Diabetologia (1986) 29: 475-480

Diabetologia

Studies on overnight insulin requirements and metabolic clearance rate of insulin in normal and diabetic man: relevance to the pathogenesis of the dawn phenomenon

P. De Feo, G. Perriello, M. M. Ventura, F. Calcinaro, G. Basta, C. Lolli, C. Cruciani, A. Dell'Olio, F. Santeusanio, P. Brunetti and G. B. Bolli

Istituto Patologia Medica, Università di Perugia, Perugia, Italy

Summary. In order to assess whether the metabolic clearance of insulin changes overnight, 11 patients with Type 1 (insulin-dependent) diabetes and low insulin antibody titre, and 6 nondiabetic subjects were studied. In these studies insulin was always infused by a Harvard pump. Initially, the nocturnal insulin requirements were assessed in the diabetic patients by an overnight feedback insulin infusion to maintain euglycaemia. The insulin requirements decreased continuously after midnight to a nadir of 0.115 ± 0.014 mU · kg^{-1} · min^{-1} at 04.30 hours, but after 05.00 hours the insulin requirements increased nearly 40 percent to a maximum of 0.16 ± 0.012 mU · kg^{-1} · min^{-1} at 07.00 hours. To assess whether plasma insulin clearance changes overnight, the diabetic patients were studied on two different occasions, from 22.00-02.30 hours and from 04.00-08.30 hours. During each of these two studies insulin was infused in sequential steps of 90 min each at the rate of 0.13, 0.40 and 0.20 mU · kg^{-1} · min^{-1}. Despite changes in plasma free insulin concentration, the metabolic clearance of insulin in the interval 22.00-02.30 hours (12.6 ± 0.17 ml · kg^{-1} · min^{-1}) was no different from that of the interval 04.00-08.30 hours (12.5 ± 0.19 ml · kg^{-1} · min^{-1}). The nondiabetic subjects were studied on two different occasions to assess whether the metabolic clearance of insulin changes overnight. Somatostatin (0.25 mg/h) and insulin (0.3 mU · kg^{-1} · min^{-1}) were infused from 22.00-02.30 hours on one occasion, and from 04.00-08.30 hours on the other. The metabolic clearance of plasma free insulin in the interval 22.00-02.30 hours was no different from that of the interval 04.00-08.30 hours (12.6 ± 0.20 vs 12.9 ± 0.25 ml · kg^{-1} · min^{-1}), nor was it different from that of the diabetic patients.

It is concluded that, first, the metabolic clearance rate of insulin does not change overnight either in diabetic or in nondiabetic subjects; second, that it is independent of plasma insulin concentration; and third, that its value is comparable in nondiabetic subjects and in diabetic patients with a low titre of insulin antibodies. Thus, changes in insulin sensitivity rather than changes in insulin clearance are implicated in the pathogenesis of the dawn phenomenon.

Key words: Type 1 diabetes mellitus, dawn phenomenon, metabolic insulin clearance, plasma free insulin, overnight insulin requirements.

It is well known that the rate of intravenous insulin infusion necessary to maintain euglycaemia overnight in subjects with Type 1 (insulin-dependent) [1-5] and Type 2 (non-insulin-dependent) diabetes mellitus [6] increases between 04.00-08.00 hours. Similarly, the rate of endogenous insulin secretion [7] as well as the peripheral plasma insulin concentration [7, 8] increases in nondiabetic subjects in the early morning. The pathogenesis of this condition, commonly referred to as the dawn phenomenon, is still controversial.

Several studies indicate that the dawn phenomenon is due to a decrease in insulin sensitivity induced by nocturnal surges of growth hormone secretion [9-11]. Nevertheless, some investigators have suggested that an increase in the metabolic clearance of insulin in the early morning, rather than a decrease in insulin sensitivity, is the primary cause of the dawn phenomenon [4, 5, 12]. However, in two recent studies the metabolic clearance of insulin did not change overnight during constant subcutaneous [13] or intravenous [14] insulin infusion in a group of diabetic patients who exhibited a dawn increase in insulin requirements. Thus, it is presently controversial whether overnight changes in metabolic insulin clearance play a role in the pathogenesis of the dawn phenomenon.

The present series of studies were undertaken to assess whether the metabolic clearance of insulin changes overnight, and the extent to which these changes eventually contribute to the increased insulin requirements at dawn in patients with Type 1 diabetes as well as in nondiabetic subjects. A Harvard pump was used in all the present studies to infuse insulin because

it has been reported that insulin delivery may wane during prolonged Biostator infusion but not during Harvard pump infusion [14-16].

