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# Comparison of human and porcine insulin therapies in children with newly diagnosed diabetes mellitus

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**Summary.** A multicenter, longitudinal study of children below the age of 16 years with newly diagnosed Type 1 (insulin-dependent) diabetes treated either with porcine monocomponent insulin ($n=26$) or semisynthetic human monocomponent insulin ($n=26$) was performed during the first 24 months after onset of diabetes. The two groups were carefully matched for age, duration of disease symptoms, initial metabolic values, islet cell antibodies and HLA-DR antigens. During the 24-month observation period there was no significant difference between the two groups in respect to the clinical course, insulin dosage, $HbA_1$ and residual B-cell activity. No child in either group had a real remission without necessitating insulin therapy. The prevalence of insulin antibodies increased slowly and was 62% in the group treated by human insulin and 52% in the porcine insulin-treated group after 24 months. The titres were generally low and there was no statistical difference between the two groups in respect to insulin antibody formation.

**Key words:** Type 1 (insulin-dependent) diabetes, children, human insulin, porcine insulin, insulin antibodies, HLA antigens.

The availability of human insulin for the treatment of children with Type 1 (insulin-dependent) diabetes mellitus has raised some hope of benefits both in respect to metabolic control and insulin antibody formation. The safety and efficacy of semisynthetic human insulin has been documented in diabetic adults [1]. Other studies published up to now have not shown conclusively whether the use of human insulin in diabetic children with Type 1 diabetes has any advantage compared to highly purified porcine insulin [2-6]. The purpose of the present work was to perform a longitudinal clinical and metabolic study in two similar groups of newly diagnosed diabetic children treated with either semisynthetic human or porcine insulin of equal purity.

## Subjects and methods

### *Patients*

The project was planned as a prospective multicenter study at four different paediatric services taking care of diabetic children (Department of Paediatrics, University of Bern, Kinderklinik Kantonspital Aarau, Kinderklinik Wildermeth Biel and a private paediatric office in Basel). Fifty-two children below the age of 16 years with a typical history of newly diagnosed and untreated Type 1 diabetes were included in the study. Half of the patients were treated by semisynthetic human insulin (Actrapid HM and Monotard HM, Novo, Copenhagen, Denmark) and the other half by porcine insulin (Actrapid MC and Monotard MC, Novo) of equal purity.

All patients received a mixture of Actrapid and Monotard in two daily injections 20-30 min before breakfast and supper. An age adapted diet containing approximately 45% of the calories as carbohydrates, 35% as fats and 20% as proteins was instructed [7] and adapted to the patients' needs. Insulin dosage was adjusted by patients or parents according to 3-4 daily urinary glucose and acetone tests [7]. The two patient groups (Table 1) were matched to be sufficiently similar in respect to sex, age, duration of diabetic symptoms, initial values of plasma glucose, urinary acetone, stable $HbA_1$, presence of islet cell antibodies and distribution of HLA-DR antigens to allow a valid comparison. All children were followed at 3-month intervals when blood was drawn for stable $HbA_1$. Insulin antibodies, were determined at 6-month intervals.

The insulin dosage was registered according to the patients' test booklets and the mean dose was calculated as units · kg body weight$^{-1}$ · day$^{-1}$.

The protocol of the study was approved by the ethical committee of the Department of Paediatrics, University of Bern.

**Table 1**

| Patients | Human insulin | Porcine insulin |
|---|---|---|
| Number (sex) | 26 (16 F, 10 M) | 26 (14 F, 12 M) |
| Age (years) | 9.3 ± 4.3 | 9.7 ± 4.2 |
| (< 5 years old) | (4 patients) | (5 patients) |
| History of symptoms (weeks) | 3.2 ± 2.7 | 4.2 ± 3.1 |
| Plasma glucose (mmol/l)[a] | 27.8 ± 10.3 | 24.5 ± 7.4 |
| Stable $HbA_1$ (%)[a] | 14.4 ± 2.5 | 12.7 ± 2.5 |
| Islet cell antibodies[a] | 21/26 patients | 20/26 patients |
| HLA antigens DR 4 | 20 | 18 |
| DR 3 | 11 | 14 |
| DR 1 | 8 | 5 |
| DR 7 | 3 | 1 |
| DR 2 | 2 | 0 |
| non DR 3 or 4 | 2 | 0 |
| HLA phenotypic combinations | | |
| DR 3.4 | 7 | 7 |
| DR 1.4 | 4 | 3 |

