

Dose-Response Relationship of Oral Insulin Spray in Healthy Subjects

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OBJECTIVE— To evaluate the pharmacodynamic and pharmacokinetic properties and the dose-ranging effects of an oral insulin spray in comparison with subcutaneous regular insulin.

RESEARCH DESIGN AND METHODS— In this randomized, five-way, cross-over study, seven healthy volunteers were assessed under euglycemic clamp and received four different doses of oral spray and one dose of subcutaneous regular insulin.

RESULTS— The time to maximum insulin concentration was shorter for oral insulin than for subcutaneous insulin (25.9 ± 9 vs. 145.7 ± 49.5 min, $P < 0.05$). Maximum serum insulin levels (C_{\max}) were comparable between the subcutaneous and 20 puffs of oral insulin (39.1 ± 19.6 vs. 34.0 ± 7.4 $\mu\text{U/ml}$, NS). The Ins-AUC₀₋₁₂₀ (area under the curve from 0 to 120 min for serum insulin) (339.8 ± 218 , 681.3 ± 407 , and $1,586.7 \pm 8$ $\mu\text{U/ml}$, $P < 0.05$) and C_{\max} (7.6 ± 2.8 , 16.4 ± 9.3 , and 39.1 ± 19.6 $\mu\text{U/ml}$, $P < 0.005$) proved a dose-response relationship for the three doses of oral insulin (5, 10, and 20 puffs, respectively). Oral insulin had an earlier onset of action (31.7 ± 12 vs. 77.8 ± 3 min, $P < 0.05$), earlier peak (44.2 ± 10 vs. 159.2 ± 68 min, $P < 0.05$), and a shorter duration of action (85.1 ± 25 vs. 319.2 ± 45 min, $P < 0.05$) compared with subcutaneous insulin. The maximum metabolic effect (1.7 ± 1.0 , 3.09 ± 1.7 , and 4.6 ± 1.5 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P < 0.05$) and the GIR-AUC₀₋₁₂₀ (amount of glucose infused from 0 to 120 min) (106.7 ± 74.3 , 162.9 ± 116.1 , and 254 ± 123 mg/kg) increased in a dose-dependent relationship for the three doses.

CONCLUSIONS— Oral insulin was absorbed in direct relation to the amount given and had a faster onset and a shorter duration of action compared with subcutaneous regular insulin. A dose-response relationship in the absorption and metabolic effect of the oral insulin was noted.

Diabetes Care 28:1353–1357, 2005

Insulin therapy is central to the treatment of people with type 1 (and some with type 2) diabetes. Intensive insulin regimen is proven to be associated with better long-term clinical outcomes; however, the inconvenience of multiple daily insulin injections might reduce compliance and possibly constitute an obstacle to obtaining good glycemic control.

Therefore, alternative, noninjectable

ways of insulin administration are being researched. One of these is oral insulin administration delivered by a spray device into the oropharyngeal cavity. It has the potential to more closely resemble the meal-stimulated physiologic insulin profile and therefore to become an attractive option and easy-to-use route for covering prandial insulin requirements.

We used an oral insulin spray system

that consists of an insulin formulation containing human recombinant insulin (Humulin R), a surfactant, a solubilizer, a micelle-creating agent, and emulsifying agents. All excipients are found on the FDA Generally Recognized as Safe list. The Generex RapidMist device, which is a proprietary pressurized Metered Dose Inhaler used to administer small- and large-molecule drugs to the buccal mucosa was used. This device is similar to that of an asthma inhaler and consists of the following components: an actuator with a dust cap, a metering valve, and an aluminum canister. The device produces an aerosol of uniform-sized particles. The mixed micellar particles result from the combination of the absorption enhancers and stabilizers, which encapsulate and protect the insulin molecules. The system introduces the aerosol at a high velocity (100 mph) into the subject's oropharyngeal cavity. The impelled micelles transverse the buccal mucosa superficial layers, and with the aid of the absorption enhancers, insulin molecules get rapidly absorbed into the bloodstream (1).

