Diabetologia (1990) 33: 688–695

Diabetologia

Partial recovery of insulin secretion and action after combined insulin-sulfonylurea treatment in Type 2 (non-insulin-dependent) diabetic patients with secondary failure to oral agents

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Summary. Metabolic control, insulin secretion and insulin action were evaluated in seven Type 2 (non-insulin-dependent) diabetic patients with secondary failure to oral antidiabetic agents before and after two months of combined therapy with supper-time insulin (Ultratard: 0.4 U/kg body weight/day) plus premeal glibenclamide (15 mg/day). Metabolic control was assessed by 24 h plasma glucose, NEFA, and substrate (lactate, alanine, glycerol, ketone bodies) profile. Insulin secretion was evaluated by glucagon stimulation of C-peptide secretion, hyperglycaemic clamp (+7 mmol/l) and 24 h free-insulin and C-peptide profiles. The repeat studies, after two months of combined therapy, were performed at least 72 h after supper-time insulin withdrawal. Combining insulin and sulfonylurea agents resulted in a reduction in fasting plasma glucose (12.9 ± 7 vs 10.4 ± 1.2 mmol/l; $p < 0.05$) and hepatic glucose production (13.9 ± 1.1 vs 11.1 ± 1.1 μmol·kg^{-1}·min^{-1}; $p < 0.05$). Mean 24 h plasma glucose was also lower (13.7 ± 1.2 vs 11.1 ± 1.4 mmol/l; $p < 0.05$). Decrements in fasting plasma glucose and mean 24 h profile were correlated ($r = 0.90$; $p < 0.01$). HbA_{1c} also improved (11.8 ± 0.8 vs $8.9 \pm 0.5\%$; $p < 0.05$). Twenty-four hour profile for NEFA, glycerol, and ketone bodies was lower after teatment, while no difference occurred in the blood lactate and alanine profile. Insulin secretion in response to glucagon (C-peptide = $+0.53 \pm 0.07$ vs $+0.43 \pm 0.07$ pmol/ml) and hyperglycaemia (freeinsulin = 13.1 ± 2.0 vs 12.3 ± 2.2 mU/l) did not change. On the contrary, mean 24 h plasma freeinsulin (13.2 ± 2.6 vs 17.5 ± 2.2 mU/l; $p < 0.01$) and C-peptide (0.76 ± 0.10 vs 0.98 ± 0.13 pmol/l; $p < 0.02$) as well as the area under the curve (19.1 ± 4.1 vs 23.6 ± 3.1 U/24 h; $p < 0.01$ and 1.16 ± 0.14 vs 1.38 ± 0.18 μmol/24 h; $p < 0.02$ respectively) were significantly increased. The ratio between glucose infusion (M) and plasma insulin concentration (I) during the hyperglycaemic clamp studies (M/I, an index of insulin sensitivity), was not statistically different (1.40 ± 0.25 vs 1.81 ± 0.40 μmol·kg^{-1}·min^{-1}/mU·l^{-1}). These data suggest that, in Type 2 diabetic patients with secondary failure to oral antidiabetic agents, the combination of supper-time longacting insulin and premeal sulfonylurea agents can improve metabolic control. This positive effect is possibly mediated through an increased secretion of insulin in response to physiologic stimuli.

Key words: insulin, sulfonylurea, combined therapy, insulin action, insulin secretion, metabolic control.

Combining insulin and sulfonylurea agents for treatment of Type 2 (non-insulin-dependent) diabetes mellitus was initially proposed by Fabrykant [1] and Lazarus et al. [2] in the late 1950's. This therapeutic approach was discontinued because it was apparently not supported by a pathophysiologic rationale. More recently, insights have been accumulated regarding the pathogenesis of glucose intolerance in Type 2 diabetes. The main role of defective Beta-cell function and impaired insulin action on the liver and peripheral tissues has been highlighted [3]. This has led to a renewed interest in the combined therapy. Sulfonylurea agents stimulate endogenous insulin secretion [4, 5] but seem also to exert extrapancreatic action on insulin target tissues [6, 7]. Normalization of glucose profile by insulin results in better Beta-cell function and an improvement in insulin sensitivity, though large amounts of insulin (> 100 U/day) are often required [8–10]. Given the ability of both sulfonylureas and insulin to improve secretion and action of insulin, a combined therapy, at least in those patients who fail to respond to oral agents or supposedly require extremely large doses of insulin, may be justified. Insulin might be employed

to correct absolute or relative hypoinsulinaemia and therefore to exert better control on the rate of hepatic glucose production, mainly during the post-absorptive phases [3]. Sulfonylurea agents may improve post-prandial insulin secretion and contribute to restrain excessive glucose fluctuation. The linkage between the two tools would be that insulin-induced improvement in plasma glucose concentration should allow sulfonylureas to exert a greater stimulation on the Beta-cell secretion, while sulfonylurea may enhance insulin action at the level of the liver as well as peripheral tissues.

