Effects of Insulin Administration on β-Cell Function in Subjects at High Risk for Type I Diabetes Mellitus

C. Rodríguez-Villar, I. Conget, J.M. González-Clemente, J. Vidal, P. Navarro, R. Casamitjana, and R. Gomis

The aim of the study was to determine the appropriate dose of subcutaneous insulin to induce "β-cell rest" without any hypoglycemic risk, as the first step in the investigation of its potential effect in preventing or delaying clinical diabetes mellitus onset in high-risk subjects. Four subjects at high risk for type I diabetes mellitus (first-degree relatives, islet cell antibodies (ICA)-positive, and with diminished first-phase insulin secretion) were compared with four healthy individuals. After hospitalization, urinary C-peptide excretion (UCP) and 24-hour serum profiles for glucose were measured before and after administration of NPH insulin 0.1, 0.2, and 0.3 U · kg body weight per day subcutaneously in a single dose on 4 consecutive days. After insulin 0.1 U · kg body weight, a significant inhibition of endogenous insulin secretion was observed in high-risk subjects, but not in control subjects. There was no further inhibition when a higher insulin dose (0.2 and 0.3) was administered. A sustained β-cell rest was obtained after 3, 6, and 12 months of treatment with 0.1 U · kg body weight per day as outpatient therapy in high-risk subjects. With this dose, no subject developed hypoglycemia (plasma glucose <50 mg/dL), whereas this adverse effect was detected after 0.2 and 0.3 U · kg body weight in both groups. In conclusion, our results indicate that administration of NPH insulin 0.1 U · kg body weight per day induces β-cell rest without the undesirable effect of hypoglycemic episodes. This is a preliminary study to investigate the potential beneficial effect of insulin in preventing or delaying type I diabetes mellitus in subjects at high risk for the disease.

INSULIN-DEPENDENT diabetes mellitus (IDDM) is considered an autoimmune disease affecting individuals with genetic susceptibility. Its clinical onset is preceded by the appearance of islet cell antibodies (ICA).[1-3] Likewise, high levels of ICA in first-degree relatives are often followed by the onset of diabetes.[4] In this case, the development of overt disease is preceded by a continuous impairment of first-phase insulin release (FPIR), reflecting a progressive loss of pancreatic islet β-cell function.[5,6]

The ability to identify subjects in the prediabetic phase supports the idea of a preventive treatment of the disease. Insulin therapy has prevented autoimmune diabetes mellitus in NOD mice and BB rats.[7,8] Early studies have shown preservation of β-cell function and prevention of IDDM with daily subcutaneous insulin and intensive intravenous insulin in subjects at high risk for the disease.[9]

Several hypotheses have been proposed to explain the beneficial effect of insulin treatment in the prediabetic state. It has been suggested that exogenous insulin induces "β-cell rest," which could decrease β-cell susceptibility to immune destruction.[10] Nevertheless, these studies have not fully defined the prophylactic insulin dose, or the best dose to achieve this beneficial effect. Furthermore, the possible role of insulin as a tolerogen or as an immunomodulator has also been proposed.[11]

The aim of this study was to determine the appropriate dose of subcutaneous insulin to induced β-cell rest with no hypoglycemic risk in subjects at high risk for IDDM, as the first step in the investigation of its potential effect in the preservation or delay of type I diabetes.

SUBJECTS AND METHODS

Subjects were recruited in our Endocrinology and Nutrition Unit after screening 711 first-degree relatives of IDDM patients. We studied four high-risk subjects: first-degree relatives of IDDM patients aged 16, 23, 30, and 42 years with a body mass index (BMI) of 22.7 ± 2.6 kg · m^2, ICA level of at least 20 Juvenile Diabetes Foundation Units (JDF U) at least twice, and first-phase (1 + 3) insulin release in an intravenous glucose tolerance test (IVGTT) under the 10th percentile of age-matched normal controls (95 ± 1.3 mU/L). An oral glucose tolerance test ([OGTT] 75 g) was performed on ICA-positive subjects at entry. This group was compared with four healthy control subjects aged 28, 30, 32, and 60 years (BMI, 23.0 ± 3.5 kg · m^2) who were ICA-negative and had normal first-phase insulin levels on IVGTT. In all subjects, hepatic and renal function parameters were within normal range. Both control and high-risk subjects were hospitalized for 4 days. The total caloric intake was adjusted for BMI and physical activity and distributed as follows: 50% as carbohydrate, 30% as fat, and 20% as protein. Physical activity was constant during this period.

