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Plasma N-Acetyl-β,D-Glucosaminidase Activities and Glycaemia in Diabetes Mellitus

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Summary. The plasma activity of the lysosomal enzyme N-acetyl-β,D-glucosaminidase (NAGase) has been shown to correlate with hyperglycaemia; their temporal relationships have been investigated. In 12 insulin-treated male diabetic patients, NAGase showed a slight diurnal variation with a nadir at 07.00 h ($F = 9.54$, $p < 0.001$). The mean plasma glucose and NAGase fluctuated similarly ($r_s = 0.88$, $p < 0.01$) but did not correlate within individual patients. Normoglycaemia was induced in eight insulin-treated diabetic patients for 4 days but the mean NAGase did not fall significantly. Glycaemic improvement over 3 months was achieved by dietary therapy in 28 newly-presenting non-insulin-dependent diabetic patients: there were significant falls in mean fasting plasma glucose (mean ± SD: 12.8 ± 3.3 to 8.3 ± 3.1 mmol/l; $p < 0.001$), glycosylated haemoglobin levels (12.4 ± 2.4 to 9.3 ± 2.3%, $p < 0.001$) and a corresponding decrease of NAGase (1.5 ± 0.5 to 1.2 ± 0.4 μmol 4-nitrophenyl-N-acetyl-β-D-glucosamide released.h^{-1}.ml^{-1}; $p < 0.001$). The change in NAGase correlated with the changes in plasma glucose and glycosylated haemoglobin levels ($r = 0.61$, $p < 0.025$; $r = 0.48$, $p < 0.05$, respectively). Plasma NAGase activity may be influenced by glycaemia in diabetes.

Key words: N-acetyl-β,D-glucosaminidase, hyperglycaemia, glycosylated haemoglobin, insulin infusion, diurnal variation.

Diabetic patients have elevated plasma or serum activities of the lysosomal enzyme N-acetyl-β,D-glucosaminidase (NAGase, E.C.3.2.1.30) [1–6]. Blood and plasma glucose levels have been shown to correlate significantly with simultaneous humoral enzyme activities [2, 4–6], but the temporal relationship between the two variables remains uncertain. We report studies on the relation of plasma NAGase to diurnal plasma glucose changes, and to improved glycaemic control over periods of 4 days to 3 months.

Patients and Methods

Diurnal Study (24 h)

Twelve male insulin-treated subjects (mean age 38 years, mean duration of diabetes 20 years) were studied. All subjects studied had a glycosylated haemoglobin (HbA_{1c}) > 10% (range 10.8–14.6%) and were less than 20% above ideal body weight (range 97%–120%) [7]. They were on no drugs other than insulin. No subject had hypertension, renal failure or liver disease. All patients were admitted, with informed consent, to a metabolic ward at 17.00 h. Venous blood samples were drawn at 17.30, 21.00, 1.00, 7.00, 12.30, 15.30 and 17.30 h through an in-dwelling cannula whilst the patients took their usual insulin doses and meals.

Insulin-Infusion Study (4 days)

Eight insulin-treated subjects (five females, three males, mean age 52 years, mean duration of diabetes 16 years), who were moderately poorly-controlled (fasting plasma glucose > 10 mmol/l and HbA_{1c} > 10%), were recruited from outpatient clinics. All patients, who gave informed consent, were admitted to a metabolic ward and were maintained on their usual insulin therapy and diet for 2 days. Patients were then put on a seven 2-hourly isocaloric-meal regimen for 4 days with a matched insulin infusion [8], the total energy content being the same as their normal diet. Venous blood samples were then taken at 08.00 h for glucose and NAGase measurements. The patients were subsequently treated with an ultralente and soluble twice-daily insulin regimen [9] (Ultratard and Actrapid, Novo, Basingstoke, UK), the doses being the same as the insulin-infusion requirements. Minor adjustments to these doses were made on the basis of pre-prandial blood glucose estimations. All patients were discharged on day 3 post-infusion and were reviewed at weeks 1, 2 and 4 after discharge.

