

Different effects of insulin and oral antidiabetic agents on glucose and energy metabolism in Type 2 (non-insulin-dependent) diabetes mellitus

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Summary. Which therapy should be used in Type 2 (non-insulin-dependent) diabetic patients with “secondary sulfonylurea failure”, insulin or a combination of sulfonylurea and metformin? To address this question, we have compared the effect of 6 months of insulin therapy twice daily with that of a combination of glibenclamide and metformin in 24 Type 2 diabetic subjects, who no longer responded to treatment with sulfonylureas. Both treatments resulted in an equivalent 30% improvement in mean daily blood glucose ($p < 0.001$), without significant effect on serum lipids. Insulin improved glycaemic control primarily by reducing basal hepatic glucose production ($p < 0.05$), but had no significant effect on peripheral glucose metabolism. The combination of glibenclamide and metformin enhanced significantly total

body glucose metabolism ($p < 0.05$), predominantly by stimulating the non-oxidative pathway. Neither insulin nor the combination therapy altered B-cell response to a test meal. Insulin therapy resulted in a 6% increase in body weight, 63% of which was accounted for by increased fat mass. Although body weight was unchanged during sulfonylurea/metformin therapy, lean body mass and energy expenditure decreased significantly ($p < 0.05$). We conclude that insulin and glibenclamide/metformin have different long-term effects on glucose and energy metabolism in Type 2 diabetes.

Key words: Type 2 (non-insulin-dependent) diabetes mellitus, sulfonylurea, metformin, insulin, secondary drug failure, energy.

Although secondary failure to treatment with sulfonylureas is a common problem in the treatment of Type 2 (non-insulin-dependent) diabetes mellitus [1–3], there is no consensus about how to handle this problem [4–7]. A recent editorial recommended starting insulin before rather than after two years of hyperglycaemic malaise [5], but this view has been challenged by others [8, 9]. The extent of the dilemma was obvious in the consensus statement by the European NIDDM Policy Group [7], suggesting that insulin therapy should be started in a patient with Type 2 diabetes “not too soon and not too late”. Whereas sulfonylureas and insulin seem to have the same hypoglycaemic properties in patients with newly diagnosed Type 2 diabetes [10, 11], the results in patients with secondary sulfonylurea failure are inconsistent [6, 8, 12, 13].

Given these conflicting results we decided to compare the metabolic effects of insulin (intermediary and short-acting insulin twice daily) with that of maximum doses of sulfonylurea and metformin in Type 2 diabetic patients with secondary failure to sulfonylureas. The second aim was to study the mechanisms by which these treatments lowered blood glucose. Thirdly, since insulin treatment has been associated with weight gain [5], we also examined the effect of treatment on energy expenditure and changes in body composition.

Subjects and methods

Twenty-four patients with Type 2 diabetes (Table 1), who no longer responded to treatment with sulfonylureas, were block-randomized to two different treatment regimes for 6 months; (1) a combination of short and intermediary acting insulin given twice daily (mean \pm SEM of daily insulin dose = 47 ± 4 IU) (Insulin); and (2) combination of 21 mg of glibenclamide and 1.5 g of metformin (O.H.A.) Patients were classified as non-responders to sulfonylurea therapy, if they after an initial good response of at least one year, were in poor glycaemic control (see below) despite maximum doses of sulfonylurea (> 15 mg day of glibenclamide or glipizide). Poor glycaemic control was defined as a fasting plasma glucose concentration (mean of last 3 measurements with 2 month intervals) > 10 mmol/l. Ten healthy, non-diabetic subjects (6 men, 4 women) served as control subjects. Their age was 49 ± 3 years, body mass index was 24.2 ± 0.9 kg/m², and their fasting plasma glucose was 4.7 ± 0.1 mmol/l. A complete medical work-up was carried out to exclude intercurrent illness or cardiac, hepatic, renal or other endocrine disease. All subjects gave their informed consent before participating. The study protocol was approved by the Ethical Committee of the Fourth Department of Medicine, Helsinki University Hospital.

Before randomization, the patients were followed by the Metabolic Outpatient Clinic for 4–6 months, during which time they were all given the same dietary advice (high fiber diet with 45% carbohydrates, 35% fat and 20% protein) and taught home-monitoring of blood glucose using a reflectance meter (Glucometer II, Ames, Elkhart, Ind, USA). All patients were admitted to hospital for commencement of treatment. After discharge, the patients were asked to measure their blood glucose at home two days a week (morning, before lunch, before dinner, evening) and to record the readings.

Insulin therapy. The patients were given intermediate and short-acting human insulin (Protaphan and Actrapid Human, Novo Industri, Copenhagen, Denmark) twice daily (before breakfast and dinner) with an insulin pen (Novopen, Novo Industri). The insulin dose was titrated to achieve mean home monitored blood glucose below 8.0 mmol/l without hypoglycaemic side effects.

O. H. A. The patients were given 6 tablets (3.5 mg per tablet) of glibenclamide (Euglucon, Orion, Helsinki, Finland) and 3 tablets (0.5 g per tablet) of slow-release metformin (Diformin retard, Medica, Helsinki, Finland) three times daily (before breakfast, before lunch, before dinner). If hypoglycaemic symptoms occurred, or the patient recorded blood glucose concentrations below 3.0 mmol/l on at least two occasions at home, the dose was reduced.