We have treated seven Type 2 diabetic patients for two months with supper-time injection of long-acting insulin to reduce the overnight liver glucose output and premeal administration of sulfonylurea to stimulate insulin secretion. The ability to recover insulin secretion or to ameliorate insulin action has been determined.

Subjects and methods

Subjects

Seven patients with Type 2 diabetes participated in the study. Patients were selected from among those who had previously failed to respond to maximal sulfonylurea dose (glibenclamide, 15 mg/day). Secondary failure was diagnosed on the basis of: (1) fasting plasma glucose concentration > 10 mmol/l for more than three months; (2) no change in body weight, and (3) no change in physical activity. There were two male and five female individuals, and they had an average age of 59 ± 2 years, were slightly overweight (BMI = 28.5 ± 2.1 kg/(m)^2), and known duration of diabetes was 12.3 ± 1.7 years (Table 1). No patient had clinical or laboratory evidence of hepatic, renal, or other endocrine diseases. All subjects were consuming a diet consisting of at least 200 g of carbohydrate per day for two weeks before the study. Before participating in the experimental protocol, the purpose, nature and possible risks of the study were explained and informed voluntary consent obtained from each subject. The protocol was approved by the local Ethical Committee.

Study protocol

All patients were admitted to the Metabolic Unit of the University of Padova for one week to obtain a basal metabolic evaluation. During this time, patients consumed a weight-maintaining diet, containing 45% carbohydrate, 35% fat, and 20% protein. Careful records of individual diets were kept. Meals were served at 08.00, 12.00, and 18.00 hours, together with their usual oral antidiabetic therapy. The following studies were performed during the seven day in-hospital period.

Table 1. Clinical parameters of the study population

Subjects	*(n)*	7
Sex	(male/female)	2/5
Age	(years)	59 ± 2
Body weight	(kg)	73.9 ± 4.3
Body mass index	(kg/m^2)	28.5 ± 2.1
Diabetes duration	(years)	12.3 ± 1.7

Glucagon-stimulated insulin secretion

On day four, at 08.00 hours, after a 10–12 h overnight fast, an i.v. catheter was inserted into an antecubital vein and kept patent with a slow infusion of 0.9% NaCl solution. Two baseline plasma samples for C-peptide, insulin, and glucose concentration were obtained. Then, 1 mg glucagon was administered i.v. over 1 min, and plasma samples were obtained after 5, 10, 15, and 30 min for glucose and hormone determination. No drug was administered before the glucagon test.

Twenty-four hour hormone and substrate profile

On day five, after an overnight fast, a catheter was inserted into a superficial vein of the forearm and blood collected at 30–240 min interval over the next 24 h (sample $n = 17$) for determination of substrates and hormones. During the test day, a carefully recorded diet was administered as described above. Antidiabetic therapy (glibenclamide, 5 mg) was administered 15 min before each meal. Patients were only allowed a limited amount of physical activity.

Hepatic glucose production

On day six, following an overnight fast and withdrawal of the morning dose of antidiabetic oral agent, a catheter was inserted into an antecubital vein for the infusion of test substances. A second cannula was inserted retrogradely into a wrist vein and the hand was placed in a box heated at 70 °C to ensure arterialization of venous blood. All plasma determinations were carried out on arterialized blood samples.

After a preliminary determination of basal plasma glucose concentration a primed-continuous (0.25 μCi/min) infusion of [6-^{3}H]-glucose (Amersham, Buckinghamshire, UK) was started. The priming dose, that for a normal euglycaemic subject approximately equals 100 times the rate of continuous infusion, was proportionally adjusted to the measured basal plasma glucose level. The continuous infusion was kept constant for 180 min. Blood samples were collected during the last 30 min of the continuous infusion for determination of [6-^{3}H]-glucose specific activity, and hormone concentration in plasma.