Improved Glycaemic Control Study (3 months)

Twenty-eight newly presenting Type 2 (non-insulin-dependent) diabetic patients without ketonuria (8 females and 20 males; mean age 52 years) and with a fasting plasma glucose > 6.0 mmol/l were studied. On average, the patients were 26% above ideal body weight (range: 87%–215%) [7]. None of the subjects studied had evidence of systemic disease, hypertension or impaired renal function or were on any drugs. They were put on an energy-restricted, low-fat, high-carbohydrate, high-fibre diet [10] for 3 months when further fasting blood samples were taken for estimation of glucose, HbA_{1c} and NAGase.

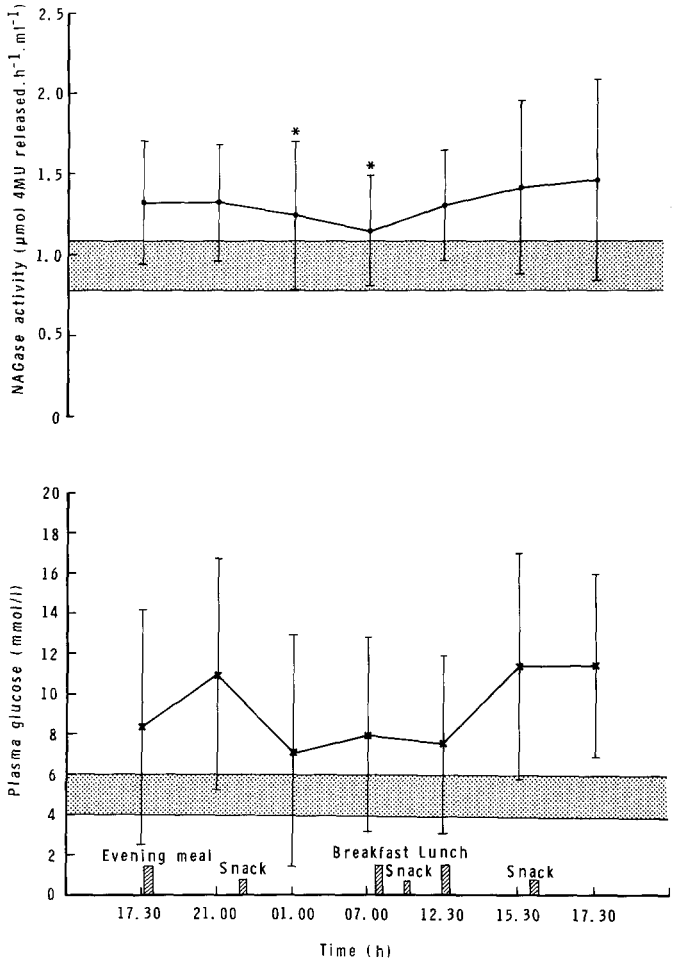

Fig. 1. Mean NAGase activities (top panel) and plasma glucose levels (lower panel) during the 24-h diurnal study in 12 insulin-treated male diabetic patients. Shaded areas represent the normal range of each variable. Values are expressed as mean ± SD (*$p < 0.05$ versus 17.30 h level)

Assays

All plasma samples were kept at −20 °C until assayed. Plasma glucose was measured by a glucose oxidase method (GOD-Perid kit, Boehringer, Lewes, UK). NAGase activity was determined fluorometrically [5] in the 24-h study and spectrophotometrically [11] in all other studies. The artificial substrates used were 4-methyl-N-acetyl-umbelliferyl-β,D-glucosaminide (4MU), (Sigma, Dorset, UK) and 4-nitrophenyl-N-acetyl-β,D-glucosaminide (PNP), (Sigma), respectively. Enzyme activities were expressed as substrate hydrolysis rate (amount of chromophore released per hour) per ml of plasma at 37 °C. The inter- and intra-assay coefficient of variations were 2.8 and 1.5% for the fluorometric assays, and 1.6 and 1.2% for the spectrophotometric assays, respectively. HbA_{1c} was estimated in duplicate using an iso-electric focussing method [12] with a 5-h incubation of a dialysed sample at 37 °C to exclude short-term glucose adducts. Normal ranges for each variable were obtained from healthy volunteers in the laboratory and the employees of a bank.

