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PRELIMINARY REPORT

Effects of Prolonged Administration of Ultralente Insulin on Fasting and Postbreakfast β-Cell Function in Normal Adults

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Treatment with small doses of subcutaneous insulin is being investigated as a possible approach to prevent type 1 diabetes in humans. The mechanism of prophylactic insulin therapy could involve the inhibition of β-cell secretory activity and/or the initiation of an active immunoregulatory process. To evaluate the pure metabolic effect of exogenous insulin, the present study assessed whether daily subcutaneous administration of ultralente insulin alters β-cell function in normal adults. Fourteen healthy adults were randomized to receive 0.2 U/kg · d ultralente insulin (Ultratard; Novo Nordisk, Bagsvaerd, Denmark) or placebo subcutaneously once daily for 30 days. Plasma glucose, C-peptide, and insulin concentrations were measured in the fasting state and 1 hour after a standardized breakfast, during treatment and during a recovery period of 10 days. Insulin administration induced a 15% to 40% decrease of fasting plasma C-peptide. In contrast, postbreakfast plasma C-peptide increased by 40% to 90% in subjects receiving insulin. Fasting and postbreakfast C-peptide concentrations were significantly different between groups during the injection period after adjustment for baseline concentrations ($P < .05$, ANOVA with repeated measures). These alterations disappeared 3 days after cessation of insulin treatment. The present regimen of exogenous insulin alters endogenous insulin secretion in normal subjects. Instead of the expected β-cell rest, the effect appeared to be dual, with insulin secretion decreasing in the basal state and increasing after meals.

IN ANIMAL MODELS for type 1 diabetes such as non-obese diabetic (NOD) mice or BioBreeding rats, the administration of exogenous insulin[1-7] prevents insulitis and diabetes.

In humans with type 1 diabetes mellitus of recent onset, intensive insulin therapy[8] was shown to increase endogenous residual β-cell function. In subjects at high risk for the disease, 2 pilot studies suggested that small doses of intravenous and subcutaneous insulin could prevent or delay the onset of diabetes.[9-11] Several intervention studies using daily injections of insulin in high-risk relatives of type 1 diabetic patients were recently launched in the United States and Europe.[12,13] The insulin doses used in the Diabetes Prevention Trial–Type 1 Diabetes and the European Prediabetes Pediatric Trial are 0.25 and 0.20 U/kg · d, respectively. These doses were chosen because they are associated with an acceptably low risk of hypoglycemia, but their effect on β-cell function is unknown.

The mechanism of the preventive action of insulin administration has been delineated in animal studies but remains unknown in humans.[14] Exogenous insulin might decrease β-cell secretory activity and reduce the expression of key antigens.[7] Alternatively, insulin, a β-cell–specific autoantigen, might trigger an active immunoregulatory process.[1,2,15-17]

To evaluate the ''pure'' metabolic effect of exogenous insulin, we assessed whether subcutaneous insulin administration alters endogenous insulin secretion in normal adults. In studying subjects with no autoimmunity toward the β cell, our purpose was to avoid interference with immune effects of the administered insulin on β-cell attack or failure.

SUBJECTS AND METHODS

Study Population

Fourteen healthy adults aged 20 to 36 years volunteered for the study (Table 1). None of them had chronic diseases or a family history of endocrine disorders or diabetes or used medications or oral contraceptives at the time of study. The protocol was approved by our institutional Committee for Medical Bioethics. Subjects received appropriate infor-

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Table 1. Initial Characteristics of the Two Groups

Characteristic	Insulin (n = 7)	Control (n = 7)	*P*
Age (yr)	29 ± 5.5	26.7 ± 5.1	NS
Sex, n (male/female)	4/3	3/4	NS
Weight (kg)	64.9 ± 2.8	63.7 ± 6.7	NS
Height (cm)	171 ± 2	171 ± 4	NS
BMI (kg/m^2)	22.2 ± 0.9	21.3 ± 1.2	NS
Glucose (mg/dL)			
T0	77 ± 3	72 ± 3	NS
T60	65 ± 7	74 ± 7	NS
Insulin (mU/L)			
T0	10 ± 1	12 ± 3	NS
T60	22 ± 3	29 ± 5	.11
C-peptide (pmol/mL)			
T0	0.29 ± 0.02	0.32 ± 0.04	NS
T60	0.59 ± 0.06	0.80 ± 0.04	<.05

Abbreviations: BMI, body mass index; T0, 0 minutes; T60, 60 minutes; NS, nonsignificant.

mation and provided informed consent for the experimental procedure. All subjects completed the study.