An essential prerequisite to proving the clinical viability of this novel insulin delivery system is showing that it can be dose titrated in a similar fashion to subcutaneous injections. Therefore, the objective of our study was to investigate the pharmacodynamic and pharmacokinetic properties of different doses of oral insulin spray in comparison with subcutaneously injected regular insulin in healthy subjects under the euglycemic clamp technique (2).

RESEARCH DESIGN AND METHODS

This study was conducted at the Diabetes Unit, Hadassah-Hebrew University Hospital, Jerusalem, Israel. The study protocol, informed consent, and poststudy assessment were approved by the local ethics committee and conducted in accordance with the principles of the Declaration of Helsinki and its amendments (to October 2000, Edinburgh, Scotland) and the Good Clinical Practice Guidelines (3).

This study included seven adult healthy male subjects. All subjects gave

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Received for publication 23 August 2004 and accepted in revised form 31 January 2005.

I.R. has received grant/research support from Generex Biotechnology.

Abbreviations: AUC, area under curve; GIR, glucose infusion rate; Ins-AUC, AUC for serum insulin.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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written informed consent before screening for possible participation in the study and after receiving detailed information about the purpose, procedures, and potential risks of the study. Screening involved physical examinations and clinical and laboratory tests conducted within 14 days before study entry. Exclusion criteria included history or evidence of diabetes, any current or previous significant medical condition or treatment, including oral lesions and/or disease involving the oral cavity, participation in another study within the previous 90 days, and any significant abnormality on electrocardiogram or routine laboratory blood screen. The subjects were instructed to consume a weight-maintenance diet and to avoid strenuous physical exercise, alcohol, or concomitant medication intake 3 days before and during the entire study period.

This was a single-center, blinded, randomized, five-way, cross-over, open-label study. On five different occasions 7 days apart, the subjects were assessed by means of euglycemic clamp technique for pharmacodynamics and pharmacokinetics characteristics of four different doses of oral spray (placebo spray: 10 puffs; insulin spray: 5 puffs, 10 puffs, and 20 puffs) and one dose of 0.1 unit/kg s.c. human regular insulin, respectively. Both the physician and the patients were blinded to the amount of insulin given by the spray device. Because oral insulin spray was an aerosolized, aqueous, regular human insulin solution, injectable regular human insulin was chosen as the comparator. To minimize the potential for errors or improper use of the device, all subjects underwent training using an identical placebo spray device.

Euglycemic clamp

After an overnight fast, the subjects were admitted to the clinic on the morning of the experiment and remained fasting and in a supine position throughout the day. A G-20 catheter needle was placed into a superficial forearm vein for the intravenous infusion of 20% glucose solution (TEVA Medical, Ashdod, Israel); 1.5 mg somatostatin (Sandostatin; Novartis Pharma, Basel, Switzerland), diluted into 0.9% NaCl solution containing 0.5% human serum albumin (Kamada, Negev, Israel) to obtain a final concentration of 0.03 mg/ml; and 20 units insulin (Actrapid HM; Novo Nordisk, Bagsvaerd, Denmark), diluted into 0.9% NaCl solu-

tion containing 0.5% human serum albumin to obtain a final concentration of 0.04 units/ml. After the catheter insertion, baseline blood samples were obtained for measurements of insulin and C-peptide. To inhibit endogenous insulin and glucagon secretion, somatostatin was infused at a constant rate of 25 ng · kg⁻¹ · min⁻¹, using a Harvard pump (Harvard Apparatus, South Natick, MA) 5 min before starting the glucose and insulin infusion administered at a constant rate of 0.2 mU · kg⁻¹ · min⁻¹. A butterfly needle was inserted retrogradely into a dorsal vein of the contralateral hand that was kept warm using a hot pad (55–60°C) for sampling of arterialized blood (4) every 5 min (and blood glucose concentration was immediately measured at bedside). The glucose infusion rate was adjusted, according to the actual blood glucose level, in order to keep the blood glucose constant at a target level of 90 ± 5 mg/dl by means of the euglycemic clamp technique (2).

