Absorption Kinetics of Semisynthetic Human Insulin and Biosynthetic (recombinant DNA) Human Insulin

F. W. KEMMER, G. SONNENBERG, H. J. CÜPPERS, AND M. BERGER

Absorption kinetics of subcutaneously injected human insulin (recombinant DNA) and semisynthetic human regular insulin were investigated and compared to the respective porcine insulin preparations in normal volunteers. The absorption of all regular human insulin preparations tested was accelerated as compared to porcine insulins. The clinical relevance of these findings in regard to the treatment of diabetes mellitus appears to be doubtful. As for mixtures of porcine regular and intermediate-acting insulin preparations, the absorption of human regular insulin is not altered when mixed with intermediate-acting human insulin. DIABETES CARE 5 (SUPPL. 2): 23–28, 1982.

During the 60 yr of clinical use of insulin in the treatment of diabetes suprisingly little attention has been paid to the absorption process of subcutaneously injected insulin. One reason for this may be the rather limited methodologic approaches by which the absorption process of subcutaneously injected insulin can be assessed accurately. Recently, we have investigated the absorption process of various porcine insulin preparations[1] using a direct method by measuring serum insulin levels at defined intervals after the subcutaneous injection. This method was used because the previously described indirect method[2] was based on several assumptions which have in part been disproved.[1] In our study we demonstrated that the absorption process of regular porcine insulin can be altered by a number of factors such as site of injection, temperature, local massage, exercise, etc. Therefore it is conceivable that the absorption kinetics of regular insulin other than that of porcine origin might also be altered. In order to address this question we have investigated the absorption process of various human insulin preparations and compared it to the absorption process of the appropriate porcine insulin preparation.

METHODS AND MATERIALS

Subjects and standard protocol. Twenty-nine healthy male volunteers, 20–30 yr old and within ±5% of ideal body weight who had never been injected with insulin before participated in the study. The subjects reported to the laboratory in the morning after an overnight fast of 14 h. On arrival in the laboratory a teflon catheter was inserted into an antecubital vein, kept patent with 0.9% saline, and was used for blood sampling at defined intervals. Throughout the experiments, which commenced at 8:00 a.m., the subjects rested in a supine position at constant room temperature and abstained from food, drink, and smoking. After an equilibration period of 30 min, insulin was injected, at time zero, into the front of a thigh using a 1-ml Plastipak syringe (Becton Dickinson, Heidelberg, West Germany). Insulin injections were always performed by the same physician to ensure comparable conditions of the injection technique as to angle, depth, and speed of the injection.

Materials. The following insulin preparations were used: Actrapid porcine and semisynthetic human, and Monotard semisynthetic human, both from Novo Industries GMBH, Mainz, West Germany; insulin Hoe 11 S (Schweineinsulin, neutral) and insulin Hoe 11 H (Humaninsulin, neutral) both from Hoechst AG, Frankfurt (Main), West Germany; regular purified porcine insulin (PPI) and regular human insulin (recombinant DNA) and mixtures of regular and NPH human insulin, all from Eli Lilly and Company, Bad Homburg, West Germany; Mixtard from Nordisk-Hormon-Chemie. The affinity of all tested insulin preparations to the antibody used in our radioimmunoassay system was the same for comparable insulin preparations.

Experimental protocols. The 29 volunteers were subdivided into four groups of 7–8 individuals and assigned to one of the protocols described below. (1) In 7 subjects 10 U of both human and porcine Actrapid were injected subcutaneously on two different occasions. On a third day 10 U of human Actrapid were mixed with 20 U of human Monotard in the same syringe and immediately injected subcutaneously. (2)

TABLE 1
Serum levels of C-peptide and blood lactate and ketone body concentrations after injection of various human and porcine insulin preparations injected subcutaneously in normal humans

