The Combined Effect of Triple Therapy With Rosiglitazone, Metformin, and Insulin Aspart in Type 2 Diabetic Patients

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OBJECTIVE — Type 2 diabetes is caused by reduced insulin secretion and insulin resistance in skeletal muscle and liver. We tested the combination therapy with insulin aspart, rosiglitazone, and metformin with the purpose of treating all three defects in order to test the hypothesis that this "triple therapy" will normalize glucose metabolism.

RESEARCH DESIGN AND METHODS — Sixteen obese type 2 diabetic outpatients on human NPH or MIX (regular + NPH insulin) insulin twice daily were randomized to either triple therapy, i.e., insulin aspart (a rapid-acting insulin analog) at meals, metformin (which improves hepatic insulin sensitivity), and rosiglitazone (which improves peripheral insulin sensitivity), or to continue their NPH or MIX insulin twice daily for 6 months. Insulin doses were adjusted in both groups based on algorithms. HbA_{1c}, insulin dose, hypoglycemic episodes, insulin sensitivity (clamp), hepatic glucose production (tracer), and diurnal profiles of plasma glucose and insulin were used in evaluating treatment.

RESULTS — In the triple therapy group, HbA_{1c} declined from 8.8 to 6.8% ($P < 0.01$) without inducing severe hypoglycemic events. Postprandial hyperglycemia was generally avoided, and the diurnal profile of serum insulin showed fast and high peaks without any need to increase insulin dose. In the control group, the insulin dose was increased by 50%, but nevertheless both HbA_{1c} and 24-h blood glucose profiles remained unchanged. Insulin sensitivity improved in both skeletal muscle and the liver in the triple therapy group, whereas no change was observed in the control group.

CONCLUSIONS — We conclude that treatment of the three major pathophysiological defects in type 2 diabetic subjects by triple therapy significantly improved glucose metabolism in obese type 2 diabetic subjects.

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Type 2 diabetes is a common fast-growing disease that affects about 5% of the population worldwide. The disease is complicated by specific cardiovascular events, and the mortality rate is 2–3 times higher than in the background population (1).

The U.K. Prospective Diabetes Study (UKPDS) clearly showed that intensive care matters because the intensive treatment group developed fewer diabetes-related end points (2). Despite that, most type 2 diabetic subjects are still in poor control and far from recommended HbA_{1c} values, i.e., <6.5–7.0% (3,4). Thus, in a multinational European study, mean HbA_{1c} values for 10 different countries were found to be ~8.1% (5). However, in several published intervention studies, the value in insulin-treated subjects is even higher, i.e., ~9–10% (6).

Pharmacological treatment is needed in >80% of type 2 diabetic subjects. In this context, there has been a tradition for many years to use only one antidiabetic drug at a time, and most patients are still treated with either insulin secretagogues or insulin alone. However, these drugs have only a minor effect on cardiovascular events and mortality (2), whereas metformin, which improves insulin sensitivity, is able to reduce the risk of myocardial infarction and reduce the mortality rate (7,8). Altogether, the UKPDS showed that for each percentage-point reduction in HbA_{1c}, diabetes complications and the mortality rate declined by 21% (9). Therefore, normalization of HbA_{1c} must be a major goal in the treatment of type 2 diabetic subjects.

Three pathophysiological components seem to be of major importance in the development of hyperglycemia in obese adults. These are peripheral insulin resistance (i.e., reduced insulin-mediated glucose uptake in skeletal muscle), insulin resistance in the liver (resulting in inappropriate glucose production), and an impaired insulin response to glucose resulting in reduced and delayed insulin peaks in relation to mixed meals (3,10,11). The purposes of this study were to *1*) evaluate whether improvement of insulin sensitivity in skeletal muscle and liver, together with restoration of the normal insulin response to a meal as applied by triple therapy (rosiglitazone, metformin, and insulin aspart), may improve glucose metabolism in type 2 diabetic subjects (12–14) and *2*) test if this therapy was better than standard therapy with NPH insulin with regard to glucose and fat metabolism.

RESEARCH DESIGN AND METHODS

— Sixteen obese type 2 diabetic outpatients treated with human

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Abbreviations: FFA, free fatty acid; HGP, hepatic glucose production; SMBG, self-monitored blood glucose; 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.

