Acute Effect of Glimepiride on Insulin-Stimulated Glucose Metabolism in Glucose-Tolerant Insulin-Resistant Offspring of Patients With Type 2 Diabetes

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OBJECTIVE — This study addressed whether acute infusion of glimepiride influences glucose metabolism independent of its effect on insulin secretion.

RESEARCH DESIGN AND METHODS — Ten healthy, glucose-tolerant but insulin-resistant probands were subjected to a placebo-controlled, double-blind, cross-over study. Each individual received infusions of either 0.15 mol/l saline or glimepiride in randomized order on two separate occasions. A three-step hyperinsulinemic (0.5, 1.0, and 1.5 mU · kg^{-1} · min^{-1})-euglycemic glucose clamp was performed on both occasions to determine insulin sensitivity. Glimepiride-induced insulin secretion was inhibited by octreotide. Endogenous glucose production and glucose elimination were measured with the "hot" glucose infusion method using U-[^{13}C]glucose as tracer. Glucose oxidation was determined from indirect calorimetry. Lipolysis was evaluated by measurements of nonesterified fatty acid (NEFA) and glycerol concentration and measurement of glycerol production.

RESULTS — Plasma glucose and insulin concentrations were not significantly different between glimepiride or saline infusions. There was a significant increase in the rate of glucose infusion necessary to maintain euglycemia during infusion of glimepiride during the low- (12.2 ± 1.1 vs. 16.1 ± 1.7 μmol · kg^{-1} · min^{-1}) and intermediate-dose insulin infusion (24.4 ± 1.7 vs. 30.0 ± 2.8 μmol · kg^{-1} · min^{-1}). This was explained by an increased rate of glucose elimination and to a lesser degree by a decrease in glucose production. Glucose oxidation rate was not different. NEFA and glycerol concentration and glycerol production were equally suppressed.

CONCLUSIONS — Glimepiride improves peripheral glucose uptake and decreases endogenous glucose production independent of its insulin secretagogue action. The effects shown in this acute study are, however, too small to be considered therapeutically beneficial for the individual patient.

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The glucose-lowering effect of the sulfonylureas may not solely be caused by their insulinotropic action but also by an insulin-independent effect. This suspicion was initially raised by the observation that after long-term treatment with sulfonylureas, insulin levels tend to decrease in type 2 diabetic patients, despite conservation of the blood glucose–lowering effect (1–3). Whereas some studies performed in type 1 diabetic patients failed to demonstrate a peripheral effect (4,5), this finding was not unanimous (6,7). Studies performed in impaired glucose tolerance (8) and type 2 diabetic patients (9–12) were not designed to differentiate between several possible mechanisms capable of inducing an increase in peripheral glucose uptake. Besides the action of insulin itself, these are also effects of plasma glucose (13,14) and free fatty acids on glucose uptake (15). A study performed in normal subjects provided circumstantial but not direct evidence for a peripheral effect of glyburide and glipizide (16).

An extrapancreatic effect has in particular been claimed to exist for the recently introduced sulfonylurea glimepiride. This postulate is based on circumstantial evidence from human and animal in vivo experiments and in vitro data. Thus, acute experiments performed in normal dogs showed that glimepiride had the highest total blood glucose–lowering activity and the lowest insulin-releasing activity during the 36-h post-treatment period when compared with glibenclamide, gliclazide, and glipizide (17). In healthy volunteers, blood glucose concentration was lower in the 2 h following a standard meal given 3 h after intravenous injection of glimepiride as compared with glibenclamide, despite higher C-peptide levels after glibenclamide (18). In vitro experiments showed that in the absence of insulin, both glimepiride and glibenclamide stimulated glucose transport in rat diaphragm and 3T3 adipocytes at low micromole-per-liter concentrations, with glimepiride exhibiting two- to threefold lower ED_{50} values than glibenclamide (17).

The present study was specifically designed to test whether glimepiride has an extrapancreatic effect on glucose metabolism. To avoid possible confounding ef-

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Abbreviations: MCR, metabolic glucose clearance rate; NEFA, nonesterified fatty acid.