Insulin preparation (s.c. injection)	Min								
	−15	0	20	40	60	90	120	150	180
10 U Actrapid, porcine									
C-peptide (ng/ml)	1.34 ± 0.2	1.24 ± 0.23	1.05 ± 0.15	—	0.71 ± 0.13	—	0.38 ± 0.06	—	0.17 ± 0.03
Blood lactate (mmol/L)	0.8 ± 0.04	0.67 ± 0.07	0.66 ± 0.07	0.67 ± 0.06	0.69 ± 0.15	0.72 ± 0.06	0.7 ± 0.07	0.8 ± 0.06	0.84 ± 0.04
Blood ketones (mmol/L)	0.14 ± 0.02	0.11 ± 0.03	0.11 ± 0.02	0.11 ± 0.02	0.08 ± 0.03	0.09 ± 0.01	0.11 ± 0.02	0.08 ± 0.02	0.1 ± 0.02
10 U Actrapid, human									
C-peptide (ng/ml)	1.13 ± 0.25	1.3 ± 0.26	1.01 ± 0.25	—	0.67 ± 0.18	—	0.37 ± 0.13	—	0.3 ± 0.14
Blood lactate (mmol/L)	0.8 ± 0.06	0.79 ± 0.07	0.89 ± 0.11	0.74 ± 0.05	0.17 ± 0.08	0.82 ± 0.08	0.94 ± 0.12	0.9 ± 0.09	0.95 ± 0.12
Blood ketones (mmol/L)	0.13 ± 0.03	0.17 ± 0.05	0.13 ± 0.04	0.11 ± 0.02	0.07 ± 0.01	0.1 ± 0.03	0.09 ± 0.02	0.11 ± 0.03	0.11 ± 0.03
10 U Hoe 11 S									
C-peptide (ng/ml)	0.88 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.51 ± 0.09	0.44 ± 0.09	0.29 ± 0.06	0.17 ± 0.03	0.13 ± 0.02	—
Blood lactate (mmol/L)	1.36 ± 0.24	1.38 ± 0.23	1.03 ± 0.12	0.96 ± 0.1	0.97 ± 0.07	1.12 ± 0.14	1.11 ± 0.18	1.2 ± 0.13	—
Blood ketones (mmol/L)	0.16 ± 0.05	0.22 ± 0.06	0.2 ± 0.05	0.15 ± 0.03	0.12 ± 0.02	0.14 ± 0.03	0.21 ± 0.11	0.15 ± 0.04	—
10 U Hoe 11 H									
C-peptide (ng/ml)	0.83 ± 0.1	0.82 ± 0.1	0.65 ± 0.13	0.48 ± 0.11	0.4 ± 0.1	0.26 ± 0.07	0.17 ± 0.05	0.14 ± 0.03	—
Blood lactate (mmol/L)	1.09 ± 0.08	1.13 ± 0.11	1.03 ± 0.05	0.98 ± 0.08	1.02 ± 0.07	1.1 ± 0.11	1.17 ± 0.06	1.34 ± 0.12	—
Blood ketones (mmol/L)	0.16 ± 0.05	0.18 ± 0.04	0.21 ± 0.05	0.12 ± 0.03	0.14 ± 0.03	0.14 ± 0.02	0.13 ± 0.03	0.18 ± 0.02	—
10 U PPI									
C-peptide (ng/ml)	0.68 ± 0.05	0.71 ± 0.14	0.61 ± 0.1	0.24 ± 0.01	0.16 ± 0.06	0.12 ± 0.05	0.04 ± 0.04	0.00 ± 0.00	0.02 ± 0.02
Blood lactate (mmol/L)	0.95 ± 0.06	0.96 ± 0.08	0.97 ± 0.05	0.96 ± 0.07	0.97 ± 0.06	1.08 ± 0.07	1.08 ± 0.09	1.08 ± 0.06	1.05 ± 0.04
Blood ketones (mmol/L)	0.11 ± 0.03	0.17 ± 0.04	0.15 ± 0.04	0.17 ± 0.03	0.11 ± 0.03	0.13 ± 0.04	0.13 ± 0.03	0.17 ± 0.03	0.16 ± 0.04
10 U human insulin (recombinant DNA)									