Table 1—*Baseline clinical and biochemical characteristics of the study groups*

	Control group	Triple therapy group	Reference material
n (male/female)	8 (4/4)	8 (3/5)	10 (8/2)
Age (years)	61 ± 2	66 ± 4	56 ± 3
BMI (kg/m^2)	31.3 ± 1.9	32.1 ± 2.1	29.0 ± 0.5
HbA_{1c} (%)	9.1 ± 0.4	8.8 ± 0.9	5.4 ± 0.1
Fasting blood glucose (mmol/l)	10.3 ± 1.1	9.3 ± 1.3	5.3 ± 0.1
Fasting serum insulin (pmol/l)	104 ± 10	104 ± 14	30 ± 6
Fasting serum C-peptide (pmol/l)	585 ± 103	715 ± 152	509 ± 50
Insulin dose per day (units/kg body wt)	0.46 ± 0.16	0.42 ± 0.16	0
Duration of diabetes (years)	7.3 ± 0.9	10.9 ± 2.6	0
Years on insulin	3.3 ± 1.2	5.1 ± 2.0	0

Data are means ± SE.

NPH or MIX insulin twice daily were recruited from our university diabetes clinic for this investigator-initiated study (Table 1). The study was approved by the local ethical committee and by the Danish Medicines Agency. All subjects gave written informed consent. The inclusion criteria were age 40–76 years, fasting serum C-peptide >250 pmol/l, BMI 25–40 kg/m^2, HbA_{1c} >7%, and insulin therapy, whereas the exclusion criteria were s-creatinine >100 μmol/l, plasma alanin aminotransferase >100 units/l, intolerance to metformin, left ventricular ejection fraction measured with echocardiography <40%, blood pressure >180/100 mmHg, severe dyslipidemia (total cholesterol >8 and triglyceride >5 mmol/l), and unawareness of hypoglycemia. A reference material of age- and BMI-matched nondiabetic control subjects (11) was used in comparison with the degree of insulin resistance (Table 1).

Design

At inclusion, subjects were matched for HbA_{1c} and age and were thereafter block randomized into two groups of eight subjects, the triple therapy group or to continued NPH or MIX insulin therapy (control group) (Table 1). During a 2-month start-up period, when the pretests were carried out, the treatment was unchanged in both groups. A total of seven patients already received metformin before randomization, three in the control group and four in the triple therapy group.

Following randomization, the control group continued their insulin regimen (and the three control group patients using metformin before randomization also continued this treatment), but the insulin dose was increased to obtain the best possible metabolic control. The aims for the control group were blood glucose within the range of 5–7 mmol/l preprandial and HbA_{1c} <7% without inducing severe hypoglycemia (i.e., events needing help from another third person). One to two mild hypoglycemic events were accepted per week. Self-monitored blood glucose (SMBG) was measured four times per day, before meals and at bedtime.

The triple therapy group started with rosiglitazone (Avandia) 4 mg/day (dose was increased to 8 mg/day after 8 weeks), metformin (Glucophage) 0.5 g twice per day (dose was increased after 4 weeks to 1 g twice daily), and insulin aspart (NovoRapid) three times per day injected just before each main meal. Initially, total daily insulin dose was reduced to one-third of the dose given during the start-up period to avoid hypoglycemia during the start of treatment with rosiglitazone and metformin, and it was divided into three equal doses. In the triple therapy group, the target SMBG values were 5–7 mmol/l postprandial (measured 1.5 h after each meal) and HbA_{1c} <7%, with no severe hypoglycemic events but one to two mild events were accepted per week.

The insulin dose in both groups was adjusted by the diabetologist at scheduled visits and at scheduled telephone contact (five outpatient clinic visits and two telephone contacts) and kept constant in the last 2 months of the study period. Thus it was possible to change insulin dosages seven times during the study. Overall, insulin dose was adjusted as follows: a reduction in total insulin dose of ~10% if mean blood glucose was <5 mmol/l, unchanged if blood glucose was 5–7 mmol/l, and increases in insulin dose of ~5–10% if blood glucose was 7–10 mmol/l, 10–20% if blood glucose was 10–13 mmol/l, and 20–30% if blood glucose was >13 mmol/l. In the control group, the morning insulin dose was changed mainly based on prelunch, predinner, and nighttime SMBG values, whereas the late insulin dose was changed mainly based on fasting SMBG. In the triple therapy group, the insulin aspart dose before a specific meal was determined by the postprandial SMBG values at that meal.

