Beneficial Effects of Insulin Versus Sulphonylurea on Insulin Secretion and Metabolic Control in Recently Diagnosed Type 2 Diabetic Patients

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OBJECTIVE — To evaluate whether treatment with insulin in recently diagnosed type 2 diabetes is advantageous compared with glibenclamide treatment.

RESEARCH DESIGN AND METHODS — β-Cell function, glycemic control, and quality of life were monitored over 2 years in 39 patients with islet cell antibody–negative type 2 diabetes diagnosed 0–2 years before inclusion in a Swedish multicenter randomized clinical trial. Patients were randomized to either two daily injections of premixed 30% soluble and 70% NPH insulin or glibenclamide (3.5–10.5 mg daily). C-peptide–glucagon tests were performed yearly in duplicate after 2–3 days of temporary withdrawal of treatment.

RESULTS — After 1 year the glucagon-stimulated C-peptide response was increased in the insulin-treated group by 0.14 ± 0.08 nmol/l, whereas it was decreased by 0.12 ± 0.08 nmol/l in the glibenclamide group, $P < 0.02$ for difference between groups. After 2 years, fasting insulin levels were higher after treatment withdrawal in the insulin-treated versus the glibenclamide-treated group ($P = 0.02$). HbA_{1c} levels decreased significantly during the first year in both groups; however, at the end of the second year, HbA_{1c} had deteriorated in the glibenclamide group ($P < 0.01$), but not in the insulin-treated group. The difference in evolution of HbA_{1c} during the second year was significant between groups, $P < 0.02$. A questionnaire indicated no difference in well-being related to treatment.

CONCLUSIONS — Early insulin versus glibenclamide treatment in type 2 diabetes temporarily prolongs endogenous insulin secretion and promotes better metabolic control.

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In type 2 diabetes, metabolic control deteriorates in most patients when the duration of diabetes increases (1). Decreased insulin secretion likely explains this deterioration in metabolic control (1,2). Decreased insulin secretion could be due to excessive secretory demands on the β-cells (1). If so, insulin treatment would be beneficial by providing relative "β-cell rest." However, sulfonylurea drugs may exert negative effects by overstimulating β-cells. No studies, to our knowledge, have been performed that rigorously compare the effects of sulfonylurea versus insulin treatment on the deterioration of insulin secretion in type 2 diabetic patients. The U.K. Prospective Diabetes Study (UKPDS) (1) randomized patients at inclusion to insulin or sulfonylurea and documented a deterioration of insulin secretion after sulfonylurea; however, endogenous insulin secretion in the insulin-treated group was not evaluated due to ongoing insulin treatment.

The main aim of this prospective study was to examine whether insulin versus glibenclamide treatment started soon after the diagnosis of type 2 diabetes is associated with better β-cell function. We furthermore tested whether the two treatments affected differently metabolic control and other clinically relevant parameters. We studied patients with recent onset of type 2 diabetes, after excluding latent autoimmune diabetes in adults (LADA) (3).

RESEARCH DESIGN AND METHODS

Patients

Women and men, 35–70 years of age, with type 2 diabetes diagnosed <2 years earlier were asked to participate. Inclusion criterion was fasting blood glucose concentrations between 7.0 and 12.0 mmol/l during screening on one occasion when on diet alone for at least 1 month. Patients were excluded if pharmacologically treated >6 months or showing low fasting plasma C-peptide concentrations (<0.2 nmol/l), significant ketonuria (more than trace amounts), BMI >35 kg/m^2, plasma creatinine >150 μmol/l, severe retinopathy (proliferative or preproliferative), severe cardiac disease (NY

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Abbreviations: GADA, GAD antibody; IA2, insulinoma-associated protein 2; IA-2A, IA2 antigen; ICA, islet cell anitbody; IAPP, islet amyloid polypeptide; LADA, latent autoimmune diabetes in adults; RIA, radioimmunoassay; UKPDS, U.K. Prospective Diabetes Study.