C-peptide (ng/ml)	0.64 ± 0.09	0.7 ± 0.19	0.43 ± 0.15	0.29 ± 0.11	0.28 ± 0.1	0.1 ± 0.06	0.04 ± 0.04	0.03 ± 0.03	0.03 ± 0.03
Blood lactate (mmol/L)	1.02 ± 0.09	0.91 ± 0.11	0.9 ± 0.03	0.93 ± 0.06	0.88 ± 0.05	0.94 ± 0.05	1.04 ± 0.06	1.08 ± 0.08	1.11 ± 0.07
Blood ketones (mmol/L)	0.14 ± 0.05	0.12 ± 0.03	0.09 ± 0.02	0.08 ± 0.02	0.13 ± 0.03	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.02	0.13 ± 0.05
20 U human insulin (recombinant DNA) mixtures regular: intermediate-acting									
20%:80%									
C-peptide (ng/ml)	0.78 ± 0.06	0.75 ± 0.04	0.64 ± 0.06	0.5 ± 0.06	0.34 ± 0.04	0.23 ± 0.04	0.17 ± 0.03	0.15 ± 0.02	0.1 ± 0.00
Blood lactate (mmol/L)	0.92 ± 0.04	0.92 ± 0.06	0.85 ± 0.04	0.86 ± 0.07	0.84 ± 0.06	0.97 ± 0.08	1.0 ± 0.06	0.99 ± 0.07	0.98 ± 0.07
Blood ketones (mmol/L)	0.09 ± 0.03	0.11 ± 0.03	0.11 ± 0.03	0.08 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.02	0.05 ± 0.01
25%:75%									
C-peptide (ng/ml)	0.78 ± 0.04	0.76 ± 0.03	0.61 ± 0.03	0.39 ± 0.04	0.29 ± 0.04	0.19 ± 0.03	0.15 ± 0.02	0.12 ± 0.02	0.1 ± 0.00
Blood lactate (mmol/L)	1.02 ± 0.07	0.95 ± 0.06	0.89 ± 0.06	0.87 ± 0.05	0.92 ± 0.06	0.97 ± 0.07	0.99 ± 0.04	0.98 ± 0.04	1.07 ± 0.05
Blood ketones (mmol/L)	0.15 ± 0.05	0.14 ± 0.05	0.12 ± 0.03	0.07 ± 0.02	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.03	0.07 ± 0.02	0.07 ± 0.01
30%:70%									
C-peptide (ng/ml)	0.7 ± 0.02	0.7 ± 0.02	0.67 ± 0.01	0.44 ± 0.04	0.32 ± 0.03	0.19 ± 0.03	0.13 ± 0.01	0.13 ± 0.01	0.1 ± 0.00
Blood lactate (mmol/L)	0.97 ± 0.07	0.95 ± 0.06	0.83 ± 0.05	0.82 ± 0.07	0.8 ± 0.05	0.93 ± 0.07	1.06 ± 0.13	1.02 ± 0.12	1.0 ± 0.08
Blood ketones (mmol/L)	0.16 ± 0.05	0.18 ± 0.05	0.19 ± 0.04	0.13 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.09 ± 0.04	0.08 ± 0.03	0.08 ± 0.03
20 U Mixtard									
C-peptide (ng/ml)	0.78 ± 0.02	0.77 ± 0.02	0.71 ± 0.03	0.59 ± 0.05	0.49 ± 0.05	0.28 ± 0.04	0.21 ± 0.02	0.16 ± 0.02	0.11 ± 0.01
Blood lactate (mmol/L)	1.04 ± 0.11	0.97 ± 0.09	0.88 ± 0.05	0.84 ± 0.04	0.89 ± 0.02	0.94 ± 0.06	0.98 ± 0.05	1.13 ± 0.09	1.07 ± 0.07
Blood ketones (mmol/L)	0.14 ± 0.03	0.12 ± 0.03	0.14 ± 0.03	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.08 ± 0.02	0.1 ± 0.03