Subjects and methods

Subjects

Informed consent was obtained from 11 patients with Type 1 diabetes mellitus and from 6 normal non-diabetic subjects. The diabetic patients (7 men, 4 women), aged 20-44 years (29 ± 3 years, mean ± SEM), had a duration of diabetes of 4-28 years (10 ± 2 years), and had no residual endogenous insulin secretion as assessed by the plasma C-peptide response to intravenous glucagon [17]. Their percent HbA_{1c} was 8.41 ± 0.44 [18] (normal range 5.1-6.9%, e.v. 3.7 ± 0.4%) and the percent plasma anti-insulin antibodies at B_o was 5.4 ± 0.6 [19]. The diabetic patients had a normal renal function (creatinine clearance 99 ± 3 ml/min). Their body mass index was 21.5 ± 0.64 kg/m^2. They were on a therapeutic regimen of 2 or 3 daily injections of insulin (Actrapid MC and Monotard MC, Novo Industries, Copenhagen, Denmark). The nondiabetic patients (all males, aged 30 ± 2 years, body mass index 22.8 ± 75 kg/m^2) belonged to a group of normal subjects in whom a dawn phenomenon had recently been demonstrated [7]. None of the diabetic patients and control subjects was receiving any drug treatment (other than insulin for the diabetics) at the time of the study.

Assessment of overnight insulin requirements and plasma free insulin clearance in the diabetic patients

The diabetic patients underwent two series of studies. Initially, the magnitude of their dawn phenomenon was defined by assessing their overnight insulin requirements. Subsequently, the possible changes in overnight metabolic insulin clearance were studied. Only these latter studies were performed in the nondiabetic subjects, in whom the pattern of overnight endogenous insulin secretion had been recently described [7].

In order to assess the overnight insulin requirements in the diabetic patients, intermediate-acting insulin was withdrawn for at least 48 h before the studies and the subjects received regular insulin (Actrapid MC) before breakfast, lunch, dinner and at 23.00 and at 03.00 hours on the basis of capillary blood glucose concentration. On the day of the study, the last subcutaneous injection of regular insulin was given at lunch. Thereafter, between 17.00-18.00 hours the subjects were placed in bed, and a G-18 catheter-needle was placed into a superficial forearm vein for infusion of insulin (Actrapid MC, diluted to a final concentration of 0.5 U/ml in 0.9% NaCl solution containing 0.5% human albumin, Immuno S.p.A., Pisa, Italy) and glucose (20% solution) by means of separate Harvard pumps (Harvard Apparatus, South Natick, Mass, USA). A G-21 butterfly needle was inserted retrogradely into a dorsal vein of the contralateral hand which was kept warm in a thermoregulated glass box at 65 °C in order to ensure arterialization of venous blood [20]. This line was used for blood sampling every 5-10 min for immediate measurement of plasma glucose concentration at bedside. Between 18.00-18.30 hours the subjects consumed a standard meal (725 kcal, 45% carbohydrate, 30% fat, 25% protein), and insulin was infused at variable rates based on an empiric feedback principle [21] in order to prevent an increase in plasma glucose concentration above 10 mmol/l with an insulin infusion rate no greater than 1.5 mU · kg^{-1} · min^{-1} for the first 2 h after the meal. Thereafter, between 20.30-09.00 hours on the next morning, the plasma glucose concentration was maintained between 4.5 and 5.5 mmol/l according to the following algorithm:

$$IR = RI\left(\frac{G-BI}{QI}+1\right)^2$$

where IR is the insulin infusion rate (mU/min), RI is the insulin infusion rate (mU/min) when the measured plasma glucose concentration (G) equals the BI (target plasma glucose = 5 mmol/l). In these experiments, RI was arbitrarily set at the 15% of the body weight expressed in kilograms. QI is a constant called static gain (1.6 mmol/l).

