Evaluation and Clinical Applications of Measurement of Urinary Growth Hormone in Diabetic Subjects

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Quantities of growth hormone (GH) excreted into the urine over 24 h were measured by the highly sensitive sandwich enzyme immunoassay in 63 non-insulin-dependent diabetes mellitus (NIDDM) subjects, 6 insulin-dependent diabetes mellitus (IDDM) subjects, and 17 age-matched nondiabetic control subjects. GH-provocative tests with intravenous infusion of arginine revealed that urinary GH levels are closely correlated with the integrated concentrations of serum GH (r = .931, n = 14, $P < .001$). Furthermore, 24-h urinary GH in control and diabetic subjects was inversely related to body mass index (r = .359, n = 80, $P < .001$). The mean 24-h urinary GH in NIDDM subjects was 11.1 ± 1.9 ng/g creatinine (Cr), which was not significantly different from that in nondiabetic control subjects (9.2 ± 2.7 ng/g Cr). By contrast, the individual values for IDDM subjects varied widely, and their mean values (42.5 ± 20.8 ng/g Cr) were much greater than those in the control and NIDDM subjects ($P < .01$). The degree of glycemic control does not seem to affect 24-h urinary GH in NIDDM. The mean 24-h urinary GH in 7 subjects with proliferative diabetic retinopathy was comparable to that in subjects without retinopathy or with background retinopathy. Thus, the measurement of 24-h urinary GH appears to provide reliable assessments of endogenous GH secretion under physiological conditions and will be a useful tool for obtaining further insight into the role of GH in diabetes. *Diabetes* 38:1567–72, 1989

Growth hormone (GH) has long been implicated as a deteriorating factor for the development of long-term complications of diabetes, especially retinopathy (1–10). Furthermore, recent studies revealed that an increased GH level can mediate metabolic derangement in diabetes (11,12) and that a nocturnal surge of the hormone might be responsible for the dawn phenomenon in patients with insulin-dependent diabetes mellitus (IDDM) (13). Therefore, GH seems to play an important role in the metabolism and long-term complications of the diabetic state.

Studies on circulating GH levels in diabetes mellitus, however, have yielded controversial results (2,3,14–20). Several investigators reported an elevated plasma GH level in diabetes mellitus (2,14–19), whereas Merimee et al. (20) found no significant difference in the mean serum GH concentrations over 24 h in clinically stable diabetic patients. Further characterization of endogenous GH secretion in diabetes mellitus is therefore needed.

Recently, Hashida et al. (21–23) developed a highly sensitive sandwich enzyme immunoassay (EIA) for GH, which enabled them to measure the GH level in unconcentrated urine. Using this assay method, Sukegawa et al. (24) found a highly significant correlation between the 24-h urinary GH level and the mean 24-h plasma GH concentration and concluded that measurement of urinary GH is a useful, simple, and practical method for evaluating endogenous GH secretion. In this study, we first evaluated the EIA method for urinary GH measurement and then determined the quantity of GH excreted into the urine over 24 h in subjects with diabetes mellitus.

RESEARCH DESIGN AND METHODS

Subjects. Sixty-three subjects with non-insulin-dependent diabetes mellitus (NIDDM), ranging in age from 21 to 82 yr (mean ± SE 58.2 ± 1.4 yr), and 6 patients with IDDM, ranging in age from 40 to 77 yr (mean 58.0 ± 5.6 yr), were selected from patients who had been admitted to the Institute for Adult Diseases, Asahi Life Foundation, in 1987 and 1988 for diabetes education and glycemic control. Seventeen control subjects, ranging in age from 30 to 84 yr (mean 56.7 ± 4.1 yr), were recruited from either healthy employees of the institution or inpatients with mild hypertension or simple obes-

Glucose 1 mM = 18 mg/dl

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ity. None of the subjects were short or had acromegalic features. Calorie intake of all the study subjects ranged from 25 to 30 kcal/kg ideal body wt, consisting of 50–60% carbohydrate, 20–25% protein, and 25–30% fat. Inpatients were encouraged to take a walk equivalent to 100–200 kcal in addition to the routine activity in the hospital. From the day after admission, the 24-h urine specimens were collected for 2 successive days from 0800 to 0800 in a 2-L plastic container with 2 ml of toluene as preservative and kept in a refrigerator (4°C).