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

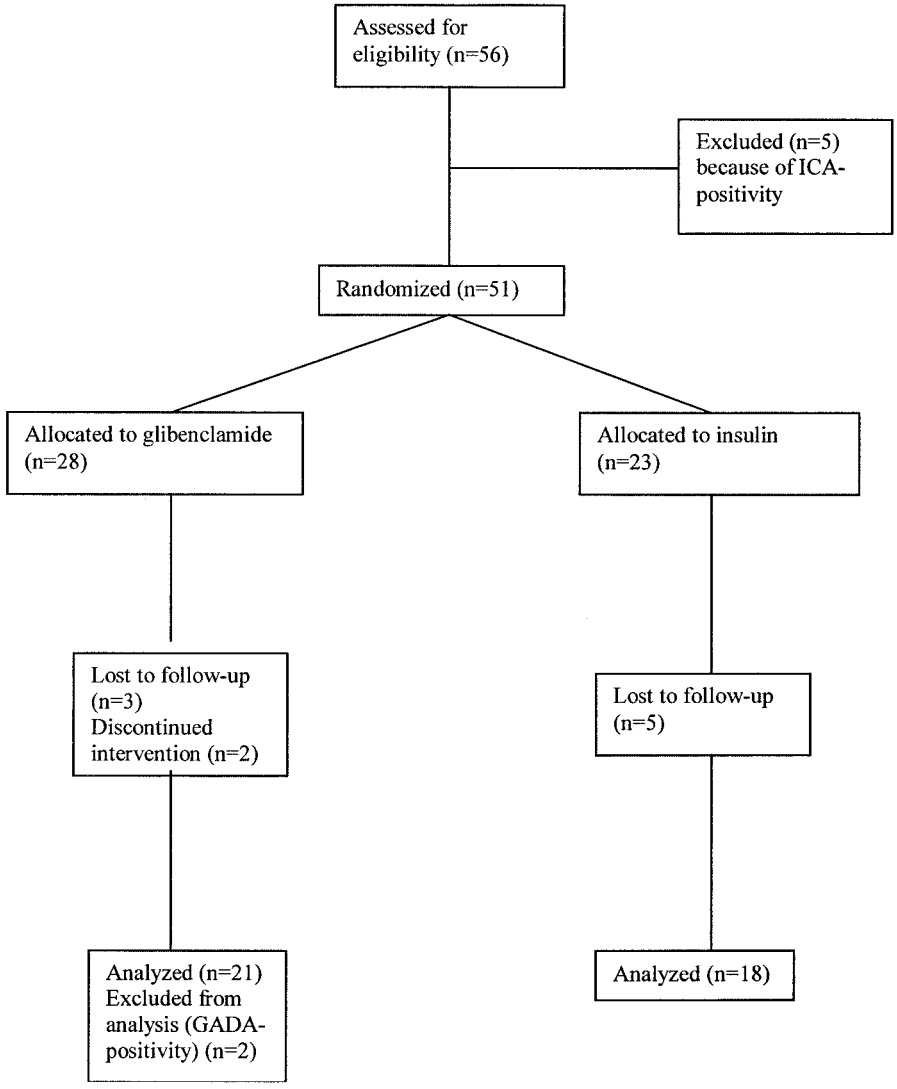

Figure 1—*Design of the study (see text for details).*

Heart Association III–IV) or other potentially life-threatening disease, or positivity for islet cell antibodies (ICAs). Six Diabetic Clinics in Sweden participated in the study, which was approved by the ethics committee at the Karolinska Institute.

Experimental design

Patients were randomized to treatment with glibenclamide or insulin (Fig. 1). Treatment with glibenclamide was started at a dose of 1.75 mg once daily. The dose was then adjusted by steps of 1.75–3.5 mg, with the aim of keeping HbA_{1c} levels within target level, i.e., ≤1% above the upper normal level of HbA_{1c}. Insulin was given twice daily as premixed insulin, i.e., a combination of 30% soluble and 70% NPH insulin (Mixtard 30/70; Novo Nordisk, Copenhagen, Denmark). The starting dose of insulin was 0.25 units · kg^{-1} · 24 h^{-1}. Two-thirds of the daily dose was given before breakfast and one-third before supper. The insulin doses were adjusted as follows: *1*) increase of total dose by 10% if mean 24-h capillary blood glucose (home glucose monitoring) was >12 mmol/l, *2*) decrease of total dose by 10% if mean capillary blood glucose at home glucose monitoring was <6 mmol/l, or *3*) decrease of individual dose by 10% if blood glucose was <4.0 mmol/l at a time point 2 h or later after the last dose.

Glucagon tests were performed yearly in duplicate on 2 consecutive days (details below) and antidiabetic treatment (glibenclamide or insulin) was withdrawn 48 h before the first day and 72 h before the second day of testing. Quality of life was assessed yearly by the SF-36 questionnaire that measures eight health concepts: physical functioning, role physical, body pain, general health, vitality, social functioning, role emotional, and mental health (4).

