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*Technical Note*

# Comparing the Biological Activity of Humulin R and Novolin R Using an Intravenous Bioassay

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## INTRODUCTION

The two characteristic manifestations of the biological effect of insulin are the time of the glucose nadir and its magnitude. Both are not determined by the USP bioassay. We have previously reported a new bioassay for insulin, which involves determination of the true glucose nadir induced by intravenously administered insulin in dogs (1). By using a continuous glucose monitoring system, we were able to compare the biological effect of different doses of standard insulin to the biological effect of the tested insulin, in the same dog during 1 day.

The improved accuracy of our bioassay for insulin is based on our ability to obtain a continuous and almost instantaneous record of the changing level of blood glucose. The continuous glucose monitor made possible an accurate determination of the insulin-induced nadir of blood glucose, even though it lasted for only a few minutes and occurred at unpredictable times. We were thus able to use in our test an intravenous, rather than a subcutaneous, mode of insulin administration, which proved to be associated with a more consistent response.

Two different preparations of synthetic human insulin are presently available in the United States. One preparation of human insulin is derived from porcine insulin by enzymatic substitution of one alanine with threonine (Novolin R). The other preparation (Humulin R) is made entirely by bacteria through recombinant DNA methodology. The chemical structure of the insulin in the two preparations of human insulin is reported to be identical. It is possible, however, that the two insulins may have subtle differences due to their different mode of preparation. Our purpose was to compare the bioactivity of the two insulins. We have used a modification of our bioassay for insulin (1) to compare the glucose nadir and time to nadir induced by intravenous injections of the "regular" form of the two preparations of human insulin. To our knowledge there are no published studies comparing the biological activity of two generic forms of insulin.

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## EXPERIMENTAL

### Animals

Six preconditioned female hound dogs (treated for worms), weighing 19–20 kg, were fasted overnight and anesthetized in the morning with intravenous sodium pentobarbital (Nembutal R) (250-mg initial dose, 25 mg about every 30 min for maintenance). Continuous glucose monitoring was then inititiated, using a nonthrombogenic catheter (Cormed, Inc.) inserted in one of the major veins of the front leg. The test was repeated two times on each dog at 2-week intervals.

### Method and Instruments

The accuracy and specificity of the glucose monitoring instrument, which was built by Dr. A. A. Kowarski, were previously described (2–4). In this study an integrated concentration was recorded every minute. All injections of insulin (1 unit/ml) were carried out using a 27-gauge, 0.5-in.-length needle and a 1-ml disposable syringe.

The statistical method for significance was the Student paired $t$ test.

### Priming Procedure

The blood concentration of glucose was monitored for at least 20 min in order to establish the baseline concentration of glucose. After establishing a baseline, the dog was given an intravenous injection of 0.5 unit Novolin R. The concentration of blood glucose fell rapidly, reaching the initial priming nadir within 30–35 min. After recording the priming nadir, the concentration of glucose was raised by the intravenous administration of 15 to 30 ml of a 25% solution of glucose. Additional glucose solution was administered in about 10-ml increments when necessary, until the blood glucose concentration stabilized within 10% of the baseline (usually within 45 min).



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Table I. Hypoglycemic Effect of 0.5 Unit of Intravenously Administered Novolin R, Then Humulin R

| | Nadir of blood glucose level (mg/dl) | | Time of nadir (min) | |
|---|---|---|---|---|
| Dog No. | Novolin R | Humulin R | Novolin R | Humulin R |
| 1 | 34 | 34 | 29 | 37 |
| 2 | 34 | 32 | 34 | 37 |
| 3 | 33 | 37 | 23 | 24 |
| 4 | 36 | 33 | 23 | 27 |
| 5 | 33 | 32 | 28 | 32 |
| 6 | 41 | 37 | 24 | 24 |
| Mean | 35.2 | 34.2 | 26.8 | 30.2 |
| ±1 SD | 3.1 | 2.3 | 4.4 | 6.1 |
| CV (%) | 8.7 | 6.8 | 16.3 | 20.1 |