Hyperglycaemic clamp

After completion of tritiated glucose infusion, plasma glucose concentration was acutely increased by 7 mmol/l above baseline. The acute elevation in plasma glucose concentration was achieved by means of a logarythmically decreasing glucose infusion [11] performed with a Harvard pump (Harvard Apparatus, Millis, Mass., USA). Once the target value was reached (~20 min), the new plasma glucose level was kept constant for 100 min by means of a variable glucose infusion automatically calculated (Biostator GCIIS, Miles Italiana, Cavenago Brianza, Italy). Plasma glucose concentration was measured on a glucose analyzer (Beckman Instruments, Fullerton, Calif., USA) at 5 min intervals on arterialized vein blood samples and Biostator readings accordingly adjusted [12].

Combined insulin and sulfonylurea therapy

After completion of the metabolic studies, patients were started on 5 mg glibenclamide three times/day (15 min before each meal) and 0.2 U/kg · day of long-acting insulin (Ultratard HM, Novo, Copenhagen, Denmark) as a single dose at supper-time. The insulin dose was then increased over 3–7 days to a maximal dose of 0.4 U/kg · day in all subjects. This treatment was maintained for two months. Pa-

tients were instructed to monitor their blood glucose level and to report any value < 3.5 mmol/l or any hypoglycaemic symptom. No attempt was made to evaluate accuracy and precision of the patients' blood glucose readings. HbA_{1c} was assessed before and after the two month treatment with combined therapy. At the end of this period all patients had been readmitted to our Metabolic Unit. On the admission day, insulin was withdrawn and, starting on day 4, all the metabolic evaluations described above were repeated. During the second in-hospital period, subjects consumed the same diet as during the initial hospital stay.

Calculations

Basal glucose turnover rate was determined by dividing the [6-^{3}H]-glucose infusion rate (counts/min) by the steady state plateau of [6-^{3}H]-glucose specific activity in plasma (counts/mg) during the last 30 min of the 3 h tracer infusion. In the presence of a steady state condition, glucose turnover rate equals the rate of hepatic glucose production (HGP), while basal glucose utilization was calculated by subtraction of urinary glucose excretion rate [12].

During the hyperglycaemic clamp, the rate of glucose infusion was used as an index of insulin action [13]. The average glucose infusion rate ($M = mg \cdot kg^{-1} \cdot min^{-1}$) during the last 60 min of the hyperglycaemic clamp was subtracted by the mean urinary loss of glucose. This value was then divided by the average plasma insulin concentration (mU/l) during the same period (M/I) to provide an index of insulin action.

Analytical procedures

Plasma glucose concentration was determined by the glucose oxidase method on a Beckman Glucose Analyzer II. Methods for the determination of plasma tritiated glucose specific activity have been published previously [12]. Plasma non-esterified fatty acid (NEFA) concentration was measured by the microenzymatic method [14]. Substrate concentrations were measured in blood after percloric acid (5%) deproteinization according to previously published enzymatic procedures [11]. Plasma levels of free-insulin and C-peptide were determined by radioimmunoassay as previously reported [11]. Glycosylated haemoglobin (HbA_{1c}; normal values = 4–6.5%) was determined by microcolumn technique.

Statistical analysis

All data are presented as mean ± SEM. Statistically significant differences were evaluated by ANOVA and Student's *t*-test. A *p* value of < 0.05 was considered as statistically significant.

Results

Clinical evaluation and metabolic control

During the two months of combined treatment there was no change in the body weight of the patients (73.9 ± 4.3 vs 74.1 ± 3.9 kg). No patient reported symptoms of hypoglycaemia. The combination of insulin with sulfonylurea therapy was followed by a significant improvement in HbA_{1c} (from 11.8 ± 0.8 to 8.9 ± 0.5%; $p < 0.05$). Figure 1 illustrates the 24 h plasma glucose profile before and after combined therapy. Both fasting (12.9 ± 0.7 vs 10.4 ± 1.2 mmol/l; $p < 0.01$) and mean 24 h plasma glucose concentration (13.7 ± 1.2 vs 11.2 ± 1.4 mmol/l; $p < 0.05$) were significantly lowered. The reduction in fasting plasma glucose concentration was correlated with the decrement in mean 24 h level ($r = 0.90$; $p < 0.01$; Fig. 2).

