Examination of the Role of the Pituitary-Adrenocortical Axis, Counterregulatory Hormones, and Insulin Clearance in Variable Nocturnal Insulin Requirements in Insulin-dependent Diabetes

DONALD A. SKOR, NEIL H. WHITE, LORRAINE THOMAS, SURESH D. SHAH, PHILIP E. CRYER, AND JULIO V. SANTIAGO

SUMMARY

In insulin-dependent diabetics, insulin requirements increase significantly after 0600 h, resulting in prebreakfast hyperglycemia with either conventional insulin therapy or constant insulin infusions with insulin infusion devices. In order to clarify the role of the pituitary-adrenocortical axis and further examine the mechanisms of the phenomenon of nocturnal variability in insulin requirements, we studied five IDDs using a closed-loop insulin infusion device (Biostator, GCIIS). The subjects were given saline (SAL) or dexamethasone (DEX) i.v. from 1800 to 0900 h on successive nights. From 2400–0300 to 0600–0900 h, mean insulin infusion rates required to maintain blood glucose values between 109 and 120 mg/dl increased by 0.21 ± 0.05 mU/kg/min during the SAL infusion, and 0.16 ± 0.04 mU/kg/min during the DEX infusion, when plasma cortisols were suppressed to ≤ 2 μg/dl. Mean free insulin concentrations did not increase and remained constant throughout both study nights in spite of the significantly higher 0600–0900-h insulin infusion rates. Growth hormone, glucagon, epinephrine, and norepinephrine concentrations showed normal nocturnal and early morning patterns during both study nights. We conclude that the nocturnal variability in insulin requirements persists despite suppression of the pituitary-adrenocortical axis, and that increased free insulin clearance or degradation may contribute to the "dawn phenomenon" of rising prebreakfast glucose despite constant insulin infusion. DIABETES 32:403–407, May 1983.

The advent of sophisticated programmable devices for insulin delivery has resulted in increased attention on the variability of overnight insulin requirements in insulin-dependent diabetics (IDDs). Nocturnal insulin requirements have been shown to increase substantially, following a 0100–0300-h nadir, at 0600–0900 h in some IDDs given insulin subcutaneously or intravenously.[1–3] If insulin replacement does not increase after 0600 h in these patients, blood glucose concentrations increase. This has been referred to as the "dawn phenomenon."[4]

The observed increase in insulin requirements is temporally related to the physiologic diurnal increase of plasma cortisol. Shamoon et al. infused cortisol into normals and IDDs maintained euglycemic with constant insulin infusions and found that the hyperglycemic effect of cortisol was more pronounced in the diabetic subjects.[5] A nocturnal increase in cortisol levels might contribute to the increased insulin requirements observed in IDDs treated with insulin infusion devices or more conventional modes of insulin replacement. However, Bright et al. observed that when IDDs were given metyrapone sufficient to block endogenous cortisol production, nocturnal variability in insulin requirements persisted,[6] thus raising doubts regarding a role for changes in plasma cortisol in the pathogenesis of the observed changes in overnight insulin requirements.

This study was designed to further examine a possible role of the pituitary-adrenocortical axis in the nocturnal variability of insulin requirements, specifically to clarify whether ACTH or an ACTH-dependent mechanism involving cortisol precursors, or changes in free insulin or counterregulatory hormone concentrations might influence overnight insulin requirements in IDDs.

METHODS

The subjects were five normal-weight IDDs between 23 and 39 yr of age, with a mean duration of diabetes of 14 ± 2 yr. Values for glycosylated hemoglobin varied from 10% to 17% in an assay where normal values are 4.0–8.3%. All had previous episodes of documented ketoacidosis. All subjects gave informed consent for participation in this study.

From the Edward Mallinckrodt Department of Pediatrics and the Department of Medicine, Washington University School of Medicine, and the Division of Endocrinology and Metabolism, St. Louis Children's Hospital, St. Louis, Missouri.