Procedural Methods

Subjects were randomly allocated to insulin or placebo treatment in a double-blind fashion. Group I received 0.2 U/kg · d ultralente insulin (Ultratard; Novo Nordisk, Bagsvaerd, Denmark) for 30 days. Group II received an equivalent volume of placebo. The placebo was completely indistinguishable from the Ultratard insulin.[18] Random assignment was made with a table of random numbers. Insulin or placebo injections were administered subcutaneously in the thigh 20 minutes precisely before breakfast.

Plasma glucose, C-peptide, and insulin concentrations were measured at 8 AM after a 12-hour overnight fast on days −1, 5, 10, 15, 20, 25, 30, 33, 35, and 40, as well as 1 hour after a standardized breakfast on days −1, 10, 20, 30, 33, 35, and 40. The standardized breakfast (386 kcal) consisted of 65 g carbohydrate, 10 g fat, and 9 g protein. Insulin antibody levels were measured on days −1 and 40.

Subjects were advised to eat a normocaloric standard diet containing 40% to 50% carbohydrate and to maintain their usual physical activity during the experimental period. Body weight was measured on days −1, 30, and 40.

Laboratory Methods

The C-peptide level was measured using M1221 antiserum (Novo Nordisk), after removal of proinsulin by polyethylene glycol precipitation.[19] The lower limit of detection was 0.02 pmol/mL. The average precision was 14% at 0.05 pmol/mL and 3% at 0.3 pmol/mL. The plasma insulin level was measured with a radioimmunoassay (CIS Biointernational, Gif-sur-Yvette, France), with a coefficient of variation within and between assays of 2% and 11%. The plasma glucose level was measured with a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Insulin antibodies were determined as previously reported.[20] All samples were analyzed in the same assay to minimize interassay variability.

Statistical Analyses

The results are expressed as the mean ± SEM and range, with the analysis based on the 2 treatment groups. Quantitative data were compared using the Mann-Whitney *U* test. C-peptide, insulin, and blood glucose concentrations during the injection period were also compared using ANOVA with repeated measures, with treatment as the between-subject variable, time as the within-subject variable, and baseline values as covariates. Statistical analysis was performed with StatView 4 software (Abacus Concepts, Berkeley, CA).

RESULTS

The two groups had comparable initial characteristics (Table 1), with the exception of postbreakfast C-peptide, which was lower in the insulin group (0.59 ± 0.15 *v* 0.80 ± 0.11 pmol/mL, $P < .05$).

In response to insulin administration, the mean fasting plasma C-peptide concentration was significantly lower in the insulin group versus the placebo group at each time point, as well as by repeated measures ($P = .02$, ANOVA with repeated measures; Fig 1A). Individual fasting C-peptide concentrations decreased to 60% to 85% of baseline values, whereas they did not change in the control group. The mean fasting plasma insulin did not change from the baseline value and remained stable in both groups.

Subjects treated with insulin had only a moderately higher postbreakfast C-peptide concentration than the placebo group, contrasting with the lower value in the insulin group at baseline (Fig 1B). However, the effect of treatment with insulin versus placebo on postbreakfast C-peptide was significant ($P = .03$, ANOVA with repeated measures) after adjustment for the C-peptide concentration at baseline. Individual postbreakfast plasma C-peptide concentrations increased to 140% to 160% of baseline values, and remained stable in the control group. Consistently,

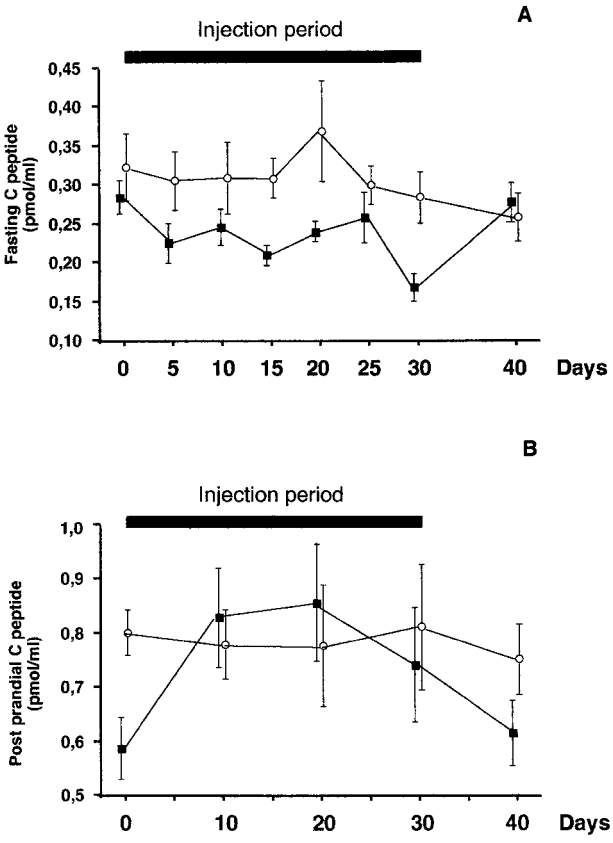

Fig 1. Evolution of (A) fasting C-peptide and (B) postprandial C-peptide in the insulin and placebo groups. ■, Insulin treatment; ○, placebo treatment. Values are the mean ± SEM.

individual postbreakfast plasma insulin concentrations increased to 150% to 190% of baseline values in the insulin group.

