**Table 1.** Characteristics (mean ± SD) of the subjects

| Males | IGT $n = 73$ | NGT $n = 100$ |
|---|---|---|
| Age (years) | 54.6 ±11 | 53.6 ±13.2 |
| Weight (kg) | 81.3 ±14.4 | 79.9 ±12.6 |
| Height (cm) | 169.0 ± 7.1 | 168.7 ± 7.9 |
| BMI (kg/m$^2$) | 28.4 ± 4.6 | 28.1 ± 4.1 |
| Waist/hip ratio | 0.95 ± 0.06 | 0.94 ± 0.06 |
| $HbA_{1c}$ (%) | 5.47 ± 0.75[b] | 4.99 ± 0.46 |
| Triglyceride (mmol/l) | 2.07 ± 1.07[a] | 1.63 ± 0.92 |
| Cholesterol (mmol/l) | 5.38 ± 1.37 | 5.59 ± 1.23 |
| C-peptide (µg/l) | 2.56 ± 0.77[a] | 2.39 ± 0.64 |
| **Females** | **IGT $n = 89$** | **NGT $n = 94$** |
| Age (years) | 49.2 ±13.4 | 47.5 ±12.3 |
| Weight (kg) | 77.2 ±14.6 | 76.1 ±14.1 |
| Height (cm) | 156.3 ± 6.27[b] | 159.3 ± 6.1 |
| BMI (kg/m$^2$) | 31.5 ± 5.6[a] | 30.0 ± 5.2 |
| Waist/hip ratio | 0.82 ± 0.06 | 0.81 ± 0.06 |
| $HbA_{1c}$ (%) | 5.34 ± 0.6[b] | 4.97 ± 0.55 |
| Triglyceride (mmol/l) | 1.65 ± 0.77[a] | 1.42 ± 0.73 |
| Cholesterol (mmol/l) | 5.45 ± 1.08 | 5.35 ± 1.2 |
| C-peptide (µg/l) | 2.80 ± 1.3[c] | 2.32 ± 0.63 |

[a] $p < 0.05$; [b] $p < 0.001$; c $p < 0.005$ compared to NGT. IGT, Impaired glucose tolerance; NGT, normal glucose tolerance

tance. We therefore confirm even in this large population of IGT patients the clustering of metabolic abnormalities [3]: hypertriglyceridaemia, hyperinsulinaemia, obesity and insulin resistance (not specifically addressed in this study but likely to be present). Interestingly, total cholesterol concentrations did not differ between IGT and NGT groups.

Female, but not male subjects with IGT are shorter than matched NGT control subjects. At present, we have no plausible explanation for the finding. Obviously, the ethnic differences may be important, as we were unable to document a difference in height between males. Since height in post-menopausal women is negatively age-related, we wondered whether the difference found could be limited to the post-menopausal age. The difference between the height of IGT and NGT females (3 cm) remained significant ($p < 0.02$) even after dividing them into pre- and post-menopausal. The measures for the female IGT group were in pre-menopause: $n = 42$; age = 37.4 ± 8.3 years; height = 158.2 ± 6 cm; in post-menopause: $n = 47$; age = 59.7 ± 6.8 years; height = 154.6 ± 6 cm.

The corresponding measures for the female NGT group were in pre-menopause: $n = 48$; age = 37.6 ± 7.9 years; height = 161.1 ± 5.6 cm; in post-menopause: $n = 46$; age = 57.9 ± 5.9 years; height = 157.4 ± 6.1 cm.

At this point, it seems best to simply state that the height of IGT females at our latitude is shorter than NGT females, but the biological significance, if any, of this finding is unclear. However, the elimination of height as an independent variable when evaluating glucose tolerance or comparing groups deserves consideration.