Address reprint requests to Julio V. Santiago, M.D., St. Louis Children's Hospital, P.O. Box 14871, St. Louis, Missouri 63178.

Received for publication 28 July 1982 and in revised form 3 December 1982.

The subjects were withdrawn from NPH or lente insulin at least 30 h before, and subcutaneous regular or crystalline insulin at least 12 h before the study period. Blood glucose was controlled with a modified closed-loop intravenous insulin delivery system before the study[7] until 2300 h, when they were connected to the Biostator GCIIS (Miles Laboratories, Elkhart, Indiana).[8] The Biostator was operated using mode 1:1, with constants set to deliver insulin at 10–25 mU/min (RI) with glucoses at 110 mg/dl (BI) so that insulin infusion rates (IR) increased twofold at a blood glucose of 122 mg/dl (QI = 30 mg/dl) according to the following algorithm:

$$IR = RI\left[\frac{G - BI}{QI} + 1\right]^2.$$

Dextrose was not given during the course of these studies. The static 1:1 mode was used instead of the dynamic 3:1 mode so as to reduce minute-to-minute variability in insulin infusion rates resulting from small changes in glucose concentrations.

The subjects ate their last meal 6 h or more before being placed under Biostator control. Either intravenous saline (SAL) or 150 μg/h dexamethasone (DEX) was infused continuously from 1800 to 0900 h on consecutive nights. In anticipation of increased insulin requirements during DEX infusion, RI was increased by 50% on the DEX night, but BI and QI were the same on both nights. Blood samples were collected through an indwelling venous catheter at 30-min intervals between 2400 and 0900 h for measurement of glucose, cortisol, glucagon, growth hormone, free insulin, epinephrine, and norepinephrine. Growth hormone, glucagon, and cortisol were measured by radioimmunoassay as previously described. Free insulin was measured by radioimmunoassay after a 2-h incubation in a metabolic shaker at 37°C and polyethylene glycol precipitation as described by Kuzuya et al.[9] Plasma epinephrine and norepinephrine were measured in duplicate in 50-μl aliquots with a single isotope derivative method.[10] Glycosylated hemoglobin was determined using a minicolumn (Isolab Inc., Akron, Ohio).

The mean glucose concentrations and insulin infusion rates were calculated as the means of minute-to-minute blood glucose concentrations and insulin infusion rates for 30-min intervals during Biostator control. The paired *t* test was used to compare data in the individual subjects from one time interval to another. Results are expressed as mean ± SEM.

RESULTS

During overnight Biostator control with the SAL infusion, mean nocturnal insulin requirements increased from 0.21 ± 0.05 mU/kg/min from 2400 to 0300 h to 0.42 ± 0.07 mU/kg/min from 0600 to 0900 h ($P < 0.01$) as plasma glucose rose from 109 ± 1 to 120 ± 3 mg/dl ($P < 0.05$) (Figure 1).

During the overnight DEX infusion, mean nocturnal insulin requirements increased from 0.38 ± 0.07 mU/kg/min from 2400 to 0300 h to 0.54 ± 0.08 mU/kg/min from 0600 to 0900 h ($P < 0.01$) in response to an increase in mean plasma glucose from 112 ± 2 to 115 ± 1 mg/dl (P = NS) (Figure 2). Mean overnight insulin requirements were 62% higher during the DEX infusion in comparison to the SAL infusion ($P < 0.001$).

