Increased Serum Levels of Advanced Glycation End Products in NIDDM Patients With Diabetic Complications

Advanced glycation end products (AGEs) are produced by a nonenzymatic reaction between proteins and sugar in patients with long-term hyperglycemia (1). AGEs accumulate with time and are irreversibly deposited in various tissues of the body, contributing to the development of diabetic complications, arteriosclerosis, and aging (1–3).

The results of the Diabetes Control and Complications Trial have shown that long-term hyperglycemia is the cause of various diabetic complications. However, the mechanisms responsible for development of these complications arising from persistent hyperglycemia have not yet been elucidated. The idea that AGEs are the ultimate causative factor of diabetic complications is highly convincing.

Histopathological studies using an anti-AGE antibody have suggested that AGEs play an important role in the development of nephropathy in diabetic animals and patients, as seen, for example, in AGE accumulation on the basement membrane in streptozocin rats (4) and AGE staining in nodular lesions of diabetic patients with nephropathy (5). In addition, an injection of the AGE-modified albumin into normal rats was reported to induce glomerulosclerosis, as manifested by basement membrane widening and an increase in mesangial extracellular matrix (6). In an in vitro investigation, exposure of mesangial cells to AGEs promoted the production of matrix protein (7). These findings strongly suggest that AGEs are associated with nephropathy.

We determined serum AGE levels in patients with NIDDM and evaluated the relationship between these levels and diabetic complications. A total of 125 patients (mean age 59.2 ± 11.1 years, duration of diabetes 11.6 ± 8.9 years, mean HbA_{1c} 6.8 ± 1.0%) and 63 healthy volunteers were studied. Serum AGEs were measured by a newly developed enzyme-linked immunosorbent assay method using anti-AGE keyhole limpet hemocyanin.

Serum AGE levels were significantly higher in the diabetic group compared with the normal control group (7.2 ± 14.6 vs. 3.3 ± 1.0 mU/ml, $P < 0.05$). Significant correlations were seen between serum AGEs and the degree of diabetic nephropathy. Serum AGE levels of diabetic patients with proliferative retinopathy were significantly higher than those of patients without retinopathy ($P < 0.05$).

Serum AGE levels reflected the severity of diabetic complications, including nephropathy and retinopathy; therefore, this method may prove to be a very useful tool for patient evaluation and follow-up, as well as for monitoring the effects of treatment in clinical practice.

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Making Things Easier Is Not So Easy

The 1997 American Diabetes Association criteria and glucose intolerance

The 1997 American Diabetes Association (ADA) diagnostic criteria for diabetes were recently compared with the criteria proposed by the World Health Organization (WHO) by applying the data from the Third National Health and Nutrition Examination Survey. Harris et al. (1) conclude that although the number of people with undiagnosed diabetes was lower when the new ADA fasting criteria were used, their extended use may result in the detection of a greater number of people with undiagnosed diabetes in clinical practice because of the simplicity and greater use of a fasting plasma glucose value versus the glucose tolerance test.

Other conclusions obtained from their results need to be emphasized. First, a poor concordance (38%) was observed between the ADA "impaired fasting glucose" category and the WHO "impaired glucose tolerance" status. In fact, 18% of the subjects with ADA impaired fasting glucose were diabetic according to the WHO criteria, and 43% had a normal glucose tolerance. These data suggest that the 2-h postchallenge plasma glucose is a complementary test needed to be done in these subjects to avoid under- or overcategorization of the cases. Second, 70% of the WHO impaired glucose tolerance cases were considered normal using the ADA criteria. These data suggest that the main purpose of the new criteria, an earlier diagnosis of diabetes and glucose intolerance (2), will not be achieved without complementary actions. Clearly, in a subset of the subjects (fasting plasma glucose <126 mg/dl), the information obtained from the fasting plasma glucose is not the same as that obtained by the 2-h postchallenge plasma glucose. We cannot leave without a proper diagnosis a large number (10.5 million according to Harris) of the glucose-intolerant subjects, knowing that they have increased cardiovascular

morbidity and a greater risk for developing diabetes (3,4). The merit of a simple test as a diagnostic tool cannot be disputed; however, some of its usefulness is lost when it is not followed by the proper use of complementary tests. A selective testing using a 2-h postchallenge plasma glucose in high-risk individuals (as defined by the Expert Committee) would be a better alternative in this subset of the population. In the U.S., according to the data from Harris et al., the vast majority of the 2.1 million cases currently unidentified as diabetic by the ADA criteria could be properly diagnosed using this approach.

In conclusion, we believe that the data reported by Harris et al. give a nice demonstration that the fasting plasma glucose and the 2-h postchallenge plasma glucose are complementary tests for diagnosing diabetes in subjects in whom a fasting plasma glucose <126 mg/dl is found.

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Hyperhomocysteinemia and Microalbuminuria in Diabetes

We read with interest the study by Hofmann et al. (1) on hyperhomocysteinemia [HH(e)] and endothelial dysfunction in patients with IDDM, and the accompanying editorial by Dr. Colwell (2). These data are compatible with previous observations that patients with IDDM without microalbuminuria or vascular disease have normal homocysteine [H(e)] metabolism (3,4). In contrast, patients with NIDDM without microalbuminuria have an increased prevalence of postload HH(e) with normal fasting plasma H(e) concentrations (4). In this context, it may be relevant that insulin plays a role in amino acid metabolism and acute hyperinsulinemia during a hyperinsulinemic-euglycemic clamp lowers plasma H(e) concentrations in normal subjects but not in insulin-resistant patients with NIDDM (5).

Dr. Colwell suggests that the HH(e) in patients with IDDM and microalbuminuria may be due to preexisting endothelial function. However, the pattern of HH(e) with both fasting and postload elevations in H(e) suggests another possible explanation. Plasma H(e) concentrations are determined by the activity of several enzymes, the two most important of which are methylene tetrahydrofolate reductase (MTHFR) and cystathionine-β-synthase. The kidney plays a pivotal role in maintaining normal plasma H(e) (6). The enzyme MTHFR is highly expressed and active in the kidney, and its dysfunction leads to HH(e) in patients with renal impairment. Decreased activity of this enzyme leads to elevated fasting plasma H(e), as in the patients of Hofmann et al. Thus, it is possible that even in the early stage of microalbuminuria, the function of this enzyme in the kidney is impaired, leading to HH(e).

Hofmann et al. suggest that HH(e) causes endothelial dysfunction by induction of oxidative stress. However, it is well recognized that diabetes itself leads to oxidative stress. We have recently established that in the presence of vascular disease, plasma concentrations of thiobarbituric acid–reactive substances (a marker of oxidative stress) are elevated in diabetic patients with vascular disease and that no further elevation occurs in the presence of coexistent HH(e) (7).

HH(e) is well established as a risk factor for macrovascular disease (8). Further investigation is required into the mechanisms of HH(e) in patients with diabetes and its role in the progression of microvascular and macrovascular disease in these patients.

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