Yours sincerely
A. Quatraro, A. Minei, N. De Rosa and D. Giugliano

## References


1. Brown DC, Byrne CD, Clark PMS et al. (1991) Height and glucose tolerance in adult subjects. Diabetologia 34: 531–533
2. Quatraro A, Consoli G, Magno M et al. (1990) Hydroxychloroquine in decompensated, treatment-refractory non-insulin-dependent diabetes mellitus. Ann Intern Med 112: 678–682
3. Reaven GM (1991) Resistance to insulin-stimulated glucose uptake and hyperinsulinemia: role in non-insulin-dependent diabetes, high blood pressure, dyslipidemia and coronary heart disease. Diabete Metab 17: 78–86



Prof. D. Giugliano
Via Emilia 1
I-80021 Afragola (NA)
Italy


# HPLC assay for serum glycated albumin

Dear Sir,

A note of caution [1] has recently been issued about the HPLC assay for glycated albumin [2] because it yields substantially higher values than a monoclonal antibody assay and conventional affinity chromatography assays.

Unfortunately the validity of the methods which establish glycation of about 1–3% of albumin in normal serum has not been discussed. This seems worthwhile because widely different results have been reported for glycated albumin affinity chromatography. A commercial assay kit, for example, yields much higher results (normal range 5.9–10.7%) [3] than the reference range of 1.5–2.6% which was (incorrectly) cited for this method. Others report reference ranges as low as 0.4–1.1, again for affinity chromatographic separation [4]. Such discordant results show that glycated albumin affinity chromatography is only a semiquantitative method.

Densitometric scanning [5] which has been mentioned as one of those methods which establish the anticipated target range might erroneously be taken for an additional method to determine glycated albumin. In fact, densitometric scanning was used only to quantitate albumin which had previously been fractionated by boronate affinity chromatography.

The mechanism by which conventional affinity chromatography operates has been studied with $^{14}C$ glycated albumin [6, 7]. Both studies showed that this method underestimates the degree of albumin glycation, apparently because only molecules carrying more than one glycated site show sufficient affinity to be tightly bound. This is in agreement with the elution of substantial amounts of glycated albumin in the unbound fraction as determined by the thiobarbiturate assay [8].

The argument that the number of bound glucose residues is not identical to the number of glycated albumin molecules is not helpful to clarify which method yields most accurate results. The only statement which can be safely made is that the amount of glycated albumin cannot exceed the amount of bound glucose. Because in non-diabetic humans about 0.37 mole glucose is attached to 1 mole serum albumin [9], not more than 37% of the albumin molecules should be glycated. Both the HPLC method and the other cited methods meet this criteria.

Lys-$^{525}$ which is the most abundantly glycated residue in human albumin carries about half of the overall glycation [10]. In normal serum 0.18 mole glucose/mole albumin would then be expected to be derived from Lys-$^{525}$ glycation alone. Because each albumin molecule contains only one Lys-$^{525}$, the glucose bound to this residue must reside on separate albumin molecules. This corresponds to a minimum estimate of about 18% glycated albumin in normal serum.

Because there is little reason to assume that the remaining glycation would be restricted to those molecules which are already glycated at Lys-$^{525}$ the true amount of glycated albumin molecules will presumably be higher than the minimum estimate. The HPLC method, yielding about 20% glycated albumin in normal serum, appears to be much more in line with these figures than those methods which estimate only about 1–3% glycated albumin.

The fructosamine assay [11], which has also been discussed in this context, measures protein glycation, not glycated albumin. Nevertheless a close relationship can be expected because albumin is the most abundant serum protein and is readily glycated. It is also known

that the fructosamine assay responds strictly proportional to increasing glycation of albumin [12], which is in effect used for the standardization of an improved version of the fructosamine assay [13].