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

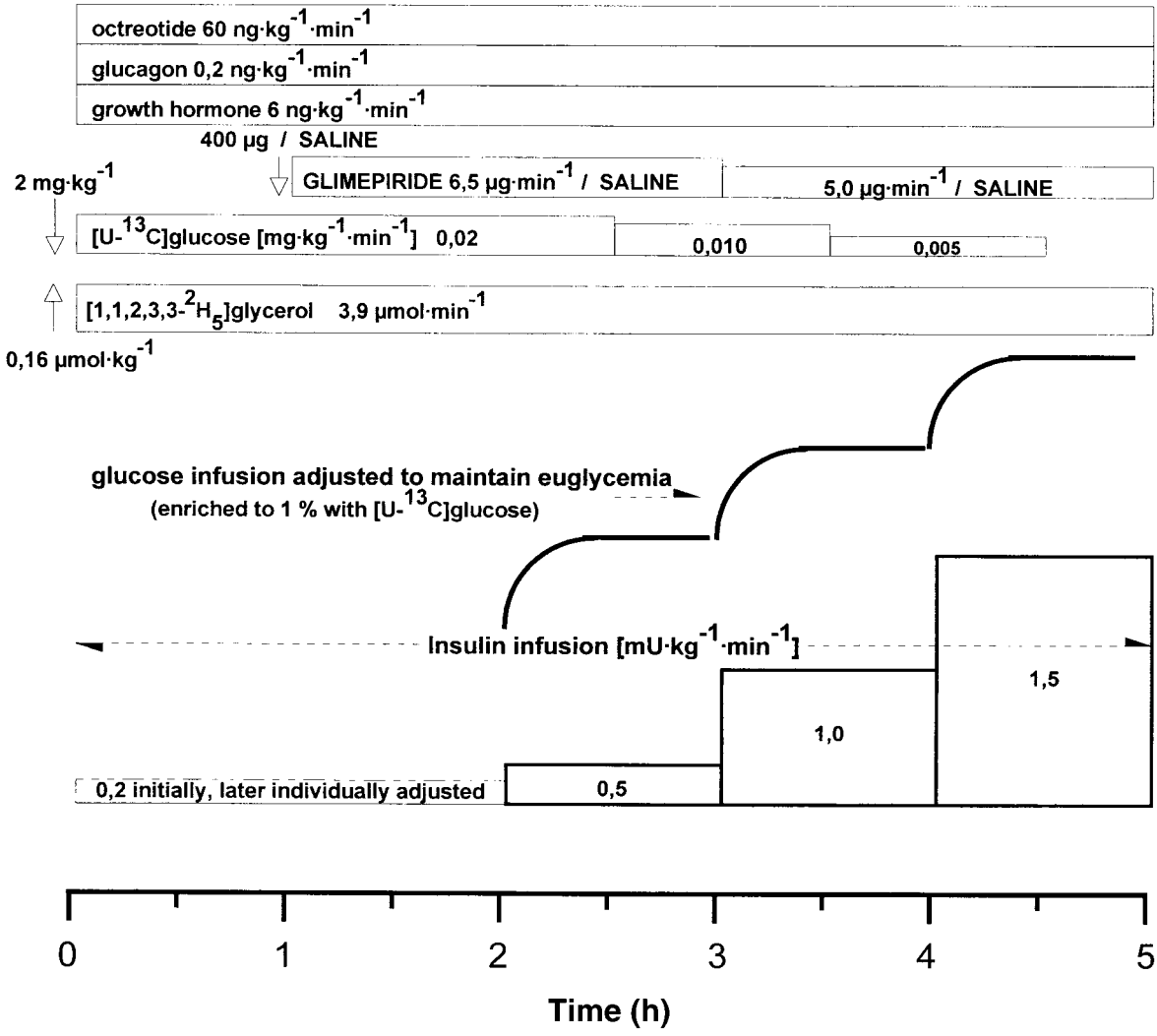

Figure 1—*Illustration of the infusion scheme.*

fects with respect to the effect of the drug on insulin secretion and plasma glucose concentration, as would have to be expected when studying patients with type 2 diabetes, the study was performed in normal glucose-tolerant but insulin-resistant subjects under the conditions of a pancreatic clamp.

RESEARCH DESIGN AND METHODS

Subjects

Written informed consent was obtained from a population of first-degree relatives of type 2 diabetic patients as previously described (19,20). Four female and six male healthy, glucose-tolerant (normal oral glucose tolerance by World Health Organization standards, HbA_{1c} 5.0 ± 0.13%) but insulin-resistant (metabolic glucose clearance rate [MCR] ≤6.5 ml · kg^{-1} · min^{-1} during a hyperinsulinemic [1 mU · kg^{-1} · min^{-1}]-euglycemic glucose clamp) probands were asked to participate in a randomized double-blind, placebo-controlled, cross-over study. Their mean age was 30 ± 2 years, BMI 27 ± 2 kg/m^2, and body fat 24 ± 3% (determined by tetrapolar impedance technique).

Study design

All participants were studied twice 1 week apart under the influence of either the active drug or placebo in randomized order. They were asked to maintain a eucaloric diet containing at least 200 g of carbohydrates at least 3 days before the study. The subjects were admitted to the research ward in the morning after an overnight fast (10–12 h) and were studied in the postabsorptive state. They were comfortably placed in a recumbent position. An 18-g venous catheter (Insite-W; Becton Dickinson Infusion Therapy Systems, Sandy, UT) was placed into a cubital vein of the nondominant arm. All infusions of glucose and hormones were applied via this catheter. A 21-g venous catheter was placed into a backhand vein of the opposite arm in a retrograde fashion in order to obtain arterialized venous blood. To guarantee oxigenation of venous blood, the hand was placed into a heat box and warmed to 55°C.

Infusions

A summary of the infusion scheme is given in Fig. 1. To block pancreatic hormone secretion, the synthetic somatostatin analog octreotide (Sandostatin; Sandoz, Nürnberg, Germany) was contin-

uously infused over 5 h at a dose of 60 ng · kg^{-1} · min^{-1}. To restore physiological concentrations of glucagon and growth hormone, glucagon (GlucaGen; Novo Nordisk Pharma, Mainz, Germany) was infused at a dose of 0.2 ng · kg^{-1} · min^{-1} and growth hormone (Genotropin; Pharmacia, Erlangen, Germany) at a dose of 6 ng · kg^{-1} · min^{-1}. Insulin (H-Insulin; Hoechst Marion Roussel, Frankfurt, Germany) was infused at an initial dose of 0.2 mU · kg^{-1} · min^{-1} and adjusted to maintain euglycemia without glucose infusions over the initial 2 h of the study. The dose was then increased in a stepwise manner to 0.5 mU · kg^{-1} · min^{-1} 2–3 h after commencement of the study, 1.0 mU · kg^{-1} · min^{-1} 3–4 h after commencement of the study, and 1.5 mU · kg^{-1} · min^{-1} 4–5 h after commencement of the study. One hour after commencement of the study, a continuous infusion of glimepiride (6.5 μg/min) was initiated after a bolus dose of 400 μg in the verum arm of the study. After 2 h, the infusion rate was reduced to 5.2 μg/min and maintained at this dose to the end of the study. In the placebo arm of the study, physiological saline was injected and infused instead of glimepiride. [U-^{13}C]glucose (99% pure; Cambridge Isotope Laboratories, Woburn, MA), filtered sterile on 0.22 μm Millipore filters, was infused in a primed (2 mg/kg) continuous (0.02 mg · kg^{-1} · min^{-1}) fashion during the initial 2.5 h of the study. The infusion rate was then reduced to 0.01 mg · kg^{-1} · min^{-1} for the ensuing 60 min and to 0.005 mg · kg^{-1} · min^{-1} for another 60 min. Starting 2 h after commencement of the study, an infusion of 40% glucose enriched with [U-^{13}C]glucose to 0.93% was initiated and adjusted to maintain euglycemia. A continuous infusion of [1,1,2,3,3-$^{2}H_5$]glycerol (3.9 μmol/min) was initiated at the start of the experiment and maintained throughout.

Sampling

Plasma glucose concentration was measured every 10 min during the initial 2 h of the study and then every 5 min. The concentrations of insulin, C-peptide, nonesterified fatty acids, glycerol, and glimepiride and the enrichments of plasma [U-^{13}C]glucose and [$^{2}H_5$]glycerol were measured every 20 min throughout the study. Gas exchange measurements were performed during the last 20 min of each hour after the start of the experiment.