In order to assess whether overnight changes in metabolic insulin clearance contribute to the increases in insulin requirements at dawn in Type 1 diabetes mellitus, the diabetic patients were restudied 1-2 months later on two separate nights, from 22.00-02.30 hours on one occasion, and a week later from 04.00-08.30 hours. The sequence of these studies was varied at random. On both occasions the subjects were withdrawn from their intermediate-acting insulin for at least 48 hours before the studies and regular insulin was given five times daily as described above. On the day of the study the last subcutaneous insulin injection was given at breakfast when the study started at 22.00 hours or at lunch when the study started at 04.00 hours. Twelve hours prior to the studies, the patients were placed in bed and two contralateral intravenous lines were started, one for infusion of insulin and glucose by separate Harvard pumps, and the other for intermittent blood sampling, as described above. Ten hours prior to the studies the patients consumed a standard meal (725 kcal, 45% carbohydrate, 30% fat and 25% protein). Insulin was infused as described above in order to maintain plasma glucose concentration lower than 10 mmol/l during the first 2 h after the meal and between 4.5 and 5.5 mmol/l thereafter. At 22.00 hours or, on the other occasion at 04.00 hours, the mean insulin infusion rate necessary to maintain euglycaemia over the previous 60 min was calculated and kept constant for the subsequent 90 min until 23.30 hours or, on the other occasion, 05.30 hours. Thereafter, the insulin infusion rate was increased to 0.4 mU · kg^{-1} · min^{-1} for an additional 90 min (from 23.30-01.00 hours or, on the other occasion from 05.30-07.00 hours). Finally, the insulin infusion rate was decreased to 0.20 mU · kg^{-1} · min^{-1} for another 90 min (from 01.00-02.30 hours, or from 07.00-08.30 hours). Glucose was infused at variable rates in order to maintain plasma glucose concentration between 4 and 8 mmol/l. Arterialized-venous blood samples were drawn for measurement of plasma free insulin after 60, 70, 80 and 90 min of each of the steps of insulin infusion.

Assessment of overnight metabolic insulin clearance in the nondiabetic subjects

In order to assess whether the metabolic insulin clearance changes overnight in normal man, the non-diabetic subjects were studied on two separate occasions, from 22.00-02.30 hours on one occasion, and a week later from 04.00-08.30 hours. The sequence of the studies was varied at random. The subjects consumed their meal 10 h prior to the studies, they were placed in bed at least 6 h prior to the studies and contralateral intravenous lines were started as described above for the diabetic subjects. At 21.30 hours (or at 03.30 hours) an infusion of somatostatin (0.25 µg/h, Stilamin, Serono, Milan, Italy) and insulin (0.3 mU · kg^{-1} · min^{-1}) was started and continued for 5 h.

Somatostatin and insulin were dissolved in 0.9% NaCl solution containing 1% human albumin and were infused by separate Harvard pumps. Glucose (20% solution) was infused at a variable rate in order to maintain plasma glucose concentration between 4 and 8 mmol/l. Arterialized-venous blood samples were drawn every 30 min for measurement of free insulin and C-peptide. Diabetic as well as nondiabetic subjects were able to sleep between midnight and 07.00 hours.

Plasma glucose was determined by a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, Calif., USA). Plasma free insulin was determined both in diabetic and in nondiabetic subjects by the method of Kuzuya et al. [22]. Polyethylene glycol precipitation was performed in stored plasma samples, after a pre-incubation at 37 °C for 2 h. In our laboratory the intra-assay coefficient of variation is 9% and the interassay coefficient of variation is 11% at plasma free insulin concentration of 20 mU/l. Plasma C-peptide was determined as previously described [23].

Statistical analysis

Data are given as mean ± SEM. Whole body metabolic clearance rate of insulin (MCR) was calculated according to the dilution principle

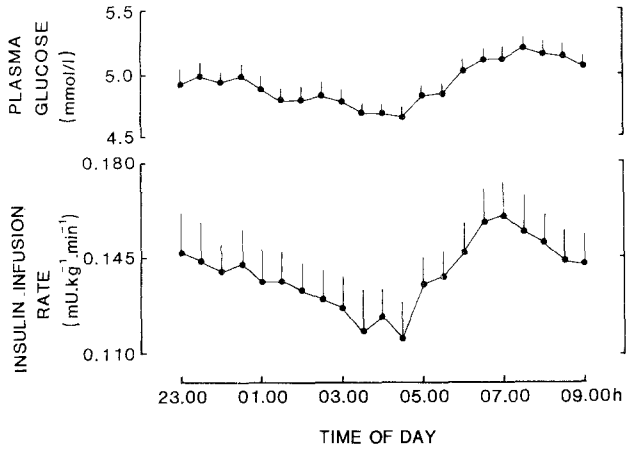

Fig. 1. Overnight insulin requirements in 11 patients with Type 1 diabetes mellitus. Insulin was infused by a Harvard pump. Data are expressed as mean ± SEM