Experimental design

Follow-up. After discharge from the hospital the patients were seen in the Metabolic Outpatient Clinic at 1 month intervals for 6 months. Body weight was recorded and blood was drawn for determination of HbA_{1c}, fructosamine, cholesterol, HDL-cholesterol, and triglycerides at each visit. In addition, the home-monitored blood glucose data were recorded at each visit.

Metabolic studies. The patients participated in each three experiments before and after therapy; (1) a 3-h test meal; and (2) a 2-h euglycaemic, hyperinsulinaemic clamp; and (3) determination of lean body mass (LBM). The metabolic studies started at 07.30 h after a 12 h overnight fast. The test meal consisted of 60 g of whole wheat bread, 60 g of cheese, 20 g of marmelade, 200 ml low-fat milk, 125 ml orange juice and 15 ml coffee (about 600 kcal). Blood samples were drawn for plasma glucose, insulin and C-peptide determinations at 30 min intervals during the test meal. Since plasma glucose had been lowered by treatment, the plasma glucose concentration was raised to pretreatment level by an infusion of glucose for 30 min before the start of the second test meal. The glucose infusions in the O.H.A. and insulin groups averaged 3.2 ± 0.8 and 5.5 ± 1.2 mg/kg · min, respectively.

Euglycaemic insulin clamp. Glucose metabolism was measured using the euglycaemic insulin clamp technique [14] whereas substrate oxidation and energy production were estimated by indirect calorimetry [15]. After obtaining 3 basal samples for glucose and insulin, a primed-constant infusion of short-acting human insulin (Actrapid) was administered at a rate of 45 mU/m² · min (about 315 pmol/m² · min). The plasma glucose concentration was determined at 5 min intervals and a variable infusion of 20% glucose was adjusted to maintain the plasma glucose concentration constant for 120 min in the control subjects. In the diabetic patients, no glucose was infused until plasma glucose had decreased to the desired level. Therefore, the pre-treatment insulin clamp lasted 165 ± 5 min in the insulin group and 164 ± 6 min in the O.H.A. group. The length of the post-treatment clamp matched the length of the pre-treatment clamp.

At unchanged plasma glucose concentration, the amount of glucose required to maintain euglycaemia equals whole-body disposal of glucose, provided that there is no entry of glucose from the liver. Hepatic glucose production was measured by the isotope dilution technique using [³H-3-]glucose (Amersham inc, Amersham, UK) administered as a primed (25 uCi) constant (0.25 uCi/min) infusion for 150 min and continued throughout the experiment. Blood samples for determination of insulin and [³H-3-]glucose specific activity were obtained in the basal state and at 15 min intervals throughout the insulin clamp.

Indirect calorimetry was employed in the basal state and during the last 60 min of the insulin clamp to estimate net rates of carbohydrate and lipid oxidation [15]. A computerized, open-circuit system was used to measure gas exchange through a transparent 25 l PVC plastic canopy (Deltatrac, Datex, Helsinki, Finland) [16]. Flow was measured by the air-dilution method, carbon dioxide concentration by a conventional infra-red detector, whereas oxygen concentration was measured by a fast differential paramagnetic oxygen sensor. The carbon dioxide concentration of room air was automatically re-measured every 30 min to avoid errors caused by drift in room air carbon dioxide concentration. The oxygen sensor maintains its ac-

curacy for up to 8 h without recalibration. The monitor has a precision of 2.6% for oxygen consumption and 1.0% for carbon dioxide production. Protein oxidation was calculated from the urinary urea nitrogen excretion obtained before and during the insulin clamp.

Lean body mass was determined with the tritiated water dilution technique [17]. Briefly, 40 uCi of tritiated water (Amersham Inc, Amersham, UK) diluted in NaCl 0.9% was given as an iv injection. Blood samples for determination of tritiated water specific activity in plasma were drawn before and 80, 100 and 120 min after the injection.

Analytical determinations. The plasma glucose concentration was measured by the glucose oxidase method on a Beckman glucose analyser II (Beckman Instruments, Fullerton, Calif, USA). Glycohaemoglobin concentration in blood was measured by microcolumn chromatography (Isolab, Akron, Ohio, USA). The reference level for the assay was 5–7%. The concentration of serum fructosamine was measured using a commercial kit (Roche Diagnostics, Basel, Switzerland). The reference range for healthy subjects was 2.0–2.7 mmol/l. Serum insulin was measured by a double-antibody radioimmunoassay (Pharmacia, Uppsala, Sweden). C-peptide was measured by radioimmunoassay using a specific guinea pig antiserum raised against human C-peptide (Cambridge Medical Diagnostics, Billerica, Mass, USA). Plasma non-esterified fatty acids (NEFA) were measured with the microfluorometric method described by Miles et al. [18]. [³H-3-]glucose specific activity was measured in duplicate on supernatants of 1 N perchloric acid extracts of plasma samples, after evaporation of radiolabelled water.