Analytical methods

Serum glimepiride concentration was measured using an established high-performance liquid chromatography method (21). The detection limit for glimepiride in serum is 5 ng/ml. Plasma glucose concentration was determined with the glucose-oxidase method on a YSI 2300 (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin concentration was measured using a microparticle enzyme immunoassay (Abboth Laboratories, Diagnostics Division, Dainabot, Tokyo, Japan) and plasma C-peptide with a radioimmunoassay (RIA-coat C-Peptid; BYK Sangtec, Dietzenbach, Germany).

Plasma nonesterified fatty acid and plasma glycerol concentrations were measured enzymatically on a BM/Hitachi autoanalyzer using reagents from Wako Chemicals and Sigma, respectively.

The ratio of [U-^{13}C]glucose to [U-^{12}C]glucose was determined by selected ion monitoring (SIM) gas chromatography-mass spectrometry as previously described (22). Plasma [$^{2}H_5$]-glycerol enrichment was determined by gas chromatography mass spectrometry using the trimethylsilyl derivative of glycerol. Electron impact ionization was applied, and the mass-to-charge ratios 205 and 208 were monitored (23).

During the last 20 min of each hour after the start of the experiment, continuous indirect calorimetry (Deltatrac MBM-100; Hoyer Medizintechnik, Bremen, Germany) with a canopy system was used to measure rates of O_2 consumption (V_{O_2}) and CO_2 production (V_{CO_2}). Nitrogen excretion was measured in a 24-h urine sample collected the day before the study. Glucose and lipid oxidation rates were determined from O_2 consumption and CO_2 production and urinary nitrogen as previously described (24,25)

Calculations

The MCR of glucose was calculated as:

$$\text{MCR} = \frac{\text{Glucose infusion rate (mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})}{\text{Blood glucose concentration (mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})}$$

where glucose infusion rate is the mean infusion rate during the last 20 min of each insulin infusion rate level and plasma glucose concentration is the mean plasma glucose concentration during the same time interval.

Endogenous glucose production (R_a) and glucose elimination (R_d) was calculated using the non–steady-state equations of Steele (26) in the form modified by deBodo (27) and adjusted to the situation of a hyperinsulinemic glucose clamp using a "hot" glucose infusate (28) from the measured enrichments of [U-^{13}C]glucose, the infusion rate of tracer glucose, the infusion rate of prelabeled glucose, and the enrichment of the prelabeled glucose infusate. To avoid spurious oscillations in calculated rates of R_a and R_d, the timecourse of enrichment was smoothed by the optimal segments method (29).

Endogenous glycerol production rate was calculated from the rate of tracer glycerol infusion ($ra_{glycerol}$) and measured glycerol enrichment ($E_{glycerol}$) starting 120 min after the commencement of the study as follows:

$$R_{a_{glycerol}} = \frac{ra_{glycerol}}{E_{glycerol}}$$

Insulin secretion was calculated by deconvolution from measured plasma C-peptide concentrations as described by Van Cauter et al. (30).

Statistical methods

All results are given as the mean ± SE unless otherwise indicated. Differences in the end points of the study were tested for statistical significance by repeated measures ANOVA using treatment arm and time as nominal fixed factors and individual subjects as a random factor. All calculations were performed on a personal computer using jmp software, version 4.0.0 (SAS Institute, Cary, NC).

RESULTS

Plasma glimepiride concentration

The plasma glimepiride concentration was 0.28 ± 0.04 μmol/ml 20 min after the initial bolus dose of 400 μg and start of the continuous infusion of glimepiride at a rate of 6.5 μg/min. It increased to a maximum concentration of 0.31 ± 0.04 μmol/ml 20 min later and then gradually decreased to 0.15 ± 0.02 μmol/ml at the end of the experiment.

Table 1—*Comparison of steady-state plasma glucose concentrations and glucose infusion rates during graded hyperinsulinemia*

Insulin infusion rate ($mU \cdot kg^{-1} \cdot min^{-1}$)	Plasma glucose concentration (mmol/l)		Glucose infusion rate ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)	
	Placebo	Verum	Placebo	Verum
0.5	5.4 ± 0.1	5.6 ± 0.1	12.2 ± 1.1	16.1 ± 1.7*
1.0	5.2 ± 0.1	5.3 ± 0.1	24.4 ± 1.7	30.0 ± 2.8*
1.5	5.3 ± 0.1	5.4 ± 0.1	38.3 ± 1.7	40.0 ± 3.3

Data are means ± SE. *Statistically significant differences.

Plasma glucose concentration

Starting with normal plasma glucose concentrations (5.3 ± 0.1 mmol/l in the verum group and 5.2 ± 0.1 mmol/l in the placebo group), there was an initial decrease in plasma glucose to a nadir of 4.7 ± 0.2 mmol/l at 50 min in the verum group and 4.2 ± 0.3 mmol/l at 40 min in the placebo group. At 120 min, plasma glucose concentration had stabilized to 5.6 ± 0.1 mmol/l in the verum group and 5.8 ± 0.1 mmol/l in the placebo group. During the rest of the experiment plasma glucose remained constant with coefficients of variation (CVs) <5% in individual patients. The mean of means of plasma glucose concentrations during the final 20 min of each level of insulin infusion is shown in Table 1. The differences between the verum and placebo group were clinically not relevant and not statistically significant at all levels of insulin infusion rate.

U-[^{13}C]glucose enrichment

In the placebo group plasma U-[^{13}C]glucose enrichment increased from 1.07 ± 0.15% 120 min after start of the experiment to a peak of 1.23 ± 0.14% at 140 min. Until the end of the experiment at 300 min, there was a continuous gradual decline in group plasma U-[^{13}C]glucose enrichment to a final value of 0.87 ± 0.02%. In the verum group, U-[^{13}C]glucose enrichment increased from 1.12 ± 0.12% at 120 min to a peak of 1.23 ± 0.13% at 140 min. During the remaining time period, there was a gradual decline parallel to that in the placebo group until a final enrichment of 0.90 ± 0.03%. The mean CV in plasma U-[^{13}C]glucose enrichment in individual probands was 15 ± 2.3% in the placebo group and 13 ± 2.4% in the verum group. By ANOVA, the difference between the treatment arms was not statistically significant (P = 0.2015). The time courses of plasma glucose concentration and U-[^{13}C]glucose enrichment are shown in Fig. 2.