Subjects were asked not to change their diet and exercise level during the study period. HbA_{1c} was measured every second month, and a 24-h profile of blood glucose, serum insulin, serum C-peptide, and plasma free fatty acid (FFA) was carried out before and after 6 months of treatment. Insulin sensitivity was measured before and after the 6-month treatment period.

Analytical procedures

Euglycemic-hyperinsulinemic clamp study. This test was performed as previously described by Hother-Nielsen et al. (15). A primed-constant intravenous infusion of 3-^{3}H-glucose (DuPont-New England Nuclear, Boston, MA) was started in the morning and continued throughout the entire study. The ratio of priming dose (10 ml) and constant infusion (0.1 ml/min) was 100 (15). To achieve a common level of basal plasma–specific activity, the tracer infusion was adjusted for surface area by adjustment of the infusate volume (16). After a 120-min basal tracer equilibration period, insulin (Actrapid Human; Novo Nordisk) was infused at a rate of 40 mU · min^{-1} per m^2 body surface area for 240 min. After initiation of the insulin infusion, the plasma glucose concentration was allowed to fall to 5.5 mmol/l, at which level it was maintained (clamped) using a variable infusion of 20% glucose enriched with 3-^{3}H-glucose to maintain the specific activity constant at baseline levels during the clamp (17).

Continuous indirect calorimetry, using a ventilated hood system (Deltatrac 2; SensorMedics, Yorba Linda, CA) was performed during the last 40 min of the basal and insulin infusion periods. Urine samples for determination of the urea excretion were obtained from the start to the

end of the euglycemic-hyperinsulinemic clamp for calculation of protein oxidation rates.

Diurnal profiles of glucose, insulin, C-peptide, and FFA. Beginning at 8:00 A.M., one catheter was inserted into an antecubital vein for blood sampling of blood glucose, serum insulin, serum C-peptide, and plasma FFA. Blood samples were obtained right before each main meal (breakfast, lunch, and dinner) and then 30 min, 1, 1.5, 2, 2.5, and 3 h later and thereafter every hour until the next meal. At night, blood samples were obtained at 3:00 and 7:00 A.M.

Assays. Plasma glucose concentration was measured using the glucose oxidation method (Glucose Analyzer 2; Beckman Instruments). HbA_{1c} was measured by a high-performance liquid chromatography method (Tosho A1c 2.2; Tosho Bioscience, Japan) (normal range 4.4–6.4%). Serum insulin and C-peptide samples were analyzed by a two-site, time-resolved immunofluorometric assay (DELFIA) (18). Insulin aspart was measured with a modification of the method described by Andersen et al. (19). Plasma FFA concentrations were measured by Itaya et al.'s method (20). Plasma total cholesterol, HDL cholesterol, and triglyceride concentrations were measured by a kit from Boehringer Mannheim (Diagnostica, Mannheim, Germany), and LDL cholesterol was calculated using the Friedewald equation. Tritiated glucose-specific activity was determined on barium/zinc deproteinized plasma samples as previously described.

Calculations

Rates of glucose appearance (R_a) and rates of glucose disappearance (R_d) were calculated using Steele's non–steady-state equations, using a distribution volume of 200 ml/kg (21) and a pool fraction of 0.65 (22). Glucose storage rate was calculated as R_d minus the glucose oxidation rate. The insulin sensitivity index (S_i) was calculated as R_d divided by incremental increase in serum insulin concentration during the insulin clamp and divided by the mean glucose concentration during the last hour of the insulin clamp. Hepatic glucose production (HGP) was calculated as: HGP = R_a − glucose infusion rate.

Statistical analysis

Results are presented as means ± SE unless otherwise stated. Differences between the groups or within a group were compared by unpaired or paired T tests. Correlation analysis was performed using Pearson correlation analysis. Statistical analysis was performed using SPSS for Windows (version 10.0). *P* values <0.05 were considered significant.