Calculations

Basal hepatic glucose production was calculated by dividing the [³H-3-] glucose infusion rate by the steady state plateau of [³H-3-]glucose specific activity achieved during the last 30 min of the basal tracer infusion period. After administration of insulin and glucose a non-steady state condition in glucose specific activity exists, and the rate of glucose appearance was calculated by a two compartment model [19]. This model is known to produce negative estimates of hepatic glucose production in the presence of high levels of insulin. Negative rates of endogenous (hepatic) glucose production were only rarely observed during the second h of the insulin clamp. These values were taken to indicate that hepatic glucose production was completely suppressed. The infusion rate of cold glucose was integrated over 20 min intervals and subtracted from the total rate of glucose appearance to obtain the hepatic glucose production rate. Total body glucose metabolism was calculated by adding the mean rate of hepatic glucose production (if a positive number) during the last 60 min of each insulin clamp step to the mean glucose infusion rate during the same period. Non-oxidative glucose metabolism was calculated as the difference between total body glucose uptake and glucose oxidation, as determined by indirect calorimetry.

Substrate oxidation. Net glucose and lipid oxidation rates were calculated from indirect calorimetric measurements in the basal state and during the last 60 min of each insulin clamp. The constants to calculate glucose, lipid and protein oxidation from gas exchange data have been described previously [15]. At a non-protein respiratory quotient > 1.0, the equation for the calculation of substrate oxidation remains valid; the remaining negative value for lipid oxidation is, in fact, equivalent to net fat synthesis [15].

Lean body mass. Total body water was calculated from steady state kinetic data assuming that 1 ml of plasma contains 93% water. Lean body mass was obtained by dividing total body water by 0.73, since the lean body mass contains approximately 73% water. Fat mass was calculated as the difference between body weight and lean body mass.

Statistical analysis

All data are expressed as mean ± SEM. Differences from baseline were tested by analysis of variance for repeated measurements using a BMDP (Biomedical Data Processing Program, Los Angeles, Calif, USA) computer programme. Differences between group means were

tested with one-way analysis of variance. Scheffe's test was applied to correct for multiple comparisons. Correlations were tested by linear regression analysis. Area under the C-peptide curves was calculated by the trapezoidal rule.

Results

Glycaemic control

Before randomization, the patients were followed by the Outpatient Clinic for 4-6 months. In the insulin group, fasting plasma glucose (from 14.3 ± 0.8 to 14.9 ± 0.7 mmol/l) and HbA_{1c} (from 11.0 ± 0.4 to $10.4 \pm 0.3\%$) did not significantly change, while there was a slight fall in fructosamine concentration (from 4.3 ± 0.2 to 3.8 ± 0.2 mmol/l; $p < 0.05$) during this period. No significant changes were observed during the same period in fasting plasma glucose (from 12.4 ± 0.6 to 13.4 ± 0.6 mmol/l), HbA_{1c} (from 10.1 ± 0.4 to $9.9 \pm 0.3\%$) and fructosamine (3.8 ± 0.1 to 3.7 ± 0.2 mmol/l), concentrations in the O.H.A. group.

There was a significant improvement in all parameters of glycaemic control (home monitored blood glucose, HbA_{1c} and fructosamine) within 4 weeks after intensified therapy was started in both groups ($p < 0.01$) (Fig. 1). Thereafter, the mean blood glucose concentration measured at home remained unchanged in the insulin and O.H.A. groups throughout the follow-up period. There was no significant difference in blood glucose, HbA_{1c} or fructosamine concentrations between the insulin and O.H.A. groups at any time.

Lipids

There was no significant change in serum cholesterol concentrations during insulin (from 7.28 ± 0.55 to 6.73 ± 0.59 mmol/l) or O.H.A. (6.42 ± 0.35 vs 6.14 ± 0.33 mmol/l). HDL-cholesterol concentrations remained virtually unchanged in the insulin (1.27 ± 0.12 vs 1.31 ± 0.12 mmol/l) and O.H.A. (1.10 ± 0.10 vs 1.17 ± 0.11 mmol/l) groups. As a consequence, the serum triglyceride concentration did not change significantly during treatment with insulin (2.35 ± 0.27 vs 2.53 ± 0.83 mmol/l) or O.H.A. (2.44 ± 0.33 vs 2.19 ± 0.27 mmol/l).

B-cell function

Basal insulin concentration did not significantly change in the insulin (from 10.2 ± 1.2 to 12.3 ± 2.0 μ U/ml) or O.H.A. (15.8 ± 3.8 vs 10.0 ± 1.3 μ U/ml) groups during the treatment period (Fig. 2). Neither was there any change in basal C-peptide concentration in the insulin- (0.48 ± 0.06 vs 0.47 ± 0.04 nmol/l) or O.H.A.-treated (0.59 ± 0.07 vs 0.63 ± 0.07 nmol/l) group.