The mean free insulin concentration during the SAL infusion was 71 ± 17 μU/ml from 2400 to 0300 h and remained

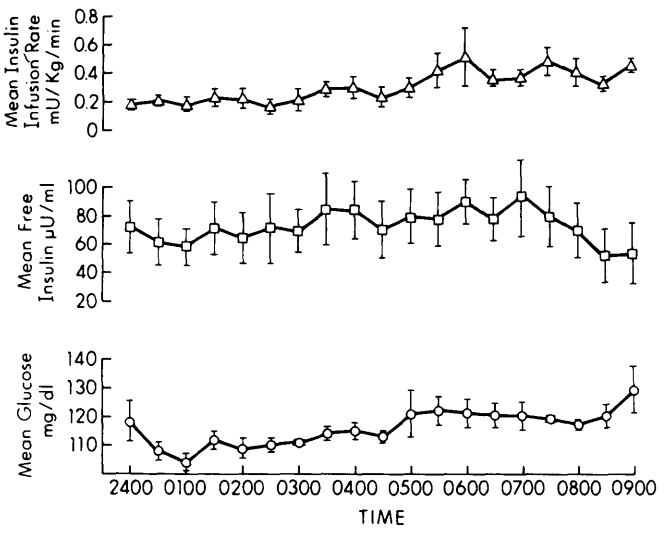

FIGURE 1. Mean ± SEM insulin infusion rates, glucose, and free insulin concentrations during overnight SAL infusion.

constant overnight with a mean concentration of 75 ± 14 μU/ml from 0600 to 0900 h (P = NS) (Figure 1). Similarly, during the DEX infusion, the mean free insulin concentration was 89 ± 15 μU/ml from 2400 to 0300 h and remained constant with a mean value of 85 ± 17 μU/ml from 0600 to 0900 h (P = NS) (Figure 2). Thus, in spite of the substantially higher insulin infusion rates from 0600 to 0900 h as compared with 2400 to 0300 h during both SAL and DEX infusions, mean plasma free insulin concentrations were not significantly different between these time periods on either study night (Figure 3).

Plasma cortisol levels showed a normal nocturnal variability during the SAL infusion with an increase to a mean level of 20 ± 2 μg/dl at 0700 h (Figure 4). During the DEX infusion, the pituitary-adrenocortical axis was suppressed as shown by plasma cortisol levels of ≤ 2 μg/dl throughout the study period (Figure 5).

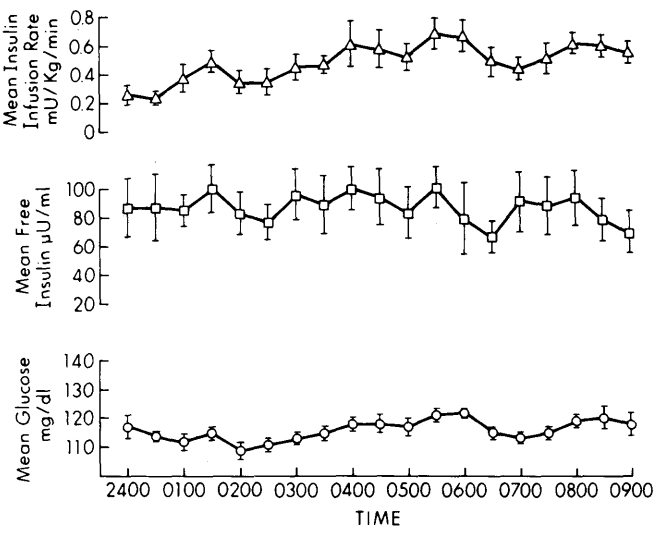

FIGURE 2. Mean ± SEM insulin infusion rates, glucose, and free insulin concentrations during overnight DEX infusion.