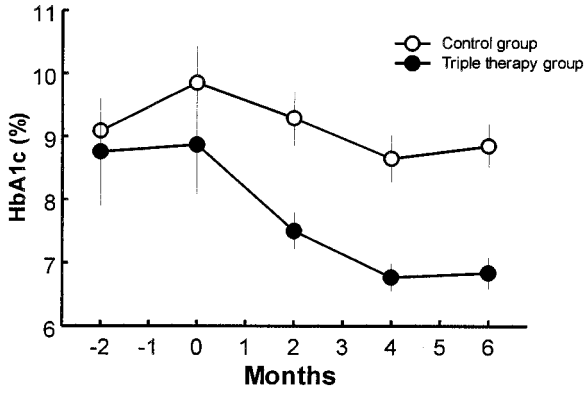

Figure 1—*Mean HbA_{1c} values before and during 6 months' intervention in type 2 diabetic patients treated with either NPH or MIX insulin twice daily (○, control group) or with insulin aspart before meals, metformin, and rosiglitazone (●, triple therapy group).*

RESULTS

Glucose metabolism (HbA_{1c} and diurnal profile of blood glucose)

HbA_{1c} did not change in the control group during the study period, whereas in the triple therapy group, a marked reduction was observed (8.8 ± 0.9 to 6.8 ± 0.3%, $P = 0.004$) (Fig. 1). Thus, at the end of the study period, HbA_{1c} levels in the triple therapy group were close to the reference value (4.4–6.4%) and were 2.0% points better than in the control group on twice daily insulin treatment ($P < 0.001$). Twenty-four–hour blood glucose profiles were sampled before and after 6 months of intervention (Fig. 2*A*). Before intervention, the two curves were superimposable (data not shown), but after intervention, blood glucose in the triple therapy group was <6 mmol/l during most of the 24-h period, and the postprandial peaks of hyperglycemia were generally eliminated. During the night, glucose values increased slightly in both groups and to a slightly higher level in the triple therapy group. However, the fasting blood glucose concentrations in the morning in the two groups after 6 months of intervention were identical (7.8 ± 0.9 and 8.2 ± 0.8 mmol/l, respectively).

Insulin dose and serum insulin values

In the control group, the insulin dose was increased gradually and reached the level of 0.67 ± 0.24 units · kg body wt^{-1} · day^{-1} (at inclusion 0.46 ± 0.16 units · kg body wt^{-1} · day^{-1}, $P < 0.03$), whereas the insulin dose following intervention was unchanged in the triple therapy group (0.29 ± 0.11 vs. 0.42 ± 0.16 units · kg body wt^{-1} · day^{-1}) despite improvement in metabolic control and being significantly lower than the insulin dose in the control group ($P < 0.002$).

The diurnal profiles of serum insulin were superimposable in the two groups before intervention (data not shown). After 6 months, the pattern for the control group was unchanged, but the diurnal level was increased (Fig. 2*B*). However, after 6 months, the triple therapy group showed clearly defined insulin peaks at meal times. The peaks rose rapidly up to 400 pmol/l after 60 min and thereafter rapidly reduced again in the pattern quite similar to that seen in nondiabetic subjects (Fig. 2*B*). The insulin peaks were mainly due to the insulin aspart injections (Fig. 2*C*). These measurements also showed that insulin aspart given with the evening meal was cleared again before midnight. Therefore, plasma insulin concentrations during the night were much lower in the triple therapy group than in the control group. This difference may be a safeguard against nocturnal hypoglycemia.