In order to achieve identical experimental conditions during the first and second test meal, the plasma glucose concentration was raised by a variable infusion of glucose before the second test meal. The basal plas-

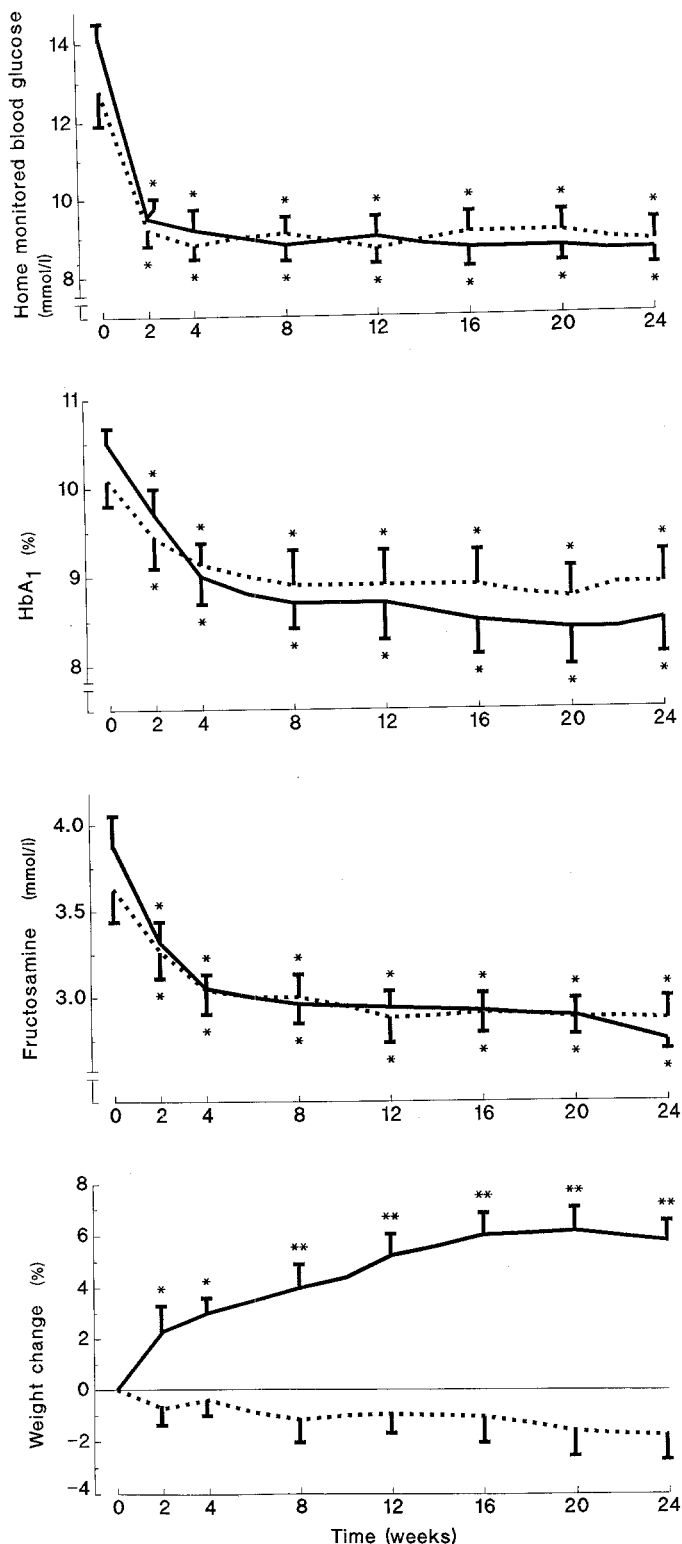


Fig. 1. Change in home monitored blood glucose (mean of 4 measurements per day during two days a week) (top panel), glycohaemoglobin (second panel), serum fructosamine (third panel) concentrations and change in body weight (bottom panel) in patients treated with insulin (unbroken line), or glibenclamide/metformin (dotted line). Values are mean \pm SEM. * $p < 0.01$, significance of difference from baseline

ma glucose concentration during the first and second test meals was 13.2 ± 0.6 vs 12.7 ± 0.7 mmol/l in the insulin group and 11.9 ± 0.5 vs 11.7 ± 0.8 mmol/l in the O.H.A. group (Fig. 2). The area under the concentra-