The glucagon test was carried out in the morning (0800) after a 10-h fast. Blood samples were taken immediately before an intravenous injection of 1 mg glucagon (Novo Nordisk, Copenhagen, Denmark) and 6 min thereafter. Samples for glucose determination were collected in tubes with fluoride and heparin, and for C-peptide in tubes with EDTA with addition of aprotinin 10,000 KIE/ml 0.1 ml/ml whole blood. Glucose samples were immediately sent to the laboratory for measurements. Other samples were frozen and kept at −70°C until assayed.

Samples for HbA_{1c} analysis were taken as capillary blood samples on filter paper (5) and sent to the Department of Clinical Chemistry, Malmö University Hospital, for analysis.

Retinopathy

Fundus photography was conducted at the patient's local hospital and centrally assessed (6).

Dropouts during the study

Of 56 patients eligible for randomization, 5 were excluded because they tested positive for ICA. Among remaining patients, 28 were randomized to glibenclamide and 23 to insulin treatment.

Ten participants left the study prematurely. Two patients randomized to insulin died (one following coronary artery bypass surgery and another because of stomach carcinoma). Six patients (three on glibenclamide and three on insulin) left the study for various personal reasons. Two in the glibenclamide group left after 1 year because they needed insulin to control their diabetes. (The guideline for a perceived need for insulin treatment was HbA_{1c} consistently >3% above the upper reference limit.) Furthermore, two patients in the glibenclamide group were excluded from analysis because of positivity for GAD 65 antibodies (GADA) (later determined). Their GADA indices were 96.6 and 157.2, respectively. Both tested negatively for ICAs and protein tyrosine phosphatase–like protein insulinoma-associated protein 2 (IA2) antibodies (IA-2A).

Table 1—*Clinical characteristics at baseline in the two treatment groups*

	Glibenclamide	Insulin
n	21	18
Age (years)	55.6 ± 1.6	51.1 ± 1.7
Sex (M/F)	15/6	11/7
BMI (kg/m^2)	27.8 ± 0.8	27.3 ± 0.7
HbA_{1c} (%)	6.9 ± 0.2	7.3 ± 0.4
AER (mg/l)	9.7 ± 3.5	8.8 ± 2.1
Systolic blood pressure (mmHg)	139 ± 3 (18)	144 ± 7 (16)
Diastolic blood pressure (mmHg)	85 ± 2	83 ± 2
Retinopathy	3/19	3/16
Total cholesterol (mmol/l)	5.39 ± 0.16	5.47 ± 0.24
LDL cholesterol (mmol/l)	3.39 ± 0.13	3.40 ± 0.20
HDL cholesterol (mmol/l)	1.06 ± 0.06	1.09 ± 0.04
Triglycerides (mmol/l)	2.25 ± 0.26	2.06 ± 0.16

Data are mean ± SEM or mean ± SEM (*n*).

Assays

ICAs were determined by a prolonged two color immunofluorescence assay (7). GADA and IA-2A were determined by radioligand binding assays (8). HbA_{1c} was determined by high-performance liquid chromatography (reference values 3.90–5.30%) (9). C-peptide was assayed by radioimmunoassay (RIA) (Euro-Diagnostica, Malmö, Sweden). The lowest detectable concentration was 0.05 nmol/l, the intraassay variation 5%, and the total variation (sum of intra- and interassay variation) 7%. Cross-reactivity with proinsulin was 41%. Proinsulin and insulin were determined by kits from Linco.

Islet amyloid polypeptide (IAPP) was assayed in 2 ml plasma acidified with acetic acid and absorbed on C-18, reversed phase Sep-Pak Cartridge (Millipore) eluted with 3 ml 50% acetonitril in 0.1% trifluoroacetic acid. Iodated porcine IAPP purchased from Amersham and anti-IAPP antiserum RAS7321 (Peninsula, Merseyside, U.K.) were used in the RIA. The antiserum reacts equally well with rat and human IAPP. The samples were analyzed in duplicate (10). The recovery after extraction was 60% and the intra-assay variation <10%.

Statistics

The Mann-Whitney *U* test was used to evaluate differences between groups, whereas Friedman's test (variance analysis) followed by Wilcoxon's paired test were used to evaluate differences within groups. $P < 0.05$ was considered significant. All statistical analyses were performed with STATISTICA 6.0 (StatSoft, Tulsa, OK). Data are presented as mean ± SEM and proportions.