GH-provocative test. Arginine tests were performed in 5 control and 9 diabetic subjects after an overnight fast. Three hundred milliliters of 10% arginine–hydrogen chloride solution was infused intravenously for 30 min, and 2-ml quantities of venous blood were collected at 0, 15, 30, 60, 90, 120, 150, and 180 min for determination of serum GH. Urine was voided just before the test and was collected at 180 min.

Measurement of urinary GH. The level of GH in dialyzed, unconcentrated urine was measured by the highly sensitive sandwich EIA kit, which was developed by Sumitomo (Osaka, Japan), according to the method described by Hashida et al. (21–23). Briefly, the urine sample (5 ml) was mixed with 50 μl of a 100 mg/ml solution of bovine serum albumin (fraction V, Armour, Kankakee, IL) and dialyzed against 10 mM sodium phosphate buffer, pH 7, supplemented with 0.4 M NaCl and 1 mg/ml of NaN_3 (buffer A) at 4°C overnight. One hundred microliters of dialyzed urine or standard hGH (Crescormon, Sumitomo) were mixed with 50 μl of 10 mM sodium phosphate buffer, pH 7, supplemented with 0.1 M NaCl (buffer B), and incubated with an anti-hGH IgG-coated polystyrene ball in a total volume of 150 μl at 37°C for 6 h with continuous shaking. The standard GH was diluted with buffer A. The incubation medium was then removed by suction, and the polystyrene ball was washed twice with 2-ml portions of buffer B. The ball was then incubated with affinity-purified–anti-hGH Fab′-peroxidase conjugate (50 μg/tube) and normal rabbit $F(ab')_2$ (100 μg/tube) in a total volume of 150 μl of buffer B, supplemented with 1 mg/ml of bovine serum albumin, at 4°C for 16 h without shaking. The medium was removed by aspiration, and the polystyrene ball was washed twice with buffer B as described above. The activity of peroxidase bound to the ball was assayed by fluorometry at 30°C for 90 min with 3-(*p*-hydroxyphenyl)propionic acid used as a substrate. The minimum detectable level of GH in the urine was 1.5 pg/ml. The coefficients of within- and between-assay variations were 6.6 and 9.3%, respectively. The mean 2-day 24-h urinary GH level was normalized with urinary Cr and was expressed as ng/g Cr.

Other methods. Serum GH was measured with commercially available radioimmunoassay (RIA) kits (Dainabot, Tokyo). Blood glucose was determined from capillary whole blood by a glucose oxidase method with a glucose autoanalyzer (Hitachi, Tokyo). Proteinuria was diagnosed by positive Rapignost (Hoechst, Tokyo) tests (urinary albumin >30 mg/ml). In patients with negative Rapignost tests, microalbuminuria was measured by RIA (Pharmacia, Uppsala, Sweden). Urinary Cr was determined by the picric acid method with commercially available kits (Wako, Osaka, Japan). Fundi were examined carefully with direct ophthalmoscopy through widely dilated pupils by an ophthalmologist experienced in ophthalmoscopy for diabetic retinopathy. The diagnosis of proliferative diabetic retinopathy was made if any of the following lesions were observed: new vessel formation, vitreous hemorrhage, or fibrous proliferation in the retina. Statistical analysis was performed by Student's *t* test or the paired *t* test and by Wilcoxon's rank-sum test for nonparametric data. Pearson's correlation coefficients were used to determine relationships among variables. The results are expressed as means ± SE.

