tures, AGEs measurement in clinical practice is still limited by lack of a simple and universal analytical procedure. No commercial kit for AGEs is available at this time, and the reference ranges in different age groups and the clinical significance of circulating LMW-AGEs have not been established. The present studies examined the reference ranges in different age groups and demonstrated that the measurement of serum LMW-AGEs emerged as an effective way to monitor the extent of tissue protein glycosylation and thus the severity of diabetic complication.

Although competitive ELISA has been the most popular method for quantifying AGEs, some technical dilemmas in making the measurement have not been resolved. The foremost is the lack of a defined calibrator and control serum for this type of measurement. In most studies, the *in vitro* prepared AGE-modified protein was assayed by fluorescence intensity and served as the calibrator. However, structural differences among different carrier proteins may generate different AGE moieties with distinctive immuno-reactivities to AGE antibodies.[22] Thus, the *in vitro* prepared AGE-protein is not appropriate to serve as a universal standard. In this study, we used naturally occurring pooled serum as the calibrator which bypassed the variability of using *in vitro* prepared AGE-modified proteins. Furthermore, we have accumulated large batches of pooled serum with both high and low AGE levels which can serve as high and low control serum in the future. With this standardization protocol and proper quality control manipulation, this study should facilitate the development of a commercial kit that can be used in clinical practice.

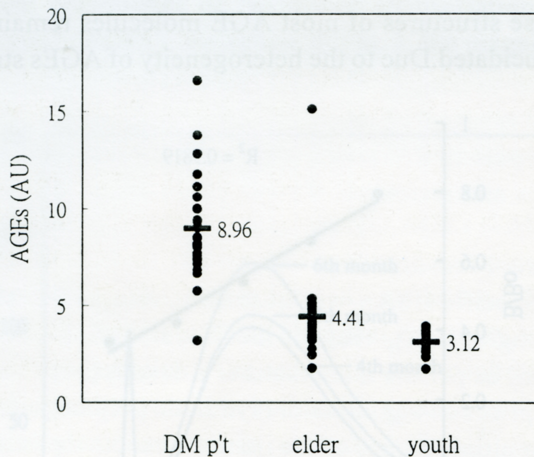

Fig. 5. Circulatory AGEs levels in diabetes comparison to those in non-diabetic groups. All samples diluted 1: 3 with dilution buffer were incubated with a BSA-AGEs antibody for 3 h, and competitive ELISA was done as described in "Materials and Methods". Data were calculated from a calibration curve and expressed as mean of AGEs units (AU) from 2 independent experiments, each done in duplicate determinations. The means ± S.E. M were 3.12 ± 0.52 (n = 30), 4.41 ± 1.1* (n = 36), and 8.96 ± 2.13** AU (n = 32) for the young group, the elderly group, and the diabetic patients, respectively. * represents $P < 0.05$; ** represents $P < 0.001$.

Traditionally, blood sugar, fructosamine and HbA_{1c} levels have been used as indicators of glycemic control. The consistent 120-day life span of RBC enables HbA_{1c} to be a good indicator of mean blood glucose control over the previous 8-10 weeks. However, whether or not this parameter is accountable for diabetic sequels has been a controversial issue.[24,25] LMW-AGEs originate from the turnover of tissue AGEs proteins which not only represent a period of glycemic control but are also directly related to the etiology of diabetic complications. The fact that the LMW-AGEs concentrations in diabetic patients are

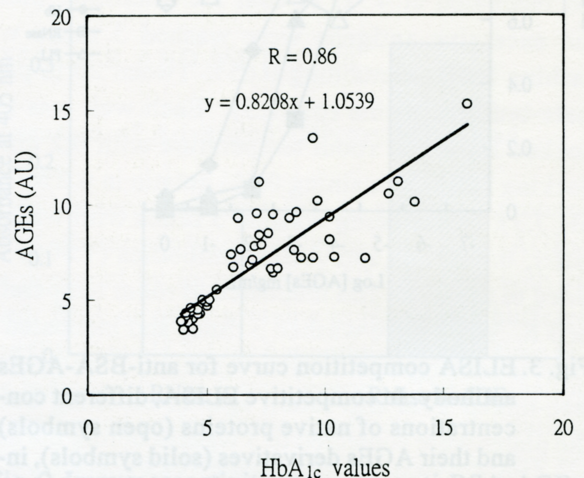

Fig. 6. Relationship between HbA_{1c} and circulatory AGEs. Values of HbA_{1c} obtained from the diabetics (n = 36) and non-diabetics (n = 50) were compared with circulatory AGEs values. The correlative coefficients were analyzed by linear regression using the Sigma Plot Software.