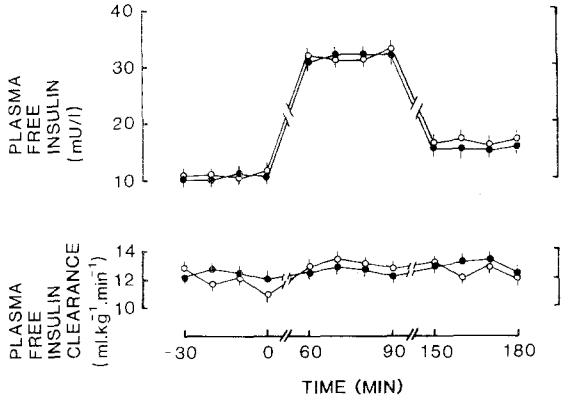

Fig. 2. Plasma free insulin concentrations and clearance during sequential stepwise infusions of insulin at 0.13, 0.40 and 0.20 mU·kg^{-1}·min^{-1} for 90 min each in 11 patients with Type 1 diabetes mellitus. The studies were performed on two different nights from 22.00-02.30 hours (●—●), and from 04.00-08.30 hours (○—○). Insulin was infused by a Harvard pump. Data are expressed as mean ± SEM

[24], as the ratio of the insulin infusion rate to the steady-state arterialized-venous plasma exogenous insulin concentration both in diabetic and nondiabetic subjects. In the nondiabetic subjects the assumption was made that suppression of endogenous insulin secretion by somatostatin would be virtually complete. Statistical analysis was performed by using the paired and unpaired Student's t-tests and the analysis of variance [25].

Results

Overnight insulin requirements in the diabetic patients

During the feedback insulin infusion, the plasma glucose concentration was relatively stable between 23.00-02.00 hours, but after 02.30 hours it decreased progressively to a nadir of 4.65 ± 0.04 mmol/l at 04.30 hours. Thereafter, plasma glucose concentration increased progressively to a peak of 5.2 ± 0.05 mmol/l at 07.30 hours ($p < 0.001$ as compared to the 04.30 hours value). The mean plasma glucose concentration over the 06.00-09.00 hour interval was greater than that over the 02.00-05.30 hour interval (5.11 ± 0.05 versus 4.74 ± 0.03 mmol/l, $p < 0.001$), but no different from that of the interval 23.00-01.30 hours. The insulin requirements decreased continuously after midnight to a nadir of 0.115 ± 0.014 mU·kg^{-1}·min^{-1} at 04.30 hours. However, after 05.00 hours the insulin requirements increased abruptly to a maximum of 0.16 ± 0.012 mU·kg^{-1}·min^{-1} at 07.00 hours. The mean insulin requirements in the interval 06.00-09.00 hours (0.15 ± 0.001 mU·kg^{-1}·min^{-1}) was approximately 18% greater thanthose of the interval 02.00-05.30 hours (0.127 ± 0.01 mU·kg^{-1}·min^{-1}, $p < 0.001$), but only 6% greater than those of the interval 23.00-01.30 hours (0.141 ± 0.01 mU·kg^{-1}·min^{-1}, $p < 0.001$) (Fig. 1).

Overnight metabolic clearance rate of insulin in the diabetic patients

In the study 21.00-02.30 hours the mean insulin infusion rate required to maintain euglycaemia between 21.00-22.00 hours was 0.13 ± 0.09 mU·kg^{-1}·min^{-1}. When such an infusion rate was maintained constant between 22.00-23.30 hours, plasma free insulin concentration was 10.6 ± 0.7 mU/l and the whole body metabolic clearance rate of insulin (MCR) was 12.3 ± 0.38 ml·kg^{-1}·min^{-1}. Subsequently, when the insulin infusion rate was increased to 0.4 mU·kg^{-1}·min^{-1} from 23.30-01.00 hours, plasma free insulin concentration increased to 32 ± 1 mU/l, but the mean value for MCR did not change (12.6 ± 0.44 versus 12.3 ± 0.38 ml·kg^{-1}·min^{-1}, p = NS). Finally, when the insulin infusion rate was decreased to 0.20 mU·kg^{-1}·min^{-1} from 01.00-02.30 hours, plasma free insulin concentration decreased to 15.6 ± 0.42 mU/l, but the MCR remained unchanged (12.9 ± 0.36 ml·kg^{-1}·min^{-1}, p = NS versus the two previous values).