After a stabilization period of 120 min, the subjects received on separate occasions and in a randomized order: 1) a single dose of 0.1 unit/kg regular insulin (Actrapid HM) (7.6 ± 1.1 units [means ± SD] that was injected subcutaneously into the umbilical region by means of a syringe (Terumo Europe N.V., Leuven, Belgium), 2) a dose of 10 puffs placebo spray, 3) a dose of 5 puffs (50 units) spray insulin, 4) a dose of 10 puffs (100 units) spray insulin, and 5) a dose of 20 puffs (200 units) spray insulin. The volunteers were instructed to relax and breathe normally; the mouthpiece of the device was placed into the mouth at the end of a normal expiration, and the subjects sprayed the insulin while holding their breath for about 5 s after each puff.

The glucose infusion rates (GIRs) continued to be monitored over the subsequent 360 min. Blood samples for estimation of plasma insulin and C-peptide concentrations were collected at 30-min intervals during the 2-hour equilibration period and at intervals of 5 min (0–60 min), 15 min (60–120 min), and 30 min (120–360 min) after treatment administration.

Analysis of blood samples

Two-milliliter samples of whole blood were drawn into tubes containing lithium heparin (Vacutainer; Beckton Dickinson, Franklin Lakes, NJ) and kept on ice. At the end of the study day, samples were centrifuged at 4°C for 10 min and plasma

for hormone analyses was stored at –28°C. Insulin and C-peptide levels were measured using a radioimmunoassay kit (Linco Research, St. Charles, MO). Human insulin and C-peptide were used as standards.

Statistical methods

The areas under the curve (AUCs) for GIR and insulin were calculated using the trapezoidal rule. Baseline GIR was calculated as the average of all GIRs recorded for the 60 min preceding the treatments. Baseline insulin was calculated as the means of values taken at 1.5, 1.0, 0.5, and 0 h before dosing. Since the sample size in this study was small, nonparametric tests were used to analyze the data. The nonparametric Friedman test was applied to check whether there was a significant trend in repeated measures, and the Bonferroni correction was applied for multiple pairwise comparisons.

Coefficients of variation (CVs) (CV = SD/mean) were used to describe the inpatient and between-patient variability of insulin kinetics for subcutaneous and oral spray insulin treatments. Due to the fact that the subjects received a certain dose of oral spray insulin only one time, we could not directly calculate the inpatient variability, and therefore we standardized the AUC values by multiplying them fourfold (for 5 puffs) and twofold (for 10 puffs). Statistical significance was assumed at *P* < 0.05.

RESULTS— Seven healthy subjects were included and completed the study: all male, age 23.6 ± 2.7 years (means ± SD), weight of 75.7 ± 12.1 kg, BMI 23.6 ± 2.5 kg/m². Fasting blood glucose levels were similar between the five visits (96.33 ± 8.5 mg/dl for subcutaneous, 92.6 ± 6.7 mg/dl for placebo, 95.9 ± 6.7 mg/dl for 5 puffs, 93.6 ± 6.8 mg/dl for 10 puffs, and 91 ± 6.8 mg/dl for 20 puffs, NS). Similarly, there were no statistically significant differences between the visits as regarding serum insulin and C-peptide levels at baseline (before treatment administration).

Pharmacodynamic and pharmacokinetic results after administration of oral spray or subcutaneous insulin are shown in Table 1 and Figs. 1 and 2.