RESULTS

Evaluation of measurement of urinary GH. Because plasma concentrations of GH are known to fluctuate widely throughout the day and presumably from day to day (2,10,14,15,17–19), we first determined the day-to-day variation of 24-h urinary GH in three diabetic inpatients whose 24-h urine was collected for 6 consecutive days. The mean coefficient of variation was 34%. Therefore, in most of the subjects, 24-h urine was collected for at least 2 consecutive days, and the results were expressed as the mean 2-day 24-h urinary GH.

It is conceivable that urinary excretion of GH is affected

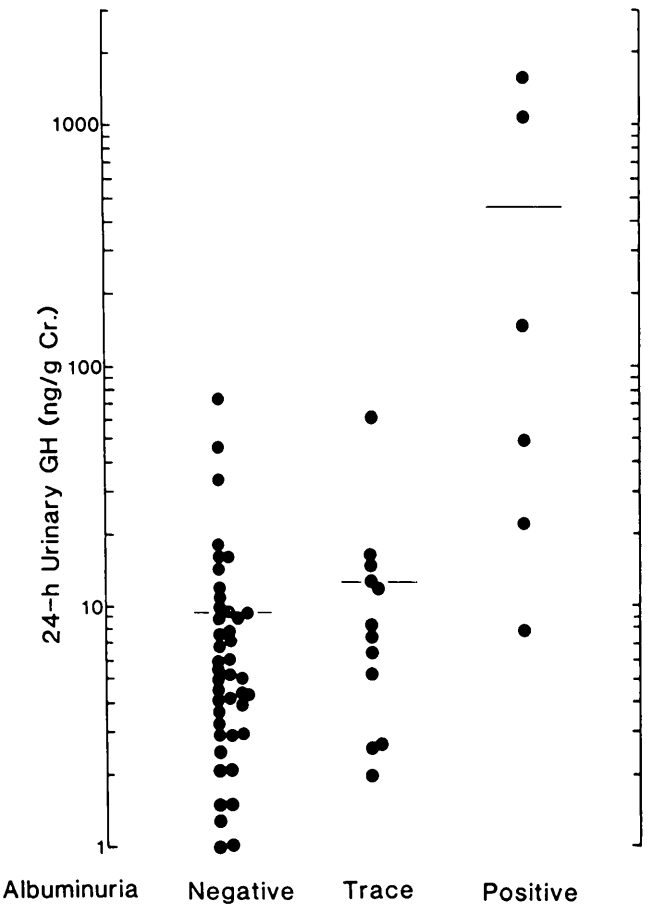

FIG. 1. Effect of proteinuria on 24-h urinary growth hormone (GH). Individual values of 24-h urinary GH in control and diabetic subjects divided into 3 groups according to their Rapignost (Hoechst) tests for urinary albumin (negative, trace, and positive) are shown. *Horizontal bars* indicate mean value of each group. Cr., creatinine. $P < .01$ negative vs. positive; $P < .05$ trace vs. positive.

by kidney function; therefore, we next determined the effect of proteinuria on 24-h urinary GH. When the diabetic inpatients were divided into three groups based on their tests for urinary albumin (negative, trace, and positive or gross), the mean 24-h urinary GH in subjects with gross albuminuria was 460.1 ± 269.8 ng/g Cr (n = 6), a value much greater than that in subjects with trace albuminuria (12.7 ± 4.6 ng/g Cr, n = 12, $P < .05$) or with negative albuminuria (9.5 ± 2.0 ng/g Cr, n = 43, $P < .01$; Fig. 1). There was no significant difference in 24-h urinary GH between the latter two groups. Furthermore, no significant correlation was found between the quantities of microalbuminuria and urinary GH levels in subjects with negative proteinuria (r = .276, n = 43, NS). The data suggest that kidney clearance of GH is enhanced in subjects with gross albuminuria, and measurement of urinary GH in these subjects may provide an erroneous estimation of endogenous secretion of GH. In this study, therefore, we excluded the subjects with gross albuminuria.