RESULTS

Baseline characteristics

The patients were middle-aged, moderately overweight, and in a fairly good metabolic control as assessed by HbA_{1c} (Table 1). Before randomization, the two treatment groups did not differ significantly in baseline clinical features including lipid parameters.

Glibenclamide and insulin dosage

After 1 year, the glibenclamide group received 2.4 ± 0.4 mg/day of glibenclamide and the insulin group 20.6 ± 2.0 IU/day. After 2 years, the dose of glibenclamide had increased significantly ($P = 0.03$) to 3.0 ± 0.5 mg/day, whereas the insulin dose was essentially unchanged (22.3 ± 2.2 IU/day).

Other medications

At baseline, seven patients in the glibenclamide-treated group and one patient in the insulin-treated group received β-blockers, lipid-lowering drugs, ACE inhibitors, or angiotensin II receptor antagonists. After 2 years, the corresponding totals were 10 patients in the glibenclamide and 6 patients in the insulin-treated group.

Body weight

Body weight increased in the glibenclamide group from 86.4 ± 2.7 to 88.1 ± 3.0 kg after 2 years ($P = 0.02$) and in the insulin group from 80.3 ± 2.4 to 83.0 ± 2.4 kg ($P < 0.01$). The increase in weight did not differ significantly between groups.

Lipids

HDL cholesterol levels increased significantly during the study in the glibenclamide group (0.15 ± 0.30 mmol/l, $P = 0.03$). Levels were unchanged in the insulin-treated patients (results not shown).

HbA_{1c}

HbA_{1c} decreased in both groups during the first year of treatment ($P < 0.01$) (Fig. 2*A*). At the end of the second year, HbA_{1c} had increased in the glibenclamide group ($P < 0.01$) but was still significantly lower compared with baseline ($P < 0.005$) in the insulin-treated patients. The difference in evolution of HbA_{1c} between groups was significant ($P = 0.02$).

Side effects

There were no obvious side effects of each treatment. In particular, there were no severe hypoglycemic episodes (coma or requiring help from others) during the study.

Concentrations of glucose before glucagon tests

After 1 year, fasting plasma glucose concentrations were lower than at baseline in both groups ($P < 0.05$) (Fig. 2*D*). After 2 years plasma glucose had increased on both day 1 ($P < 0.05$) and day 2 ($P = 0.02$) in the glibenclamide-treated group. In contrast, an increase in fasting glucose in the insulin-treated group occurred only between day 1 and 2 ($P = 0.002$).

Glucagon tests: C-peptide and IAPP

Fasting C-peptide levels did not differ between the insulin and glibenclamide groups during the study. In the insulin group, the C-peptide response to glucagon increased significantly during the study ($P = 0.02$); the effects were seen on the second day (first year, $P = 0.03$; second year, $P < 0.01$) (Fig. 2*C*). In contrast, the C-peptide response to glucagon in the glibenclamide group was unchanged. Importantly, the C-peptide response to glucagon in relation to baseline was significantly higher ($P = 0.02$) in the insulin than in the glibenclamide group on day 2 after 1 year in the study.

At baseline, the levels of IAPP increased during glucagon stimulation from 0.54 ± 0.12 to 1.54 ± 0.15 fmol/l in the

