Assay of Serum Fructosamine: Internal vs External Standardization

To the Editor:

In their paper on estimation of fructosamine (1), Howey et al. propose some changes to our original assay (2, 3), including internal standardization with deoxynorpholinofructose (DMF), correction for albumin concentration, and a shorter reaction time. Internal standardization is meant to overcome problems of matrix and buffer pH, but we question whether this has been entirely achieved. In our experience with the procedure of Howey et al., an increase of buffer pH from 10.35 to 10.80 decreases the fructosamine value by about 5%. Such a discrepancy is much less than that found with external DMF standards, although we have reported similar results with glycated albumin standards as calibrators (3).

Use of DMF as a calibrant and a shorter reaction time such as 5–7 min increases sample fructosamine values by about 20% as compared with those at 10–15 min. This is true whether the conditions of Howey et al. (1) are used or those of our original proposal (2), illustrating the arbitrary calibration of this test (2, 4). Thus, fructosamine values generated by Howey et al. depend on accurate timing of the 5–7 min measurement interval. By contrast, our results based on secondary standard materials of human serum or albumin were less affected by minor alterations in measurement interval (5).

Their use of buffer of pH 10.80 is unexpected in view of their acknowledgment that "this was later shown and admitted (by others) to be incorrect." Both this change and use of a shorter reaction time bring dangers of interference that they seem not to have considered.

Their correction for albumin appears to be based on their assertion that "fructosamine originates from the non-enzymic glycation of albumin," whereas other serum proteins are also known to be reactive (4). They found a weak relationship between fructosamine and albumin concentrations in a group including severely hypoalbuminemic individuals; we found no such relationship in patients with albumin concentrations >90 g/L (6) and we have evidence that routine correction for albumin concentration is inappropriate (7).

In summary, Howey et al. would have us replace a single determination with three. The benefit is in a lesser dependence of calibration value on pH at a cost of greater time spent, greater imprecision (cf. ref. 5), and potential confusion among clinicians because of two sets of fructosamine values. Any cost in terms of greater interference or poorer discrimination of individuals with diabetes mellitus remains to be determined.

References


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The authors of the paper in question respond:

To the Editor:

Johnson and Baker have raised a number of questions about our work on assays of fructosamine in serum (1), but quoted only their own published work to support their arguments. In response, we refer to the published work of others to refute their comments, and to consider the wider and more important question of whether laboratories should add a fructosamine assay to their repertoire.

A major question raised was whether our approach entirely overcame matrix effects. The title of our paper and the detailed data presented clearly show that the problems were simply minimized, not eliminated. However, our results do verify that pH has no effect on our assay.

The actual fructosamine concentrations measured may depend on the accurate (and precise) timing of the 5–7 min measurement interval. But, more importantly, Blair et al. have shown (2) that the interference by many analytes is dependent on the time of analysis, and that the interferences documented by Baker et al. (3) may not be the same for all discrete analyzers. Moreover, Oosterom et al. (4) have suggested that the values for serum fructosamine measured in diabetics were higher with shorter pre-incubation times, whereas the values for healthy individuals were unchanged; this implies that the use of short pre-incubation times would improve the discrimination between these two populations.

We do not find the use of pH 10.80 surprising. Initially, until we became aware of the methodological deficiencies, we used the original method of Johnson et al. (5). When the description of the reagent composition was shown and admitted to be incorrect (6), we studied the effect of pH on our assay, and demonstrated that the results obtained at pH 10.80 were identical to those at pH 10.35.

We did not find a "weak" relationship between fructosamine and albumin concentrations, but a highly significant statistical correlation (r = 0.57, n = 60, t = 3.01, P < 0.005). Interestingly, this has also been documented by research groups in Holland (7), Korea (8), Switzerland (9), and Denmark (10), all of whom have suggested that correction of fructosamine concentration for albumin is important, particularly in the hypalbuminemic diabetic. Indeed, Johnson et al. themselves recommend that control of