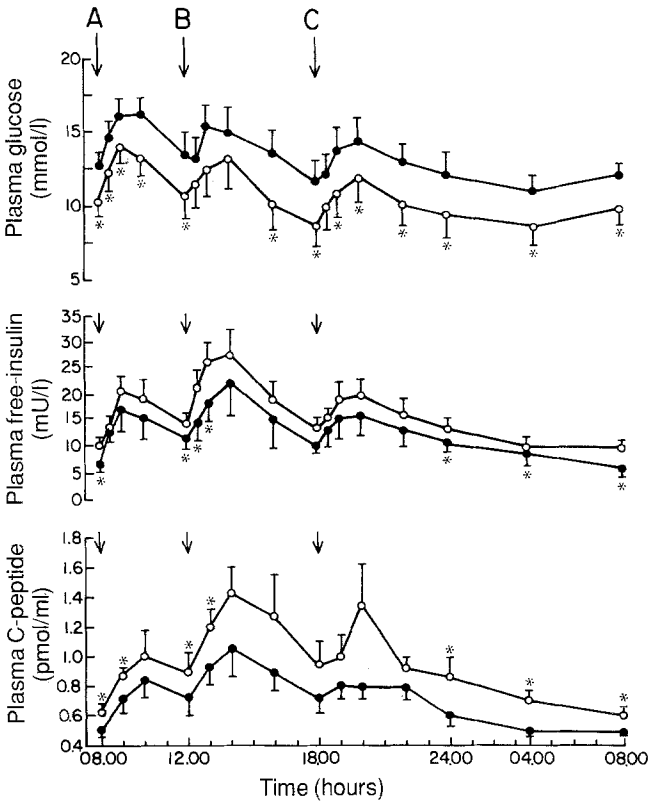

Fig. 1. Twenty-four hour plasma glucose, free insulin, and C-peptide profile in seven Type 2 diabetic patients before (●) and after (O) two months of treatment with supper-time insulin and pre-meal glibenclamide. *$p < 0.05$. Arrows indicate the time of meals; A = breakfast; B = lunch; C = dinner

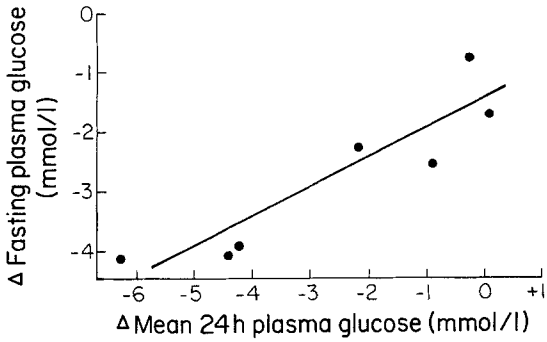

Fig. 2. Correlation between decrements in fasting plasma glucose concentration and changes in mean 24 h plasma glucose levels after two months of treatment with supper-time insulin and pre-meal glibenclamide

Basal plasma levels of NEFA were not affected by two months of combined therapy (0.67 ± 0.07 vs 0.65 ± 0.05 mmol/l). Following breakfast, there was a prompt decline in plasma NEFA concentration that persisted all day (Fig. 3). Mean 24 h plasma concentration of NEFA was significantly lower after combination of insulin

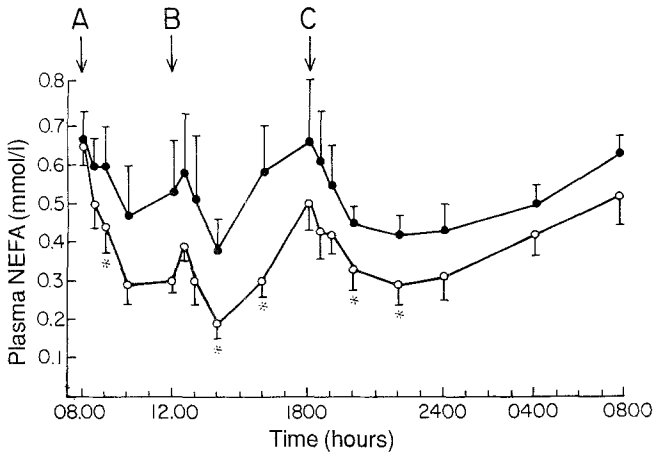

Fig. 3. Twenty-four hour plasma NEFA in seven Type 2 diabetic patients before (●) and after (O) two months of treatment with supper-time insulin and premeal glibenclamide. *$p < 0.05$. Arrows indicate the time of meals; A = breakfast; B = lunch; C = dinner

