study of 48 subjects above 50 years old, sub-divided into three age groups, Arnetz et al. observed significant differences in HbA₁c levels between the groups, the oldest having the highest values.¹ In contradiction to this, Kabadi found no significant relationships between age and fasting plasma glucose (FPG), glycated haemoglobin, glycated protein or glycated albumin.² Yet another group of workers reported that haemoglobin A₁c (HbA₁c) showed a positive linear relationship with age in non-diabetic individuals, whereas fructosamine did not.³ If such a relationship exists, one implication is that reference ranges for HbA₁c would need to be age-specific.

While the evidence seems strongly in favour of age influencing HbA₁c, most of these studies can be criticised either for selecting subjects in a way that would include some with abnormal glucose tolerance,¹⁴ or for not excluding individuals whose diabetes or impaired glucose tolerance (IGT) was discovered during the study.³ We decided, therefore, to re-examine the situation in subjects who had been classified by means of an oral glucose tolerance test (OGTT) performed and interpreted according to World Health Organization recommendations.⁵

Methods

Our subjects comprised 399 patients who had been routinely referred for OGTT. The test was performed and interpreted according to WHO protocol with the classification of the patient being based on the 2-h glucose result.⁵ Blood for glucose assay was collected by finger prick into fluoride preservative, and plasma from this was used for measurement on an Analox GM7 glucose analyser (Analox Instruments) or a Beckman Glucose Analyzer (Beckman Instruments). The same blood samples were used for HbA₁c measurement by means of Daiichi HA-8121 or HA-8140 high performance ion-exchange chromatography (HIPEC) analysers.
(Biomen) with between-batch CV of less than 2% at HbA\textsubscript{1c} levels of 4.4% and 8.2%. The reference range for this method is 3.8 to 5.5%, as established by ourselves, and in agreement with the manufacturer’s published values. Comparability between the two HbA\textsubscript{1c} analysers was checked by exchanging samples. Agreement was good, the measured HbA\textsubscript{1c} differing on average by only 0.05% (CV 2.2%).

**Results**

After performing the OGTT, the 399 patients were categorized into the following three groups: normal (127), IGT (94) and diabetic (178). Figure 1 shows HbA\textsubscript{1c} results for these three groups plotted against age and there is no significant positive relationship with age for any of the groups. In the case of HbA\textsubscript{1c}, versus age for the normal cohort, \( r = 0.112; \) NS and for the diabetic group a weak negative correlation is suggested \( (r = -0.153; p < 0.05). \) It is clear, however, that there is a greater concentration of subjects in the diabetic group between the middle and older end of the age range, and that the mean HbA\textsubscript{1c} level is higher than for the other groups.

A weak correlation \( (r = 0.265; p < 0.01) \) is detectable when FPG is plotted against age for the normal subjects (Figure 2), but 2 h plasma glucose against age does not show a significant correlation \( (r = 0.026; \) NS) (Figure 3).

If we had taken all individuals with FPG < 6.4 mmol/l as our normal group, the number would have increased to 194 by including a proportion of patients with abnormal 2 h plasma glucose, but also excluding some with normal 2 h plasma glucose whose FPG was > 6.4 mmol/l. A plot of

**Figure 1.** HbA\textsubscript{1c} results plotted against age separately for normal, IGT and diabetic subjects.
HbA\textsubscript{1c} against age would then have shown some correlation ($r = 0.300$, $p < 0.001$) (Figure 4), as would a plot of 2 h plasma glucose versus age ($r = 0.309$; $p < 0.001$), but this also shows that 26 had diabetes and 55 had IGT and were, therefore, not normal (Figure 5). In total, almost 42% of the subjects who would have been classified as normal by FPG $< 6.4$ mmol/l were either diabetic or had IGT, according to their responses in the OGTT.

**Discussion**

The findings of our study do not lend support to the suggestion that HbA\textsubscript{1c} increases with age per se. None of the three groups of patients (non-diabetic, IGT and diabetic) showed any significant positive correlation between HbA\textsubscript{1c} and age within the groups, but in the diabetic cohort there was a greater concentration of subjects from the middle to the older end of the age range, as would be predicted. Our data for normal subjects hinted at a very weak correlation for FPG with age. Although it might be expected that the OGTT would emphasize any such relationship, no correlation was observed between 2 h plasma glucose and age.