Pharmacokinetics

The basal serum insulin levels before treatment administration were similar across the five visits (Table 1). The maxi-

Table 1—Summary of pharmacokinetic and pharmacodynamic parameters after administration of subcutaneous and spray insulin

Parameters	Subcutaneous	Placebo	5 puffs	10 puffs	20 puffs	P*
Pharmacokinetics						
Basal serum insulin ($\mu\text{U/ml}$)	18.82 \pm 4	15.71 \pm 3.8	18.57 \pm 7	15.41 \pm 5.8	19.75 \pm 6.2	0.43
C_{max} ($\mu\text{U/ml}$)†	34.03 \pm 7.4	5.85 \pm 4.2	7.57 \pm 2.8	16.43 \pm 9.3	39.1 \pm 19.6	<0.001
Ins-AUC ₀₋₁₂₀ ($\mu\text{U} \cdot \text{kg}^{-1} \cdot 120 \text{ min}^{-1}$)†	2,529.73 \pm 725.8	105.03 \pm 78	339.82 \pm 218.2	681.38 \pm 407.7	1,586.78 \pm 824	<0.001
Ins-AUC ₀₋₂₄₀ ($\mu\text{U} \cdot \text{kg}^{-1} \cdot 240 \text{ min}^{-1}$)†	5,641.16 \pm 976.3	473.82 \pm 382.8	543.39 \pm 307.7	1,079.01 \pm 644.5	2,018.57 \pm 2,056.1	<0.001
Ins-AUC ₀₋₃₆₀ ($\mu\text{U} \cdot \text{kg}^{-1} \cdot 360 \text{ min}^{-1}$)†	7,571.87 \pm 815.3	751.1 \pm 579	831.07 \pm 384	1,464.7 \pm 775.4	2,423.57 \pm 1,409.6	<0.001
t_{max} (min)	145.71 \pm 49.5	182.14 \pm 66.1	26.42 \pm 8	25 \pm 9.5	26.42 \pm 13.4	<0.001
Pharmacodynamics						
Basal GIR ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	2.13 \pm 1.4	1.78 \pm 1	1.91 \pm 0.7	1.57 \pm 0.7	2.24 \pm 1.4	0.507
GIR _{max} ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	6.82 \pm 1.3	2.47 \pm 0.89	1.72 \pm 1.03	3.09 \pm 1.6	4.61 \pm 1.5	<0.001
GIR-AUC ₀₋₁₂₀ ($\text{mg} \cdot \text{kg}^{-1} \cdot 120 \text{ min}^{-1}$)†	305.56 \pm 150.3	48.89 \pm 42.6	106.69 \pm 74.3	162.87 \pm 116.1	253.99 \pm 122.9	0.002
GIR-AUC ₀₋₂₄₀ ($\text{mg} \cdot \text{kg}^{-1} \cdot 240 \text{ min}^{-1}$)†	891.74 \pm 319.1	151.22 \pm 110.9	237.95 \pm 399.6	252.36 \pm 184	445.41 \pm 204.8	0.001
GIR-AUC ₀₋₃₆₀ ($\text{mg} \cdot \text{kg}^{-1} \cdot 360 \text{ min}^{-1}$)†	1,340.16 \pm 321.6	327.45 \pm 177.9	399.6 \pm 205.2	456.74 \pm 194.1	569.46 \pm 239.9	0.002
t_{max} (min)	159.28 \pm 66.9	182.14 \pm 89.3	45 \pm 13.2	50 \pm 14.4	44.28 \pm 10.1	0.002
Early $t_{50\%}$ (min)	77.85 \pm 28.5	160.14 \pm 82.5	27.71 \pm 8.8	35.57 \pm 17	32 \pm 8.6	0.003
Late $t_{50\%}$ (min)	319.28 \pm 45.1	287 \pm 91.6	81.42 \pm 30.3	89.28 \pm 21.8	84.71 \pm 27.4	<0.001

Data are means \pm SD. *The analysis refers to the comparison of all five treatments. †After baseline subtraction.

imum serum insulin levels (C_{max}) as well as AUC of the serum insulin (Ins-AUC) showed a clear dose-response relationship for the three doses of oral insulin (Table 1 and Fig. 1).