The 24-hour urinary C-peptide excretion (UCP) was measured beginning at 9 AM and was considered an indicator of β-cell function.[12,14] Twenty-four-hour profiles for glucose were determined in serum obtained via any indwelling intravenous catheter at 9 AM, 11 AM, 1, 3, 7, and 9 PM, and 2 AM every day. These serum profiles and UCP were measured before (day 0) and after administration of 0.1 U · kg (day 1), 0.2 U · kg (day 2), and 0.3 U · kg (day 3) body weight per day of subcutaneous NPH insulin (Novo Nordisk, Bagsvaerd, Denmark) on consecutive days. Insulin was always administered as a single dose at 9 AM. Hypoglycemia was considered if typical symptoms appeared and/or serum glucose was less than 50 mg/dL (2.7 mmol · L^{-1}).

ICA were determined by an indirect immunofluorescence technique on sections of human frozen pancreas.[15] Titers were converted to JDF U. IVGTT was performed as follows. Glucose 0.5 g · kg body weight (maximum dose, 35 g) as a 25% glucose solution was infused over 3 minutes. Two baseline samples (−10 minutes and 0 minutes) were obtained before glucose was administered. Further samples were obtained at 1, 3, 5, and 10 minutes after glucose administration was completed. The sum of insulin levels at

From the Endocrinology and Nutrition Unit and the Hormonology Unit, Hospital Clínic, Barcelona, Spain.

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Address reprint requests to R. Gomis, MD, PhD, Endocrinology and Nutrition Unit, Hospital Clínic, Villarroel 170, 08036 Barcelona, Spain.

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minutes 1 and 3 yielded an immunoreactive insulin (IRI) of 1 + 3, and this was considered the FPIR.

Glucose level was measured in serum by the glucose oxidase method. Serum insulin levels were measured by a radioimmunoassay method (IRI-CIS; Biointernational, Gif-Sur-Yvette, France). The coefficient of variation within and between assays was 2% and 11%, respectively. To measure UCP, urine was collected in chilled plastic containers at 4°C, and aliquots were adjusted to pH 7.0 with dilute NaOH. C-peptide in urine was determined by radioimmunoassay (RIA-coat C-Peptid; Byk-Sangtec Diagnostica, Dietzenbach, Germany). UCP excretion was determined as the product of the urine volume per 24 hours and the concentration of C-peptide in the urine. The coefficient of variation within and between assays was 5.5% and 11.8%, respectively.

After clinical, biochemical, and hormonal analysis of data obtained during hospitalization, NPH insulin 0.1 U/kg body weight per day was continued as outpatient therapy for 12 months. UCP excretion was determined in high-risk subjects at 3, 6, and 12 months, and a dietetic meal plan was maintained unmodified throughout the follow-up period.

The study was approved by the Ethics Committee of the Hospital Clinic of Barcelona, and written consent was obtained from participants.

Statistical Analysis

Results are expressed as the mean ± SD, and P less than .05 was considered statistically significant. The data were compared using parametric tests: Student's t test and ANOVA for repeated measurements using the Statistical Package for the Social Sciences (SPSS, Chicago, IL).

RESULTS

There were no abnormalities on OGTT in first-degree ICA-positive relatives, according to World Health Organization criteria.

When IVGTT values were analyzed, an impairment in FPIR was found in the ICA-positive subjects (IRI 1 + 3, 33 ± 5 mU/L) as compared with ICA-negative healthy individuals (IRI 1 + 3, 181 ± 67 mU/L; $P < .03$).

UCP excretion on day 0 was significantly higher in high-risk subjects than in the control group (Table 1). This was also the case when glucose levels were analyzed (6.0 ± 0.4 mmol · L^{-1} in high-risk subjects v 4.1 ± 0.4 in the control group, $P < .05$). There were no differences for UCP and glucose values on days 1, 2, and 3 after insulin administration.

When the UCP to mean serum glucose (MSG) ratio was analyzed in both groups, no differences were found under all experimental conditions (Table 2).

The percentage decrease of 24-hour UCP excretion after different insulin doses is shown in Table 3. After 0.1 U NPH insulin, there was a 53% decrease in UCP excretion in high-risk subjects; however, there was no further decrease with higher doses of insulin.

A long-term preservation of β-cell rest in ICA-positive subjects was observed at 3, 6, and 12 months of follow-up study (Table 4).