Figure 2 shows the relationship between the sum of serum GH levels during arginine tests in five control and nine diabetic subjects and the quantity of GH excreted into the urine during the 3-h tests. In accordance with the previously reported data (23), there was a strong correlation between the two variables (r = .931, n = 14, $P < .001$), indicating that urinary GH levels reflect the integrated serum GH concentrations.

In adulthood, endogenous GH secretion has been shown to be inversely related to age and adiposity (25–29). Figure 3 shows individual values of 24-h urinary GH of control and diabetic subjects that were divided into four groups according to body mass index (BMI; group A, BMI < 19 kg/m²; group B, 19 ≤ BMI < 23 kg/m²; group C, 23 ≤ BMI < 27 kg/m²; group D, BMI ≥ 27 kg/m²). Despite a large variation, it is apparent from this figure that the mean 24-h urinary GH tended to be low as BMI increased (36.9 ± 13.3 ng/g Cr, n = 12; 11.7 ± 2.3 ng/g Cr, n = 36; 7.2 ± 2.0 ng/g Cr, n = 19; and 5.2 ± 1.1 ng/g Cr, n = 13, in groups A–D, respectively). Furthermore, a significant inverse correlation was found between BMI and 24-h urinary GH (r = −.359, n = 80, $P < .001$).

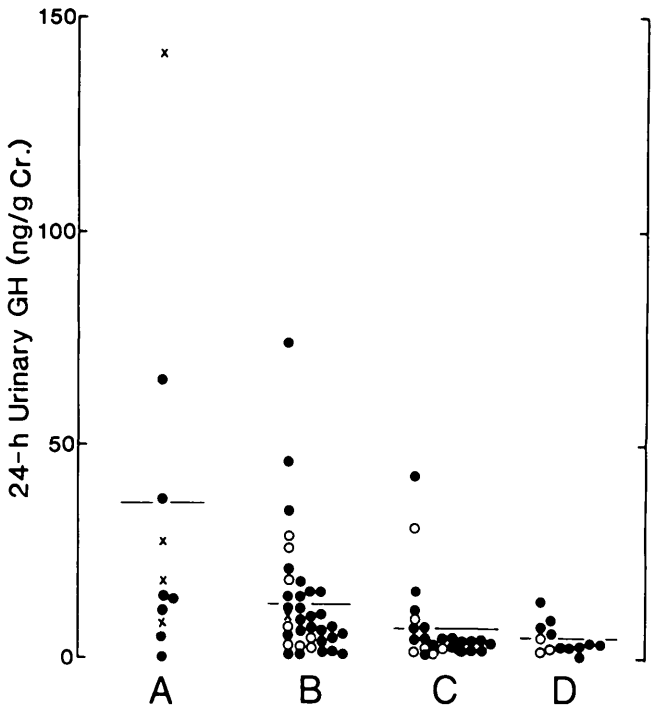

FIG. 3. Effect of adiposity on 24-h urinary growth hormone (GH). Individual levels of 24-h urinary GH in control (○), non-insulin-dependent (●), and insulin-dependent (×) diabetic subjects divided into 4 groups based on body mass index (kg/m²) are shown. *Horizontal bars* indicate mean value of each group. Statistical significance was analyzed by nonparametric Wilcoxon's rank-sum test. *A*, BMI < 19 kg/m²; *B*, 19 ≤ BMI < 23 kg/m²; *C*, 23 ≤ BMI < 27 kg/m²; *D*, BMI ≥ 27 kg/m². $P < .05$ *B* vs. *D*; $P < .01$ for all other comparisons. Cr., creatinine.

