The Acute Effect of Metformin on Glucose Production in the Conscious Dog Is Primarily Attributable to Inhibition of Glycogenolysis

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Although metformin has been used worldwide to treat type 2 diabetes for several decades, its mechanism of action on glucose homeostasis remains controversial. To further assess the effect of metformin on glucose metabolism, 10 42-hour-fasted conscious dogs were studied in the absence ([Con] n = 5) and presence ([Met] n = 5) of a portal infusion of metformin (0.15 mg · kg^{-1} · min^{-1}) over 300 minutes. Hepatic glucose production was measured by both arteriovenous-difference and tracer methods. All dogs were maintained on a pancreatic clamp and in a euglycemic state to ensure that any changes in glucose metabolism would result directly from the effects of metformin. The arterial metformin level was 21 ± 3 μg/mL during the test period. Net hepatic glucose output (NHGO) decreased in Met dogs from 1.9 ± 0.2 to 0.7 ± 0.1 mg · kg^{-1} · min^{-1} ($P < .05$). NHGO remained unchanged in Con dogs (1.7 ± 0.3 to 1.5 ± 0.3 mg · kg^{-1}min^{-1}). Tracer-determined glucose production paralleled NHGO. The net hepatic glycogenolytic rate decreased from 1.0 ± 0.2 to −0.3 ± 0.2 mg · kg^{-1} · min^{-1} ($P < .05$) in Met dogs, but remained unchanged in Con dogs (0.8 ± 0.2 to 0.8 ± 0.3 mg · kg^{-1} · min^{-1}). No significant change in gluconeogenic flux was found in either the Met group (1.2 ± 0.3 to 1.3 ± 0.3 mg · kg^{-1} · min^{-1}) or the Con group (1.3 ± 0.4 to 1.0 ± 0.3 mg · kg^{-1} · min^{-1}). No significant changes were observed in glucose utilization or glucose clearance in either group. In conclusion, in the normal fasted dog, (1) the primary acute effect of metformin on glucose metabolism was an inhibition of hepatic glucose production and not a stimulation of glucose utilization; and (2) the inhibition of glucose production was attributable to a decrease in hepatic glycogenolysis and not to an alteration in gluconeogenic flux.

METFORMIN (*N*,*N*-dimethylbiguanide) is an antihyperglycemic agent that has been used widely in the treatment of type 2 diabetes since 1957. Although it has been proven to be effective in clinical studies, its direct mode of action on glucose metabolism is still poorly understood. Early studies[1,2] suggested that the antihyperglycemic effect of metformin in type 2 diabetes is primarily mediated through an increase in insulin sensitivity in peripheral tissues and thus an increase in glucose uptake. However, recent studies in humans[3-6] have suggested that the primary action of metformin occurs in the liver and results in a reduction of endogenous glucose production (EGP). Some studies[4,7] have indicated that the effect of metformin on glucose production occurs primarily as a result of a reduction of gluconeogenesis, whereas others[3,5] have suggested that gluconeogenesis is not affected.

The determination of the extent of metformin action on the different facets of glucose metabolism has been complicated by the changes in pancreatic hormone levels and hyperglycemia that occur during long-term treatment with metformin. DeFronzo et al,[3] Nosadini et al,[8] and Reaven et al[9] reported that endogenous circulating insulin levels decrease in response to metformin. Glucagon can also change during metformin treatment, and Argaud et al[10] showed in vitro that glucagon can inhibit the effect of metformin on glycolysis. In addition, as shown in our recent study,[11] it is known that hyperglycemia per se can inhibit hepatic glycogenolysis. Since all in vivo studies assessing the long-term effects of metformin were conducted such that the endogenous pancreatic hormone and glucose levels changed, it is difficult to assess the extent of metformin's direct action on glucose production or utilization. Furthermore, all studies[1,3,4-6,8,9] that examined the effect of metformin on hepatic gluconeogenesis in humans used a technique to measure gluconeogenesis that has many limitations. It is based on a combination of assumptions,[4] including the assumption that lactate accounts for 60% of overall gluconeogenesis and that the incorporation of labeled lactate into labeled glucose underestimates lactate conversion to glucose by 40%. Thus, it is difficult to draw conclusions regarding the response of the latter process to metformin.