Insulin antibody was not detected at day 0 or during the study period.

Fasting and postbreakfast serum glucose levels were similar and stable in the 2 groups during the whole study period ($P > .05$) for insulin *v* placebo; fasting, 73 ± 5 *v* 72 ± 5 mg/dL on day 30; postbreakfast, 71 ± 13 *v* 71 ± 13 mg/dL on day 30).

Three days after interruption of insulin treatment, plasma C-peptide concentrations returned to baseline. During the recovery period, fasting plasma C-peptide reverted to similar concentrations between groups (Fig 1A), whereas postbreakfast plasma C-peptide returned to lower concentrations in the insulin group ($P < .05$; Fig 1B), consistent with the values observed at baseline.

During treatment, the mean body weight increased by 2.2 ± 0.5 kg (+3.5% ± 0.9%) in the insulin group and 0.54 ± 0.5 kg (+1.1% ± 0.6%) in the control group ($P < .05$ between groups). Despite a small weight loss after cessation of the injections in the insulin group (−0.4 ± 0.4 kg from day 30 to day 40), the weight gain was still significant at day 40 ($P < .05$).

Mild symptoms of hypoglycemia (shakiness and hunger) were reported by 4 of 7 insulin-treated subjects, of whom each had 1 to 3 episodes before the evening meal. Comparable symptoms were reported by 1 of 7 placebo-treated subjects.

DISCUSSION

To investigate the putative mechanisms of action of prophylactic insulin therapy, we measured fasting and postprandial plasma C-peptide levels in adults during insulin administration. The present data support a more complex effect than simple β-cell rest. In the fasting state, plasma C-peptide was consistently lower in individuals receiving insulin. In contrast, a paradoxic increase of plasma C-peptide was observed after breakfast. These alterations disappeared as soon as 3 days after interruption of insulin treatment.

Comparable experiments have been reported in the literature, but only in the short term. Rodriguez-Villar et al[21] found no modification of urinary C-peptide during 1 to 3 days of administration of NPH insulin at similar doses in 4 normal adults. The small number of subjects in this study might not allow a valid detection of a consistent difference. Alternatively, the dual effect of exogenous insulin on plasma C-peptide observed in our study could result in unchanged daily urinary C-peptide, since basal and meal-elicited insulin secretion each represent approximately 50% of total 24-hour insulin production.[22-24] In 4 adult subjects a high risk for type 1 diabetes, the same group found that urinary C-peptide was 2-fold higher than in the controls, while the first-phase insulin response to intravenous glucose was decreased. This suggested marked β-cell hyperactivity in the basal state. Urinary C-peptide decreased by 53% after administration of 0.1 to 0.3 U/kg NPH insulin per day.[21] This decrease could reflect the effect of exogenous insulin on fasting rather that postbreakfast insulin secretion. Complex alterations of β-cell function are also found in prediabetic NOD mice.[25]

Subjects treated with insulin had only a moderately higher postbreakfast C-peptide concentration than the placebo group. This could be due to the lower postbreakfast C-peptide level at baseline and after the interruption of insulin treatment in the insulin group. To account for the differences between groups at baseline, we performed an ANOVA with repeated measures with baseline C-peptide as a covariate. This analysis showed that the increase in postbreakfast C-peptide in the insulin group was significant. Potential determinants of postbreakfast C-peptide at baseline, such as gender, age, body mass index, physical activity, diet, glycemic values, and renal or liver function, were comparable in both groups.

The mechanism responsible for the alteration of C-peptide secretion in response to insulin administration is unknown. Exogenous insulin administration might induce some degree of insulin resistance. It could interfere with the pulsatility of endogenous insulin secretion, or lead to portal hypoinsulinemia, decreasing the hepatic sensitivity to insulin.[22,26-28]

In conclusion, administration of exogenous insulin at the doses currently used in type 1 diabetes prevention trials alters endogenous insulin secretion. Rather than the expected β-cell rest, insulin secretion showed 2 apparently opposite effects: it decreased in the basal state and increased after meals. Whether these alterations could be relevant to the putative preventive effect of insulin remains to be determined.

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