In 7 different subjects 10 U of both human and porcine neutral (Hoechst) were injected on two different occasions. (3) In a third group of 7 volunteers 10 U of both regular PPI and regular human insulin (recombinant DNA) were injected subcutaneously in the same person on different occasions. (4) In each of the remaining 8 subjects 20 U of Mixtard and 20 U of the three mixtures of human insulin (recombinant DNA) were injected in randomized order. The ratios of regular and intermediate-acting human insulin in the mixtures were 20%:80%, 25%:75% and 30%:70%.

Processing of blood samples. Venous blood samples were collected in chilled tubes. One portion of blood was im-

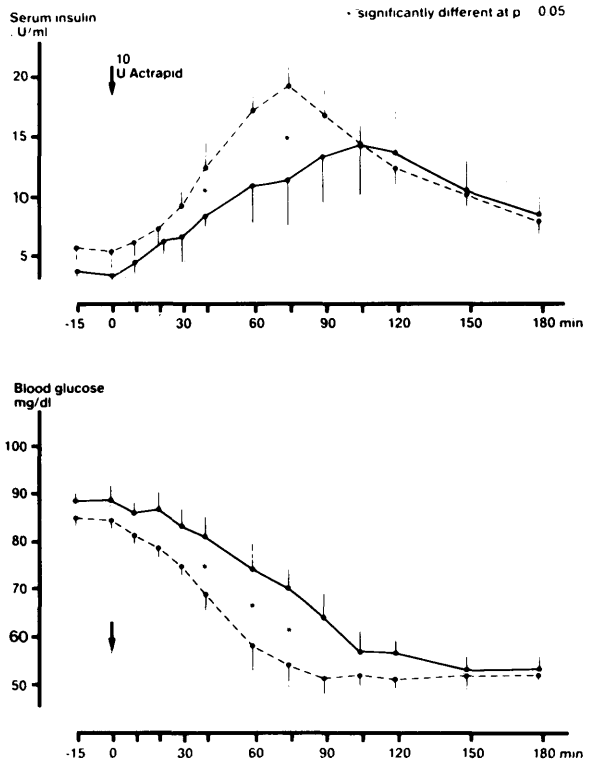

FIG. 1. Serum insulin concentrations and blood glucose levels after subcutaneous injection of 10 U of Actrapid, semisynthetic human (●----●) or porcine (●—●). N = 7.

mediately added to ice-cold perchloric acid (2 mol/L) for deproteinization, mixed, centrifuged, and the neutralized supernatant assayed for lactate, acetoacetate, and 3-hydroxybutyrate.[3] A second portion of the blood was centrifuged and serum was stored at −20°C for later analysis of insulin and C-peptide as described previously.[4] Blood glucose levels were measured from a third portion of blood using a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, California).

Calculations and statistical analysis. In the text, tables, and figures data are given as means ± SEM. For statistical evaluations of experimental conditions in the same subjects, paired analysis was applied. $[IRI]_{max}$ is the peak insulin level observed following the administration of exogenous insulin; $T_{[IRI]_{max}}$ is the time interval between the insulin injection and the time at which $[IRI]_{max}$ was reached.

RESULTS

Under all conditions tested, serum C-peptide concentrations decreased promptly after the subcutaneous injection of insulin (Table 1), indicating an instantaneous inhibition of endogenous insulin secretion. There were no differences between human and porcine insulin preparations with respect to blood lactate and ketone body concentrations (Table 1).

Absorption kinetics of regular porcine and human insulins. When semisynthetic human insulin (Actrapid) was injected, serum insulin levels rose sharply and reached maximal levels of 26.1 ± 7.7 μU/ml while $[IRI]_{max}$ was only 18.0 ± 3.3 μU/ml following the injection of the porcine insulin. However, this difference did not reach statistical significance (Figure 1 and Table 2). The maximal insulin levels were reached approximately 45 min earlier after administration of the human Actrapid as compared to the porcine Actrapid ($P < 0.05$). Plasma glucose levels started to decrease earlier after injection of human insulin, but the nadir reached was similar after both types of insulin (Figure 1).

Injection of neutral, semisynthetic human insulin (Hoe 11 H) resulted in marginally higher serum insulin levels as compared to the neutral porcine insulin (Hoe 11 S) (Figure 2). $[IRI]_{max}$ and $T_{[IRI]_{max}}$ were similar for both insulin preparations (Table 2). However, when comparing the absorption curves of both insulins, plasma insulin levels were significantly higher 135 min after the injection ($P < 0.05$). Blood glucose levels fell slightly lower after the injection of the human insulin, but this difference did not reach statistical significance.

When regular human insulin (recombinant DNA) was injected, maximal insulin levels were approximately 4 μU/ml higher than after injection of regular PPI ($P < 0.05$; Table 2 and Figure 3). However, $T_{[IRI]_{max}}$ was similar after injection of both types of insulin. Although higher insulin levels were reached after the injection of human insulin, the blood glucose responses were the same.

Effects of mixing regular and intermediate-acting human insulin preparations. When a mixture of 20 U human Monotard and of 10 U human Actrapid was injected subcutaneously, serum insulin levels rose sharply and reached similar concentrations as after administration of the regular insulin alone. Consequently, blood glucose levels declined as rapidly as after injection of regular insulin alone (Figure 4).

TABLE 2
Absorption kinetics of various human and porcine insulin preparations injected subcutaneously in normal man

Type and dose of insulin injected	$[IRI]_{max}$ (μU/ml)	$T_{[IRI]_{max}}$ (min)
10 U Actrapid human	26.1 ± 1.8	67.9 ± 6.9*
10 U Actrapid porcine	18.0 ± 3.3	112.9 ± 15.8
10 U Hoe 11 H	27.2 ± 2.0	120.0 ± 10.9
10 U Hoe 11 S	21.8 ± 1.9	117.9 ± 8.9
10 U human insulin (recombinant DNA) regular	24.3 ± 2.5*	70.0 ± 10.0
10 U PPI regular	20.4 ± 2.0	84.0 ± 16.0
20 U human insulin (recombinant DNA) reg/NPH		
20%:80%	25.7 ± 1.7	149.0 ± 23.0
25%:75%	28.7 ± 3.4	161.0 ± 23.0
30%:70%	23.2 ± 2.4	150.0 ± 25.0
10 U Mixtard	29.4 ± 2.3	163.0 ± 37.0

*Significant difference from respective porcine insulin at $P < 0.05$.