Table 2. Basal and mean 24 h concentration of hormones and substrates before and after two months of combined insulin/sulfonylurea therapy

	Before	After	$p <$
Plasma glucose (mmol/l)			
Basal	12.9 ±0.7	10.4 ±1.2	0.05
Mean 24 h	13.7 ±1.2	11.1 ±1.4	0.05
Plasma insulin (mU/l)			
Basal	6.8 ±1.3	10.6 ±1.0	0.001
Mean 24 h	13.2 ±2.6	17.5 ±2.2	0.01
Plasma C-peptide (pmol/ml)			
Basal	0.49 ± 0.06	0.63 ± 0.07	0.05
Mean 24 h	0.76 ± 0.10	0.98 ± 0.13	0.02
Plasma NEFA (mmol/l)			
Basal	0.67 ± 0.07	0.65 ± 0.5	NS
Mean 24 h	0.54 ± 0.08	0.38 ± 0.03	0.02
Blood lactate (mmol/l)			
Basal	1.28 ± 0.24	1.21 ± 0.18	NS
Mean 24 h	1.42 ± 0.26	1.43 ± 0.22	NS
Blood alanine (mmol/l)			
Basal	0.35 ± 0.04	0.33 ± 0.03	NS
Mean 24 h	0.39 ± 0.05	0.37 ± 0.03	NS
Blood glycerol (mmol/l)			
Basal	0.08 ± 0.01	0.08 ± 0.05	NS
Mean 24 h	0.09 ± 0.02	0.07 ± 0.01	0.05
Blood ketones (mmol/l)			
Basal	0.49 ± 0.11	0.34 ± 0.08	0.05
Mean 24 h	0.28 ± 0.07	0.20 ± 0.05	0.05

and sulfonylurea therapy (0.54 ± 0.08 vs 0.38 ± 0.03-mmol/l; $p < 0.02$). The behaviour of plasma NEFA was substantiated by the blood concentration of glycerol and ketone bodies (Table 2). No difference was apparent in the 24 h profile of blood lactate and alanine concentration (Table 2).

Basal hepatic glucose production

Basal HGP was 13.9 ± 1.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ before patients were started on insulin plus sulfonylurea therapy. After two month treatment there was a significant drop in basal HGP to 11.1 ± 1.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ ($p < 0.05$). Plasma glucose clearance rate did not change.

Insulin secretion

Glucagon test. Glucagon-stimulated C-peptide secretion was assessed on day 4. Both fasting plasma glucose (13.2 ± 0.3 vs 8.9 ± 1.4 mmol/l) and C-peptide concentrations (0.69 ± 0.13 vs 0.46 ± 0.10 pmol/ml; both $p < 0.05$) were lower after the two month combined therapy. This difference persisted after glucagon challenge (Fig. 4), with a peak value of 1.22 ± 0.17 vs 0.83 ± 0.13 pmol/ml ($p < 0.01$). However, the increment above baseline did not differ on either occasion (+0.53 ± 0.07 vs +0.43 ± 0.07-pmol/ml).

Hyperglycaemic (+7 mmol/l) clamp

A +7 mmol/l hyperglycaemic clamp was performed before and after two month therapy with insulin and sulfonylurea (Table 3). Fasting plasma glucose was lower after combined therapy (11.4 ± 0.8 vs 9.2 ± 0.8 mmol/l; $p < 0.05$) and it raised to 17.7 ± 1.0 and 16.4 ± 0.7 mmol/l. Coefficient of variation during the clamp was < 6% in all studies. In response to an abrupt increase in plasma glucose concentration, plasma levels of both free-insulin and C-peptide were slightly increased. The two month combined treatment had no effect on both the first phase (0–20-min = 11.4 + 1.7 vs 10.0 + 1.3 mU/l, and 0.61 ± 0.10 vs 0.59 ± 0.09 pmol/ml for free-insulin and C-peptide respec-