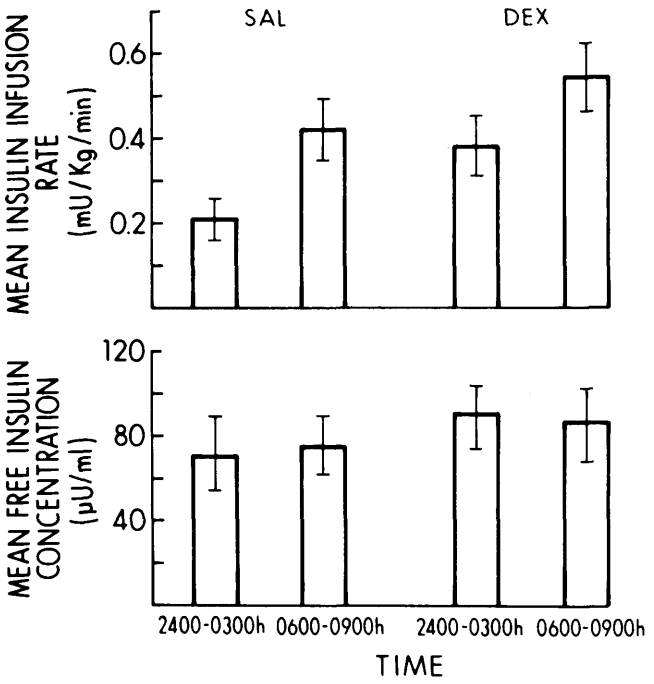

FIGURE 3. Comparison of mean ± SEM insulin infusion rates and free insulin concentrations between 2400 and 0300 h and 0600 and 0900 h during SAL and DEX infusions. The increase in insulin infusion rates was significant ($P < 0.01$) from 2400–0300 h to 0600–0900 h during both SAL and DEX infusions. Free insulin concentrations were not significantly different.

Plasma glucagon concentrations remained relatively constant throughout both study nights, and there was no difference in the mean overnight values between the SAL (189 ± 2 pg/ml) and the DEX (190 ± 4 pg/ml) infusions. The serum growth hormone values showed normal nocturnal variability on both study nights (Figures 4 and 5) with spikes in growth hormone concentrations of 5–45 ng/ml during the early hours of sleep between 2400 and 0300 h.

Mean plasma epinephrine values were 37 ± 9 pg/ml from 2400 to 0300 h during the SAL infusion, and increased slightly to 47 ± 16 pg/ml from 0600 to 0900 h (P = NS) (Figure 6). During the DEX infusion, mean plasma epinephrine values were 33 ± 18 pg/ml from 2400 to 0300 h and also increased slightly to 48 ± 24 pg/ml from 0600 to 0900 h (P = NS) (Figure 7). There was, however, a significant diurnal pattern noted with norepinephrine, with an increase from 141 ± 17 pg/ml from 2400–0300 h to 174 ± 20 pg/ml from 0600–0900 h

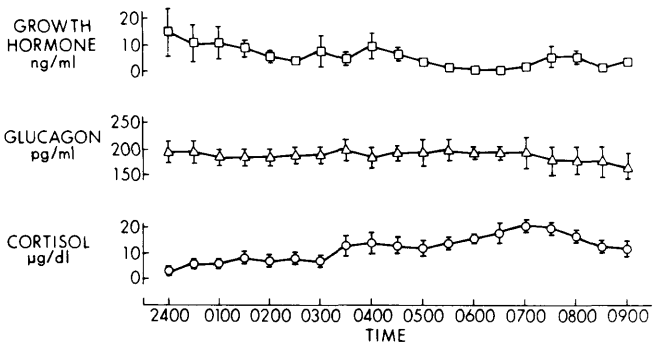

FIGURE 4. Mean ± SEM concentrations of cortisol, glucagon, and growth hormone during overnight SAL infusion.

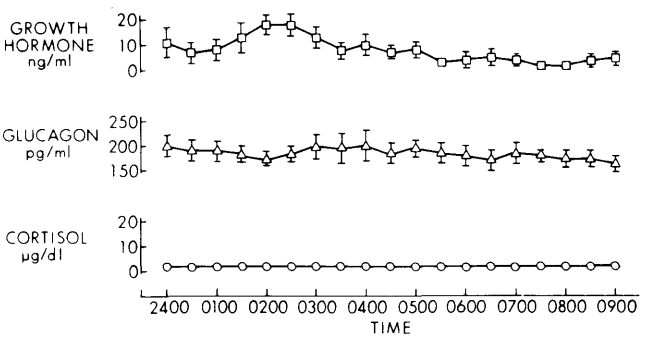

FIGURE 5. Mean ± SEM concentrations of cortisol, glucagon, and growth hormone during overnight DEX infusion.