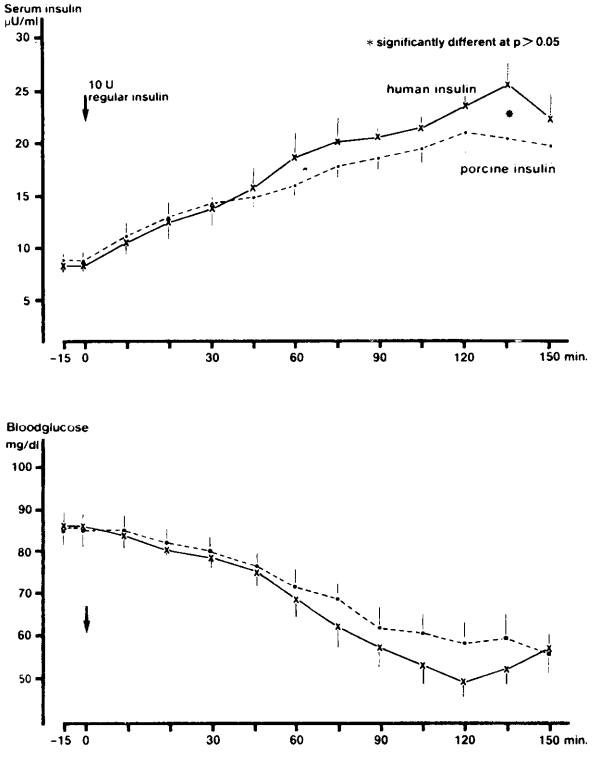

FIG. 2. Serum insulin concentrations and blood glucose levels after subcutaneous injection of 10 U of regular human (Hoe 11 H) or 10 U regular porcine (Hoe 11 S). N = 7.

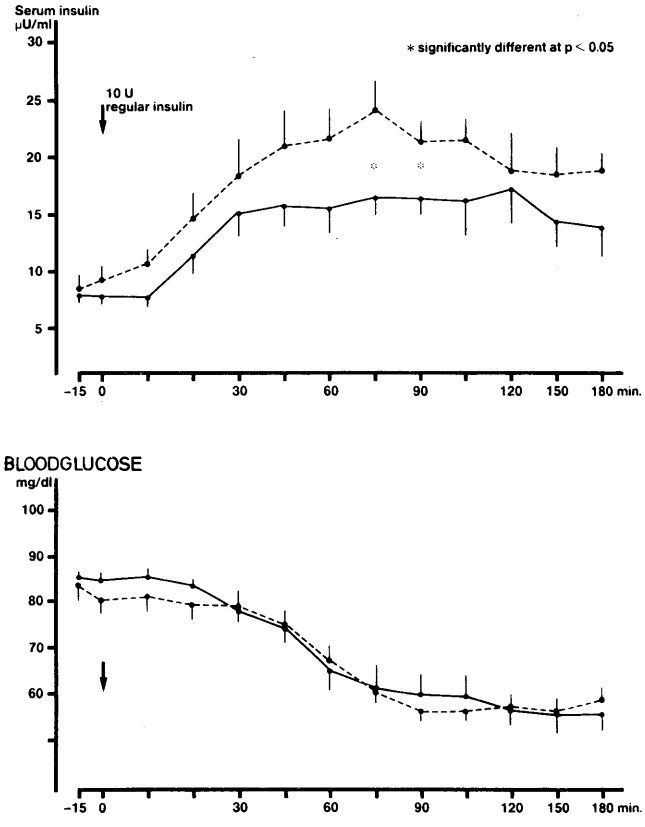

FIG. 3. Serum insulin concentrations and blood glucose levels after subcutaneous injection of 10 U regular human insulin (recombinant DNA) (●----●) or 10 U regular PPI (●—●). N = 7.