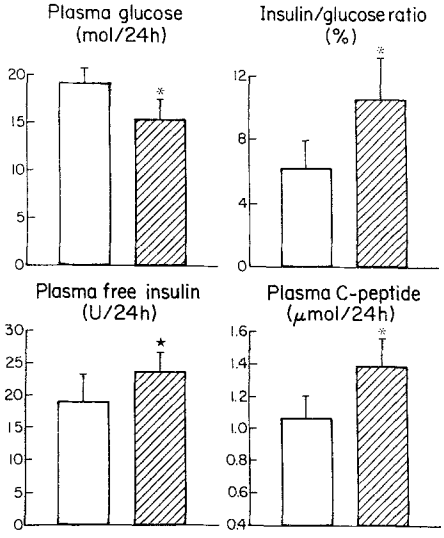

Fig. 4. Plasma glucose, free-insulin, and C-peptide areas under the curve during the 24 h profile and insulin/glucose ratio before (open bars) and after (cross hatched bars) two months of treatment with supper-time insulin and pre-meal glibenclamide. *$p < 0.05$; $p < 0.01$

Table 3. Plasma concentrations of glucose, free-insulin, and C-peptide in the basal state and during hyperglycaemic (+ 7 mmol/l above baseline) clamp studies performed before and after two months of combined insulin/sulfonylurea therapy. The rate of glucose infusion and the M/I[a] ratio (an index of insulin action) is given as well

	Before	After
Fasting plasma glucose (mmol/l)	11.4 ± 0.8	9.2 ± 0.8[b]
Clamp plasma glucose (mmol/l)	17.7 ± 1.0	16.4 ± 1.1
Fasting plasma insulin (mU/l)	6.7 ± 1.2	6.5 ± 0.6
0′–20′ plasma free-insulin (mU/l)	11.4 ± 1.7	10.0 ± 1.3
20′–120′ plasma free insulin (mU/l)	13.1 ± 2.0	12.3 ± 2.2
Glucose infusion rate ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	17.2 ± 2.8	21.1 ± 2.8
M/I ratio[a] ($\mu mol \cdot kg^{-1} \cdot min^{-1}/mU \cdot l^{-1}$)	142 ± 25	181 ± 40

[a] Ratio between the rate of glucose infusion ($mg \cdot kg^{-1} \cdot min^{-1}$) and the plasma insulin concentration (mU/l) during the period 60–120 min of the hyperglycaemic clamp.
[b] $p < 0.05$

tively) and second phase of insulin secretion (13.1 ± 2.0 vs 12.3 ± 2.2 mU/l, and 0.78 ± 0.13 vs 0.78 ± 0.15 pmol/ml, respectively).

Twenty-four hour profile of plasma C-peptide and free-insulin

Both fasting plasma levels of free-insulin (6.8 ± 1.3 vs 10.6 ± 1.0 mU/l) and C-peptide (0.49 ± 0.07 vs 0.63 ± 0.07 pmol/ml; $p < 0.05$) were higher after combined treatment (Fig. 1). At variance to what was observed in response to glucagon and acute hyperglycaemia, the response to mixed meals, a more physiologic stimulus, was increased after two months insulin and sulfonylurea therapy (Fig. 1). Areas under the curve of plasma C-peptide (1.16 ± 0.14 vs 1.38 ± 0.18 μmol/24 h; $p < 0.02$) and free-insulin (19.1 ± 4.1 vs 23.6 ± 3.1 U/24 h; $p < 0.01$) were higher (Fig. 4).

The ratio between the 24 h areas under the curve of plasma free-insulin and glucose concentration was significantly higher after combined therapy (1.42 ± 0.25 vs 1.81 ± 0.40; $p < 0.05$; Fig. 4).

Insulin action

The rate of exogenous glucose infusion during the hyperglycaemic clamp was only slightly higher after than before combined therapy (17.2 ± 2.8 vs 21.1 ± 2.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; Table 3). The M/I ratio also was not significantly different before and after combined therapy (142 ± 25 vs 181 ± 40 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; Table 3).

Discussion

Our results suggest that the addition of a single dose of long-acting insulin (0.4 U/kg · day) at supper-time to sulfonylurea (5 mg three times a day) can be a useful procedure for ameliorating glucose control in Type 2 diabetic patients with secondary failure to oral hypoglycaemic agents.