($P < 0.05$) during the SAL infusion (Figure 6), and a similar increase from 159 ± 16 pg/ml from 2400–0300 h to 201 ± 15 pg/ml from 0600–0900 h ($P < 0.001$) during the DEX infusion (Figure 7). There was no significant difference in mean epinephrine and norepinephrine concentrations between the two study nights.

DISCUSSION

Several studies have now documented a nocturnal variability in overnight basal insulin requirements in IDDs.[1-4] Previously, since overnight levels of glucagon were found to be unchanged, and since fluctuations of growth hormone correlated poorly with changes in insulin requirements during the day,[11] it was logical to suspect that this variability might be secondary to the diurnal variation of plasma cortisol. However, normal adults maintain constant plasma glucose, insulin, and C-peptide concentrations in spite of normal diurnal variations of plasma cortisol.[12,13] The observations of Bright et al. suggested that a process other than cortisol might be responsible for the observed changes in insulin requirements.[6] Indeed, it was particularly interesting in this latter study that the prebreakfast increase in insulin infusion rates was more pronounced when the subjects were given metyrapone. Since suppression of endogenous cortisol release would otherwise be expected to decrease basal insulin requirements from 0600 to 0900 h, then either cortisol precursors or an ACTH-dependent process might contribute to the increased insulin requirements at this time.

The results of the present study demonstrate that the pre-

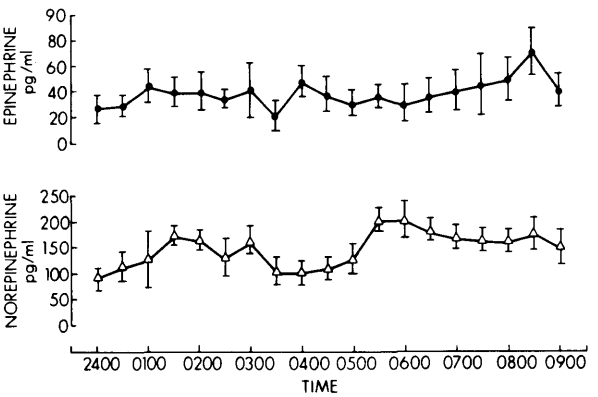

FIGURE 6. Mean ± SEM concentrations of epinephrine and norepinephrine during overnight SAL infusion.

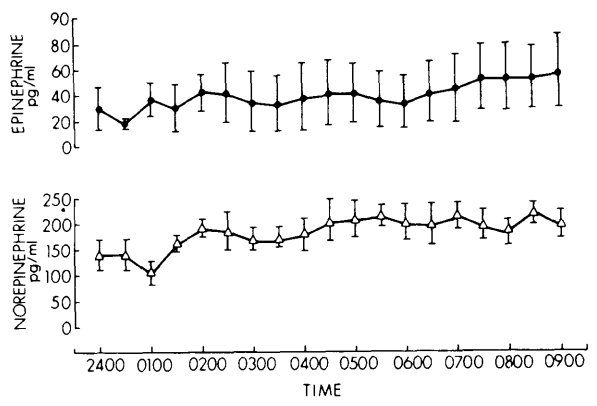

FIGURE 7. Mean ± SEM concentrations of epinephrine and norepinephrine during overnight DEX infusion.