Three different mixtures of regular and intermediate-acting human insulin (recombinant DNA) were tested. As expected, serum insulin levels rose sharply after administration of all insulin mixtures, indicating that the absorption of the regular insulin was not affected by the addition of intermediate-acting insulin. Maximal insulin levels were similar for all mixtures of human insulin (recombinant DNA). Since there were no differences in the absorption of these insulins, blood glucose fell at the same rate and to the same nadir after the administration of each type of insulin (Figure 5 and Table 2). Comparison of the absorption curve of the human insulin (recombinant DNA) mixtures to a widely used commercial mixture of porcine insulin also did not reveal differences among the absorption kinetics of these insulins (Figure 6 and Table 2).

The slightly accelerated absorption of human insulin (recombinant DNA) and semisynthetic human insulin from its subcutaneous depot as demonstrated in our experiments has been observed previously in several studies[5-9] in normal man. However, it may be questionable whether the biologic effects of subcutaneously injected insulin in normal subjects can be extrapolated to diabetic subjects. In insulin-dependent diabetic patients several other factors such as insulin binding to antibodies or alterations in the subcutaneous tissue structure may influence the absorption and biologic action of the insulin. Thus, it remains rather doubtful whether the small acceleration of human insulin absorption observed in normal subjects would be of any clinical relevance in the treatment of type I diabetes. We have therefore investigated the efficacy of human semisynthetic and porcine insulin in a double-blind crossover study in type I diabetic subjects on continuous subcutaneous insulin infusion treatment.[10] The study was carried out over 6 wk and near normoglycemia was maintained throughout this period. Insulin requirements were the same whether human or porcine insulin was infused. Thus, it can be concluded that under the conditions tested the small acceleration of the absorption of human insulin is of no clinical relevance to the maintenance of normoglycemia in type I diabetic patients.

In the routine therapy of diabetic patients very often regular and intermediate-acting insulin preparations are mixed in the syringe before injection. As demonstrated previously, regular and intermediate-acting porcine insulins are miscible in the same syringe prior to administration without affecting the absorption kinetics of regular insulin.[1] It was therefore of interest to investigate whether human insulins may also be mixed without altering the absorption of the regular insulin. The results of this study indicate that the absorption of regular human insulin was not altered by the addition of

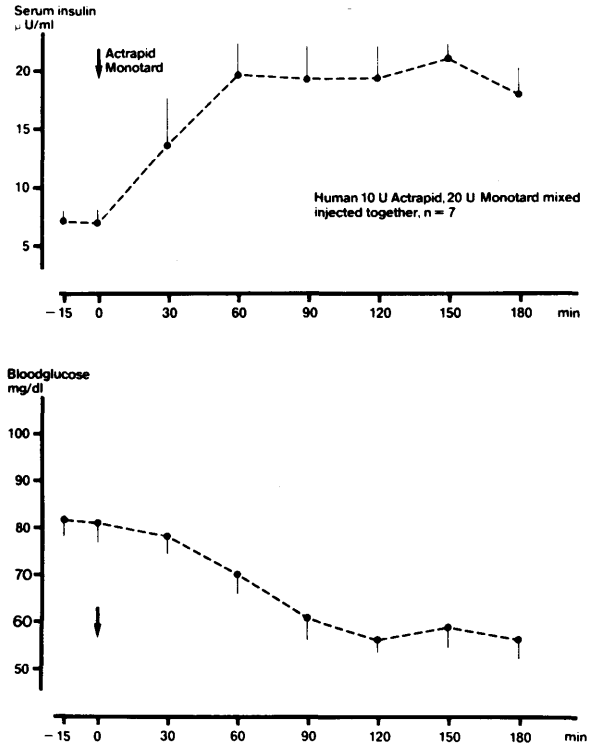

FIG. 4. Serum insulin levels and blood glucose concentrations after subcutaneous injection of a mixture of 10 U human Actrapid and 20 U human Monotard. N = 7.