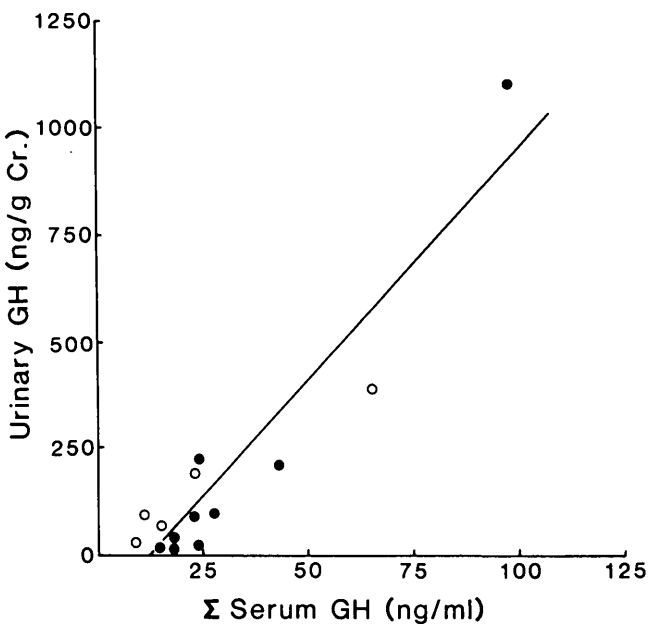

FIG. 2. Correlation between integrated concentrations of serum growth hormone (GH) and urinary GH after intravenous infusion of arginine. Thirty grams of arginine–hydrogen chloride were infused into 5 control (○) and 9 diabetic (●) subjects in 30 min, and serum levels of GH at 0, 15, 30, 60, 90, 120, 150, and 180 min were determined by the conventional radioimmunoassay. Quantities of GH excreted into urine during 3-h tests were determined by sandwich enzyme immunoassay. Cr., creatinine. r = .931; $P < .001$.

The effect of aging on 24-h urinary GH is shown in Fig. 4. Because some of the lean subjects showed unusually high urinary GH levels (Fig. 3), those with BMI <19 kg/m² were excluded from Fig. 4 to eliminate the effect of adiposity. The mean BMI values in subjects in the third to eighth decades were in a range of 23.3–24.5 kg/m², and no significant difference was found among the groups. In disagreement with the previously reported data (25–29), there was no significant difference in the mean 24-h urinary GH among all age-groups. Notably, even some subjects aged ≥70 yr showed high urinary GH levels. Furthermore, consistent with the data of Lang et al. (28), who showed that postmenopausal women had GH responses to GH-releasing hormone (GHRH) similar to those of age-matched men, the mean 24-h urinary GH values were comparable for both sexes at ages 50–69 yr (7.7 ± 2.2 ng/g Cr, n = 22, and 11.3 ± 3.0 ng/g Cr, n = 25, for men and women, respectively; NS).

Twenty-four-hour urinary GH in diabetes mellitus. The mean 24-h urinary GH value for NIDDM subjects was