The argument that the fructosamine assay correlates well with the HPLC method [2] because both assays might be similarly sensitive to non-specificity or interferences does not take into account that these two assays operate by completely different principles. Glucose is one metabolite where common interference seems possible because it has reducing properties and binds to boronate affinity columns. However, apart from any resulting interference which would be expected to have the opposite result, free glucose is known to have little effect on the fructosamine assay [14]. Therefore, the probability that two basically different methods correlate well because of similar non-specificity seems extremely low. It appears much more likely that both assays correlate well because they measure closely related quantities, glycated albumin and protein glycation.

In summary there are strong arguments that the HPLC method might yield more accurate results for glycated albumin than those methods whose value has not been questioned. If accurate quantitation can be rigorously shown, then the HPLC method might serve as a reference for less complex and costly routine methods.

Yours sincerely,
E. Vorberg

## References


1. Cohen MP (1991) Caution: interpretation of results of HPLC assay for serum glycated albumin. Diabetologia 34: 766 (Letter)
2. Shima K, Ito N, Abe F et al. (1988) High-performance liquid chromatographic assay of serum glycated albumin. Diabetologia 31: 627–631
3. Rosenthal MA, Dearth KA (1991) Evaluation of an affinity chromatographic method for glycated protein and albumin as an indicator of diabetic control. Clin Chem 37: 962 (Abstract)
4. Woo J, Weinstock RS, Ozark C, Sunderji S (1987) Glycated albumin affinity chromatography and radioimmunoassay in the management of diabetes mellitus. J Clin Lab Anal 1: 163–169
5. Austin GE, Mullins RH, Morin LG (1987) Non-enzymatic glycation of individual plasma proteins in normoglycemic and hyperglycemic patients. Clin Chem 33: 2220–2224
6. Johnson RN, Baker JR (1988) Inaccuracy in measuring glycated albumin concentration by thiobarbituric acid colorimetry and by boronate chromatography. Clin Chem 34: 1456–1459
7. Vidal P, Deckert T, Hansen B, Welinder BS (1989) Low affinity chromatographical efficiency for the separation of glycated and non-glycated serum albumin. Diabetologia 32: 553A (Abstract)
8. Rendell M, Kao G, Mecherikunnel P et al. (1985) Use of aminophenylboronic acid affinity chromatography to measure glycosylated albumin levels. J Lab Clin Med 105: 63–69
9. Olufemi S, Talwar D, Robb DA (1987) The relative extent of glycation of haemoglobin and albumin. Clin Chim Acta 163: 125–136
10. Garlick RL, Mazer JS (1983) The principal site of nonenzymatic glycosylation of human serum albumin in vivo. J Biol Chem 258: 6142–6146
11. Johnson RN, Metcalf PA, Baker JR (1983) Fructosamine: a new approach to the estimation of serum glycosylation. An index of diabetic control. Clin Chim Acta 127: 87–95
12. Schleicher ED, Vogt BW (1990) Standardization of serum fructosamine assays. Clin Chem 36: 136–139
13. Henrichs HR (1990) European Fructosamine workshop report. Wien Klin Wochenschr [Suppl] p 180
14. Workshop report Fructosamine (1989) Boehringer Mannheim GmbH, Mannheim, p 43



Dr. E. Vorberg
F. Hoffmann-La Roche AG
Grenzacherstraße 124
CH-4002 Basel
Switzerland


# *Erratum*

*Diabetologia, Volume 35, Number 3, March 1992*

pp 267–271, A. Goday et al.: Incidence of Type 1 (insulin-dependent) diabetes in Catalonia (Spain)

On page 267, under the heading, should read:
A. Goday[1].
[1] Servei d'Endocrinologia, Hospital Clinic, Servei d'Endocrinologia, Hospital de l'Esperança, Barcelona. Comissió d'Epidemiologia del Consell Assessor sobre la Diabeis a Catalunya.

On page 270, Acknowledgements: Should be mentioned:
Alberto Goday wishes to express his thanks for the facilities, support and encouragement given by Drs. E. Vilardell and R. Gomis, of the Servei d'Endocrinologia, Hospital Clinic, Barcelona.