respectively.

Characterization of the Competitive ELISA

By defining 1 AGEs unit as the inhibition that results from 1:5 diluted pooled serum in competitive ELISA, the pool serum was serially diluted with PBS in varying ratios to result in 1 to 5 AGEs units. The linearity up to 5 AGEs units of the assay is demonstrated in Fig. 4. In subsequent experiments, all specimens were diluted 3-fold before the assay. Thus, using the best fit linear regression program we were able to obtain a linear calibration curve ranging from 3 to 15 AGEs units (R^2 = 0.9819) (Fig. 4). Data lower than 3 AU or higher than 15 AU were repeated with lower or higher dilutions, respectively. This methodology was used to determine the AGEs values in 2 different age groups of non-diabetic individuals and 1 diabetic group. We found that the circulatory AGEs levels in the young group as well as in the elderly group of non-diabetic individuals fit a normal distribution, and the reference ranges were 3.12 ± 0.52 (n = 30) and 4.41 ± 1.1 AU (n = 36), respectively. The circulation AGEs levels in the diabetic patients were 8.96 ± 2.13 AU (n = 32) which are significantly higher than those of both age groups of non-diabetics (p < 0.001) (Fig. 5). The presence of hemolysis in serum specimens may interfere with the measurement of LMW-AGEs (data not shown). Thus, all hemolytic specimens were excluded from the sampling.

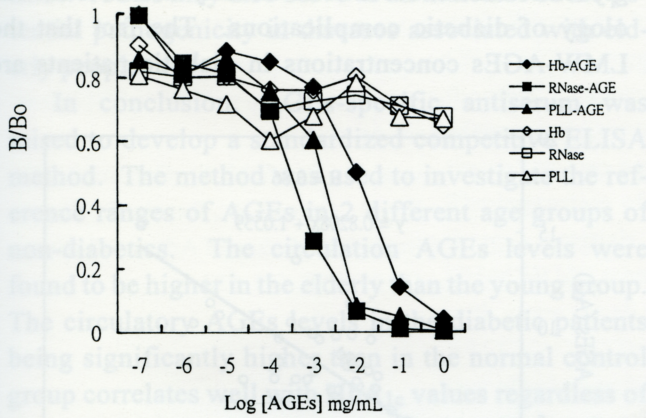

Fig. 3. ELISA competition curve for anti-BSA-AGEs antibody. In competitive ELISA, different concentrations of native proteins (open symbols) and their AGEs derivatives (solid symbols), including hemoglobin (diamonds), RNase (squares), or poly-L-lysine (triangles), were incubated with anti-BSA-AGEs antibody for 3 h. The mixtures were then added to the RNase-AGEs-coated (0.1 g/well) ELISA plates as indicated in "Materials and Methods". Data represent the mean of B/Bo from 2 independent experiments each done, in duplicate determinations.

Correlation of HbA_{1c} and Plasma LMW-AGEs

The HbA_{1c} level and plasma LMW-AGEs values in 50 normal individuals and 36 diabetics regardless of their ages were compared (Fig. 6). The circulation AGEs data correlated well with HbA_{1c} values. The slope equalled 0.82, and the correlation coefficient (r) was 0.86 among the 86 diabetic and non-diabetic individuals.

DISCUSSION

AGEs are derived from different proteins, and the precise structures of most AGE molecules remain to be elucidated.Due to the heterogeneity of AGEs struc-

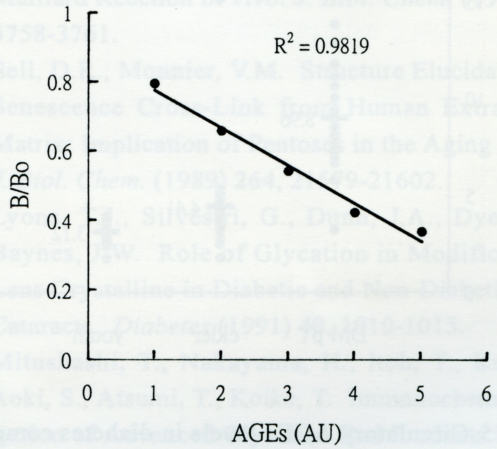

Fig. 4. Calibration curve of competitive ELISA using diluted pooled serum. Pooled serum was prepared from a combination of more than 500 apparently normal human samples. Different dilutions of the pooled serum were used as calibrators to calibrate the competitive ELISA. The standard curve was set up in every measurement and the slope of the calibration curve calibrated from the least squares linear regression line. Data shown are a representative calibration curve which has been reproduced more than 10 times.