There was no effect of the oral insulin dose on the time to maximum insulin concentration, which was similar across the dosage range studied ($P = 0.63$). The time to maximum insulin concentration was significantly shorter for oral insulin compared with subcutaneously injected insulin ($P = 0.018$) (Table 1 and Fig. 1).

Variability between subjects for serum insulin at AUC 120, 240, and 360 min were 0.28, 0.17, and 0.11, respectively, for subcutaneous insulin and 0.58, 0.55, and 0.52, respectively, for oral spray insulin.

The within-subject CVs for AUC 120, 240, and 360 min were 0.48 (± 0.13), 0.5 (± 0.16), and 0.51 (± 0.12), respectively for oral insulin spray.

Pharmacodynamics

The baseline glucose requirements before treatment administration were similar between the five visits (Table 1). The maximum metabolic effect (GIR_{max}) and the amount of glucose infused (GIR-AUC) increased in a dose-dependent fashion for the three doses of oral insulin studied (Table 1 and Fig. 2). The time to maximum metabolic effect (t_{max}), the time to early half-maximum effect (early $t_{50\%}$), and the time to late half-maximum effect (late $t_{50\%}$) were similar between the three doses of oral insulin: 5, 10, and 20 puffs ($P: 0.772, 0.707,$ and 0.764 , respectively).

Oral insulin administration was associated with a more rapid increase in glucose consumption, as indicated by smaller t_{max} values compared with those obtained for subcutaneous insulin ($P = 0.018$). Similarly, early $t_{50\%}$ and late $t_{50\%}$ also occurred significantly sooner compared with subcutaneous insulin ($P = 0.018$) (Table 1 and Fig. 2). After the first 120 min and 360 min following administration of the three doses of oral insulin, the plotted data (mean Ins-AUC versus GIR-AUC) was shown to lie on a linear dose-response curve.

The continuous insulin and somatostatin infusions (and orally administered insulin) resulted in a reduction of the mean serum C-peptide values throughout the experiments from mean values of $1.5 \pm 0.7 \text{ ng/ml}$ for subcutaneous, $1.5 \pm 0.4 \text{ ng/ml}$ for placebo, $1.8 \pm$

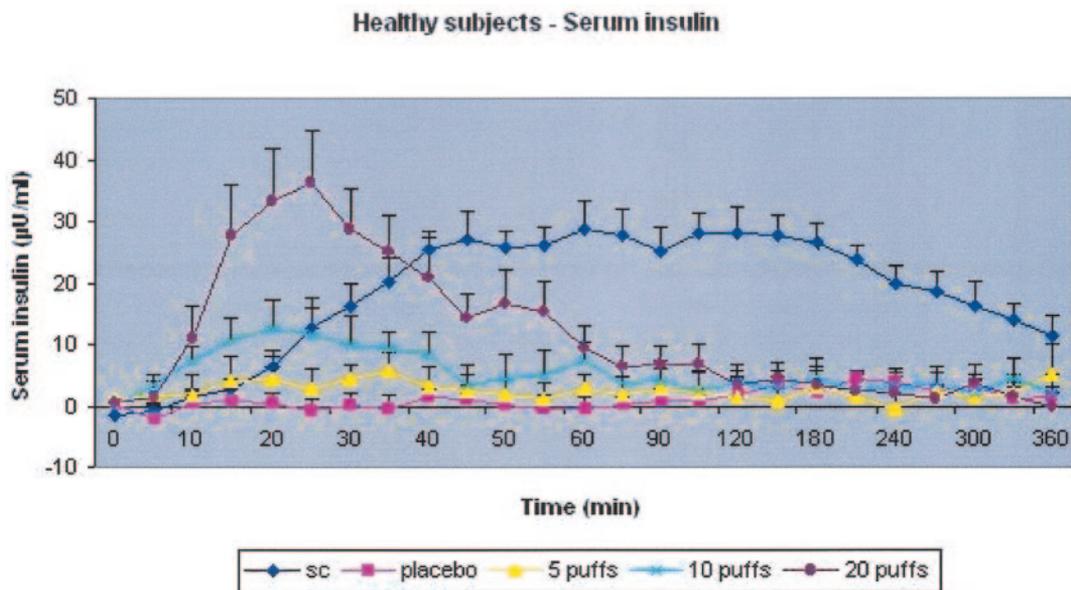


Figure 1—Mean (SE) serum insulin levels ($\mu\text{U/ml}$) over 360 min following administration of subcutaneous insulin and oral spray insulin in healthy subjects.