Selection of subjects may be the key to the differences in findings between our study and those where age was considered to have an influence on HbA\textsubscript{1c}.\textsuperscript{1,3,4} In our case, they were all non-pregnant individuals referred, mostly by general practitioners, for OGTT because of a suspicion that they might...
Figure 4. HbA\textsubscript{1c} plotted against age for subjects with FPG < 6.4 mmol/l. ($n=194$, $r=0.300$; $p<0.001$).

Figure 5. Two-hour plasma glucose against age for subjects with FPG < 6.4 mmol/l. ($n=194$, $r=0.309$; $p<0.001$).

have diabetes. The reasons for this suspicion were very varied, but included family history, glycosuria and other symptoms possibly attributable to diabetes. Nevertheless, our group of normal subjects did have convincing evidence, in the form of the OGTT result, that they were indeed normal, i.e. neither diabetic nor IGT. The subjects in the study of Kilpatrick et al.\textsuperscript{4} were classified as normal simply on the basis of FPG < 6.4 mmol/l, and this alone is not a sufficiently rigorous criterion to exclude all diabetics and individuals with IGT.\textsuperscript{6,7} Consequently, it is likely that their cohort contained some people with diminished glucose tolerance. This is even more likely in the study of Arnetz et al., where a cut-off for FPG as high as 7.2 mmol/l was used for the selection of normal subjects.\textsuperscript{1} As the incidence of non-insulin-dependent diabetes increases with age, it would be expected that such individuals, inadvertently and incorrectly included in the ‘normal’ group, would be predominantly at the older end of the age spectrum and would produce an apparent correlation between HbA\textsubscript{1c} and age \textit{per se}. Indeed, this phenomenon was demonstrated in our own patients when all with FPG < 6.4 mmol/l were grouped together. Similarly, significant positive correlation between 2 h plasma glucose and age became apparent when all subjects with FPG < 6.4 mmol/l were included, but some of these had diabetes and others had IGT. The large Telecom population study was also compromised by the inclusion of people with impairment of glucose tolerance.\textsuperscript{3} Although previously-known diabetics were excluded, over 7% of individuals...
included were found to have IGT or diabetes, and these would be expected to be mostly in the older age groups, enhancing the apparent correlation of HbA1c with age.

This does not, however, provide an explanation of why fructosamine and FPG did not correlate with age in the study of Kilpatrick et al. if they had included some subjects with diminished glucose tolerance. We did not measure fructosamine; our findings with FPG suggested a weak correlation existed between this parameter and age in our normal subjects classified by OGTT, but the relationship was not very convincing.

There is a fundamental difference in the ways that HbA1c and fructosamine results are expressed. HbA1c is given as a percentage of the total haemoglobin present, whereas fructosamine is reported as concentration in a volume of plasma, e.g. μmol/l. In theory, this implies that the total haemoglobin concentration would not affect the HbA1c result, but changes in plasma albumin would be expected to influence fructosamine. In practice, the latter does not necessarily apply, as it has been shown that low plasma albumin is associated with an increase in specific glycation of albumin, presumed to be due to a prolonged albumin half-life. As this infers that fructosamine concentration is still valid at different albumin levels, it is argued that correction of results for albumin concentration leads to inaccuracy. In a separate study comparing fructosamine, HbA1c, glycated serum proteins and glycated albumin in pregnant diabetics, fructosamine correlated least well with glucose profile results and its specificity was called into question.

Such uncertainties make it difficult to elucidate the influence of age on fructosamine, but it also has to be recognised that HbA1c can be affected by factors such as altered red cell life, uraemia and even drugs such as high-dose salicylate therapy. Nevertheless, our findings suggest that previously-reported positive correlations between HbA1c and age have been enhanced by the inadvertent inclusion of some subjects who were either diabetic or in the IGT category, and who would be likely to be present in greater numbers at the older end of the age range.

In conclusion, we were unable to detect any direct relationship between age and HbA1c measured by ion-exchange chromatography in groups of subjects carefully categorized by OGTT. Hence, we cannot support claims that age-related reference ranges may be needed for HbA1c. It is certainly true that there is a greater incidence of impaired glucose tolerance and diabetes among older people, and the reported relationship between age and HbA1c is probably secondary to this.

References