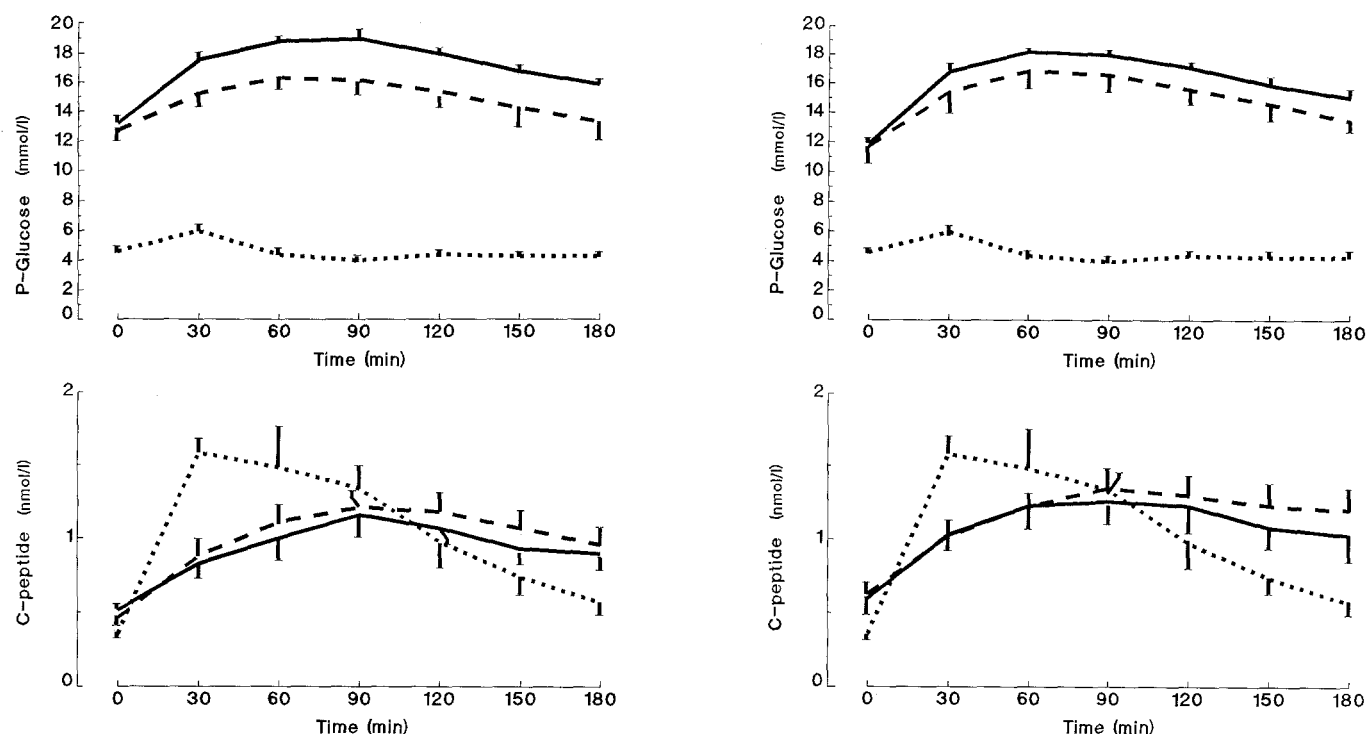


Fig. 2. Plasma glucose (top panel) and C-peptide (bottom panel) responses to test meal before (unbroken line) and after treatment (broken line) with insulin alone (to the left) or a combination of glibenclamide and metformin (to the right). The dotted lines represent data from healthy control subjects. Values are mean \pm SEM

tion curve was similar before and after treatment in the insulin (5.42 ± 0.65 vs 6.16 ± 0.70 nmol/l \times 180 min; $p = \text{NS}$) and O.H.A. (6.64 ± 0.77 vs 7.03 ± 0.69 nmol/l \times 180 min; $p = \text{NS}$) groups.

Glucose metabolism

Hepatic glucose production. At the beginning of the study, the basal rate of hepatic glucose production (HGP) was enhanced in both the insulin (4.58 ± 0.29 mg/kg LBM \cdot min) and O.H.A. group (4.40 ± 0.36 mg/kg LBM \cdot min) compared with healthy control subjects (2.62 ± 0.1 mg/kg LBM \cdot min; both $p < 0.001$). The enhanced basal rate of HGP was significantly reduced with insulin (to 3.72 ± 0.30 mg/kg LBM \cdot min; $p < 0.05$), and less so with sulfonylurea/metformin (to 3.90 ± 0.19 mg/kg LBM \cdot min) (Fig. 3). However, even after treatment, basal HGP remained 40–50% higher in the diabetic patients than in healthy control subjects. During the insulin clamp, hepatic glucose production was completely suppressed in control subjects. Suppression of HGP by insulin was not significantly influenced by insulin or O.H.A. therapy (Fig. 3).

Total glucose metabolism. In the basal state total glucose metabolism equalled the rate of appearance of glucose, i.e. HGP, and was calculated from the isotopic data

minus glucose loss in the urine. Insulin-stimulated glucose disposal increased by 32% in the O.H.A. group (5.07 ± 0.31 vs 6.68 ± 0.82 mg/kg LBM \cdot min; $p < 0.05$) (Fig. 3). Despite this improvement, total glucose metabolism was still reduced by 21% in the O.H.A. group compared with the age-matched control subjects ($p < 0.05$). There was no significant increase in total glucose disposal during insulin therapy (from 5.09 ± 0.25 to 5.59 ± 0.55 mg/kg LBM \cdot min).

Glucose oxidation. The basal rate of glucose oxidation did not significantly change during insulin (from 1.64 ± 0.18 to 1.82 ± 0.23 mg/kg LBM \cdot min) or O.H.A. (1.59 ± 0.23 vs 1.84 ± 0.25 mg/kg LBM \cdot min) treatment

Table 1. Clinical characteristics of subjects treated with insulin, a combination of sulfonylurea and metformin (OHA) and in healthy control subjects