Table II. Hypoglycemic Effect of 0.5 Unit of Intravenously Administered Humulin R, Then Novolin R

| | Nadir of blood glucose level (mg/dl) | | Time of nadir (min) | |
|---|---|---|---|---|
| Dog No. | Humulin R | Novolin R | Humulin R | Novolin R |
| 1 | 62 | 60 | 18 | 21 |
| 2 | 39 | 38 | 24 | 26 |
| 3 | 40 | 37 | 29 | 30 |
| 4 | 46 | 42 | 23 | 19 |
| 5 | 51 | 47 | 22 | 25 |
| 6 | 48 | 50 | 23 | 23 |
| Mean | 47.7 | 45.7 | 23.2 | 24.0 |
| ±1 SD | 8.4 | 8.6 | 3.5 | 3.9 |
| CV (%) | 17.6 | 18.9 | 15.3 | 16.3 |

### Bioassay Procedure

After maintaining the glucose concentration at a consistent concentration for 10 min, the first dose of 0.5 unit Novolin R was administered intravenously in one rapid bolus. The first nadir was recorded by the glucose monitor within 45 min after the injection of Novolin R. As soon as an upturn in the concentration of blood glucose was established, the concentration of blood glucose was raised using a 25% glucose solution, as previously described.

After reestablishing a constant glucose concentration within 10% of the baseline, the procedure was repeated, using 0.5 unit Humulin R. The second nadir of blood glucose was recorded within 45 min after the injection of Humulin R.

In order to eliminate the possible effect of the order of injection, the study was repeated, at least 2 weeks later, in the reverse order starting with 0.5 unit Humulin R as the priming dose and using 0.5 unit Humulin R as the first dose and 0.5 unit Novolin R as the second dose.

## RESULTS AND DISCUSSION

The results of the six repeated comparative bioassays of 0.5 unit Novolin R (first dose) and 0.5 unit Humulin R are depicted in Table I. The mean blood glucose concentration at the nadir of Novolin R was 35.2 ± 3.1 mg/dl (mean ± SD). The coefficient of variation of the six consecutive studies of Novolin R was 8.7%. The mean time to reach nadir and its coefficient of variation of the Novolin R were 26.8 ± 4.4 min and 16.3%. The mean blood glucose concentration at the nadir of Humulin R was 34.2 ± 2.3 mg/dl. The coefficient of variation of the six consecutive studies of Humulin R was 6.8%. The mean time to reach nadir and its coefficient of variation of the Humulin R were 30.2 ± 6.1 min and 20.1%.

Table III. Paired $t$ Test on the Difference Between Novolin R and Humulin R (df = 11)

| Variable | Nadir of blood glucose level (mg/dl) | Time of nadir (min) |
|---|---|---|
| Mean difference | 0.5 | 1.2 |
| SE of mean difference | 2.9 | 3.4 |
| $t$ value | 0.6 | 1.3 |
| Significance of the difference | NS | NS |

The results of the six repeated comparative bioassays of 0.5 unit Humulin R (first dose) and 0.5 unit Novolin R are shown in Table II. The mean blood glucose concentration at the nadir of Humulin R was 47.7 ± 8.4 mg/dl. The coefficient of variation of the six consecutive studies of Humulin R was 17.6%. The mean time to reach nadir and its coefficient of variation of the Humulin R were 23.2 ± 3.5 min and 15.3%. The mean blood glucose concentration at the nadir of Novolin R was 45.7 ± 8.6 mg/dl. The coefficient of variation of the six consecutive studies of Novolin R was 18.9%. The mean time to reach nadir and its coefficient of variation of the Novolin R were 24.0 ± 3.9 min and 16.3%. The accuracy of the bioassay was not affected by the intraassay variability, since the bioactivities of the two insulins were compared in each assay.

Table III depicts the results of a paired $t$ test on the difference between the effect of Novolin R and that of Humulin R. There was no significant difference between the effects of the two preparations of insulin at the nadir concentration of glucose as well as the time of the nadir. This study demonstrates the usefulness of our previously published bioassay of insulin (1). The results of this study indicated that the bioactivity of intravenously administered Novolin R was equivalent to the bioactivity of intravenous administered Humulin R. This is the only documented independent comparative evaluation of the bioactivity of the two preparations.

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