In the study 04.00-08.30 hours, the mean insulin infusion rate required to maintain euglycaemia between 03.00-04.00 hours was 0.127 ± 0.01 mU·g^{-1}·min^{-1} (p = NS as compared to the insulin infusion rate between 21.00-22.00 hours of the previous study). When this infusion rate was kept constant, plasma free insulin concentration was 10.8 ± 0.8 mU/l and the MCR 12 ± 0.77 ml·kg^{-1}·min^{-1}. When the insulin infusion was increased to 0.4 mU·kg^{-1}·min^{-1} from 05.30-07.00 hours, the plasma free insulin concentration was 31.7 ± 1.3 mU/l and the MCR 12.9 ± 0.6 ml·kg^{-1}·min^{-1}. Finally, when the insulin infusion rate was decreased to 0.20 mU·kg^{-1}·min^{-1} from 07.00-08.30 hours, plasma free insulin concentration was 16.2 ± 0.6 mU/l and the MCR 12.5 ± 0.5 ml·kg^{-1}·min^{-1}. All these values were not significantly different from those observed in the intervals 22.00-23.30 hours, 23.30-01.00 hours, 01.00-02.30 hours of the previous study. The overall MCR in the interval 22.00-02.30 hours (12.6 ± 0.2 ml·kg^{-1}·

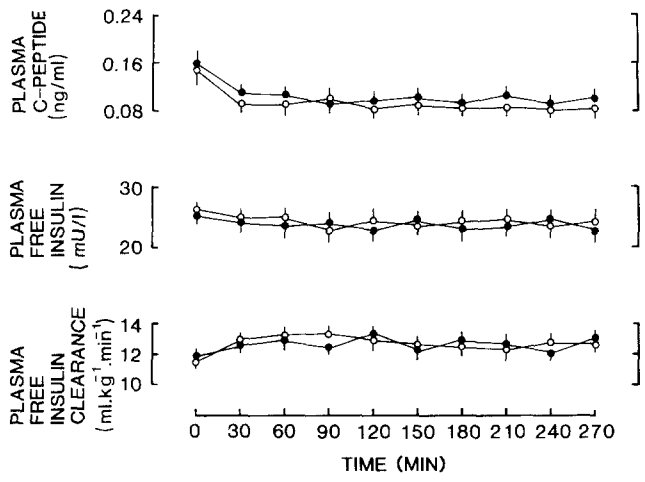

Fig. 3. Plasma C-peptide and free insulin concentration and plasma free insulin clearance in 6 non-diabetic subjects during an infusion of somatostatin (0.25 mg/h) and insulin ($0.30\,mU \cdot kg^{-1} \cdot min^{-1}$). The studies were performed on two different nights, from 20.00-02.30 hours (●—●) and from 04.00-08.30 hours (○—○). Insulin was infused by a Harvard pump. Data are expressed as mean ± SEM

min^{-1}) was no different from that of the interval 04.00-08.30 hours ($12.4 \pm 0.3\,ml \cdot kg^{-1} \cdot min^{-1}$, $p = NS$) (Fig. 2).

Overnight metabolic insulin clearance in the non-diabetic subjects

In the non-diabetic subjects, the infusion of somatostatin resulted in a virtually complete suppression of endogenous insulin secretion, as shown by the plasma C-peptide concentration in both studies, between 22.00-02.30 hours (0.10 ± 0.01 ng/ml) and between 04.00-08.30 hours (0.09 ± 0.002 ng/ml, $p = NS$). Insulin infusion resulted in a plateau plasma free insulin concentration of 23.9 ± 0.37 mU/l between 22.00-02.30 hours and 23.3 ± 0.43 mU/l between 04.00-08.30 hours ($p = NS$). The mean value for MCR in the interval 22.00-02.30 hours ($12.6 \pm 0.20\,ml \cdot kg^{-1} \cdot min^{-1}$) was no different as compared to that of the interval 04.00-08.30 hours ($12.9 \pm 0.25\,ml \cdot kg^{-1} \cdot min^{-1}$ $p = NS$). Finally, the overall value for MCR determined overnight in the non-diabetic subjects was no different from that of the diabetic patients (12.7 ± 0.7 and $12.5 \pm 0.16\,ml \cdot kg^{-1} \cdot min^{-1}$, respectively, $p = NS$) (Fig. 3).

Discussion

The present study demonstrates, first, that the MCR of insulin does not change overnight either in patients with Type 1 diabetes mellitus, or in non-diabetic individuals studied at physiological plasma insulin concentration.

Second, in the diabetic patients the MCR of insulin did not change despite fluctuations in plasma insulin concentration in the therapeutic range (10-40 mU/l). This finding indicates that the MCR of insulin in diabetic patients with a low titre of insulin antibodies is linear over the physiological range of the plasma hormone level, i.e. that the MCR of insulin is independent of plasma insulin concentrations. Although two studies [26, 27] have suggested that MCR of insulin decreases with increasing plasma insulin concentrations, our results that MCR is independent of concentration are in agreement with all the other studies which have examined insulin kinetics in vivo [28-33]. The discrepancy between the results of the studies in which MCR of insulin has been found to be independent of plasma insulin concentration [28-33, and the present study] and the others [26, 27] are probably explicable on the basis of methodological differences [26] as well as the way MCR_I was calculated [27] which leads to an overestimation of MCR_I at low plasma insulin concentration (the subtraction of basal insulin from the steady-state arterial insulin concentration during insulin infusion in the denominator of the MCR_I formula in this last study does not seem to be appropriate since the contribution of endogenous insulin production is part of the numerator).