Plasma insulin concentration

Starting from a moderately elevated fasting concentration of 58 ± 8 pmol/l in the verum group and 49 ± 5 pmol/l in the placebo group, there was a slight decrease to 38 ± 12 pmol/l in the verum group and to 28 ± 8 pmol/l in the placebo group at 60 min. Plasma insulin then gradually increased to 131 ± 17 pmol/l in the verum group and 100 ± 18 pmol/l in the placebo group at 120 min. The plasma insulin concentration was 203 ± 16 pmol/l in the verum group and 197 ± 11 pmol/l in the placebo group 60 min after increasing the insulin infusion rate to 0.5 $mU \cdot kg^{-1} \cdot min^{-1}$. The plasma insulin concentration was 468 ± 47 pmol/l in the verum group and 500 ± 52 pmol/l in the placebo group 60 min after increasing the insulin infusion rate to 1.0 $mU \cdot kg^{-1} \cdot min^{-1}$. At the end of the experiment at 300 min (60 min after increasing the insulin infusion rate to 1.5 $mU \cdot kg^{-1} \cdot min^{-1}$), the plasma insulin concentration was 655 ± 46 pmol/l in the verum group and 677 ± 30 pmol/l in the placebo group. By ANOVA, the plasma insulin concentrations were not statistically different between the groups.

Plasma C-peptide concentration

Fasting plasma C-peptide concentration was slightly elevated in both groups (0.83 ± 0.10 nmol/l in the verum group and 0.72 ± 0.10 nmol/l in the placebo group). After initiation of the somatostatin infusion, there was a prompt decrease in plasma C-peptide concentration to 0.18 ± 0.03 pmol/l in the verum group and 0.13 ± 0.03 pmol/l in the placebo group. After initiation of the glimepiride/saline infusion at 60 min, there was a further continuous decrease in plasma C-peptide in the placebo group to 0.05 ± 0.01 pmol/l at 300 min. In the verum group there was a slight increase in C-peptide to a maximum of 0.24 ± 0.05 pmol/l at 100 min. Thereafter, there was a continuous decrease in plasma C-peptide to 0.12 ± 0.03 pmol/l at 300 min.

Insulin secretion

Insulin secretion, as derived by deconvolution after adjustment for sex, height, weight, and age, started from a basal value of 210 ± 34 pmol/min in the placebo group and 234 ± 34 pmol/min in the verum group. It then decreased to negative values after 20 min in both groups. In

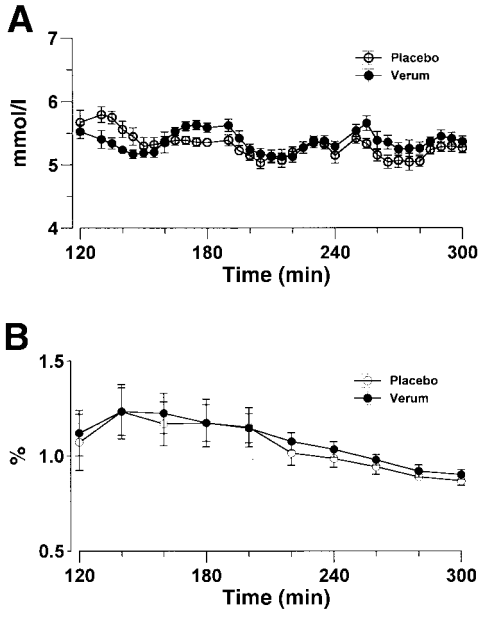

Figure 2—A: *Plasma glucose concentration.* B: *Plasma U-[^{13}C]glucose enrichment.*

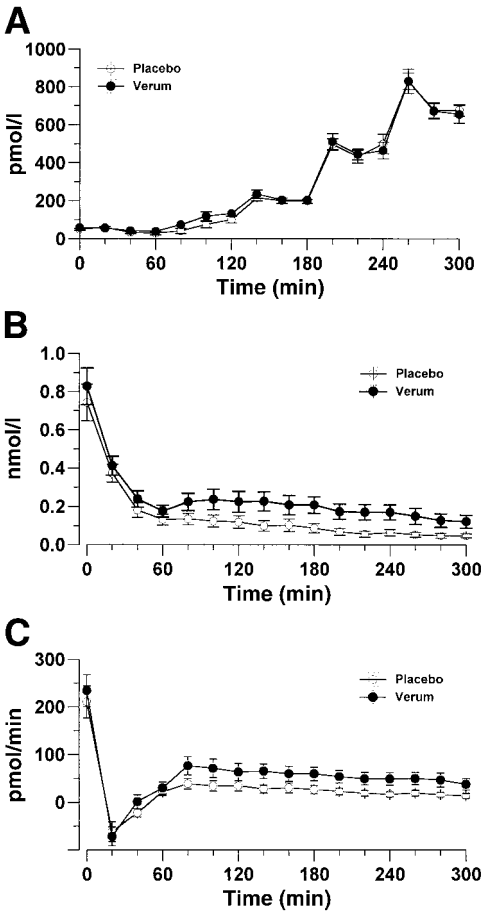

Figure 3—*A: Graded infusion of insulin (~0.2 mU · kg^{-1} · min^{-1} from 0 to 120 min, 0.5 mU · kg^{-1} · min^{-1} from 120 to 180 min, 1.0 mU · kg^{-1} · min^{-1} from 180 to 240 min, and 1.5 mU · kg^{-1} · min^{-1} from 240 to 300 min). B: Plasma C-peptide concentration after hormonal blockade of pancreatic secretion with octreotide starting at 0 min. C: Calculated (from deconvolution) insulin secretion rates.*

the placebo group, insulin secretion then recovered to a transient maximum of 38 ± 11 pmol/min at 80 min and thereafter gradually decreased to 14 ± 5 pmol/min at 300 min. In the verum group, insulin secretion was calculated to increase to a maximum of 76 ± 19 pmol/min at 80 min and then gradually decreased to 38 ± 12 pmol/min at 300 min. The difference between the secretion rates was statistically significant ($P < 0.0001$). A synopsis of plasma insulin and C-peptide concentrations and insulin secretion rate is shown in Fig. 3.