Statistical Analysis

All data are expressed as mean ± SD. Student's paired t-test, Pearson's correlation, one-way analysis of variance, Q test [13] and Z-transformation [14] for parametric data, Spearman rank correlation for non-parametric data were used.

Results

Diurnal Study

The plasma glucose levels and NAGase activities of the 12 insulin-treated diabetic subjects are shown in Figure 1. Mean plasma NAGase values were 1.3 ± 0.1 μmol 4MU released.h^{-1}.ml^{-1} with six patients exceeding the normal range of 0.7–1.2 μmol 4MU released.h^{-1}.ml^{-1}. Plasma NAGase activity appeared to relate to time of day (one-way analysis of variance, $F = 9.54$, $p < 0.001$). The post-hoc Q test [13] showed that the mean plasma NAGase levels at 01.00 and 07.00 h were significantly lower than that at 17.30 h ($p < 0.05$).

The mean plasma glucose and NAGase levels fluctuated similarly during the 24 h and the mean for each time point correlated significantly ($n = 7$, $r_s = 0.88$, $p < 0.01$). The plasma NAGase activities of all patients' samples correlated with their simultaneous plasma glucose levels (after a Z-transformation [14], $n = 81$, $r = 0.22$, $p < 0.05$), but there was no discernable short-term change of plasma NAGase activities with changing plasma glucose levels within individuals.

Insulin-Infusion Study

The 08.00 h plasma NAGase activities and glucose measurements before, during and after insulin infusion are shown in Figure 2. Mean glucose levels were significantly lowered during the infusion period (paired t-test, $p < 0.01$) and there was a significant fall of HbA_{1c} levels over the 5-week period in these patients (13.1 ± 1.4 to 10.4 ± 0.5%; paired t-test, $p < 0.001$). The mean plasma NAGase activity fell by 15% during the infusion period but was only significantly decreased on one of the 4 infusion days ($p = 0.04$).

Improved Glycaemic Control Study (3 Months)

Mean fasting plasma glucose and HbA_{1c} fell significantly over the 3 months in the 28 newly presenting non-insulin-dependent diabetic patients. This was accompanied by a corresponding fall in the plasma NAGase activity in all subjects studied (Fig. 3). The change in plasma NAGase activity over the 3-month period correlated significantly with both the change in fasting plasma glucose ($n = 25$, $r = 0.61$, $p < 0.025$) and the change in HbA_{1c} levels ($n = 25$, $r = 0.48$, $p < 0.05$).

Discussion

During recovery from episodes of gross metabolic decompensation, rapid falls in NAGase activity have been reported [2, 15]. The present studies suggest, however, that in response to glycaemic changes in moderately controlled ambulant diabetic patients, the relationship between plasma glucose and NAGase activity has a rel-