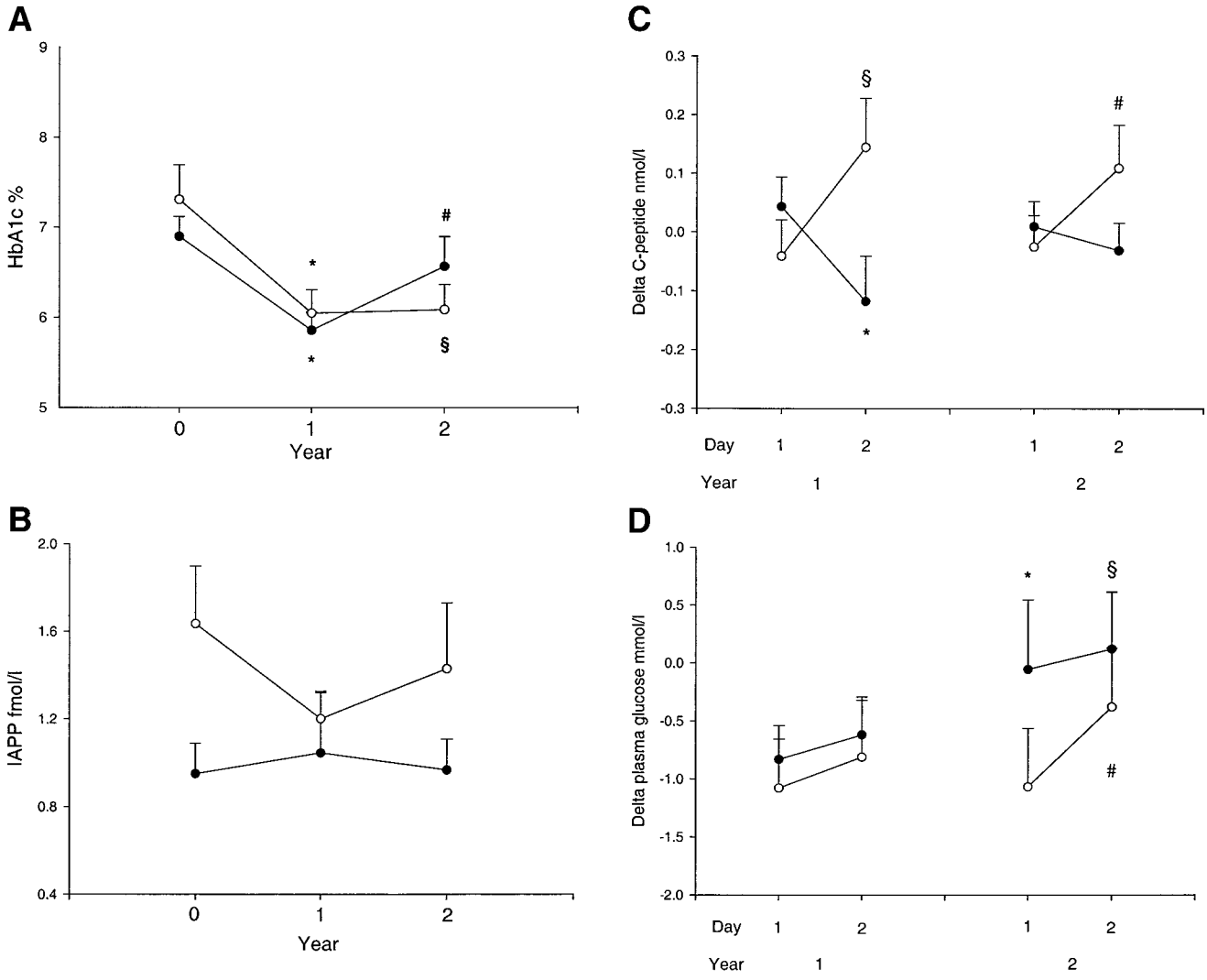

Figure 2—*Results (mean ± SEM) at start and during study for HbA_{1c} levels* (A) (*P < *0.01 year 0 vs. 1,* §P < *0.005 year 0 vs. 2,* #P < *0.01 year 1 vs. 2), IAPP responses to glucagon day 2* (B)*, incremental or decremental values for C-peptide responses to glucagon versus baseline* (C) (*P = *0.02 glibenclamide vs. insulin,* §P < *0.05 year 1 day 1 vs. 2;* P < *0.01 year 1 day 1 vs. year 2 day 2), and incremental or decremental values for fasting plasma glucose concentrations versus baseline* (D) (*P < *0.05 day 1 year 1 vs. 2,* §P = *0.02 day 2 year 1 vs. 2,* #P < *0.01 year 2 day 1 vs. 2).* ●*, glibenclamide;* ○*, insulin.*

glibenclamide group and from 0.44 ± 0.12 to 2.08 ± 0.34 fmol/l in the insulin group. After the first year the stimulatory effect of glucagon on IAPP was nonsignificantly decreased in the insulin, whereas it was unaltered in the glibenclamide group (−0.43 ± 0.31 vs. 0.25 ± 0.31 fmol/l) (Fig. 2*B*). After 2 years there were no apparent differences between groups (−0.21 ± 0.45 vs. 0.11 ± 0.16 fmol/l).