Plasma nonesterified fatty acids

In the verum group, plasma nonesterified fatty acid (NEFA) concentration decreased from 0.53 ± 0.04 mmol/l to a nadir of 0.33 ± 0.03 mmol/l at 20 min after the start of the experiment. NEFA concentration then increased to a peak concentration of 0.52 ± 0.09 mmol/l at 80 min. After this point, the concentration decreased to a final concentration of 0.03 ± 0.01 mmol/l at the end of the experiment. In the placebo group, NEFA concentration decreased from a basal concentration of 0.57 ± 0.06 mmol/l to a nadir of 0.30 ± 0.03 mmol/l at 40 min. It then increased to a peak concentration of 0.70 ± 0.12 mmol/l at 100 min. Thereafter, NEFA concentration decreased to a minimum of 0.03 ± 0.01 mmol/l at 300 min. The difference between the treatment arms was statistically significant ($P = 0.012$)

Plasma glycerol

Starting from a basal concentration of 0.12 ± 0.01 mmol/l in both groups, plasma glycerol concentration decreased to a transient nadir of 0.07 ± 0.01 mmol/l in the placebo group after 20 min. In the verum group, a nadir of 0.07 ± 0.01 mmol/l was reached after 60 min. Plasma glycerol then increased to a peak concentration of 0.11 ± 0.12 mmol/l at 100 min in the placebo and 0.09 ± 0.12 mmol/l at 80 min in the verum group. Thereafter, there was a gradual decrease in plasma glycerol concentration to 0.04 ± 0.01 mmol/l in both groups at 300 min. The difference between the treatment arms was statistically significant ($P < 0.001$)

Glycerol production rate

In the placebo group, glycerol production rate was 1.69 ± 0.29 μmol · kg^{-1} · min^{-1} 120 min after the start of the experiment. It then gradually declined to a nadir of 0.63 ± 0.07 μmol · kg^{-1} · min^{-1} at the end of the experiment. In the verum group, glycerol production was somewhat lower at 120 min after the start of the experiment with 1.21 ± 0.18 μmol · kg^{-1} · min^{-1} and then also gradually declined to 0.60 ± 0.06 μmol · kg^{-1} · min^{-1} at 300 min. After 120 min, following the start of the experiment, the difference between the treatment groups was not statistically significant ($P = 0.065$). A synopsis of plasma NEFA and glycerol concentrations and glycerol production rate is shown in Fig. 4.

Glucose infusion rate

The mean of means of the glucose concentrations and rates of glucose infusion during the last 20 min of each dose level of insulin infusion is shown in Table 1. Whereas the glucose concentrations were not significantly different, significantly higher glucose infusion rates were necessary to maintain euglycemia in the active drug group ($P = 0.0007$). Contrasting the infusion rates between verum and placebo during the last 20 min of each dose level of insulin infusion revealed that the glucose infusion rate during the low ($P = 0.023$) and intermediate ($P = 0.0046$) insulin dose was significantly different, whereas it was not during the high insulin dose ($P = 0.35$).

MCR of glucose

A comparison between the verum and placebo group of the MCRs at each insulin infusion rate level is shown in Fig. 5. The MCRs increased almost linearly from 2.32 ± 0.28 over 4.77 ± 0.37 ml · kg^{-1} · min^{-1} to 7.38 ± 0.35 ml · kg^{-1} · min^{-1} in the placebo group and from 3.13 ± 0.31 over 5.61 ± 0.57 ml · kg^{-1} · min^{-1} to 7.52 ± 0.67 ml · kg^{-1} · min^{-1} in the verum group. By ANOVA, the difference between the levels of insulin infusion was highly significant ($P < 0.001$). Across all insulin levels, the difference between the MCRs observed in the verum and placebo group was statistically significant ($P = 0.0055$). Contrasting the MCRs between verum and placebo during the last 20 min

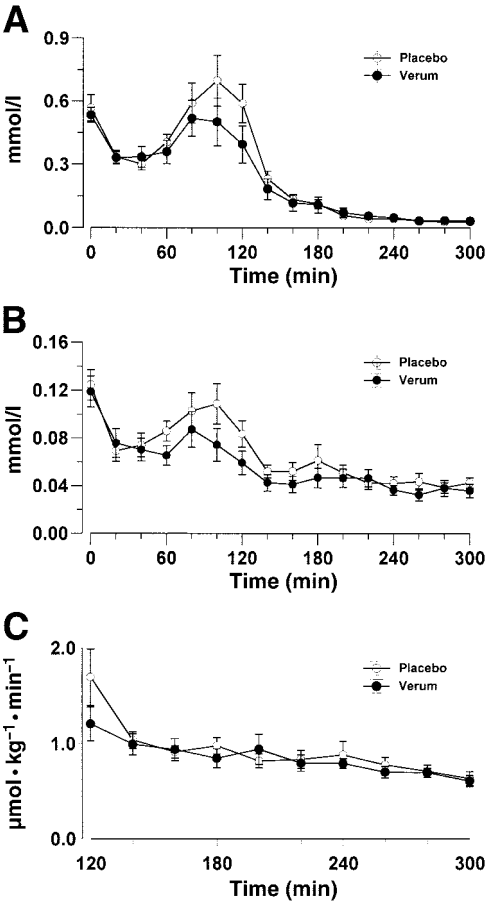

Figure 4—A*: Plasma NEFA concentration.* B*: Plasma glycerol concentration.* C*: Glycerol production rate.*

of each dose level of insulin infusion revealed that the MCR during the low (P = 0.0272) and intermediate (P = 0.0228) insulin dose was significantly different, whereas it was not during the high insulin dose (P = 0.686).