In the last few years there has been a renewed interest in combined therapy [15–33]. Among this large body of literature, our study differs in many respects. Our study population consists of patients with ascertained secondary failure to oral hypoglycaemic agents, while no such criteria was commonly used in other papers. Our therapeutic approach differs as well. Groop et al. [15, 19], Schwartz et al. [24], Castillo et al. [25], and Gutniak et al. [30] have chosen to give insulin in two or more doses a day. Holman et al. [29] have used a similar approach to ours. However, in their study the time of insulin administration (i.e. morning or evening) was not evident and the insulin dose was adjusted to reduce fasting plasma glucose below a target value of 6.1 mmol/l.

We have favoured the use of a fixed dose of 0.4 U/kg · day given at supper-time. This choice was based on the assumption that absolute or relative overnight hypoinsulinaemia is responsible for glucose overproduction by the liver [3]. The use of an ultralente insulin preparation should provide a basal plasma insulin concentration designed to avoid excessive elevations in plasma insulin concentration during the first hours of the night [34], prevent the dawn phenomenon [35], and restrain hepatic glucose production (HGP) [3].

The risk of hypoglycaemic events in aged patients, often affected by macroangiopathy, may be of concern. In our study, the combination of supper-time insulin and pre-meal sulfonylurea was followed by a reduction in the fasting and 24 h plasma glucose profile, and improvement in HbA_{1c} without variation in body weight. No hypoglycaemic reactions were reported by our patients. However, in the absence of laboratory reference measurements, the accuracy and precision of patients' blood glucose readings remains uncertain and it makes it difficult to ascertain the actual frequency of low glucose levels. This may be critical at night, following the injection of long-acting insulin. According to Holman et al. [29] hypoglycaemic events occurred in 7% of patients treated with sulfonylurea and in 47% of those treated with sulfonylurea + insulin. Nevertheless, none of these hypoglycaemic episodes was as severe as to result in incapacitation of the patients. Furthermore, once stable glucose control was achieved there were no further hypoglycaemic reactions.

The beneficial effect on plasma glucose level can be related to increased insulin secretion, HGP suppression, improved insulin action or a combination of these processes. Insulin secretion has been evaluated in response to pharmacologic (i.e. glucagon), paraphysiologic (i.e. acute hyperglycaemia) and physiologic (i.e. mixed meal) stimuli. The mean basal plasma free-insulin and C-peptide concentrations for the three studies did not change after two months of combined therapy. In previous

studies, the plasma levels of C-peptide were found to be increased [15, 16, 21–23, 28, 31, 33], unchanged [17, 18, 20, 24, 29], or even reduced [36, 37]. These results reflect the heterogeneity of the study populations, but may be a direct consequence of therapy as well. Exogenous insulin may act via a negative feedback to reduce endogenous secretion [38]. This interpretation does not fit our data since they were obtained after 72 h insulin withdrawal. In spite of insulin withdrawal, the fasting plasma glucose concentration was significantly lower after combined therapy. Overall plasma glucose concentration is the major determinant of insulin secretion. It may be argued that a certain improvement in Beta-cell function must have occurred in order to ensure the same plasma free-insulin and C-peptide concentration in the presence of a lower plasma glucose level. The same kind of reasoning applies to the C-peptide response to i.v. glucagon. Plasma C-peptide concentrations in response to glucagon were lower after the two month combined treatment. Glucose potentiates the effect of glucagon on C-peptide. Therefore, it is likely that a better Beta-cell function must have allowed a similar response of C-peptide in the presence of lower plasma glucose concentration. Insulin response to mixed-meals was increased (Fig. 1). The improved insulin secretory function is also supported by a larger insulin/glucose area ratio during the test day (Fig. 4), suggesting that more insulin was secreted through the day. The reason for such a difference is not readily apparent. Glucose and amino acids stimulate insulin secretion via different mechanisms [39]. Chronic hyperglycaemia has been shown to hamper glucose but not arginine-mediated insulin secretion [40]. Normalization of the plasma glucose concentration can improve Beta-cell sensitivity to glucose. However, the degree of glucose control we obtained was still far away from normalization. Since amino acids stimulate insulin secretion via a mechanism (change in membrane potential) which is not as specific as glucose stimulation (possibly via the generation of an active metabolite) it may be conceivable that the Beta-cell might regain a response to amino acids at a lower degree of improvement in metabolic control.