0.5 ng/ml for 5 puffs, 1.7 ± 0.4 ng/ml for 10 puffs, and 1.6 ± 0.3 ng/ml for 20 puffs, respectively, to values <0.4 ng/ml.

Tolerability of insulin inhalation

The oral insulin spray was generally well tolerated by all subjects; nevertheless, five subjects complained of dizziness while taking the spray (both placebo and insulin spray). The reported dizziness was mild to moderate, self-limited, and transient (1–2 min). We did not consider it to be clinically relevant.

CONCLUSIONS— Novel noninvasive delivery systems have been researched to overcome some of the limitations of conventional insulin therapy, mainly the fear and discomfort of using needles (5,6) and the mismatch between the time-action profile of the administered insulin and the postprandial glucose excursions (7). Even with the new insulin analogs, there is still a less-than-ideal synchronizing of insulin action and glucose absorption from a meal.

One of the new routes of insulin de-

livery that has shown some success is the pulmonary inhaled insulin, which offers some advantages over the injected insulin (8,9). However, even with this method of delivery, there are some disadvantages and concerns, mainly related to its long-term safety and tolerability (10,11).

Therefore, in this study we evaluated orally administered insulin via a spray device, which might offer an attractive alternative in terms of convenience, ease of use, time-action profile, and acceptability. To our knowledge, this is the first re-

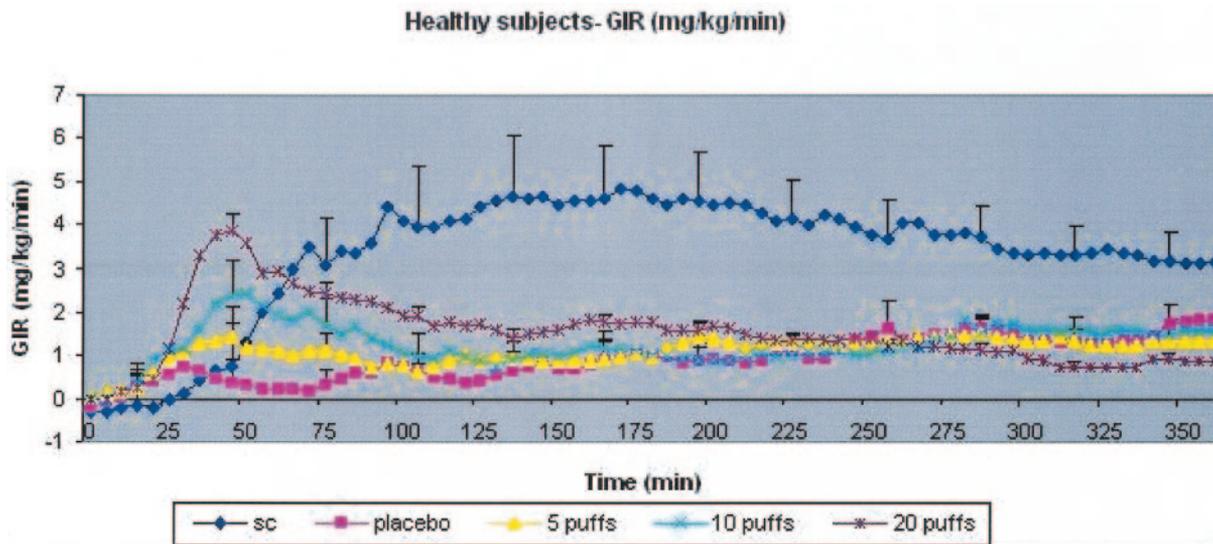


Figure 2—Mean (SE) GIR (in milligrams per kilogram per min) over 360 min following administration of subcutaneous insulin and oral spray insulin in healthy subjects.

port of the dose response of an oral insulin formulation in comparison with subcutaneous insulin under glucose clamp conditions in healthy volunteers. In the present study, we used an orally administered insulin by means of a Rapid-Mist Insulin Device, the insulin being mainly absorbed through the mucosa of the oropharynx.