Insulin resistance, glucose oxidation, and HGP

After 6 months of intervention, glucose infusion rates in the triple therapy group rose significantly from 101.8 ± 22.0 to

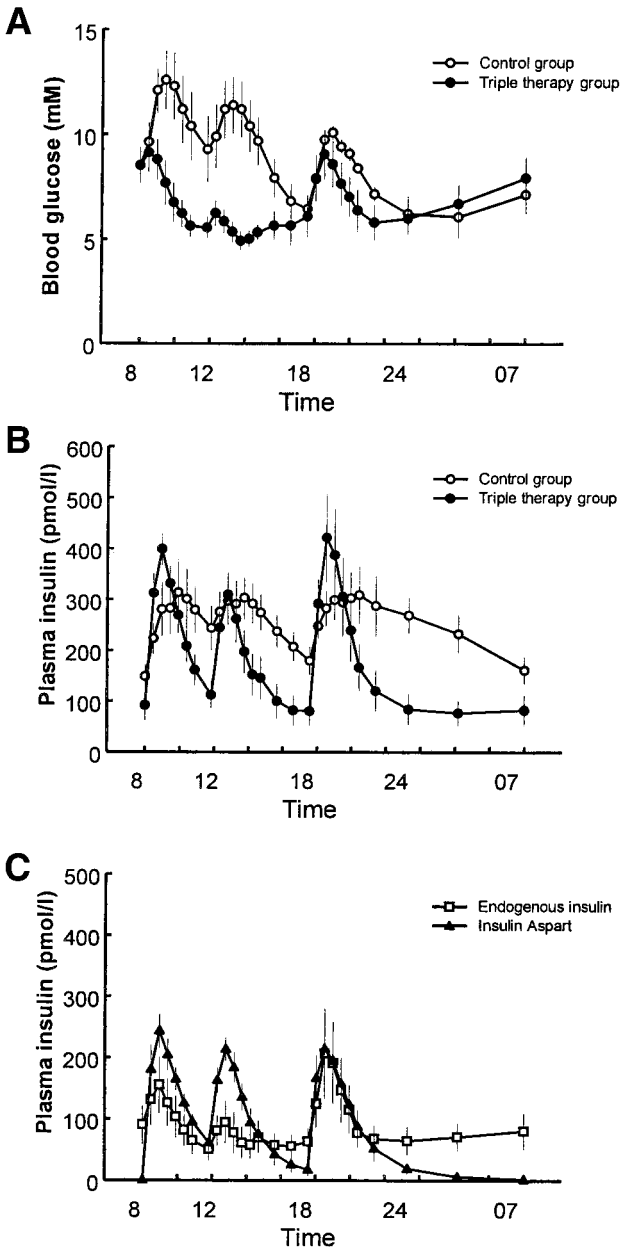

Figure 2—*Diurnal profiles (from 8:00 A.M. to 7:00 A.M. the next morning) of blood glucose (*A*) and serum insulin (*B*) in type 2 diabetic patients treated with either NHP or MIX insulin twice daily (○, control group) or with insulin aspart before meals, metformin, and rosiglitazone (●, triple therapy group). C: Diurnal profiles (from 8:00 A.M. to 7:00 A.M. the next morning) of endogenous and exogenous insulin (insulin aspart) in the triple therapy group.*

166.5 ± 11.8 mg · min^{-1} · m^{-2}, ($P <$ 0.02); however, insulin sensitivity was still below the level for the nondiabetic reference group (~270 ± 20 mg · min^{-1} · m^{-2}). When insulin sensitivity was estimated by the "tracer technique," S_i for the triple therapy group was found to increase too, and this increase was mainly due to an increase in glucose oxidation and not in glucose storage (Table 2). In accordance with this, lipid oxidation during the insulin clamp decreased significantly (Table 2). Interestingly, lipid oxidation during the clamp correlated with mean diurnal FFA concentration in the combined group of patients (R = 0.65, $P < 0.01$).

During intervention in both groups, HGP was suppressed to the normal level (nondiabetic control group: 21 ± 3 mg · min^{-1} · m^{-2}) but at lower serum insulin values in the triple therapy group than in the control group, indicating improved liver insulin sensitivity during treatment in the triple therapy group (Table 2).

Body weight, blood pressure, lipids, and safety

Body weight increased nonsignificantly by 1 kg in the control group and 3 kg in the triple therapy group ($P > 0.1$). Blood pressure and lipids did not change significantly (Table 2). Fasting plasma FFA values were identical before and after intervention in the triple therapy group. However, during the 24-h profile mean diurnal plasma FFA values were lower in the triple therapy group compared with the control group (22.7 ± 1.4 vs. 33.5 ± 4.7 mmol/l, $P = 0.057$).