Whatever the mechanism for improved insulin secretion, the net result is a better insulinisation of the organism. The result becomes even more substantiated if one takes into consideration the demonstration by Gutniak et al. [30] that post-meal insulin action was also improved. To further support the increased availability of circulating insulin after two months of combined therapy, plasma NEFA concentrations were significantly reduced (Fig. 3), particularly following meal ingestion. Plasma NEFA concentration is mainly regulated by the rate of lipolysis, a process which is extremely sensitive to minute changes in plasma insulin levels [41]. The drop in plasma NEFA concentration is also expected to have a beneficial effect on glucose metabolism [3], by improving insulin-mediated glucose disposal [42] and reducing gluconeogenesis [43].

If larger insulin availability can account for the amelioration in post-prandial glucose levels, the reduction in fasting plasma glucose concentration occurred in the face of an unchanged plasma free-insulin level. Fasting hyperglycaemia is a function of the rate of HGP [3]. Following two month insulin plus sulfonylurea therapy, HGP was lowered. This effect can be the result of: (1) increased portal concentration of insulin and (2) increased liver sensitivity to the suppressive effect of insulin. On average, no significant changes in basal plasma concentrations of C-peptide and free insulin were apparent after combined therapy. Increased insulin action at the level of the liver has been suggested by Castillo et al. [25]. These authors monitored their patients with an artificial endocrine pancreas and reported a reduced overnight insulin requirement. This effect may be even more pronounced after the meal, when insulin secretion is stimulated. Groop et al. [41] have recently shown that a rise in plasma insulin concentration comparable to that observed in the post-prandial phase in our patients can induce a 50% inhibition of HGP without promoting peripheral glucose disposal. The effect on the liver may also explain the correlation we found between fasting plasma glucose concentration and the 24 h mean plasma glucose value (Fig. 2). This correlation may be accounted for by the fact that overnight reduction in HGP lowers plasma glucose concentration over the nocturnal period which occupies one third of the daily cycle. Taskinen et al. [44] have recently shown that the reduction in fasting and overnight blood glucose concentration is due to the suppression of glucose production that follows the small increment in overnight plasma insulin concentration following bedtime insulin injection. Since our results were obtained after insulin withdrawal, the hypothesis that improvement of fasting plasma glucose concentration may allow partial recovery of insulin secretion and tissue response, as originally proposed by Riddle [45], may be put forth.

In the present study, insulin action was evaluated during a hyperglycaemic clamp. The M/I ratio, which simply provides an index of insulin sensitivity, was not increased following two months of combined therapy. In the literature the matter is controversial. Some authors have reported an improved insulin-mediated glucose metabolism [20, 25], others did not [19, 25, 27, 30]. It is likely that the degree of glucose control to be attained with the combined therapy directly modulates the capacity to restore insulin action to a certain extent. The data by Castillo et al. [25] are particularly supportive of this point of view. In fact, they could observe an increase in insulin sensitivity only on those patients who actually displayed an improved glucose control. Conversely, in the paper by Simonson et al. [27] Type 2 diabetic patients treated with insulin plus sulfonylurea, which had no favourable effect on their glucose profile, exhibited no improvement in insulin action. It can be argued that in order to elicit any beneficial effect on insulin action a longer period of treatment and/or more strict metabolic control may be required. This is also supported by the fact that normalization of glucose control by intensive insulin therapy can ameliorate insulin sensitivity [8–10], while the simple improvement in glucose profile obtained with combined therapy for as long as 85 days does not seem to affect insulin action [30].

In conclusion, our study suggests that the combination of a maximal dose of 0.4 U ultralente insulin per kg body

weight per day injected once a day at supper-time to three pre-meal doses of sulfonylurea is free of hypoglycaemic reactions, and is well accepted by patients.

Maintenance of this scheme for two months can improve metabolic control and exert a beneficial effect on insulin secretion and action.

Acknowledgements. We thank Drs. E. Iori, M. C. Marescotti, and A. Valerio for their laboratory assistance, Ms. D. Joung for expert secretarial help, and the nurses of Divisione Malattie del Ricambio, Ospedale Civile di Padova for care of the patients. This work was supported in part by a grant from Novo Italia e CNR grant n. 84.02551 56.

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Received: 1 November 1989
and in revised form: 8 June 1990

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