Endogenous glucose production and glucose elimination

Despite taking all precautions recommended to avoid calculating negative endogenous glucose production rates, R_a evaluated to negative figures in 18% of all in the placebo group and in 27% of all in the verum group. To not distort the results by unequal censoring of the data by setting the glucose production rates at these time points to 0, negative production rates were not excluded. Endogenous glucose production was 10.7 ± 1.4 μmol · kg^{-1} · min^{-1} in the placebo and 10.0 ± 1.0 μmol · kg^{-1} · min^{-1} in the verum group 120 min after start of the experiment. Under infusion of 0.5 mU · kg^{-1} · min^{-1} of insulin, endogenous glucose production decreased to 3.5 ± 1.2 μmol · kg^{-1} · min^{-1} in the placebo group and 2.6 ± 1.2 μmol · kg^{-1} · min^{-1} in the verum group. Under infusion of 1.0 mU · kg^{-1} · min^{-1} of insulin, endogenous glucose production decreased to 2.5 ± 0.8 μmol · kg^{-1} · min^{-1} in the placebo group and 0.8 ± 0.9 μmol · kg^{-1} · min^{-1} in the verum group. There was a slight increase in endogenous glucose production under infusion of 1.5 mU · kg^{-1} · min^{-1} of insulin (4.3 ± 1.0 μmol · kg^{-1} · min^{-1} in the placebo and 2.9 ± 1.3 μmol · kg^{-1} · min^{-1} in the verum group). By ANOVA, the difference between the treatment groups was statistically significant (P = 0.001).

Glucose elimination was assumed to equal glucose production after the initial 120 min of the experiment. Under infusion of 0.5 mU · kg^{-1} · min^{-1} of insulin, R_d then increased to 15.1 ± 1.0 μmol · kg^{-1} · min^{-1} in the placebo group and 16.9 ± 1.5 μmol · kg^{-1} · min^{-1} in the verum group. There were further increases in R_d under infusion of 1.0 mU · kg^{-1} · min^{-1} of insulin (27.5 ± 1.4 in the placebo group and 29.2 ± 2.6 μmol · kg^{-1} · min^{-1} in the verum group) and 1.5 mU · kg^{-1} · min^{-1} of insulin (40.7 ± 2.5 in the placebo group and 42.5 ± 3.9 μmol · kg^{-1} · min^{-1} in the verum group). By ANOVA, the difference between the treatment groups was statistically significant (P = 0.0298). Glucose MCR, endogenous glucose production and elimination rates, and the differences between the treatment arms in glucose R_a and R_d are shown in Fig. 5.

Glucose oxidation

Glucose oxidation as derived from indirect calorimetry was 7.1 ± 2.1 μmol · kg^{-1} · min^{-1} in the placebo group and 7.0 ± 1.9 μmol · kg^{-1} · min^{-1} in the verum group 60 min after the start of the experiment. Glucose oxidation then decreased to 4.4 ± 1.7 μmol · kg^{-1} · min^{-1} in the placebo group and 5.5 ± 2.2 μmol · kg^{-1} · min^{-1} in the verum group 120 min after start of the experiment. Thereafter, there was a gradual increase in glucose oxidation over 6.6 ± 1.7 μmol · kg^{-1} · min^{-1} at 180 min, 8.2 ± 1.4 μmol · kg^{-1} · min^{-1} at 240 min, to 10.6 ± 1.4 μmol · kg^{-1} · min^{-1} at 300 min in the placebo group and 8.5 ± 1.7 μmol · kg^{-1} · min^{-1} at 180 min, 10.2 ± 1.8 μmol · kg^{-1} · min^{-1} at 240 min, to a final value of 10.1 ± 1.7 μmol · kg^{-1} · min^{-1} at 300 min in the verum group. By ANOVA, the difference between the treatment arms was not statistically significant (P = 0.0943), whereas there was a strong linear dependence on insulin dose (P < 0.0001).

CONCLUSIONS

Despite 40 years of study, the issue of a putative extrapancreatic effect of sulfonylurea drugs still seems to be unsettled. The divergence in study results may be due to the different pharmacodynamic profiles of different sulfonylurea compounds and to differing study conditions.

To exclude possible confounding variables, such as different prestudy plasma glucose, insulin, and lipid concentrations, the present study was performed in normal glucose-tolerant subjects. Insulin-resistant offspring of patients with type 2 diabetes were selected in order to look at a cohort at increased risk for the future development of type 2 diabetes. As the influence of endogenous insulin had to be minimized as completely as possible, the study had to be performed as an acute experiment with

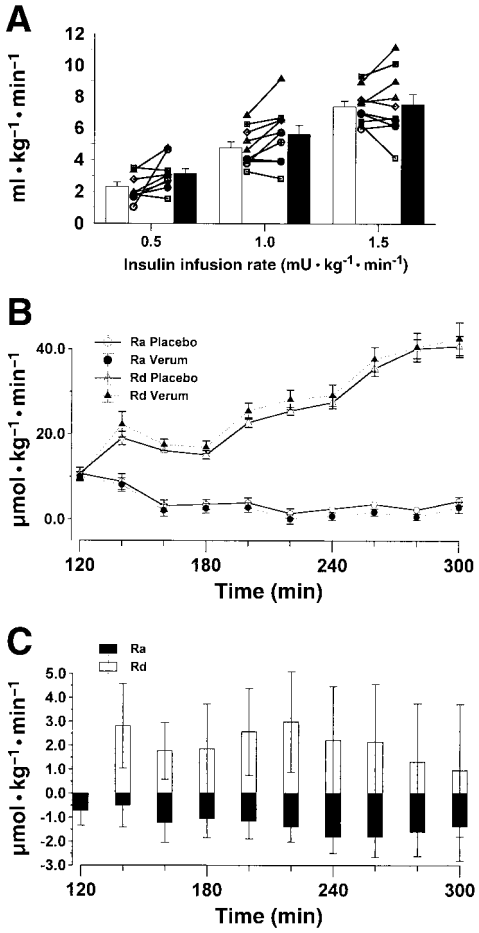

Figure 5—A*: MCR of glucose during the last 20 min of each level of insulin infusion indicated on the abscissa. The individual symbols connected by solid lines represent data from individual patients under placebo (left hand from respective insulin infusion rate indicated on the abscissa) or verum (right hand from respective insulin infusion rate indicated on the abscissa). The open bars represent the respective means ± SE of data obtained under the influence of placebo, whereas closed bars represent the respective means ± SE of data obtained under the influence of the active drug.* B*: Endogenous glucose production (solid and open circles) and peripheral glucose elimination rates (solid and open triangles) calculated from U-[^{13}C]glucose tracer data using non–steady-state equations.* C*: Differences ± SE between the verum and placebo experiment in* R_d *(open bars) and* R_a *(closed bars).*

relatively little time for the drug to develop its effects.

Using a primed continuous infusion regime, we achieved a drug concentration in the range of the maximum concentration observed 2.3 ± 0.5 h after oral administration of 1 mg glimepiride to healthy volunteers (31).