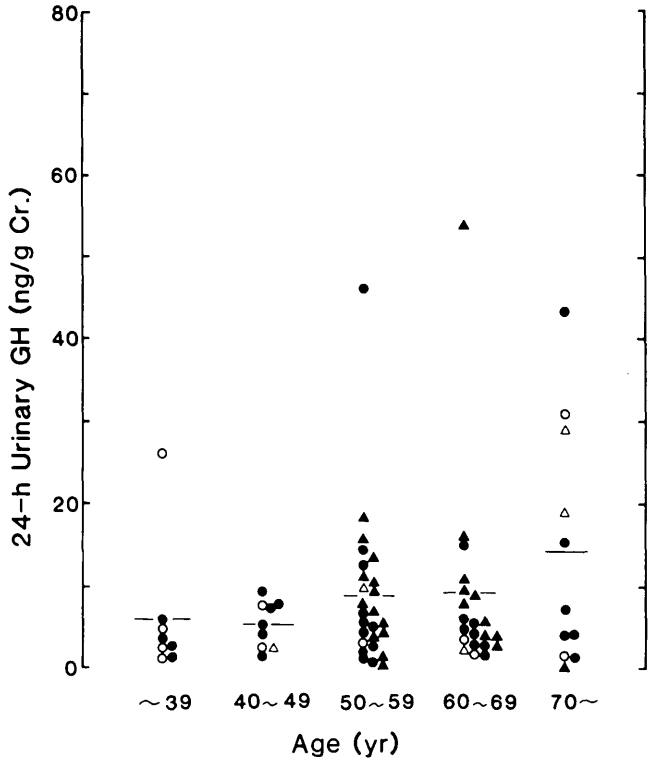

FIG. 4. Effect of age on 24-h urinary growth hormone (GH). Individual levels of 24-h urinary GH in control male (○) and female (△) and diabetic male (●) and female (▲) subjects divided into 5 groups based on age. *Horizontal bars* indicate mean of each group. Data from subjects with body mass index <19 kg/m² not shown. Cr., creatinine.

11.1 ± 1.9 ng/g Cr (n = 63), which was not significantly different from that for nondiabetic control subjects (9.2 ± 2.7 ng/g Cr, n = 17). By contrast, the individual data for IDDM subjects varied widely, ranging from 8.4 to 142 ng/g Cr, and the mean values (42.5 ± 20.8 ng/g Cr, n = 6) were much greater than those for nondiabetic and NIDDM subjects (P < .01 by Wilcoxon's rank-sum test). These data, however, should be interpreted with caution, because the mean BMI value for IDDM subjects was significantly less than that for the other two groups (17.4 ± 0.7 vs. 24.0 ± 0.8 and 23.2 ± 0.5 kg/m² in nondiabetic and NIDDM subjects, respectively; P < .01). The greater values for subjects with IDDM may therefore be solely due to their being less adipose.

Pulsatile secretion of GH frequently occurs at night (2,10,14,15,17,18); therefore, we next determined GH levels in urine collected from 0800 to 2200 and from 2200 to 0800 the next morning for 34 NIDDM subjects. Unexpectedly, there was no significant difference between the two determinations (4.0 ± 0.8 vs. 4.9 ± 0.8 ng/g Cr, in the day and night, respectively; NS by paired t test).

Glycemic control does not seem to affect endogenous secretion of GH in NIDDM subjects. When the subjects were divided into three groups based on their fasting blood glucose ([FBG] group G: FBG < 120 mg/dl, mean HbA_1 9.5 ± 0.8%, n = 12; group F: 120 ≤ FBG < 180 mg/dl, mean HbA_1 10.9 ± 0.9%, n = 35; and group P: FBG ≥ 180 mg/dl, mean HbA_1 12.8 ± 0.4%, n = 16), the mean 24-h urinary GH levels were identical in the three groups (13.7 ± 6.2, 7.6 ± 1.3, and 14.3 ± 4.3 ng/g Cr, in groups G, F, and P, respectively; NS). Neither the mean age nor the mean BMI values were significantly different among the groups. Furthermore, 24-h urinary GH in 11 NIDDM subjects was not decreased after an average of 13 days of hospitalization (8.7 ± 1.6 vs. 8.4 ± 2.3 ng/g Cr in early and late stages of hospitalization, respectively; NS by paired t test) even though FBG levels were markedly improved from 178 ± 12 to 111 ± 5 mg/dl (P = .01). Likewise, in the 6 IDDM patients, neither the quantity of GH excreted into the urine during 24 h or that excreted during the overnight period (from 2200 to 0800) was not related to FBG levels taken on the days beginning or ending urine collection. However, in one IDDM subject to whom intermediate-acting insulin was given twice daily (i.e., at morning and evening), a significant positive correlation was found between 24-h urinary GH, which was repeatedly determined for 11 consecutive days, and FBG levels the next morning (r = .623, n = 11, P < .05). No such correlation was found in the other IDDM subject, who was given intermediate-acting insulin at bedtime in addition to the mixtures of intermediate- and short-acting insulins in the morning and evening and whose urinary GH was determined for 15 successive days.