[a] Initial values prior to insulin therapy. All variables are not statistically different

## *Methods*

Plasma glucose was measured by an automated hexokinase method. Stable $HbA_1$ was estimated by a commercialised, strict-temperature regulated microcolumn method (Boehringer Mannheim, FRG) after removal of the labile component. Interassay coefficient of variation was 4.4% at $HbA_1$ 7%, 3.4% at 10% and 2.6% at 16%. Reference values in 20 normal children were 5.8–8.0%.

Plasma C-peptide was determined by a commercialised RIA (Novo, Copenhagen, Denmark) with an interassay coefficient of variation of 15% at 125 pmol/l, 7.5% at 250 pmol/l and 10.4% at 500 pmol/l. Fasting reference values in 12 lean young adults were 178–630 pmol/l.

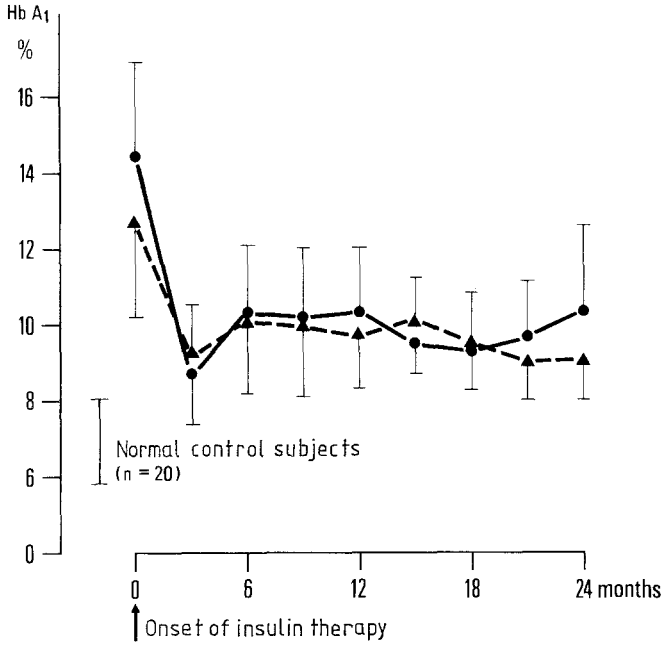


**Fig. 1.** Glycosylated haemoglobin during the first 24 months in children treated with human insulin ($n=26$) and porcine insulin ($n=26$). ●—● Human MC insulin; ▲ – – ▲ porcine MC insulin

**Table 2.** Insulin dose and plasma C-peptide

| Patients (26 in each group) | Insulin | 1st year | 2nd year | |
|---|---|---|---|---|
| Mean insulin dose ($U \cdot kg^{-1} \cdot day^{-1}$) | Human | $0.53 \pm 0.23$ | $0.67 \pm 0.23$ | NS |
| | Porcine | $0.52 \pm 0.26$ | $0.63 \pm 0.22$ | |
| Maximal plasma C-peptide (pmol/l) | Human | 0–898 (106)[a] | 0–436 (93)[a] | NS |
| | Porcine | 0–686 (150)[a] | 0–498 (80)[a] | |

[a] Range (median)

Insulin antibodies were primarily determined by using 125-J ox insulin (Novo) with amberlite separation according to a modified method by Melani et al. [8]. The interassay coefficient of variation was 7.9%. Results are given as % binding of 125-J insulin. Values below 1.7% were considered to be negative. For comparison the classical immunoelectrophoresis technique was used [9] with values below 0.1% considered to be negative. In addition, parallel determinations were done using 125-J pork and human insulin (Novo) with amberlite separation.

Islet cell antibodies were assessed by an indirect immunofluorescene technique on ultrathin ($<4$ μm) sections of freshly frozen human pancreas [10]. Reproducibility of assays was determined by including both a positive control serum at a titre of 1:64 with an interassay variation of not more than one dilution step and a negative control serum.

HLA typing was performed using the standardised microlymphocytotoxicity technique [11].