	Insulin	O.H.A.	Control subjects
N (females/males)	12 (6/6)	12 (6/6)	10 (56/56)
Age (years)	59 \pm 2	60 \pm 2	49 \pm 3
Duration (years)	11 \pm 1	12 \pm 2	
Body weight (kg)	76.4 \pm 3.3	77.7 \pm 3.6	72.6 \pm 3.7
Body mass index (kg/m ²)	27.7 \pm 0.9	26.5 \pm 1.0	24.2 \pm 0.9
Fat mass (%)	38.5 \pm 2.2	35.4 \pm 2.0	29.4 \pm 2.3
Fasting plasma glucose (mmol/l)	14.3 \pm 0.7	12.5 \pm 0.4	4.7 \pm 0.1
Glycohaemoglobin (%)	10.7 \pm 0.2	10.4 \pm 0.4	5.9 \pm 0.3
Fasting insulin (μ U/ml)	10.2 \pm 1.2	15.8 \pm 3.8	6.0 \pm 0.5
Cholesterol (mmol/l)	7.3 \pm 0.5	6.4 \pm 0.4	5.8 \pm 0.3
HDL-Cholesterol (mmol/l)	1.3 \pm 0.1	1.1 \pm 0.1	1.5 \pm 0.1
Triglycerides (mmol/l)	2.35 \pm 0.27	2.44 \pm 0.33	1.0 \pm 0.5

Values are mean \pm SEM

(Fig. 3). During the insulin clamp, there was a 2-fold increase in the rate of glucose oxidation compared with baseline. However, insulin-stimulated glucose oxidation was not significantly altered by insulin (3.30 ± 0.27 vs 3.62 ± 0.33 mg/kg LBM·min) or by sulfonylurea/metformin (3.05 ± 0.17 vs 3.33 ± 0.31 mg/kg LBM·min) therapy.

Non-oxidative glucose metabolism. The basal rate of non-oxidative glucose disposal was enhanced most likely as a consequence of the mass action effect of higher plasma glucose concentrations. Lowering of the fasting plasma glucose concentration by insulin and O.H.A. resulted in a reduced basal rate of non-oxidative glucose disposal (Fig. 3). Insulin-stimulated non-oxidative glucose disposal accounted for 35% of total body glucose disposal in the diabetic patients compared with 50% in healthy control subjects ($p < 0.05$). Insulin therapy did not significantly alter the rate of non-oxidative glucose disposal. On the other hand, treatment with sulfonylurea/metformin caused a 75% improvement in non-oxidative glucose metabolism (from 1.98 ± 0.30 to 3.46 ± 0.72 mg/kg LBM·min; $p = 0.07$), which was no longer significantly different from the value observed in healthy control subjects (4.41 ± 0.19 mg/kg LBM·min). There was a large variation in the individual responses to treatment (7 increased, 2 unchanged, 2 decreased), which explains the failure to observe a statistically significant difference (at $p < 0.05$ level) during treatment.

NEFA and net lipid oxidation. Basal NEFA concentrations were similar before and after treatment with insulin or O.H.A. (Table 2). NEFA concentrations were markedly suppressed during the insulin clamp in both control subjects and diabetic patients, and this suppression was further enhanced in diabetic patients with sulfonylurea/metformin treatment ($p < 0.01$ vs before O.H.A. treatment).

The basal rate of net lipid oxidation was significantly reduced by sulfonylurea/metformin treatment (from 1.53 ± 0.09 to 1.31 ± 0.07 mg/kg LBM·min; $p < 0.02$) but not with insulin (1.64 ± 0.09 vs 1.49 ± 0.16 mg/kg LBM·min; $p = \text{NS}$). During the insulin clamp, lipid oxidation was reduced by 70% in control subjects (from 1.30 ± 0.11 to 0.48 ± 0.11 mg/kg LBM·min; $p < 0.001$). Suppression of lipid oxidation by insulin was not significantly altered by O.H.A. (0.91 ± 0.08 vs 0.79 ± 0.10 mg/kg LBM·min) or insulin (1.0 ± 0.08 vs 0.84 ± 0.15 mg/kg LBM·min) therapy.

Protein oxidation. The rate of protein oxidation was unaltered by insulin (0.98 ± 0.07 vs 1.07 ± 0.10 mg/kg LBM·min) and sulfonylurea/metformin (1.05 ± 0.18 vs 1.11 ± 0.17 mg/kg LBM·min) therapy, and did not significantly differ from the value observed in control subjects 1.07 ± 0.12 mg/kg LBM·min.

Body composition and energy expenditure. The insulin treated patients gained on average 6% (5 kg) in weight over a 4-month period, after which the weight appeared to reach a new steady state (from 76.4 ± 3.3 to