Levels of proinsulin and insulin

At baseline, fasting levels of proinsulin and proinsulin-to-insulin ratios did not differ significantly with respect to treatment (55 ± 16 pmol/l and 29 ± 2% for glibenclamide group vs. 35 ± 5 pmol/l and 30 ± 3% for insulin group) during the study. There was a tendency in the insulin group for an increase in fasting insulin levels day 2 after the first year ($P < 0.10$ vs. the glibenclamide-treated group); this effect was significant after 2 years ($P = 0.02$). Effects on proinsulin levels were similar to those of insulin (Fig. 3*A* and *B*).

Quality of life as assessed during treatments

The SF-36 score did not change during the study period for the group as a whole, nor were there any differences between the glibenclamide- and the insulin-treated patients during the 2 years (data not shown).

CONCLUSIONS — Two key findings emerge from the present study. First, parameters of β-cell function were better preserved in the insulin than in the glibenclamide-treated patients. Most importantly, there was a more pronounced C-peptide response to glucagon in the insulin group 1 year after the start of the study. Additionally, the levels of fasting insulin were reactive to increases in fasting blood glucose only in the insulin group. The nonsignificant decrease in glucagon-stimulated IAPP levels that we observed after 1 year in the insulin group

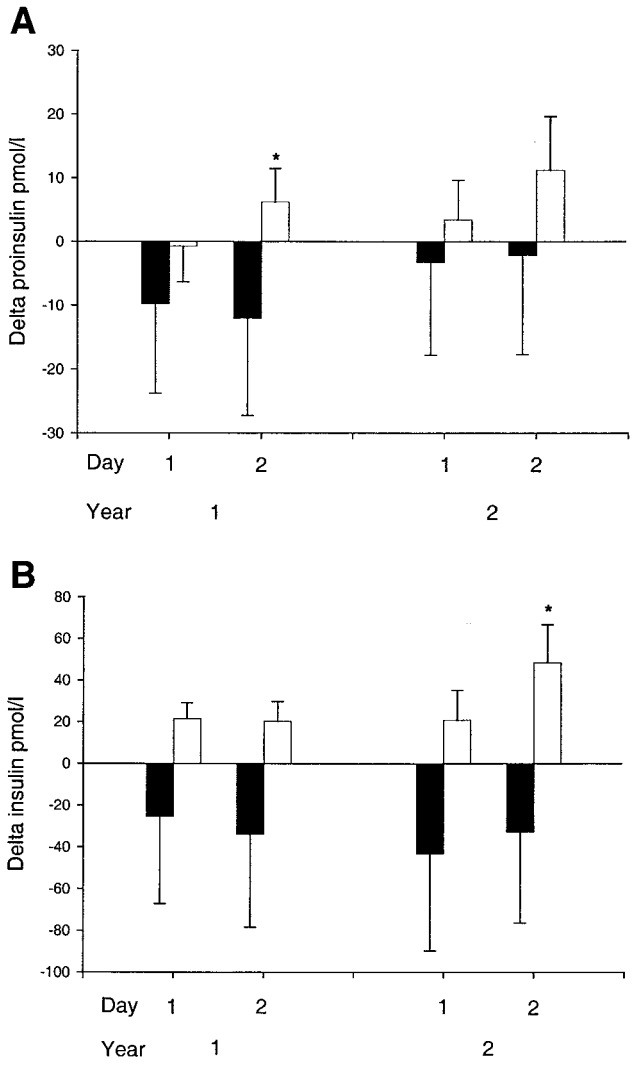

Figure 3—*Incremental or decremental values for fasting levels of proinsulin versus baseline,* (A) *(*P = 0.02 year 1 day 1 vs. 2) and insulin versus baseline,* (B) *(*P = 0.02 glibenclamide vs. insulin). Data are mean ± SEM.* ■*, glibenclamide;* □*, insulin.*

could also indicate lesser β-cell overstimulation (11). Second, improved metabolic control was achieved in both treatment groups initially, but later deteriorated in patients treated with glibenclamide, whereas patients treated with insulin did not deteriorate. Hence, early insulin treatment in type 2 diabetic patients had favorable effects compared with glibenclamide treatment.