## *Statistical analysis*

All values are given as mean ± 1 standard deviation (SD) if not otherwise stated. For statistical analysis the $X^2$-test, Fisher's exact test, Wilcoxon test and Spearman rank test were applied.

## Results

The clinical course of the two groups of children treated by semisynthetic human or porcine insulin was similar. No patient in either group entered a full remission with normalisation of plasma glucose levels and glycosylated haemoglobin values while receiving no insulin. This similar clinical course was also reflected by an almost identical mean insulin dosage (Table 2) together with a similar mean $HbA_1$ (Fig. 1) during the whole observation period.

Severe hypoglycaemic episodes with loss of consciousness necessitating either the administration of glucagon or i.v. glucose during the first 2 years of therapy occurred in three patients on porcine, and six patients on human insulin. This difference was not significant.

Residual B-cell activity as estimated by casual plasma C-peptide measurements (Table 2) also showed no difference between the two groups.

The prevalence of insulin antibodies increased slowly during the 24-month observation period when it was higher in patients treated by human than in those treated by porcine insulin (Table 3). The titres were generally very low in both groups showing a gradual

**Table 3.** Prevalence of insulin antibody response ($n=26$ patients in each group)

| Method | Antibody titre | Insulin | Duration (months) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 6 % | 12 % | 18 % | 24 % | |
| 125-J ox insulin/amberlite | >1.6% | Human | 23 | 35 | 54 | 62 | NS |
| | | Porcine | 31 | 48 | 46 | 52 | |
| | >3.0% | Human | 8 | 15 | 15 | 35 | NS |
| | | Porcine | 8 | 28 | 25 | 28 | |
| 125-J ox insulin/immuno-electrophoresis | >0.1% | Human | | 62 | | 73 | NS |
| | | Porcine | | 56 | | 70 | |
| 125-J pork insulin/amberlite | >1.6% | Human | | 35 | | 65 | NS |
| | | Porcine | | 56 | | 64 | |
| 125-J human insulin/amberlite | >1.6% | Human | | | | 58 | |

increase over the first 24 months (Fig. 2). The prevalence of responders (titre > 1.6%) and moderate responders (titre > 3.0%) was not significantly different in the two groups (Table 3). There was only one high responder in each group (titre > 20%). Applying different species of labelled insulin including 125-J human insulin and different methods of separation also did not show any statistically significant difference between both groups (Table 3). Furthermore, insulin antibody titres did not correlate with either metabolic control ($HbA_1$) or insulin dosage.

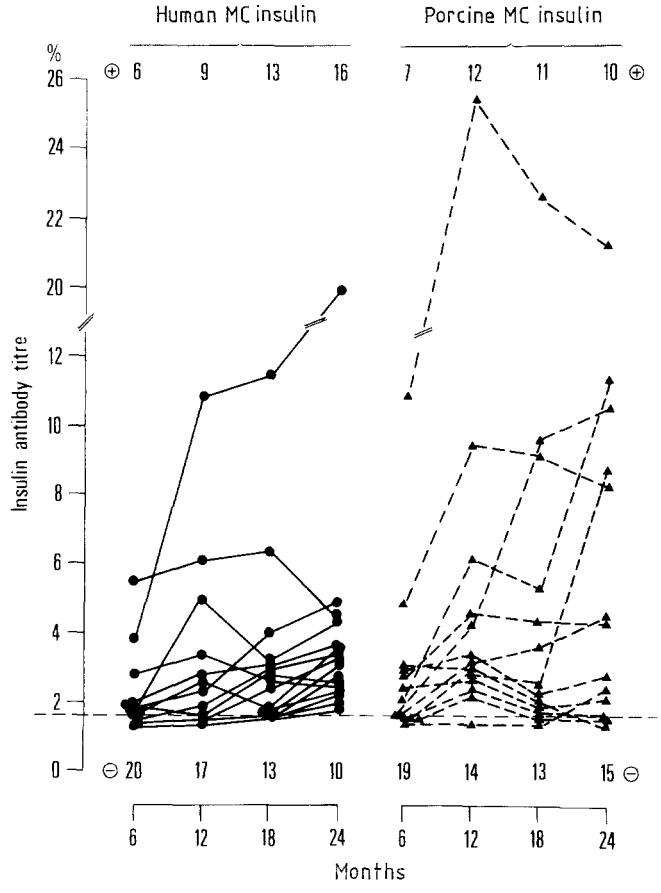


**Fig. 2.** Development of insulin antibodies in children with newly diagnosed diabetes treated with human insulin ($n = 26$) or porcine insulin ($n = 26$). Values consistently below 1.7% are not given