The first aim of the present study was therefore to determine whether metformin has an acute effect on glucose metabolism in vivo in the absence of changes in arterial pancreatic hormone levels or the plasma glucose concentration. The second aim was to determine the mechanism of the effect. In view of the difficulty encountered in direct assessments of hepatic gluconeogenesis in the human, we used a combined tracer and arteriovenous-difference technique to assess glycogenolysis and gluconeogenesis directly in the dog.

MATERIALS AND METHODS

Experiments were performed on 10 42-hour-fasted conscious mongrel dogs (20 to 30 kg) of either sex that were fed a standard diet of meat and chow as described elsewhere.[11,12] The animals were housed in a facility that meets the guidelines of the American Association for the Accreditation of Laboratory Animal Care, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee.

A laparotomy was performed 16 to 18 days before each experiment to implant catheters and Doppler flow probes in or around the appropriate blood vessels as described previously.[11,12] Each dog was used for only

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one experiment. All dogs had the following characteristics: (1) leukocyte count less than 18,000/μL, (2) hematocrit greater than 35%, (3) good appetite, and (4) normal stools.

Each experiment consisted of a 90-minute tracer equilibration and hormone adjustment period, a 30-minute basal period, and a 300-minute test period. In all studies, a priming dose of purified [3-^{3}H]glucose (42 μCi) was administered at 0 minutes, followed by a constant infusion of [3-^{3}H]glucose (0.35 μCi/min), [U-^{14}C]alanine (0.35 μCi/min), and indocyanine green (0.1 mg/m^2 · min). An infusion of somatostatin (0.8 μg · kg^{-1} · min^{-1}) was started at the beginning of the study to inhibit endogenous insulin and glucagon secretion. Concurrently, intraportal replacement infusions of insulin (300 μU · kg^{-1} · min^{-1}) and glucagon (0.5 ng · kg^{-1} · min^{-1}) were started. The plasma glucose level was monitored every 5 minutes and euglycemia was maintained by adjusting the rate of insulin infusion. The final alteration in the insulin infusion rate was made at least 30 minutes before the start of the basal period, and the rate of insulin infusion (mean, 228 μU · kg^{-1} · min^{-1}) remained unchanged thereafter. The study included two groups (Fig 1). In one group (Met), metformin (Groupe Lyonnaise Industrielle Pharmaceutique [LIPHA], Lyon, France) dissolved in saline was infused at 0.15 mg · kg^{-1} · min^{-1} (total dose, 45 mg/kg) into the portal circulation via the splenic and jejunal vein catheters during the test period. In the control group (Con), saline was infused via the same catheters instead of metformin. Euglycemia was maintained in both groups by an infusion of 20% glucose into the right cephalic vein.

Arterial plasma drug/concentrations were determined using high-performance liquid chromatography (Groupe LIPHA). Plasma and blood glucose, plasma [^{3}H]glucose and [^{14}C]glucose, blood lactate, glycerol, β-hydroxybutyrate (βOHB), acetoacetic acid (AcAc), alanine, glutamine, glutamate, glycine, serine, and threonine, and plasma free fatty acids (FFAs) were determined using previously described methods.[11,12] Insulin, glucagon, cortisol, epinephrine, and norepinephrine levels were also determined as described elsewhere.[11,12]

Transonic flow probes and indocyanine green dye were used to estimate total hepatic blood flow.[11,12] The data in the figures and tables were calculated with transonic-measured flows. The net hepatic balance and fractional extraction of blood glucose, lactate, glycerol, βOHB, alanine, other gluconeogenic amino acids, and plasma FFA in the present study were calculated using arteriovenous-difference methods described elsewhere.[11,12]