A slight decrease in the hemoglobin concentration in the triple therapy group was found (Table 2), and interestingly, plasma alanin aminotransferase values decreased (but not statistically significantly), which may be caused by a reduction of fat content in the liver.

One subject in the triple therapy group stopped treatment due to subjective sensation of fluid retention but no peripheral edema was demonstrated and X-ray of the chest was without sign of pulmonal edema.

Hypoglycemia

Despite the increased insulin dose in the control group and the improvement of metabolic control in the triple therapy group, no severe hypoglycemic attacks were reported in either group. In the last 2 months, when the insulin dose was maintained constant and glucose levels were stable, one mild hypoglycemic event was reported in the control group and a total of seven events (in two subjects only) in the triple therapy group ($P > 0.1$). No nocturnal episodes were registered.

CONCLUSIONS — In this study of poorly controlled, long-term type 2 diabetic subjects with severe insulin resistance and abolished first-phase insulin response, we found that a new concept of treatment with insulin aspart, metformin, and rosiglitazone based on pathophysiological knowledge was able to significantly improve the HbA_{1c} level without inducing severe hypoglycemia. In fact, the regimen may be a safeguard against nocturnal hypoglycemia. No changes in

Table 2—*Clinical and biochemical variables and glucose and lipid metabolic parameters obtained before and after 6 months of treatment*

	Control group		Triple therapy group	
	Before	After	Before	After
Body weight (kg)	86 ± 5	87 ± 5	87 ± 7	91 ± 9
Systolic blood pressure (mmHg)	129 ± 7	132 ± 3	140 ± 7	147 ± 6
Diastolic blood pressure (mmHg)	76 ± 4	79 ± 2	76 ± 3	78 ± 3
Fasting plasma FFA (mmol/l)	0.67 ± 0.06	0.51 ± 0.05*†	0.70 ± 0.04	0.67 ± 0.06
Fasting plasma LDL cholesterol (mmol/l)	3.0 ± 0.4	3.5 ± 0.4‡	2.8 ± 0.3	3.4 ± 0.5
Fasting plasma HDL cholesterol (mmol/l)	1.14 ± 0.07	1.07 ± 0.08	1.46 ± 0.15	1.53 ± 0.16†
Fasting plasma triglycerides (mmol/l)	2.6 ± 0.5	2.1 ± 0.3	1.1 ± 0.2	1.3 ± 0.2†
Hemoglobin (mmol/l)	8.1 ± 0.3	8.1 ± 0.4	8.7 ± 0.4	8.0 ± 0.4‡
Alanin aminotransferase (units/l)	27 ± 6	24 ± 5	29 ± 9	17 ± 4.0
Alkaline phosphatase (units/l)	180 ± 17	214 ± 19	194 ± 13	152 ± 8*
Basal serum insulin (pmol/l)	104 ± 12	100 ± 24	187 ± 58	76 ± 28
Clamp serum insulin (pmol/l)	504 ± 47	400 ± 25	635 ± 102	397 ± 36
Basal HGP mg · min^{-1} · m^{-2}	81.6 ± 3.7	88.7 ± 5.2	82.1 ± 5.6	86.7 ± 6.3
Clamp HGP mg · min^{-1} · m^{-2}	30.2 ± 2.7	21.4 ± 4.5	37.4 ± 7.3	22.0 ± 4.0
S_i, 10^{-2} mg · min^{-1} · m^{-3} per pmol/l per mmol/l	6.5 ± 1.1	6.3 ± 0.5	8.5 ± 1.3	10.3 ± 1.0‡§
Basal glucose oxidation mg · min^{-1} · m^{-2}	59.9 ± 7.4	66.1 ± 9.8	43.8 ± 5.6	52.0 ± 7.7
Clamp glucose oxidation mg · min^{-1} · m^{-2}	84.5 ± 11.0	82.7 ± 11.5	83.7 ± 10.0	106.8 ± 5.5‡
Clamp glucose storage mg · min^{-1} · m^{-2}	53.2 ± 12.3	68.9 ± 17.2	51.3 ± 16.8	67.5 ± 7.1
Basal lipid oxidation mg · min^{-1} · m^{-2}	30.3 ± 4.7	34.1 ± 3.2	41.7 ± 4.7	39.3 ± 4.4
Clamp lipid oxidation mg · min^{-1} · m^{-2}	22.8 ± 4.7	27.0 ± 2.6	23.6 ± 6.2	15.5 ± 3.7‡†