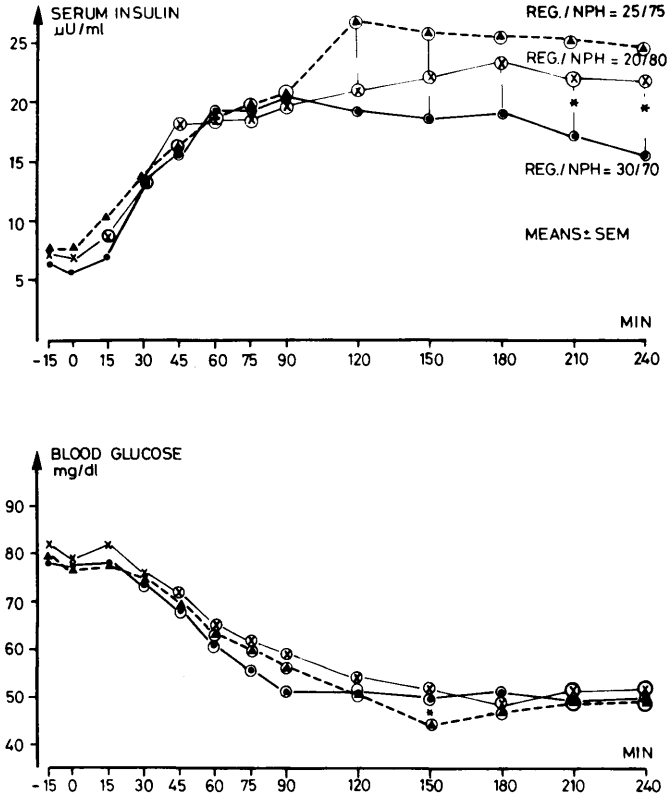

FIG. 5. Serum insulin concentrations and blood glucose levels after subcutaneous injection of 20 U of three different mixtures of human insulin (recombinant DNA) regular and NPH. N = 8.

NPH human insulin, whether the insulins were mixed prior to injection or long before use by the manufacturer. Furthermore the absorption of human Actrapid was unaltered when injected together with human Monotard immediately following the mixing procedure.

DISCUSSION

In the present study we have investigated the absorption kinetics of three different regular human insulin preparations in comparison to the respective porcine insulins. Secondly we have addressed the question whether the absorption of regular human insulin is altered by the addition of intermediate-acting human insulin.

In order to evaluate the absorption kinetics of subcutaneously injected regular insulin, unlabeled insulin was injected into the thigh and plasma levels of immunoreactive insulin were measured. Details as to the validity and practicability of this method have been discussed elsewhere.[1] In principle, a sharp rise of plasma insulin concentrations ensues from the subcutaneous injection, causing an almost instantaneous drop in glycemia. Presumably as a consequence thereof, endogenous insulin secretion is suppressed, as indicated by a prompt decline in C-peptide levels. Thus, except for approximately the first 15 min after the insulin injection, serum insulin originates almost exclusively from the subcutaneous depot of exogenous insulin.

The absorption of all three insulin preparations tested was somewhat accelerated. However this reached statistical significance only for one of the human insulins, whereas higher serum insulin concentrations were observed for all three human insulin preparations in comparison to the respective porcine insulins. There are several possibilities that might explain these small differences between the absorption curves of human and porcine insulin. First, it could have been that the affinity to the antibody used in our assay system was not identical for human and porcine insulin. This could be excluded, because no differences in affinity were demonstrated. Secondly, inter- or intraindividual variations in insulin absorption kinetics may occur. This possibility, however, was very unlikely because only paired testing was performed and insulins were tested in randomized order. Finally, the tertiary structure and/or the solubility of human insulin might differ from that of porcine insulin. In fact, on crystallographic analysis distinct differences between the human and the porcine insulin molecules became evident (G. Dodsen, communication to the Steno Memorial Symposium, Copenhagen, Denmark, May 26, 1981). Thus the use of mixtures of human regular and intermediate-acting insulin in the therapy of type I diabetes mellitus appears to be practicable.

It was surprising that the injection of the three different human (recombinant DNA) insulin mixtures did not result