Figure 5 shows the individual values of 24-h urinary GH for diabetic subjects without retinopathy or with background or proliferative diabetic retinopathy. We restricted the group without retinopathy to subjects with a duration of diabetes >10 yr. None of the subjects with proliferative retinopathy showed an elevated urinary GH level, and no significant difference was found in the mean 24-h urinary GH among the three groups (20.7 ± 9.5 ng/g Cr, n = 16; 11.0 ± 3.4

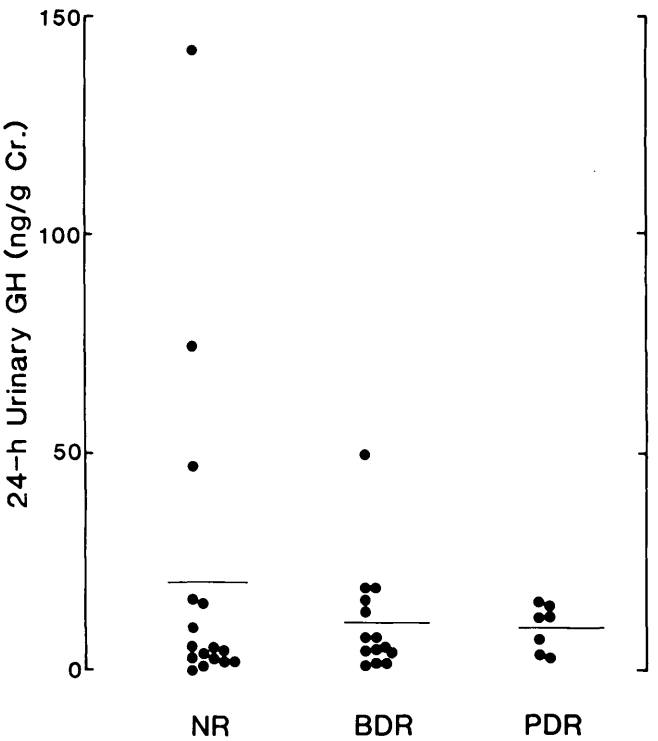

FIG. 5. Individual levels of 24-h urinary growth hormone (GH) in diabetic subjects without retinopathy (NR) or with background diabetic retinopathy (BDR) or proliferative diabetic retinopathy (PDR). Subjects in NR group restricted to those with duration of diabetes >10 yr. *Horizontal bars* indicate mean of each group. Cr., creatinine.

ng/g Cr, n = 14; and 9.8 ± 1.9 ng/g Cr, n = 7, in the groups without retinopathy and with background or proliferative diabetic retinopathy, respectively; NS by Wilcoxon's rank-sum test). The mean age and BMI values were also comparable among the three groups.

DISCUSSION

Because plasma levels of GH fluctuate widely with time (2,10,14,15,17–19), a single determination of plasma GH concentration does not provide any reliable information about endogenous GH secretion. Furthermore, the GH-provocative tests with various pharmacological stimuli do not always reflect endogenous secretion of GH (30,31). Therefore, the most reliable method for assessing endogenous GH secretion would be determination of the integrated concentrations of GH in blood samples collected every 15–20 min for 24 h. This method, however, requires frequent and multiple blood samplings over 24 h and is not suitable to use for a large number of subjects. By contrast, measurement of 24-h urinary GH by the newly developed EIA is a simpler and less-invasive method than measurement of integrated plasma GH levels. This EIA is sensitive enough to measure GH levels in 100 μl of unconcentrated urine and is shown to be specific for 22,000-M_r authentic GH in urine (22).

In agreement with previously reported data (23), we found that the quantity of GH excreted into the urine is strongly correlated with the integrated serum GH concentrations during 3-h arginine tests (Fig. 2). By use of the same EIA method, Sukegawa et al. (24) also found close correlations between 24-h urinary GH and mean 24-h plasma GH concentrations (r = .81, P < .001) and plasma somatomedin C levels (r = .67, P < .001) in four subjects with hypopituitarism and 25 healthy children with short stature. Thus, measurement of 24-h urinary GH provides a reliable index of endogenous GH secretion despite the fact that only a minute fraction (<0.01%) of secreted GH is estimated to reach the final urine (32–34). However, this method cannot be applied to subjects with gross albuminuria, because kidney clearance of plasma GH appears to be enhanced in these subjects (Fig. 1).