The present data indicate a more rapid increase in maximum serum insulin levels and consequently a faster onset of action (early $t_{50\%}$, t_{max}) of oral insulin compared with subcutaneous administration. This effect can be explained by a rapid rate of insulin absorption across the buccal and pharyngeal mucosae, due to the large available and relatively permeable absorption area. From a therapeutic point of view, this characteristic of oral insulin spray allows insulin to be dosed closer to the start of a meal, so that the onset of postprandial glucose control is faster and optimized. The possibility of using preprandial insulin just a few minutes before meals represents a substantial improvement in quality of life.

A recent report showed dose-dependent time to peak insulin levels with a right shift of the time-action profile for subcutaneously injected insulin (12). In contrast, we found that for oral insulin the time to maximum insulin concentrations, as well as time to peak metabolic effects, appear to be independent of the administered dose. From a clinical perspective, this might be a distinct advantage of orally administered insulin spray over subcutaneous insulin.

The AUC for GIR and serum insulin showed that oral insulin is mainly absorbed and effective in the first 2 h after its administration, which is the crucial time period for postprandial glucose control. A major advantage of oral insulin over injected insulin is that its maximum metabolic activity occurs earlier and has a shorter run-off. More importantly, its effect covers the 2 h after food intake, mimicking more closely the physiological insulin secretion in response to meals. Due to this time-action profile, there could potentially be a reduced risk of postprandial hypoglycemic events and of the need for between-meal snacks.

The present data indicate that increasing doses of oral insulin resulted in a dose-dependent increase in serum insulin concentrations and subsequent glucose-lowering effects; thus, a plot of Ins-AUC versus GIR-AUC demonstrated a clear dose-response relation for oral insulin. This finding is important in clinical practice because it allows dose adjustments for oral insulin as for other types of insulin. On the other hand, the CV between patients of serum insulin after oral spray was higher than after subcutaneous insulin injection.

Another important advantage of oral insulin spray is the convenience of administration by the patient. It is very easy to take and, as most of the patients have more than three food intakes per day, oral insulin can easily be used up to six to eight times a day depending on food ingestion. Patient satisfaction and acceptance of a formulation/delivery system are very important in routine clinical practice. Therefore, the ease of use of oral insulin spray may result in potentially better compliance and, in addition, may provide a better noninvasive alternative in special groups like children, adolescents, the elderly, or patients who are otherwise unresponsive to multiple injection regimens.

The present study had some limitations. First, the subjects received only one dose of subcutaneous insulin; therefore, a direct head-to-head comparison of the dose-response curves of oral versus injected insulin was not possible. Second, the number of subjects included in the study was small. Long-term clinical studies to evaluate the effectiveness of oral insulin spray in patients with diabetes, utilizing change in HbA_{1c} as the primary outcome variable along with some quality of life measures, will be required to establish oral insulin spray as a realistic noninvasive therapy for achieving adequate glycemic control.

Acknowledgments— We kindly acknowledge Yoheved Mizrahi for her valuable technical assistance, Rebecca Sprung, MS, for her editorial assistance, and Tali Bdolach-Abram, MS, for her skillful help with statistical analysis.

This study was presented in part at the Annual Meeting of the American Diabetes Association, Orlando, Florida, 4–8 June 2004 and at the 40th Annual Meeting of the European Association for the Study of Diabetes, Munich, Germany, 5–9 September 2004.

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