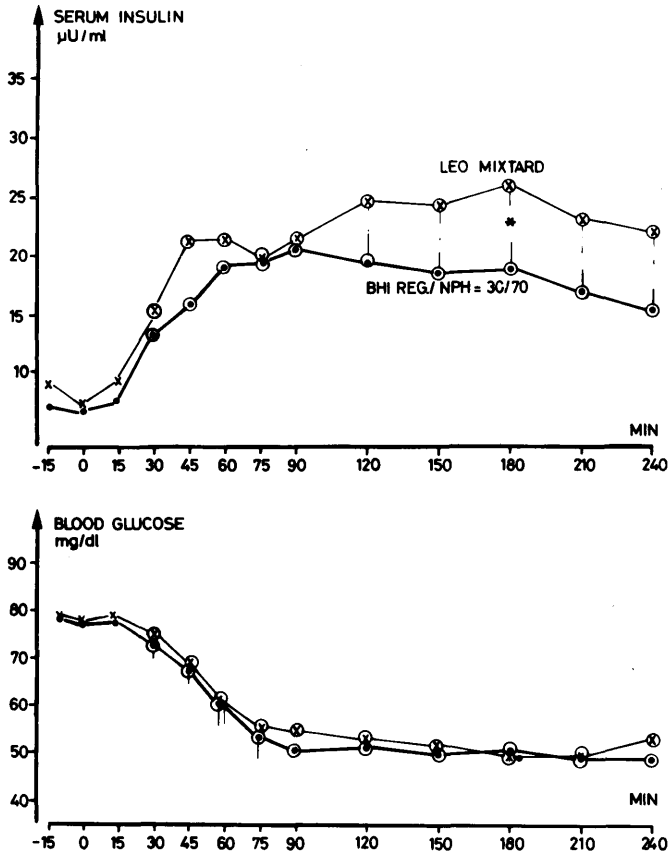

FIG. 6. Serum insulin concentrations and blood glucose levels after subcutaneous injection of 20 U of Mixtard and 20 U of human insulin (recombinant DNA) regular/NPH = 30%/70%. N = 8.

in discriminate maximal serum insulin levels. Considering the actual amount of regular insulin administered, i.e., 4, 5, and 6 U, respectively, these results were, however, to be expected. Any anticipated differences in serum insulin levels would have been masked by the interindividual variability in insulin absorption kinetics.[1]

ACKNOWLEDGMENTS: The expert technical help of C. Broermann and M. Schütte is gratefully acknowledged. The studies were financially supported by the companies Eli Lilly, Novo, Nordisk, and Hoechst.

From the Department of Medicine E, University of Düsseldorf, 4 Düsseldorf, West Germany.

Address reprint requests to Prof. Dr. M. Berger, Medizinische Klinik E, University of Düsseldorf, Moorenstr. 5, 4 Düsseldorf, West Germany.

REFERENCES

[1] Berger, M., Cüppers, H. H., Hegner, H., Jörgens, V., and Berchtold, P.: Absorption kinetics and biologic effects of subcutaneously injected insulin preparations. Diabetes Care 5: 77–91, 1982.

[2] Binder, C.: Absorption of injected insulin. Acta Pharmacol. Toxicol. (Copenh.) *27* (Suppl. 2): 1–87, 1969.

[3] Berger, M., Berchtold, P., Cüppers, H. J., Drost, H., Kley, H. K., Müller, W. A., Wiegelmann, W., Zimmermann-Telschow, H., Gries, F. A., Krüskemper, H. L., and Zimmermann, H.: Metabolic and hormonal effects of muscular exercise in juvenile-type diabetics. Diabetologia *13*: 355–65, 1977.

[4] Kemmer, F. W., Berchtold, P. Berger, M., Cüppers, H. J., Starke, A., Gries, F. A., and Zimmermann, H.: Exercise induced fall of blood glucose unrelated to alteration of insulin mobilization. Diabetes *28*: 1131–37, 1979.

[5] Laube, H., Velcovsky, H. G., and Federlin, K.: Semisynthetiches Humaninsulin-vergleichende Untersuchung über Stoffwechseleffekte beim Menschen. Akt. Endokrin. *2*: 102, 1981. Abstract.

[6] Federlin, K., Laube, H., and Velcovsky, H. G.: Biologic and immunologic in vivo and in vitro studies with biosynthetic human insulin. Diabetes Care 4: 170–74, 1981.

[7] Gyaram, H., and Bottermann, P.: Pharmakokinetik von biosynthtische hergestelltem Humaninsulin. Akt. Endokrin. Stoffw. *3*: 97, 1982.

[8] Pickup, J. C., Bilous, R. W., Viberti, G. C., Keen, H., Jarrett, R. J., Glynne, A., Cauldwell. J., Root, M., and Rubenstein, A. H.: Plasma insulin and C-peptide after subcutaneous and intravenous administration of human insulin (recombinant DNA) and purified porcine insulin in healthy men. Symposium on Human Insulin of Recombinant DNA Origin. Diabetes Care 5 (Suppl. 2): 29–34, 1982.

[9] Owens, D. R., Jones, M. K., Hayes, T. M., Heding, L. G., Alberti, K. G. M. M., Home, P. D., Burrin, J. M., and Newcombe, R. G.: Human insulin: study of safety and efficacy in man. Br. Med. J. *282*: 1264–66, 1981.

[10] Sonnenberg, G., Chantelau, E., Sundermann, S., Hauff, C., and Berger, M.: Human and porcine regular insulins are equally effective in subcutaneous replacement therapy. Results of a double blind crossover study in type I diabetic patients with continuous subcutaneous insulin infusion. Diabetes *31*: 600–602, 1982.