## Discussion

Care was taken in this multicenter prospective study to achieve an optimal match of the two groups of children with newly diagnosed diabetes treated with either semisynthetic human or porcine insulin. The similar age range is of importance as younger children have a more rapid decline of B-cell residual activity than older children [12]. The distribution of the HLA-DR genotypes showing no significant difference is of importance in view of the dissimilar clinical course in different HLA genotypes [12-14].

The insulin requirement of human insulin in contrast to another study [15] was not superior to the one of porcine insulin and the metabolic control was not worse in the human insulin group [3]; but rather was it equal in both groups in confirmation of others [15]. Residual B-cell activity decreased rapidly and equally in both groups over the 24-month observation period which is in accordance with the usual clinical course [14].

The immunological response to exogenous insulin is generally more marked in children than in adults [16]. The overall prevalence of insulin antibody responders in our study was rather high and gradually increased up to 24 months after onset of therapy. For comparison insulin antibody determinations were performed by immunoelectrophoresis showing a prevalence in all 52 children of 58% at 12 months which is similar to that found by others [6, 17]. In contrast to other studies [4, 6], both prevalence and degree of insulin antibody response was not significantly smaller in our patients treated with human insulin. The reason for this discrepancy is not clear. Methodological differences were excluded by performing parallel determinations (Table 3) applying the same methods as in other studies [4, 6]. As the responder rate in our study continued to increase slowly with duration of therapy and the follow-up was shorter in the other studies [4, 6] a longer observation and a larger number of patients may be necessary for clarification.

As in adult diabetic patients [18, 19] our children bearing the HLA-DR 3 antigen had a significantly

**Table 4.** Relation of insulin antibody formation to HLA DR-antigens

| | Responders | Non-responders | | Moderate and high responders | Non- and low responders | |
|---|---|---|---|---|---|---|
| Insulin antibodies | > 1.6% | ≦ 1.6% | | > 3% | ≦ 3% | |
| Patients *(n)* | 29 | 23 | | 18 | 34 | |
| HLA antigens | | | $X^2$-Test | | | $X^2$-Test |
| DR 4 | 23 | 15 | NS | 13 | 25 | NS |
| DR 3 | 9 | 16 | $p < 0.01$ | 4 | 21 | $p < 0.01$ |
| DR 1 | 10 | 3 | NS | 9 | 4 | $p < 0.005$ |
| HLA phenotypic combinations | | | | | | |
| DR 3.4 | 5 | 9 | NS | 1 | 13 | $p < 0.025$ |
| DR 1.4 | 7 | 0 | $p < 0.001$[a] | 6 | 1 | $p < 0.005$ |

[a] Fisher's exact test

lower prevalence of insulin antibody formation than with other DR antigens (Table 4). Prevalence of HLA-DR 3 in our children treated with porcine insulin was somewhat higher than in the group treated with human insulin (Table 1) which may have had some influence on insulin antibody formation. The DR 4 antigen in confirmation with some earlier studies [18, 19] had no influence on antibody formation to insulin, therefore not confirming the tendency to develop insulin antibodies more rapidly in DR 4 antigen positive children observed by others [4, 17, 20].

In our group, insulin antibody formation, especially at higher titres, both in response to human and porcine insulin correlated significantly with HLA-DR 1 and DR 1.4 (Table 4). This significantly increased prevalence of DR 1.4 phenotype has already been described as a new DR specificity recognised either by monoclonal antibodies [21] or by alloantisera [22]. This specificity was found significantly associated with rheumatoid arthritis [21]. The increased frequency of both DR 1 and DR 1.4 specificities in our patients with Type 1 diabetes was not found in other populations and could possibly be ascribed to ethnical differences.

In spite of the multicenter nature of our study, with the possible disadvantage of different clinical guidance of patients and their families, our controlled study revealed no discernable difference in clinical course, metabolic control and development of insulin antibodies in children treated with semisynthetic human insulin in comparison to porcine insulin of equal purity.

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