Table 1. UCP Excretion (μg/d)

Day	High-Risk Group	Controls	P
0	160.0 ± 54.7	83.6 ± 38.6	<.05
1	75.5 ± 24.7	72.6 ± 53.0	NS
2	72.0 ± 18.1	73.8 ± 69.5	NS
3	78.9 ± 34.3	71.2 ± 42.1	NS

NOTE. Values are the mean ± SD.

Table 2. UCP/MSG Ratio in High-Risk Group and Controls

Day	High-Risk Group	Controls	P
0	1.46 ± 0.49	1.32 ± 0.54	NS
1	0.75 ± 0.20	1.14 ± 0.58	NS
2	0.79 ± 0.12	1.24 ± 1.17	NS
3	0.81 ± 0.31	1.27 ± 0.80	NS

NOTE. Values are the mean ± SD.

DISCUSSION

Firstly, the metabolic alterations found in high-risk subjects should be described. As already mentioned, a typical FPIR impairment was found in ICA-positive subjects as compared with the control group. This phenomenon indicates an alteration in insulin secretion in response to an acute supraphysiological stimulus, but little is known about β-cell function in daily physiological conditions in the prediabetic state. To further clarify this subject, we analyzed 24-hour UCP excretion in such conditions in both high-risk and control subjects. On day 0, UCP excretion was significantly higher in high-risk subjects (ICA-positive) than in the control group (ICA-negative). Likewise, at the same time, mean serum glucose levels were higher in high-risk subjects than in the control group, but were always within the normal range. These latest findings could be related, at least in part, to an insulin resistance state, a phenomenon that has already been described at the onset of IDDM.[16,17] It should be emphasized that when the UCP/MSG ratio was considered, there were no differences between the two groups. Therefore, taken as a whole, our results support the view that despite an alteration in insulin release following a supraphysiological stimulus, the β-cell mass in the prediabetic state is still able to adapt its functional capacity to the metabolic demand. According to our results, a similar phenomenon was observed "in vitro" when islets of prediabetic NOD mice with impaired insulin secretion on an IVGTT were challenged with glucose after culture at high concentrations of the hexose.[18] In addition, and from a pathophysiological point of view, β-cell hyperactivity in the prediabetic state should be considered, since it has been claimed that the increased β-cell activity found in nondiabetic HLA-identical siblings of IDDM patients predisposes to β-cell damage.[19]

Secondly, to determine the appropriate dose of insulin to induce β-cell rest, we considered the decrease in 24-hour

Table 3. Percentage Decrease of 24-Hour UCP Excretion

Comparison	High-Risk Group	Controls
Day 1 (0.1 U NPH) v day 0 (0 U NPH)	53%	13%
Day 2 (0.2 U NPH) v day 1 (0.1 U NPH)	2%	1%
Day 3 (0.3 U NPH) v day 2 (0.2 U NPH)	4%	3%

Table 4. UCP Excretion and UCP/MSG Ratio at 3, 6, and 12 Months in High-Risk Subjects

Time	UCP	UCP/MSG	*P*
3 mo	75.5 ± 21.4	0.74 ± 0.19	NS
6 mo	62.5 ± 51.9	0.62 ± 0.41	NS
12 mo	73.2 ± 43.2	0.78 ± 0.39	NS

NOTE. Values are the mean ± SD.

UCP excretion under a prefixed insulin dose. After NPH insulin 0.1 u · kg body weight per day, a significant decrease was observed in UCP excretion in high-risk subjects, but this was not the case in the control group. In addition, there was no further decrease in UCP excretion with either 0.2 or 0.3 U · kg body weight per day.

Finally, our study dealt with the safety of the insulin dose to be administered as a prophylactic treatment in high-risk subjects. It should be emphasized that no hypoglycemic episodes were identified after administration of 0.1 U insulin throughout the study, with sustained β-cell rest throughout the follow-up period. However, serum glucose values less than 2.7 mmol · L^{-1} occurred on two occasions with 0.2 U, once in a prediabetic and once in a control subject. The same phenomenon was observed in four patients, two from each group, after administration of insulin 0.3 U · kg body weight on more than one occasion. NPH insulin as a single dose in the morning was selected to control daytime postprandial glycemia. In addition, this pharmacological form of insulin allows administration by a pen injector. Moreover, as previously mentioned, NPH insulin produced a sustained β-cell rest despite its pharmacokinetics.

In summary, our results indicate that administration of NPH insulin 0.1 U · kg body weight per day induces β-cell rest without the undesirable effect of hypoglycemic episodes. These findings are the starting point for future clinical trials to investigate the potential beneficial effect of insulin in preventing or delaying IDDM in subjects at high risk for the disease.

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