Third, in the present study the diabetic patients who had a low titre of insulin antibodies ($5.4 \pm 0.9\%$ at B_o) showed a value for MCR comparable to that of non-diabetic subjects in whom the endogenous insulin secretion was suppressed by somatostatin (approximately $12\,ml \cdot kg^{-1} \cdot min^{-1}$ in both groups). It is important to note, however, that the value for MCR of insulin depends on the titre of circulating insulin antibodies [34]. Specifically, diabetic patients with binding greater than 10% have values of MCR which are considerably greater than those of nondiabetic subjects and of diabetic patients with a binding lower than 10% [34, 35].

In the present experiments, somatostatin was used to suppress endogenous insulin secretion in the non-diabetic subjects. Since somatostatin decreases the splanchnic blood flow [36], it could theoretically decrease the hepatic insulin clearance and thereby invalidate any comparison of MCR with the diabetic patients not receiving somatostatin. However, it has been shown in dogs [37] and in humans [30, 38] that somatostatin does not effect the splanchnic extraction ratio of insulin. Thus, the results of the present study indicate that the values for MCR of insulin in non-diabetic and in diabetic subjects are virtually identical, at least over a physiological range of insulin concentration.

Several studies have suggested that MCR of insulin increases in the early morning in Type 1 diabetes mellitus [3-5, 9, 10, 12]. Since in two of these studies insulin sensitivity did not appear to change overnight [5, 12], it was concluded that the increase in the insulin requirements in the early morning in Type 1 diabetes is due solely to an increase in MCR of insulin [5, 12].

In some of these studies [3, 9, 10] a closed-loop insulin infusion device (Biostator) was used to infuse insulin, whereas in the present studies a Harvard pump was

used for all the insulin infusions. This difference in manner in which insulin was infused may explain the discrepancy between the results of studies using this closed-loop device and the present study. Three recent reports [14-16] indicate that insulin delivery by the closed-loop device wanes over time. Brennan et al. [15] found that the immunoreactivity and bioactivity of insulin in the infusate of the closed-loop device decreases more than 50% over 6 h and that this was apparently due to pump-induced aggregation of insulin at the low infusion rates used clinically (approximately 2 ml/h). This was not observed at infusion rates greater than 16 ml/h or when a Harvard pump was used. Such a decrease in Biostator insulin delivery would result in a decrease in circulating insulin concentrations and thus an apparent increase in MCR of insulin. In two different centers, however, an overnight increase in MCR of insulin was observed during an infusion of insulin by a syringe pump [4, 5, 12]. In the studies by Dux et al. [5] and Skor et al. [12], however, the plasma free insulin levels were nearly two-fold greater than those expected from the nominal insulin infusion rates used [39-41]. Furthermore, the rates of insulin clearance in those two studies were markedly different from those generally found. Taken together these observations suggest that the finding of overnight increase in MCR of insulin in those two studies [5, 12] was probably due to some unprecision of the free insulin assay. Consistent with this interpretation is the observation that in those two studies [5, 12] there was no change in insulin sensitivity, which one would have expected to decrease if MCR of insulin had increased significantly and contributed to the dawn phenomenon. Kerner et al. [4] reported an early morning increase in MCR of insulin in some of the eight diabetic patients infused overnight with insulin by a syringe pump [4]. However, these authors observed a meaningful increase in blood glucose at dawn only in the two subjects in whom MCR of insulin increased by more than 15%, thus suggesting that an overnight increase in MCR of insulin might well be the exception rather than the rule in diabetic patients.

The conclusion of the present study, that MCR of insulin does not change overnight in Type 1 diabetes, is in agreement with the study of Koivisto et al. [13] as well as with that of Campbell et al. [14], who found an increase in insulin requirements at dawn in the absence of an increase in MCR of insulin in a group of subjects with Type 1 diabetes treated with continuous subcutaneous [13] or intravenous [14] infusion of insulin at a constant rate.

In conclusion, the present experiments demonstrate that the metabolic clearance rate of insulin does not change overnight either in patients with Type 1 diabetes mellitus, or in nondiabetic subjects, despite changes in plasma insulin concentration in a therapeutic and physiologic range. Thus, since in all the diabetic and nondiabetic subjects of the present experiments the insulin requirements increased in the early morning, it is concluded that the dawn phenomenon is explained solely by an abrupt change in insulin sensitivity.