The insulin secretagogue effect of glimepiride was considerably but not completely blunted by the octreotide infusion, as can be seen from the plasma C-peptide concentrations (Fig. 3). Starting from slightly elevated fasting levels, there was an exponential decrease in plasma C-peptide concentration to values well below fasting during the first hour after initiation of the octreotide infusion in both treatment groups. In the placebo group, this decrease continued almost linearly, whereas in the verum group there was an increase in plasma C-peptide concentration beginning immediately after the initiation of the drug infusion and exhibiting peak concentrations in the low normal range 40 min later. Thereafter, there was a linear decrease in plasma C-peptide parallel to that in the placebo group. The increase in plasma C-peptide in the verum group shows that relevant concentrations of glimepiride were reached.

At first sight, it might appear tempting to interpret all of the following results as a consequence of the difference in insulin secretion undoubtedly present between the treatment arms. We would like to put forward the following arguments against a major physiological significance of the differences in insulin secretion observed regarding glucose metabolism. First, the small difference in peripheral plasma insulin concentration observed between 60 and 120 min after the start of the experiment was capable of modulating the degree of lipolysis as reflected by different peripheral NEFA and glycerol concentrations. An influence of the different insulin secretion rates on endogenous glucose production cannot be ruled out, although we feel that at least during the two higher insulin infusion rates the difference in endogenous portal insulin concentration is most likely overplayed by exogenous insulin infusion, and peripheral insulin concentrations were not different after 120 min.

Second, on the basis of both measured C-peptide concentration and the rates of insulin secretion, one can calculate that the small increase in insulin secretion observed in the verum group would only be sufficient to explain an increase in peripheral insulin concentration in the range of 18 pmol/l. Changes in insulin concentration in this range are not known to influence peripheral glucose uptake (32), although we cannot totally exclude a priming effect of the different insulin concentration between 60 and 120 min on the glucose uptake rates observed thereafter. With the insulin infusion rates used, we obtained plasma insulin concentrations in the high physiological (insulin infusion rate of 0.5 and 1.0 mU • kg^{-1} • min^{-1}) and low pharmacological range (insulin infusion rate of 1.5 mU • kg^{-1} • min^{-1}). Statistically significant differences were observed between the MCRs of glucose measured under the influence of either glimepiride or placebo (Fig. 5). The differences between the verum and placebo groups observed at each insulin dose level were rather small (0.81 ± 0.36 ml • kg^{-1} • min^{-1} at an insulin infusion rate of 0.5 mU • kg^{-1} • min^{-1} and 0.84 ± 0.30 ml • kg^{-1} • min^{-1} at an insulin infusion rate of 1.0 mU • kg^{-1} • min^{-1} and 0.14 ± 0.38 ml • kg^{-1} • min^{-1} at an insulin infusion rate of 1.5 mU • kg^{-1} • min^{-1}) when compared with the differences in MCR brought about by the different insulin concentrations. The difference in the glucose infusion rates needed to maintain euglycemia between the treatment groups (Table 1) appears to be explained both by a decrease in endogenous glucose production and an increased glucose elimination in the verum group. The mean difference in endogenous glucose production (−1.25 ± 0.26 µmol • kg^{-1} • min^{-1}) in the verum group is smaller than the mean increase in peripheral glucose elimination (1.72 ± 1.65 µmol • kg^{-1} • min^{-1}). The effect of glimepiride on peripheral glucose metabolism appears to be independent of differences in lipolysis, as both NEFA and glycerol concentrations and glycerol production were identical during the last 3 h of the exper-

iment where the measurements of glucose turnover were made.

In keeping with in vitro data obtained in both muscle and adipose tissue preparations of glimepiride-stimulated glycogenesis and lipogenesis (33), glimepiride in vivo appears to influence only nonoxidative aspects of glucose metabolism. In accordance with this, the rate of glucose oxidation as measured by indirect calorimetry was not different between the treatment arms.

The apparent dependence of the effect of glimepiride on the plasma insulin concentration leaves room for speculation about the mode of action of the drug (rev. in 34). If glimepiride augmented the glucose-transporting capacity of insulin-inducible glucose transporters, one would expect the effect of the drug to be more pronounced the more glucose transporters were recruited to the cell surface by insulin. Therefore, the most pronounced effect would be expected at the highest plasma insulin concentration. This does not appear to be the case in the present investigation. The drug effect is more pronounced at the two lower levels of insulin infusion, thus making it possible that glucose elimination is enhanced by a mechanism separate from the glucose transport–enhancing effects of insulin. In in vitro experiments, glimepiride has been shown to increase myocardial glucose transport and GLUT1 and GLUT4 protein expression independent of the effect of insulin (33).

In conclusion, we believe to have presented evidence for a peripheral effect of the sulfonylurea glimepiride on glucose metabolism in insulin-resistant but glucose-tolerant offspring of patients with type 2 diabetes. The differences are, however, too small to be considered therapeutically beneficial for the individual patient. We would rather this study to be considered as pointing to the principle capacity of the drug studied to alter glucose turnover independent of its insulin secretagogue action.