Data are means ± SE. S_i is calculated as R_d/(I × PG), where R_d is the tracer calculated rate of disappearance, I is the incremental serum insulin during the clamp, and PG is plasma glucose concentration. *$P < 0.01$ before versus after; †$P < 0.05$ triple therapy versus control; ‡$P < 0.05$ before versus after; §$P < 0.01$ triple therapy versus control.

body weight, blood pressure, or lipid profile were observed. The only side effect recognized was a 7% reduction of the hemoglobin concentration. This new concept shows interesting potential, but of course long-term studies are needed to prove this (these studies have already been initiated in Denmark).

Test of hypothesis

Our primary aim was to test the hypothesis that normalization of the three major defects in the pathophysiology of type 2 diabetes may result in near normalization of blood glucose. We found that the HbA_{1c} levels in the triple therapy group after 6 months improved significantly. Furthermore, the 24-h glucose values measured at home (SMBG) and during a 24-h profile in the hospital (Fig. 2*A*) showed a glucose pattern close to that found in normal subjects with geometric mean values of about 7 and 6 mmol/l, respectively. Thus, our study seems to confirm the hypothesis that restoration of the three pathophysiologic defects in type 2 diabetic subjects may improve the blood glucose profile.

Insulin sensitivity was not completely normalized in the triple therapy group compared with a nondiabetic population, but insulin aspart at mealtime was able to mimic a normal insulin profile with rapid and high insulin peaks. This resulted in no postprandial glucose elevation after breakfast and lunch and minor elevation after dinner. These findings demonstrate the importance of a normal insulin profile but also show that improvement of postprandial glucose values has an impact on the 24-h glucose profile as well as on fasting blood glucose. Moreover it shows the putative impact of postprandial hyperglycemia on HbA_{1c}.

With respect to nocturnal hypoglycemia, triple therapy seems to be safe because the risk is very low. The reason for this is insulin aspart, which disappears from the blood during the evening and therefore only endogenous insulin is present at night. If blood glucose declines at night, endogenous insulin secretion declines as well.

In the control group, HbA_{1c} values did not improve significantly despite a 50% increase in NPH or MIX insulin dose. The explanation for this is primarily that these subjects have severe insulin resistance; some received 100 units/day of insulin. The reason for the high HbA_{1c} values despite high doses of NPH insulin could be that postprandial hyperglycemia is not avoided by 1–2 injections of NPH insulin per day, as indicated in a recent study by Yki Järvinen et al. (23). Thus, NPH insulin once or twice daily seems unable to normalize blood glucose values. It could be argued that the dose of insulin in the control group was still not high enough to produce a normal glycemic response. However, our study demonstrates that with triple therapy postprandial glucose and HbA_{1c} decreased without an increase in insulin dose, which is completely opposite to what is seen in the control group. Therefore, the answer to our secondary aim is that triple therapy is associated with a lower HbA_{1c} level than treatment with NPH or MIX insulin, at least in the present study setup.

Clinical experience with combination therapy in type 2 diabetes

In the literature, we found 25 studies on combination therapy in type 2 diabetes, i.e., a combination of NPH insulin and either metformin, sulphonylureas, acarbose, or glitazone running for time peri-

ods of 3–12 months. Overall, after treatment in these studies, HbA_{1c} levels only decreased to a value of ~9% (6). Thus, the results of the combination of NPH insulin and one oral antidiabetic drug have not been encouraging. The combination of NPH insulin at night with metformin twice daily seems the most appropriate treatment for the moment, but even in the best of these studies, the HbA_{1c} levels are 1.2% above the upper level for control subjects (6).

Only two studies on the effect of rapid-acting insulin before meals have been carried out in type 2 diabetic subjects. In a controlled study, the combination of regular insulin at meals and a sulphonylurea was not better than NPH insulin alone (24). A similar result was obtained with the combination of insulin lispro at meals with a sulphonylurea in comparison with the combination of NPH insulin with a sulphonylurea (25). Altogether, the literature and our results suggest that triple therapy seems superior to other regimes, yet it has to be tested in larger studies against different combinations of treatment.