Endogenous secretion of GH, assessed by either multiple blood samplings or GH-provocative tests with various stimuli, has been shown to be inversely correlated with adiposity and age after the second decade of life (25–29). It was also shown by Ho et al. (27) that premenopausal women have higher 24-h integrated concentrations of GH, whereas postmenopausal women have concentrations similar to those of age-matched men, although Zadik et al. (26) did not find any differences in nonobese male and female subjects 7–65 yr of age.

Measurement of 24-h urinary GH gave data consistent with these findings except that there was no age-related decline in urinary GH as shown in Fig. 4. The reason for this discrepancy is not clear. It might be caused by alterations in kidney clearance of plasma GH in older subjects, although they showed no albuminuria, and their serum Cr levels were within normal range. Alternatively, because our data were derived mostly from NIDDM subjects, not from healthy subjects, the disparity could be caused by a bias in subject selection. However, the fact that we did not find any differences in 24-h urinary GH between nondiabetic control and NIDDM subjects apparently argues against the latter explanation.

In diabetes mellitus, 24-h plasma GH concentrations and GH response to physical exercise are reportedly elevated (2,14–19), especially in poorly controlled subjects (16,18). These abnormalities can be normalized after correction of glycemic control (16,18), indicating that the raised GH levels are the consequence rather than the cause of metabolic derangements. Recently, however, Merimee et al. (20) showed that the mean serum GH concentrations during 24 h were similar in clinically stable diabetic subjects to those in age-matched control subjects. Our data obtained from NIDDM subjects were compatible with those of Merimee et al. By contrast, for subjects with IDDM, the individual values of 24-h urinary GH varied from the normal range to extremely high levels, and the mean values were significantly greater than those for nondiabetic and NIDDM subjects. Worse glycemic control does not account for the elevated urinary GH level in the IDDM group; we found no relationship between 24-h urinary GH and glycemic control. Although the mechanism is not clear, some of the lean nondiabetic subjects also showed high urinary GH (Fig. 3), and therefore the abnormalities in these subjects could be caused by their being less adipose than the NIDDM subjects, Therefore, our data do not appear to support the implicated role of GH in metabolism in diabetes. However, note that in one IDDM patient with conventional insulin therapy the 24-h urinary GH was positively related to FBG. The data suggest that GH may deteriorate glycemic control under conditions in which circulating insulin levels are extremely low.

The view that GH may contribute to the development of diabetic microangiopathy, especially retinopathy, is based on the following observations: *1*) diabetic retinopathy in one patient regressed after postpartum putuitary infarction (1); *2*) pituitary ablation can arrest or slow the progression of severe diabetic retinopathy (4); and *3*) diabetic retinopathy was absent in a large group of GH-deficient dwarfs with diabetes (6). This view is supported by findings that the circulating GH concentrations or GH responses to either muscular exercise or GHRH are indeed higher in diabetic subjects with retinopathy than in subjects without retinopathy (3,9,10,19). Merimee et al. (7) also found higher serum levels of insulinlike growth factor I, a GH-dependent growth factor, in seven IDDM subjects with rapidly deteriorating proliferative retinopathy than in IDDM subjects with no retinopathy or less-severe retinopathy. Furthermore, a direct effect of GH on the proliferation of arterial myomedial cells has been documented in vitro (5).

In this study, we did not find any relationship between the severity of diabetic retinopathy and 24-h urinary GH levels (Fig. 5). However, because only a cross-sectional aspect of endogenous GH secretion has been shown in this and in other studies (3,9,10,19), further studies including longitudinal data on GH secretion in the course of the development of retinopathy are needed to elucidate the possible role of GH in diabetic microangiopathy. The measurement of 24-h urinary GH by the highly sensitive EIA method will be a useful tool for obtaining additional insight into the role of GH in diabetes.

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