Acknowledgements. The editorial assistance of Ms. P. Boyce is gratefully acknowledged. This work was supported by the Consiglio Nazionale delle Richerche (C. N. R. finalized project, Complications of Diabetes, Ob-46; and C. N. R. grant 86.00021.04).

References

1. Clarke WL, Haymond MW, Santiago JV (1980) Overnight basal insulin requirements in fasting insulin-dependent diabetics. Diabetes 29: 78-80
2. Bright GM, Melton TW, Rogal AD, Clarke WL (1980) Failure of cortisol blockade to inhibit early morning increases in basal insulin requirements in fasting insulin-dependent diabetics. Diabetes 29: 662-664
3. Skor DA, White HN, Thomas L, Shah SD, Cryer PE, Santiago JV (1983) Examination of the role of the pituitary-adrenocortical axis, counterregulatory hormones and insulin clearance in variable nocturnal insulin requirements in insulin-dependent diabetics. Diabetes 32: 403-407
4. Kerner W, Navascués I, Torres AA, Pfeifer EF (1984) Studies on the pathogenesis of the dawn phenomenon in insulin-dependent diabetic patients. Metabolism 33: 458-464
5. Dux S, White NH, Skor DA, Santiago JV (1985) Insulin clearance contributes to the variability of nocturnal insulin requirement in insulin-dependent diabetes mellitus. Diabetes 34: 1260-1265
6. Bolli GB, Gerich JE (1984) The "Dawn Phenomenon" - a common occurrence in both non insulin-dependent and insulin-dependent diabetes mellitus. N Engl J Med 310: 746-750
7. Bolli GB, De Feo P, De Cosmo S, Perriello G, Ventura MM, Calcinaro F, Lolli C, Campbell P, Brunetti P, Gerich JE (1984) Demonstration of a dawn phenomenon in normal human volunteers. Diabetes 33: 1150-1153
8. Schmidt MI, Lin QX, Gwynne JT, Jacobs S (1984) Fasting early morning rise in peripheral insulin: evidence of the dawn phenomenon in nondiabetes. Diabetes Care 7: 32-35
9. Campbell PJ, Bolli GB, Cryer PE, Gerich JE (1985) Pathogenesis of the dawn phenomenon in patients with insulin-dependent diabetes mellitus. N Engl J Med 312: 1473-1479
10. Arias P, Kerner W, Pfeiffer EF (1984) Suppression of the dawn phenomenon by somatostatin. Diabetologia 27: 252A
11. Orskov H, Schmitz O, Christiansen J (1985) More evidence of growth hormone's role in the dawn phenomenon. Diabetes Res Clin Pract (Suppl 1): 426
12. Skor DA, White NH, Thomas L, Santiago JV (1984) Relative roles of insulin clearance and insulin sensitivity in the prebreakfast increase in insulin requirements in insulin-dependent diabetic patients. Diabetes 33: 60-63
13. Koivisto VA, Yki-Järvinen H, Helve E, Karonen SL, Pelkonen R (1986) Pathogenesis and prevention of the dawn phenomenon in diabetic patients treated with CSII. Diabetes 35: 78-82
14. Campbell P, Cryer P, Gerich JE (1986) Occurrence of the dawn phenomenon without a change in insulin clearance in patients with insulin-dependent diabetes mellitus. Diabetes 35: 749-752
15. Brennan JR, Gebhart SSP, Blackard WG (1985) Pump-induced insulin aggregation: a problem with the Biostator. Diabetes 34: 353-359
16. Harris M, Davidson M, Rosenberg C (1986) A simple solution to the problem of Biostator-induced insulin aggregation. Clin Res 34: 59A
17. Faber OK, Binder C (1977) C-peptide response to glucagon: a test for the residual β-cell function in diabetes mellitus. Diabetes 26: 605-610
18. Trivelli LA, Ranney HM, Lai HT (1971) Hemoglobin components in patients with diabetes mellitus. N Engl J Med 284: 353-357