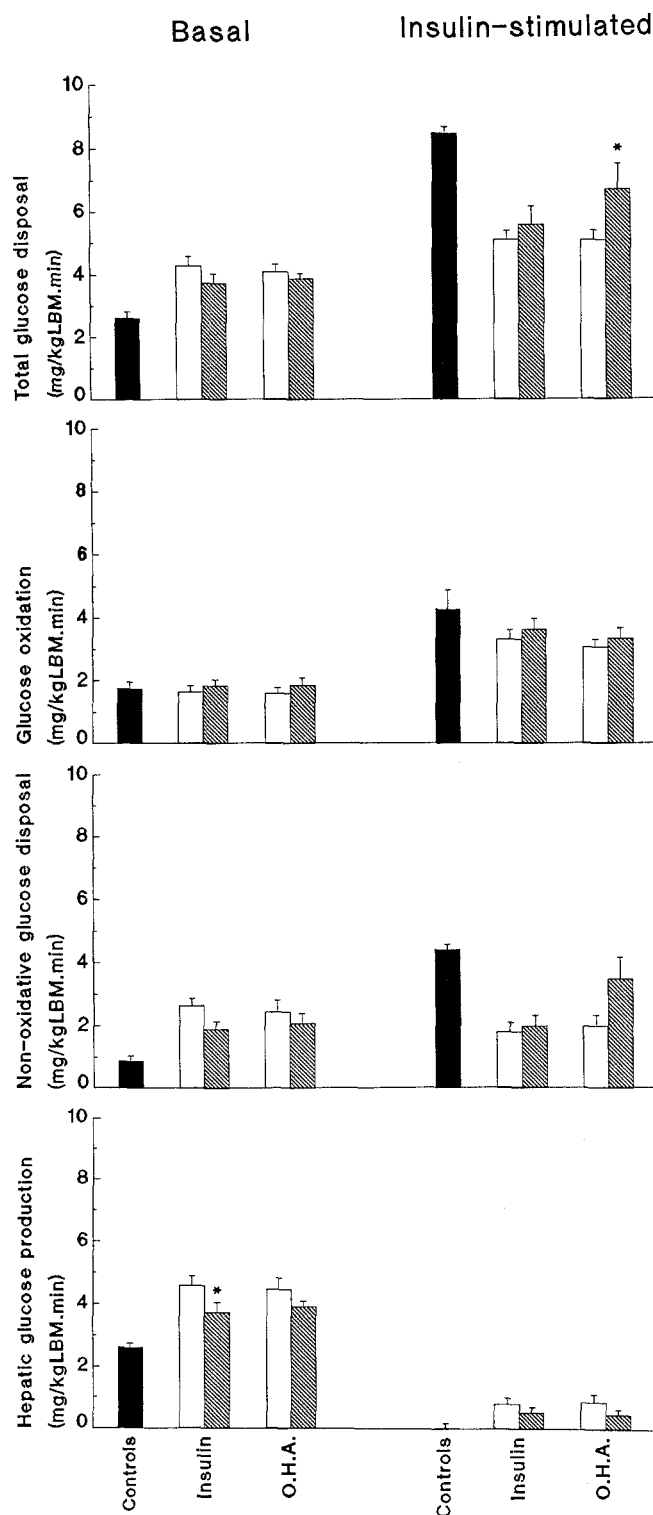


Fig. 3. Insulin-stimulated total glucose metabolism (top panel), glucose oxidation (second panel), non-oxidative glucose metabolism (third panel) and hepatic glucose production (bottom panel) in the basal and insulin-stimulated state before (open bars to the left) and after (shaded bars to the right) treatment with insulin alone or a combination of glibenclamide and metformin. Black bars represent data from healthy control subjects. Values are mean \pm SEM

80.5 ± 3.7 kg; $p < 0.001$) (Fig. 1). Similarly, BMI rose from 27.3 ± 1.0 to 28.8 ± 1.1 kg/m² ($p < 0.01$). There was no change in body weight or BMI in patients treated with sulfonylurea/metformin (77.7 ± 3.6 to 76.4 ± 3.9 kg

Table 2. Metabolic characteristics of the euglycaemic insulin clamp before and after treatment with insulin twice daily (Insulin) or a combination of maximum doses of glibenclamide and metformin (O. H. A.)

	Insulin		O. H. A.		Control subjects
	Before	After	Before	After	
Basal glucose (mmol/l)	13.2 ± 0.4	9.1 ± 0.6	13.1 ± 0.7	10.1 ± 0.8	4.6 ± 0.1
Clamp glucose (mmol/l)	5.5 ± 0.2	5.7 ± 0.2	6.1 ± 0.5	6.2 ± 0.5	4.6 ± 0.1
CV of clamp glucose	6.8 ± 1.0	3.6 ± 0.5	4.5 ± 0.8	6.2 ± 0.9	4.5 ± 0.5
Basal insulin (μU/ml)	9 ± 1	16 ± 6	14 ± 3	9 ± 2	6 ± 1
Clamp insulin (μU/ml)	74 ± 2	81 ± 7	89 ± 5	81 ± 5	78 ± 3
CV of clamp insulin	8 ± 2	7 ± 1	9 ± 2	7 ± 2	6 ± 1
Basal RQ	0.78 ± 0.01	0.79 ± 0.01	0.78 ± 0.01	0.80 ± 0.01	0.79 ± 0.01
Clamp RQ	0.84 ± 0.01	0.86 ± 0.01	0.84 ± 0.01	0.86 ± 0.01	0.89 ± 0.01
Urea nitrogen (mmol/l)	6.7 ± 0.8	7.9 ± 0.6	8.1 ± 1.2	8.4 ± 1.3	8.6 ± 0.02
Basal NEFA (μmol/l)	674 ± 54	818 ± 71	857 ± 41	784 ± 72	599 ± 76
Clamp NEFA (μmol/l)	97 ± 15	117 ± 12	137 ± 15	95 ± 9 ^a	76 ± 9

Values are mean ± SEM. ^a $p < 0.01$ vs pre-treatment value. CV = coefficient of variation; NEFA = non-esterified fatty acid; RQ = respiratory quotient

and 26.3 ± 1.1 to 25.8 ± 1.1 kg/m²). Increase in fat mass contributed more than the increase in LBM (63% vs 37%) to the increased body weight in insulin treated patients. In the O. H. A. treated patients, LBM decreased significantly from 50.9 ± 2.4 to 48.2 ± 2.5 kg ($p < 0.05$). This, in turn, was associated with an increased percentage of fat mass from 35.4 ± 1.9 to $37.3 \pm 1.8\%$ ($p < 0.05$).