breakfast increase in insulin requirements persists despite pharmacologic suppression of the pituitary-adrenocortical axis in IDDs. The greater 0600–0900-h increase in glucose concentrations during the SAL infusion compared with the DEX infusion suggests that the 0.21 ± 0.05 mU/kg/min increment in insulin requirements during the SAL infusion was inadequate to prevent the prebreakfast rise in blood glucose. A further increase in the 0600–0900-h insulin infusion rate sufficient to maintain a more constant blood glucose may have resulted in a larger difference in the increments in insulin infusion rates between the SAL and DEX infusions. This suggests that suppression of the pituitary-adrenal axis may moderately blunt the prebreakfast increase in insulin requirements. However, the 0.16 ± 0.04-mU/kg/min increment during the DEX infusion remains a significant finding, suggesting that the physiologic variability of ACTH and cortisol is not the only determinant of the "dawn phenomenon." The 62% increase in mean insulin requirements overnight during the DEX infusion compared with the SAL infusion is consistent with the known hyperglycemic effect of chronic cortisol excess in both normals and diabetics,[14,15] and is consistent with the observations of Shamoon.[5]

Although ACTH levels were not measured, the continuous infusion (150 μg/h) of a supraphysiologic dose of 2.25 mg of dexamethasone overnight should have been more than sufficient to suppress ACTH secretion.[16] The totally suppressed levels of plasma cortisol to less than 2 μg/dl throughout the study period demonstrate adequate cortisol suppression. Although dexamethasone levels were not measured, the plasma level should have been stable since the constant infusion was begun 6 h before beginning the study.

The mean free insulin concentrations failed to increase despite the significantly higher insulin infusion rates from 0600 to 0900 h during both study nights. Although metabolic clearance rates of insulin could not be assessed precisely using this study design involving small minute-to-minute variations in insulin infusion rates, a nocturnal variability in free insulin clearance might contribute to the observed prebreakfast increase in insulin requirements in these patients (Figure 3). The data also suggest that the variability in insulin clearance is unrelated to changes in plasma cortisol or ACTH, since the free insulin concentrations did not increase in response to higher 0600–0900-h insulin infusion rates during the DEX infusion. Further studies are needed to evaluate possible changes in insulin degradation and kinetics of insulin binding to antibodies during the night since either could account for apparent variations in free insulin clearance from plasma.

Previous studies have failed to show any correlation between overnight variability in insulin requirements and plasma glucagon levels.[1,6] In fact, glucagon levels have been found to remain relatively constant overnight in IDDs.[2,6,11] The constant levels of plasma glucagon during both the SAL and DEX infusions corroborate these earlier findings.

Growth hormone concentrations during both study nights demonstrated a normal nocturnal pattern with spikes[11,17] occurring during early sleep in each patient, resulting in significantly higher mean values noted from 2400 to 0300 h than from 0600 to 0900 h. There was no significant difference in the mean overnight growth hormone concentrations between the SAL or DEX infusions. MacGorman et al. demonstrated that the hyperglycemic effects of growth hormone may be delayed by several hours after a growth hormone infusion, and occur after an initial insulin-like, hypoglycemic effect seen during the first 2.5 h of hormone infusion.[18] Thus, the physiologic nocturnal increase in growth hormone may contribute to the observed pattern of variable insulin requirements, perhaps contributing to both the 0100–0300-h nadir as well as the 0600–0900-h increase in plasma glucose concentration and insulin requirements. Further studies are needed to evaluate this issue.

The observed overnight patterns of epinephrine and norepinephrine are consistent with previous studies showing an increase in norepinephrine during the waking hours.[19–22] Although the mean epinephrine concentrations increased slightly, it seems unlikely that these minor changes and relatively low absolute values of epinephrine contribute significantly to the nocturnal variability in insulin requirements. Clutter et al. have previously shown that increments in plasma glucose concentration and glucose production occur at threshold values of 150–200 pg/ml for epinephrine, which exceed all values obtained during this study.[23] The observed diurnal increase in mean plasma norepinephrine concentrations from 0600 to 0900 h in these subjects is consistent with the observations of Stene et al., who noted an abrupt rise in norepinephrine upon awakening at this time in normal subjects.[22] Since normal subjects have neither an increase in plasma glucose nor an increase in insulin concentrations in response to waking,[12,13] despite similar increments in plasma norepinephrine concentrations, it seems unlikely that norepinephrine alone is a major hyperglycemic factor in the dawn phenomenon.