19. Bolli GB, Dimitriadis GD, Pehling GB, Baker BA, Haymond MW, Cryer PE, Gerich JE (1984) Abnormal glucose counterregulation after subcutaneous insulin in insulin-dependent diabetes mellitus. N Engl J Med 310: 1706-1711
20. McGuire E, Helderman J, Tobin J, Andres R, Berman M (1976) Effects of arterial versus venous sampling on analysis of glucose kinetics in man. J Appl Physiol 41: 565-573
21. Bolli GB, De Feo P, Perriello G, De Cosmo S, Ventura M, Campbell P, Brunetti P, Gerich JE (1985) Role of hepatic autoregulation in defense against hypoglycaemia in humans. J Clin Invest 75: 1623-1631
22. Kuzuya H, Blix PM, Horwitz DL, Steiner DF, Rubenstein AH (1977) Determination of free and total insulin and C-peptide in insulin-treated diabetics. Diabetes 26: 22-29
23. Faber O, Binder C, Markussen J, Heding L, Naithani V, Kuzuya H, Blix P, Horwitz D, Rubenstein A (1978) Characterization of seven C-peptide antisera. Diabetes 27 (Suppl 1): 170-177
24. Shipley RA, Clark RE (1972) Tracer methods for in vivo kinetics. Theory and applications. Academic, New York
25. Zar JH (1984) Biostatistical analysis. Prentice-Hall, Englewood Cliffs, New Jersey
26. Sönksen P, Tompkins C, Srivastava M, Nabarro J (1973) A comparative study on the metabolism of human insulin and porcine proinsulin in man. Clin Sci Mol Med 45: 633-645
27. Waldhäusl WK, Gasić S, Bratusch-Marrain P, Korn A, Nowotny P (1982) Feedback inhibition by biosynthetic human insulin of insulin release in healthy human subjects. Am J Physiol 243: E476-E482
28. Sherwin RS, Kramer KJ, Tobin JD, Insel PA, Liljenquist JE, Berman M, Andres R (1974) A model of the kinetics of insulin in man. J Clin Invest 53: 1481-1492
29. Traneberg KG, Dencker H (1978) Modeling of plasma disappearance of unlabelled insulin in man. Am J Physiol 235: E577-E585
30. Ferranini E, Wahren J, Faber OK, Felig P, Binder C, De Fronzo RA (1983) Splanchnic and renal metabolism of insulin in human subjects: a does-response study. Am J Physiol 244: E517-527
31. Polonsky K, Jaspan J, Emmanouel D, Holmes K, Moossa AR (1983) Differences in the hepatic and renal extraction of insulin and glucagon in the dog: evidence for saturability of insulin metabolism. Acta Endocrinol 102: 420-427
32. Bratusch-Marrain PR, Waldhäusl WK, Gasić S, Hofer A (1984) Hepatic disposal of biosynthetic human insulin and porcine C-peptide in humans. Metabolism 33: 151-157
33. Cobelli C, Mari A, Ferranini E (1986) On the linearity of insulin kinetics. Am J Physiol (Endocrinol Metab) E 247-E 248
34. Van Haeften TW, Bolli GB, Dimitriadis GD, Gottesman IS, Horwitz DL, Gerich JE (1986) Effect of insulin antibodies and their kinetic characteristics on plasma free insulin dynamics in patients with diabetes mellitus Metabolism 35: 649-656
35. Waldhäusl W, Bratusch-Marrain P, Kruse V, Jensen I, Nowotny P, Vierhapper H (1985) Effect of insulin antibodies on insulin pharmacokinetics and glucose utilization in insulin-dependent diabetic patients. Diabetes 34: 166-173
36. Wahren J, Felig P (1976) Influence of somatostatin on carbohydrate disposal and absorption in diabetes mellitus. Lancet 2: 1213-1216
37. Jaspan JB, Polonsky KS, Lewis M, Pensler J, Pugh W, Moossa AR, Rubenstein AH (1981) Hepatic metabolism of glucagon in the dog: contribution of the liver to overall metabolic disposal of glucagon. Am J Physiol 240: E233-E244
38. Donner CC, Chen Y-DI, Fraze E, Moore J, Reaven GM (1985) Metabolic clearance rate of insulin in patients with noninsulin dependent diabetes mellitus. Clin Res 33: 59A (Abstract)
39. De Fronzo R, Hendler R, Simonson D (1982) Insulin resistance is a prominent feature of insulin-dependent diabetes. Diabetes 31: 795-801
40. Kolterman O, Insel J, Saekow M, Olefsky J (1980) Mechanisms of insulin resistance in human obesity: evidence for receptor and postreceptor defects. J Clin Invest 65: 1272-1284
41. Rizza R, Mandarino L, Gerich J (1981) Dose-response characteristics for the effects of insulin on production and utilization of glucose in man. Am J Physiol 240: E630-E639

Received: 28 February 1986
and in revised form: 16 June 1986

Dr. Geremia B. Bolli
Istituto di Patologia Medica
Via E. Dal Pozzo
I-06100 Perugia
Italy