Any claim for a treatment effect on insulin secretion necessitates validation of the testing procedure. The C-peptide–glucagon test is the most standardized one for testing endogenous insulin secretion (12). It has been validated in several respects (13–16). Ambient blood glucose potentially influences C-peptide responses, but only in the hypoglycemic range (17). In our study, any influence of ambient blood glucose would be negligible, since *1*) hypoglycemia was not encountered and *2*) patient concentrations of blood glucose were similar between test occasions. C-peptide–glucagon tests are usually performed during ongoing treatment with sulfonylureas or other antidiabetic medications. Such medication, however, influences endogenous insulin secretion. We therefore stopped pharmacological treatment for a minimum of 48 h (first of the two successive tests) and a maximum of 72 h (second of the two successive tests). Other studies have refrained from such measures for fear of rapid metabolic deterioration. Major deterioration was, however, not encountered in this study. To our knowledge, our experimental setting for glucagon–C-peptide testing is unique.

Our design with repeated C-peptide–glucagon tests, performed on successive days, was initially set up to reduce the influence of day-to-day variability on responses of individual patients. Thus, we aimed to combine the results from the 2 test days for each individual patient. The results, however, revealed important differences between tests performed after 48 vs. 72 h of treatment withdrawal. In tests performed after 1 year of study, the insulin group increased their responses from day 1 (48 h after treatment withdrawal) to day 2 (72 h after treatment withdrawal) of testing. In contrast, no such effect was seen in glibenclamide-treated patients. A likely interpretation is that β-cells are not performing at a maximal capacity during insulin treatment (because of relative β-cell rest) but are able to successively increase their responses to higher demands brought about by a slight increase in blood glucose due to withdrawal of exogenous insulin.

The beneficial effect of early insulin treatment on stimulated C-peptide was most pronounced after the first year of the study. It seems possible that a more profound β-cell rest by intensified insulin treatment could have prolonged the beneficial effect. Such treatment was not included here, since we wished to test a modality of insulin treatment that would be acceptable to a large segment of the type 2 diabetic population.

The increase in HbA_{1c} values in the glibenclamide-treated group after the initial improvement after the first year is in line with the evolution with time of metabolic control in the UKPDS (1). However, in sharp contrast to the present study, the insulin-treated subjects in the UKPDS experienced the same increase in HbA_{1c} as sulfonylurea-treated subjects. Our insulin regimen was different, and our goals of treatment stricter than in the UKPDS. These differences may account for the discrepancies between the studies.

The question arises whether a higher dosage of glibenclamide than that employed here could have delayed the increase in HbA_{1c} seen during the second year in the glibenclamide group. A maximally effective dose of glibenclamide is sometimes given as 10–20 mg/day; however, dose-response studies indicate max-

imal effects at ~7 mg/day using the present micronized preparation of the drug (18,19). Furthermore, an observational study reports better effects on metabolic control with doses below than above 5 mg/day (19). Hence, we consider it unlikely that increased dosage of glibenclamide would have improved metabolic control in our study.

The favorable effect of insulin treatment on glycemic control in our study could be due to better endogenous insulin secretion, although this was only evidenced by a trend for difference in C-peptide responses between groups. Another possibility is that β-cell secretory capacity may have diminished in all patients to a degree that would render supplementary insulin necessary to curb hyperglycemia.

What is the clinical significance of our findings? As previously mentioned, insulin treatment in patients with type 2 diabetes is typically instituted only after failure of peroral medications due to the cumbersome nature of insulin treatment with perceived risk of hypoglycemia. The observation that insulin treatment in the UKPDS was not associated with benefits in comparison to other treatment alternatives (20) may be taken as another argument against early insulin treatment in type 2 diabetic patients. Our findings of preserved endogenous insulin secretion and improved metabolic control, however, provide arguments for insulin treatment early in the disease. In further support, we found no difference in well-being between glibenclamide- and insulin-treated subjects and no difference in the frequency of hypoglycemia, which was rare in both groups.

Insulin treatment has been said to promote hyperlipidemia (21); however, this was not found in this study. Furthermore, the regulation of insulin doses in our patients conducted on an outpatient basis did not pose any problems and did not necessitate more consultations with doctors and nurses than did the control of blood glucose and treatment in the glibenclamide-treated group. Indeed, the weight gain in insulin-treated patients after 2 years did not differ from glibenclamide-treated patients. Weight gain, as observed in previous studies (22), does not seem to be a problem in our recently diagnosed type 2 diabetic patients started early on insulin treatment. One reason for this could be that the daily insulin dose in the current study was low compared with other studies.

In conclusion, an initial treatment with insulin in patients with type 2 diabetes had a favorable outcome with regard to endogenous insulin secretion and metabolic control compared with conventional sulfonylurea treatment.

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