Thus, these studies demonstrate that the nocturnal variability in insulin requirements in IDDs occurs despite overnight patterns of cortisol, glucagon, growth hormone, epinephrine, and norepinephrine similar to those previously described in normal subjects. However, in spite of the peripheral venous hyperinsulinemia noted in our subjects and those previously reported by others,[24] it is unlikely that our patients were receiving sufficient insulin to reduce hepatic glucose production rates to normal values when blood glucoses were maintained at 110–120 mg/dl. This relative hepatic underinsulinization may be common in insulin-treated diabetics and, as observed by Shamoon et al.,[5] might make

IDDs increasingly responsive to the hyperglycemic effects of cortisol, glucagon, epinephrine, and other counterregulatory factors. Under conditions of mild to moderate hepatic underinsulinization, small increments in one or more counterregulatory hormones or neurotransmitters could result in exaggerated hyperglycemic effects in IDDs, which do not occur in normal subjects in whom insulin is delivered directly to the liver through the portal circulation.

In summary, the present studies demonstrate that the prebreakfast increase in insulin infusion rates required to maintain plasma glucose concentrations between 110 and 120 mg/dl in many IDDs persists despite suppression of the pituitary-adrenocortical axis with dexamethasone infusions. The physiologic nocturnal variability of ACTH or cortisol secretion is not the only determinant of this pattern. Further studies are needed to evaluate the possible role of growth hormone, changes in the metabolic clearance rate of insulin, and the effect of variable degrees of glycemic control using more physiologic routes for insulin delivery on the variability of nocturnal insulin requirements in IDDs.

ACKNOWLEDGMENTS

We gratefully acknowledge the support of the nursing staff of the Washington University Clinical Research Center. This study was supported in part by National Institutes of Health grants RR-00036, AM20579, and AM27085, and by a grant from the St. Louis Affiliate of the American Diabetes Association. D.A.S. was the recipient of the Jules and Joyce Pass Research Fellowship from the St. Louis Affiliate of the American Diabetes Association.

REFERENCES

[1] Clarke, W. L., Haymond, M. W., and Santiago, J. V.: Overnight basal insulin requirements in fasting insulin-dependent diabetics. Diabetes 1980; 29:78–80.

[2] Deckert, T., and Lorup, B.: Regulation of brittle diabetics by a preplanned insulin infusion program. Diabetologia 1976; 12:573–79.

[3] Schmidt, M. I., Hadji-Georgopoulos, A., Rendell, M., Margolis, S., Kowarski, D., and Kowarski, A. A.: Fasting hyperglycemia and associated free insulin and cortisol changes in "somogyi-like" patients. Diabetes Care 1979; 2:457–64.

[4] Schmidt, M. I., Hadji-Georgopoulos, A., Rendell, M., Margolis, S., and Kowarski, A.: The dawn phenomenon, an early morning glucose rise: implications for diabetic interday blood glucose variation. Diabetes Care 1981; 4:579–85.

[5] Shamoon, H., Hendler, R., and Sherwin, R. J.: Altered responsiveness to cortisol, epinephrine and glucagon in insulin-infused juvenile onset diabetics. Diabetes 1980; 29:284–91.

[6] Bright, G. M., Melton, T. W., Rogol, A. D., and Clarke, W. L.: Failure of cortisol blockade to inhibit early morning increases in basal insulin requirements in fasting insulin-dependent diabetics. Diabetes 1980; 29:662–64.

[7] White, N. H., Skor, D., and Santiago, J. V.: Practical closed loop insulin delivery: a system for the maintenance of overnight euglycemia and the calculation of basal insulin requirements in insulin-dependent diabetics. Ann. Intern. Med. 1982; 97:210–13.

[8] Clarke, W., Thomas, L., and Santiago, J.: Clinical evaluation and preliminary studies on the use of an artificial pancreatic beta cell in juvenile diabetes mellitus. J. Pediatr. 1977; 91:590–96.