Basal and insulin-stimulated energy expenditure did not change during insulin therapy (1.22 ± 0.05 vs 1.21 ± 0.06 kcal/min and 1.22 ± 0.06 vs 1.23 ± 0.07 kcal/min). In contrast, during treatment with sulfonylurea/metformin basal energy expenditure decreased significantly from 1.14 ± 0.05 to 1.07 ± 0.05 kcal/min ($p < 0.05$). Sulfonylurea/metformin therapy did not significantly alter insulin-stimulated energy expenditure (1.12 ± 0.06 vs 1.08 ± 0.05 kcal/min). Energy expenditure correlated with lean body mass in all subjects ($r = 0.71$; $p < 0.001$).

Discussion

Treatment with insulin or a combination of glibenclamide and metformin for 6 months resulted in an equivalent 30% improvement in mean blood glucose concentrations in Type 2 diabetic patients, who had failed to respond to treatment with sulfonylurea alone. In insulin-treated patients, this improvement in glycaemic control was associated with a 6% increase in body weight, mainly fat mass. Although body weight remained unchanged in the O. H. A. treated patients, their LBM (i.e. muscle mass) decreased significantly during the follow-up period.

The data provide some new insights into the mechanisms by which insulin and sulfonylurea/metformin improve glycaemic control in patients with secondary failure to sulfonylureas. The effect of insulin therapy was primarily explained by enhanced suppression of basal HGP by insulin, while insulin had no significant effect on peripheral glucose uptake. At first glance, these data seem to disagree with a number of previous studies, which reported an average improvement in

total body glucose disposal of 47% [13, 19–24] after short-term intensified insulin therapy in Type 2 diabetic patients. There are, however, several differences between the present and previous study designs. In previous reports, the patients were re-studied after an average period of 3 weeks, compared with 6 months in the present study. During such a short period of insulin therapy, there is usually no significant weight gain. In contrast, the insulin-treated patients in the current study gained on average 5 kg in weight. It is well described that insulin therapy in Type 2 diabetic patients results in weight gain [5, 11], but it has not been known to what extent this weight gain is due to an increase in muscle or fat mass. Our data demonstrate that $\frac{2}{3}$ of the increase in weight is due to increased fat mass. It is possible, that the metabolic consequences of an increased fat mass have counteracted the possible favourable effect of insulin on peripheral glucose metabolism. There is at least indirect evidence to support this view. In contrast to the O. H. A. group, in which lipid oxidation was reduced by treatment, lipid oxidation remained enhanced during insulin therapy. In addition, suppression of plasma NEFA concentration during the insulin clamp was enhanced by sulfonylurea/metformin treatment. One could therefore postulate the following hypothetical sequence of events; increased fat mass, increased supply of non-esterified fatty acids (NEFA), increased lipid oxidation and through the activation of the Randle cycle, decreased glucose utilization [25].

In contrast to insulin, the addition of metformin to glibenclamide, resulted in enhanced peripheral glucose disposal, mainly enhanced non-oxidative glucose disposal. It has previously been shown, that 3 months of treatment with insulin or tolazamide resulted in a similar improvement in insulin action [10]. The difference between insulin and the combination therapy, must therefore be attributed to the presence of metformin.

In vitro and in vivo studies have shown, that metformin can enhance peripheral glucose uptake [26], especially the oxidation of glucose [27] and decrease gluconeogenesis [28]. In addition, metformin can alter the intestinal absorption of glucose [29]. Interestingly,

biguanides have also been shown to inhibit NEFA oxidation in rat diaphragm [30]. In the present study, the basal rate of net lipid oxidation was significantly reduced during sulfonylurea/metformin therapy, and this, in turn, was associated with a significant reduction in the basal energy production. Oxidation of lipids produces more energy than oxidation of glucose [15], and reduced lipid oxidation should therefore result in reduced energy expenditure. Furthermore, lean body mass (i.e. muscle mass) decreased during treatment with sulfonylurea/metformin and the decrease in LBM was associated with a decrease in energy expenditure, ($r=0.71$; $p<0.01$). It is evident, that the patients were in a negative energy balance during treatment with sulfonylurea/metformin. The reasons are unknown, but there are several possibilities. First, it could reflect the natural course of the diabetic disease. Second, it could be a consequence of the inhibitory effect of metformin on intestinal glucose absorption [28]. Third, it could reflect a general inhibition of oxidative processes as suggested for phenformin [30].

In summary, the data suggest, that long-term insulin and glibenclamide/metformin therapy result in an equivalent improvement in glycaemic control by different mechanisms.

But there was also a reverse side to this coin, while insulin resulted in positive, sulfonylurea/metformin resulted in negative energy balance with a decrease in muscle mass.

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