Peripheral insulin resistance

Type 2 diabetic subjects are insulin resistant, i.e., insulin-mediated glucose disposal is significantly reduced, as also demonstrated in this study (10). This defect seems to be allocated to skeletal muscle, where glucose uptake, disposal, and oxidation are reduced. In this study, glucose oxidation during treatment in the control group was unchanged, whereas glucose oxidation improved significantly in the triple therapy group (Table 2). This makes sense because an increase in glucose oxidation is mandatory for a decline in blood glucose values. The increase in glucose oxidation may be mainly explained by rosiglitazone treatment (26). In accordance with this, FFA levels during the day were reduced during treatment with triple therapy, and the mean FFA concentration correlated with lipid oxidation during the clamp. This may be mainly explained by the treatment with rosiglitazone.

HGP

Basal HGP has been reported to be slightly increased (by about 15%) in untreated type 2 diabetic subjects (11,27). In this study, fasting HGP was slightly increased compared with nondiabetic reference material. After 6 months, fasting HGP was still within the normal range despite a decline in fasting serum insulin in the triple therapy group, indicating that other variables also play an important role in fasting HGP. The action of HGP during the insulin clamp was estimated at only two insulin concentrations in this study, but the results indicate that the liver is also insulin resistant and that the treatment increases hepatic insulin sensitivity in the triple therapy group. The improvement of insulin sensitivity in the liver may be related mainly to metformin treatment (26).

Body weight, blood pressure, liver function, and safety

Body weight increased slightly but nonsignificantly. Improved metabolic control can by itself account for an increase in body weight. Yki Järvinen (6) calculated that a 1% decrease in HbA_{1c} level results in a 2-kg increase in body weight. Thus, there is no reason to anticipate that rosiglitazone should have increased body weight specifically. However, if so, this effect may have been counterbalanced by metformin treatment.

Blood pressure and plasma triglyceride did not change significantly, and there was no difference between the groups. Specifically, there was no increase in LDL cholesterol in either group. Rosiglitazone did not induce any degree of liver toxicity whatsoever. One subject stopped the triple therapy after several months due to a subjective feeling of edema, but objective signs of edema were not found and X-ray of the chest was normal. The only recognized side effect was the well-known reduction in hemoglobin concentration.

Design

The main aim in this study was to test a hypothesis, and this does not involve a controlled blinded protocol. However, a subaim was to study if the chosen concept, i.e., triple therapy, was better than treatment with NPH or MIX insulin twice daily. Such a study should ideally be blinded, but that is impossible if you choose to give both groups the benefit of changing insulin dosages during the study period. Furthermore, in a blinded trial, syringes with saline should have been used, from which ethical considerations arise. By offering both groups identical care, applying identical goals for the metabolic control in both groups, and using insulin algorithms, we feel that we have compensated for the missing blinding, and therefore a bias in favor of the triple therapy group has been minimized. One year after completion of the study, five subjects still receive triple therapy, and these subjects' glycemic control is nearly identical to what it was at the end of the study (6.7 ± 0.4 vs. 6.6 ± 0.3%), whereas seven subjects in the control group have continued conventional treatment with no improvement in HbA_{1c} over time (8.8 ± 0.4 vs. 9.2 ± 0.8%)

In conclusion, we have shown that 6 months of treatment with a new concept based on our available pathophysiological knowledge (rosiglitazone, metformin, and insulin aspart) significantly reduced the 24-h glucose profile and the HbA_{1c} level. No significant increase in body weight and no impairment in plasma lipids, blood pressure, and safety parameters (hemoglobin was an exception) were observed in the triple therapy group.

These results strongly indicate that normalization of the three major pathophysiological defects of type 2 diabetic subjects, namely peripheral insulin resistance, hepatic insulin resistance, and reduced insulin response following meals can significantly improve glucose metabolism. Triple therapy seems a promising treatment for hyperglycemia in type 2 diabetic subjects, but long-term studies are necessary. However, if these results are confirmed, diabetes complications may be dramatically reduced.

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