[9] Kuzuya, H., Blix, P. M., Horwitz, D. L., Steiner, D. F., and Rubenstein, A. H.: Determination of free and total insulin and C-peptide in insulin-treated diabetics. Diabetes 1979; 26:22–29.

[10] Cryer, P. E., Santiago, J. V., and Shah, S. D.: Measurement of norepinephrine and epinephrine in small volumes of human plasma by a single isotope derivative method. Response to the upright position. J. Clin. Endocrinol. Metab. 1974; 39:1025–29.

[11] Hansen, A. A., and Johansen, K.: *Diurnal patterns of blood glucose*, serum free fatty acids, insulin, glucagon, and growth hormone in normals and juvenile diabetics. Diabetologia 1970; 6:27–33.

[12] Santiago, J. V., Haymond, M. W., Clarke, W. L., and Pagliara, A. J.: Glucagon, insulin, and glucose responses to physiologic testing in normal and massively obese adults. Metabolism 1977; 26:1115–22.

[13] Tasaka, Y., Sekine, M., Wakatsuki, M., Ohgawara, H., and Shizume, K.: Levels of pancreatic glucagon, insulin and glucose during twenty-four hours of the day in normal subjects. Horm. Metab. Res. 1976; 7:205–206.

[14] Schade, D. S., and Eaton, R. P.: Dose response to insulin in man: differential effects on glucose and ketone body regulation. J. Clin. Endocrinol. Metab. 1977; 44:1038–53.

[15] Schade, D., Eaton, R. P., and Standefer, J.: Glucocorticoid regulation of plasma ketone body concentration in insulin-deficient man. J. Clin. Endocrinol. Metab. 1977; 44:1069–79.

[16] Liddle, G. W.: Tests of pituitary-adrenal suppressibility in the diagnosis of Cushing's syndrome. J. Clin. Endocrinol. Metab. 1960; 20:1539–60.

[17] Hunter, W. M., Friend, J. A. R., and Strong, J. A.: The diurnal pattern of plasma growth hormone concentration in adults. J. Endocrinol. 1966; 34:139–46.

[18] MacGorman, L. R., Rizza, R. A., and Gerich, J. E.: Physiological concentrations of growth hormone exert insulin-like and insulin antagonistic effects on both hepatic and extrahepatic tissues in man. J. Clin. Endocrinol. Metab. 1981; 53:556–59.

[19] Karki, N. T.: The urinary excretion of noradrenaline and adrenaline in different age groups, its diurnal variation, and the effect of muscular work on it. Acta Physiol. Scand. 1956; 39 (Suppl. 132):1–96.

[20] Elmadjian, F., Lamson, E. T., and Neri, R.: Excretion of adrenaline and noradrenaline in human subjects. J. Clin. Endocrinol. Metab. 1956; 16:222–34.

[21] Prinz, P. N., Halter, J., Benedetti, C., and Raskind, M.: Circadian variation of plasma catecholamines in young and old men: relation to rapid eye movement and slow wave sleep. J. Clin. Endocrinol. Metab. 1979; 49:300–304.

[22] Stene, M., Panagiotis, N., Tuck, M. L., Sowers, J. R., Mayes, D., and Berg, G.: Plasma norepinephrine levels are influenced by sodium intake, glucocorticoid administration and circaidan changes in normal man. J. Clin. Endocrinol. Metab. 1980; 51:1340–45.

[23] Clutter, W. E., Bier, D. M., Shah, S. D., and Cryer, P. E.: Epinephrine plasma metabolic clearance rates and physiological thresholds for metabolic and hemodynamic actions in man. J. Clin. Invest. 1980; 66:94–101.

[24] Horwitz, D. L., Zeidler, A., Gonen, B., and Jaspan, J. B.: Hyperinsulinism complicating control of diabetes mellitus by an artificial beta cell. Diabetes Care 1980; 3:274–77.