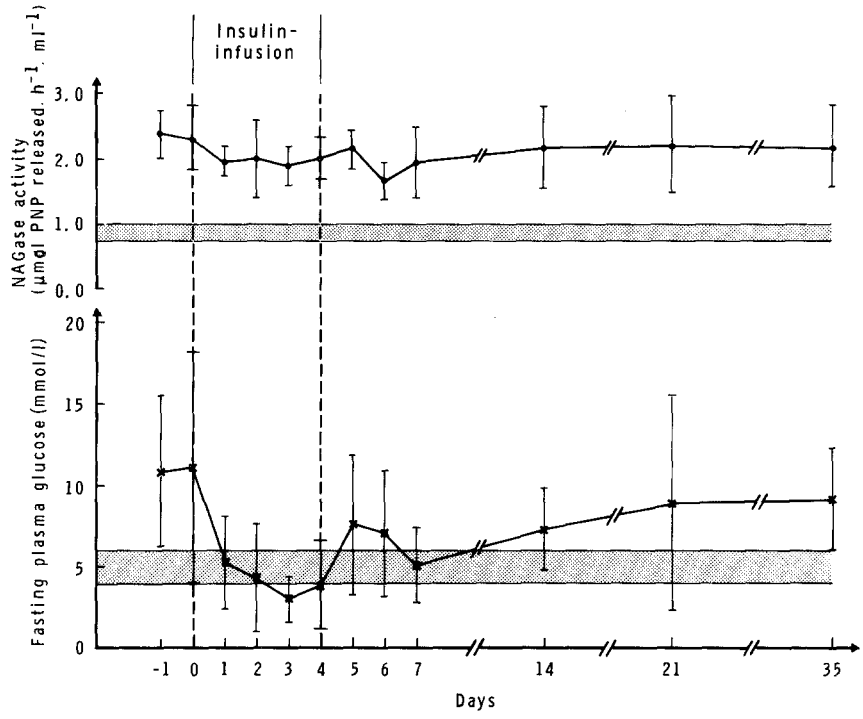

Fig. 2. Mean NAGase activities (top panel) and plasma glucose levels (lower panel) before, during and after the 4-day insulin-infusion period. Shaded areas represent the normal range of each variable. Values are expressed as mean ± SD

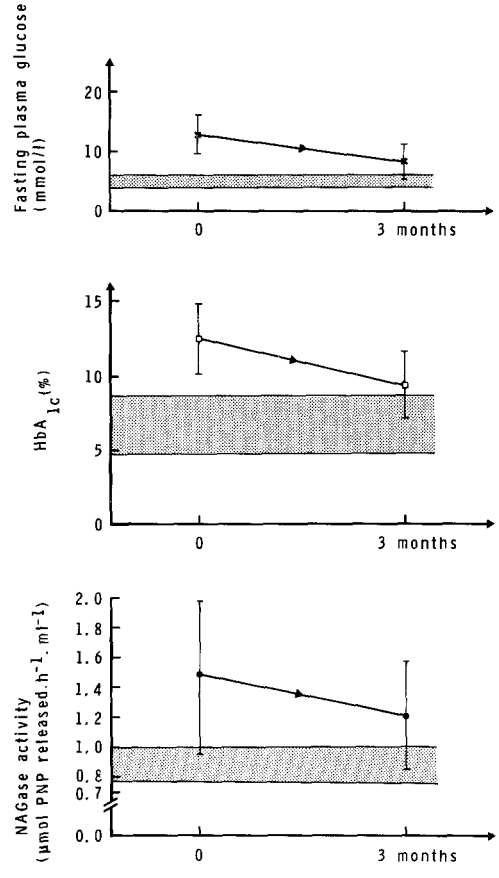

Fig. 3. Mean change of fasting plasma glucose (top panel) HbA_{1c} (middle panel) and NAGase activities (lower panel) during the 3 months improved glycaemic control study. Shaded areas represent the normal range of each variable. Values are expressed as mean ± SD. All mean changes are statistically significant ($p < 0.001$, paired t-test)

atively long time course, in the order of weeks rather than hours or days. The small diurnal variation of NAGase activity did not relate to the simultaneous plasma glucose values in individual subjects. In the insulin-infusion study there was a slight decrease in plasma NAGase activity when the plasma glucose levels were lowered to the normal range for 4 days. During this period the mean NAGase activity remained twice the normal level. In the 3 months study of dietary therapy on the newly-presenting non-insulin-dependent diabetic patients, the improved glycaemia was accompanied by a 18% fall in the enzyme activity, which was however still above the normal range. The decrease in NAGase activity correlated with the reduction in both the fasting glucose and HbA_{1c} levels.

The present findings are in accord with the report that changes similar to those shown by NAGase also occur for other lysosomal enymes, suggesting a general lysosomal involvement in diabetes [16]. Lysosomes have been shown to be the probable sites of intracellular glycoprotein catabolism [17–19]. In diabetes, thickening of capillary basement membrane is associated with vascular lesions, with their well-known morbidity and mortality [20, 21]. Alteration in the lysosomal hydrolases activity in tissue may play a role in the pathophysiology of diabetic microangiopathy. An elevation of serum enzyme levels occurs pari passu with decreased activities in kidney and retina in diabetic rats [22, 23]. Studies on insulin-treated diabetic subjects, however, showed a weak trend [2, 5, 6] between the severity of diabetic retinopathy and the degree of elevation in the lysosomal enzyme activity. Hence, the synthesis, secretion and/or turnover of the lysosomal enzymes may be affected

independently by glycaemia and by diabetic microangiopathy. The relationship between the enzyme changes and microangiopathy remains to be clarified.

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