

## Letter to the editor

### Caution: interpretation of results of HPLC assay for serum glycosylated albumin

Dear Sir,

The exceedingly high values for glycosylated albumin (normal,  $20.2 \pm 1.6\%$ , diabetic,  $39.6 \pm 5.4\%$ ) reported with the high performance liquid chromatographic (HPLC) assay described by Shima et al. [1] are far in excess of values established with any other method, including boronate affinity chromatography (normal range, 1.5–2.6% [2]), densitometric scanning of agarose gel electrophoresis (normal range, 0.4–2.0% [3]), or monoclonal antibody based enzyme linked immunosorbent assay (normal,  $2.3 \pm 0.29\%$  [4, 5]). Careful examination of the principles underlying the HPLC assay and the interpretation of its results are therefore necessary in order to avoid erroneous assignment of the acceptable range of values for non-diabetic populations and for assessment of glycaemic control.

The principal determinants of the extent of nonenzymatic glycosylation of any protein are the glucose concentration to which it is exposed, the half-life of the involved protein, and the accessibility of free amino groups within the protein for condensation with glucose, the latter in turn dependent on local factors such as pKa and structural considerations. Thus, not every epsilon amino group of lysine residues becomes glycosylated; the principal site of albumin glycosylation in vivo is lysine-525, which has been reported to account for about 33% of the overall glycosylation of this protein [6]. Glucose adducts continue to form as a function of time until equilibrium is reached and, in general, there is a positive relation between protein half-life and extent of in vivo glycosylation; the dominant factor at low to moderate glucose concentrations appears to be half-life, those proteins with lower half-lives showing greater glycosylation [7, 8].

The number of glucose residues in a molecule of albumin is not the same thing as the percent of total number of albumin molecules that become glycosylated. In other words, glycosylation of 20% of the lysine residues in a single molecule does not mean that 20% of the total number of albumin molecules are glycosylated (at one or more sites), and this point is apparently one of confusion in the report by Shima et al. The authors interpret the amount of albumin glycosylation of 0.37 mol/mol protein reported by other investigators [9] as equivalent to glycosylation of 37% of the molecules and thereby conclude that other methods, which find considerably lower percentages, underestimate glycosylated albumin levels. They also interpret their value of 12.5 nmol/mg of protein as equivalent to glycosylation of 87% of the albumin molecules, which is clearly not the case. One mg of albumin, at a molecular weight of 69,000, is about 14.4 nmol; this means that they found 0.87 mol of glucose per mol of albumin, and not glycosylation of 87% of the total number of albumin molecules.

Since HPLC estimation of glycosylated albumin uses a boronate column to separate glycosylated from nonglycosylated species, it is important to know whether only glycosylated species bind to boronate. Austin et al. [3] point out that the boronate affinity resin contains nonspecific binding sites which, when very small amounts of protein are applied, lead to an overestimation of the proportion of glycosylated protein compared with that obtained when larger protein loads are applied. This could be a source of error in the HPLC assay, since only 5 µl of sample are injected. Additionally, confirmation is needed that the albumin peak from the first column, which is an ion exchange resin, does not contain other serum proteins; obviously, other glycosylated proteins contained within the albumin peak that is subsequently applied to the boronate column would bind to the resin just as would

glycosylated albumin, thus spuriously elevating the values. Evidence confirming that the glycosylated albumin peak from the second HPLC column does not contain other glycosylated proteins is lacking.

The finding by Shima et al. of a significant correlation between fructosamine values and glycosylated albumin levels determined by the HPLC assay is also curious, given that the fructosamine assay is now known to be lacking in methodologic reliability and clinical usefulness [10]. One possible explanation is that there are similar inherent methodologic imperfections regarding specificity and/or susceptibility to interfering substances in the HPLC and the fructosamine assays. Thus, despite good analytical precision (CV of 1–5%), the clinical meaning of HPLC glycosylated albumin results would be subject of the same concerns as those raised for the fructosamine assay which, with an estimated CV of 2–3%, is analytically precise but of questionable clinical usefulness or relevance [10].

Yours sincerely,  
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### References

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