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References

1. Yalow RS, Black H, Villason M, Berson SA: Comparison of the plasma insulin levels following administration of tolbutamide and glucose. *Diabetes* 9:356–362, 1960
2. Feldman JM, Lebovitz HE: Endocrine and metabolic effects of glibenclamide: evidence for an extrapancreatic mechanism of action. *Diabetes* 20:745–755, 1971
3. Kolterman OG, Gray RS, Shapiro G, Scarlett JA, Griffin J, Olefsky JM: The acute and chronic effects of sulfonylurea therapy in type II diabetic patients. *Diabetes* 33:346–354, 1984
4. Keller U, Müller R, Berger W: Sulfonylurea treatment fails to diminish insulin resistance in type 1 diabetic subjects. *Horm Metab Res* 18:599–603, 1986
5. Leblanc H, Thote A, Chatelier G, Passa P: Effect of glipizide on glycemic control and peripheral insulin sensitivity in type 1 diabetics. *Diabet Metab* 16:93–97, 1990
6. Pontiroli E, Alberetto M, Bertoletti A, Baio G, Pozza G: Sulfonylureas enhance in vivo the effectiveness of insulin in type 1 (insulin dependent) diabetes mellitus. *Horm Metab Res* 16:167–170, 1984
7. Pernet A, Trimble ER, Kuntschen F, Assal JP, Hahn C, Renold AE: Sulfonylureas in insulin-dependent (type 1) diabetes: evidence for an extrapancreatic effect in vivo. *J Clin Endocrinol Metab* 61:247–251, 1985
8. Schulz B, Ratzmann KP, Heinke P, Besch W: A stimulatory effect of tolbutamide on the insulin-mediated glucose uptake in subjects with impaired glucose tolerance (IGT). *Exp Clin Endocrinol* 82:222–231, 1983
9. Kolterman OG, Olefsky J: The impact of sulfonylurea treatment upon the mechanism responsible for the insulin resistance in type II diabetes. *Diabetes Care* 7 (Suppl. 1):81–88, 1984
10. Greenfield MS, Doberne L, Rosenthal M, Schulz B, Widstrom A, Reaven GM: Effect of sulfonylurea treatment on in vivo insulin secretion and action in patients with non-insulin-dependent diabetes mellitus. *Diabetes* 31:307–312, 1982
11. Kolterman OG: Longitudinal evaluation of the effects of sulfonylurea therapy in subjects with type II diabetes mellitus. *Am J Med* 79:23–33, 1985
12. Ward GM, Harrison LC, Proietto J, Aitken P, Nankervis A: Gliclazide therapy is associated with potentiation of postbinding insulin action in obese, non-insulin-dependent diabetic subjects. *Diabetes* 34: 241–245, 1985
13. Unger RH, Grundy S: Hyperglycemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance: implications for the management of diabetes. *Diabetologia* 28:119–121, 1985
14. Rossetti L: Glucose toxicity: the implications of hyperglycemia in the pathophysiology of diabetes mellitus. *Clin Invest Med* 18:255–260, 1995
15. Nuutila P, Koivisto VA, Knuuti J, Ruotsalainen U, Teräs M, Haaparanta M, Bergman J, Solin O, Voipio-Pulkki L-M, Wegelius U, Yki-Järvinen H: Glucose-free fatty acid cycle operates in human heart and skeletal muscle in vivo. *J Clin Invest* 89:1767–1774, 1992
16. Groop L, Luzi L, Melander A, Groop P-H, Ratheiser K, Simonson DC, DeFronzo RA: Different effects of glyburide and glipizide on insulin secretion and hepatic glucose production in normal and NIDDM subjects. *Diabetes* 36:1320–1328, 1987
17. Müller G, Satoh Y, Geisen K: Extrapancreatic effects of sulfonylureas: a comparison between glimepiride and conventional sulfonylureas. *Diabetes Res Clin Pract* 28: S115–S137, 1995
18. Raptis SA, Hatziagelaki E, Dimitriadis G, Draeger KE, Pfeiffer C, Rapitis AE: Comparative effects of glimepiride and glibenclamide on blood glucose, C-peptide and insulin concentrations in the fasting and postprandial state in normal man. *Exp Clin Endocrin Diab* 107:350–355, 1999
19. Koch M, Rett K, Maerker E, Volk A, Haist K, Deninger M, Renn W, Haring HU: The PPARgamma2 amino acid polymorphism Pro 12 Ala is prevalent in offspring of type II diabetic patients and is associated to increased insulin sensitivity in a subgroup of obese subjects. *Diabetologia* 42:758–762, 1999
20. Koch M, Rett K, Volk A, Maerker E, Haist K, Weisser M, Rettig A, Renn W, Haring HU: The tumor necrosis factor alpha-238 G→A and -308 G→A promoter polymorphisms are not associated with insulin sensitivity and insulin secretion in young healthy relatives of type II diabetic patients. *Diabetologia* 43:181–184, 2000
21. Lehr KH, Damm P: Simultaneous determination of the sulfonylurea glimepiride and its metabolites in human serum and urine by high-performance liquid chromatography after pre-column derivatization. *J Chromatogr* 526:497–505, 1990
22. Pickert A, Overkamp D, Renn W, Liebich H, Eggstein M: Selected ion monitoring gas chromatography/mass spectrometry using uniformly labeled (13C)glucose for determination of glucose turnover in man. *Biomed Mass Spectrom* 20:203–209, 1991
23. Campbell PJ, Carlson MG, Hill JO, Nurjhan N: Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification. *Am J Physiol* 263:E1063–E1069, 1992
24. Frayn KN: Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* 55:628–634, 1983

25. Jéquier E: Direct and indirect calorimetry in man. In *Substrate and Energy Metabolism*. Garrow JS, Halliday D, Eds. London, Libbey, 1985, p. 91
26. Steele R, Wall JS, De Bodo RC, Altszuler N: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol* 187:15–24, 1956
27. De Bodo RC, Steele R, Altszuler N, Dunn A, Bishop JS: On the hormonal regulation of carbohydrate metabolism: studies with C14 glucose. *Recent Prog Horm Res* 19:455–488, 1963
28. Finegood DT, Bergman RN, Vranic M: Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps: comparison of unlabeled and labeled exogenous glucose infusates. *Diabetes* 36:914–924, 1987
29. Finegood DT, Bergman RN: Optimal segments: a methods for smoothing tracer data to calculate metabolic fluxes. *Am J Physiol* 244:E472–E479, 1983
30. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41: 368–377, 1992
31. Malerczyk V, Badian M, Korn A, Lehr KH, Waldhäusl W: Dose linearity assessment of glimepiride (Amaryl®) tablets in healthy volunteers. *Drug Metabol Drug Interact* 11:341–357, 1994
32. Stumvoll M, Jacob S: Multiple sites of insulin resistance: muscle, liver and adipose tissue. *Exp Clin Endocrin Diab* 107:107–110, 1999
33. Müller G, Wied S, Wetekam EM, Crecelius A, Unkelbach A, Pünter J: Stimulation of glucose utilization in 3T3 adipocytes and rat diaphragm in vitro by the sulfonylureas, glimepiride and glibenclamide, is correlated with modulations of the cAMP regulatory cascade. *Biochem Pharmacol* 48:985–996, 1994
34. Müller G: The molecular mechanism of the insulin-mimetic/sensitizing activity of the antidiabetic sulfonylurea drug amaryl. *Molecular Medicine* 6:907–933, 2000