It should be noted that to the extent that there is hepatic glucose uptake (HGU), net hepatic glucose output (NHGO) slightly underestimates total hepatic glucose release (NHGO + HGU). Total glucose production and utilization were determined using both 1- and 2-compartment models as previously described.[11,12] The results were similar regardless of which approach was used because the deviations from steady state were minimal. The data in the figures and tables were calculated using the 2-compartment method. It should also be noted that the rate of EGP determined by the tracer method slightly (~0.2 mg · kg^{-1} · min^{-1}) overestimates total hepatic glucose release, since the kidneys produce a small amount of glucose.[13] However, this overestimate should have been equal in the two groups prior to metformin treatment. It was not possible to assess the effect of metformin on renal gluconeogenesis. The hepatic gluconeogenetic rate from circulating gluconeogenetic precursors was calculated using methods described previously.[11,12] Briefly, the net hepatic balance of the gluconeogenetic precursors alanine, glycine, serine, threonine, glutamine, glutamate, lactate, and glycerol was measured. The net hepatic balance of pyruvate was assumed to be 10% of lactate balance. The gluconeogenetic flux from phosphoenolpyruvate to glucose 6-phosphate was estimated by dividing the above-mentioned uptake rates by 2 to account for the incorporation of C-3 precursors into the C-6 glucose molecule. This value has been shown to be similar to the rate of hepatic gluconeogenesis measured by an independent tracer method.[14] Net hepatic glycogenolysis was calculated[11,12] as net hepatic glycogenolysis = NHGO + NHLO + G_{oxid} − GNG, where NHLO is net hepatic lactate output. When net hepatic lactate uptake occurred, its net output was considered to be zero; G_{oxid} is hepatic glucose oxidation, which was estimated to equal 15% of NHGO as suggested by our recent study,[15] and GNG is hepatic gluconeogenic flux.

Statistical Analysis

All statistical comparisons were made using repeated-measures ANOVA with post hoc analysis by univariate F tests or the paired Student's *t* test where appropriate. Statistical significance was accepted at a *P* level less than .05. Data are expressed as the mean ± SE.

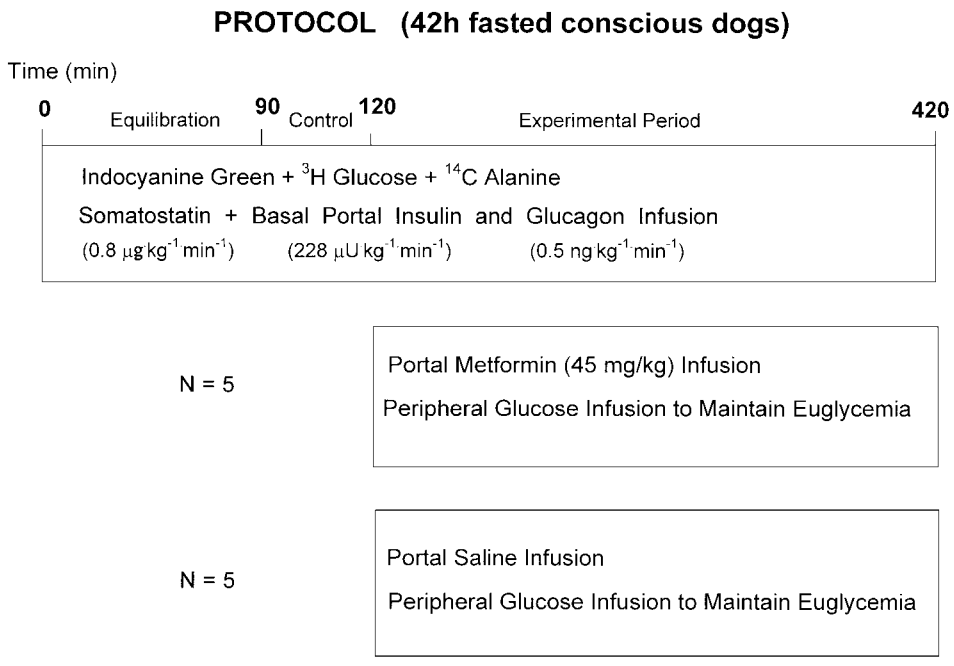

Fig 1. Study protocol.

RESULTS

Drug Concentration, Hormone Levels, and Hepatic Blood Flow

The arterial plasma level of metformin was maintained at 21.4 ± 4 μg/mL in the Met group during the test period (Fig 2). Arterial plasma insulin, glucagon, norepinephrine, epinephrine, and cortisol remained at basal values in both groups throughout the study (Fig 3). Hepatic blood flow remained stable in both groups (Table 1).

Arterial Blood or Plasma Level and Net Hepatic Balance of Glycerol, FFAs, βOHB, and AcAc

The arterial blood level and net hepatic uptake of glycerol did not change significantly in Met dogs (99 ± 7 to 77 ± 12 μmol/L and 1.7 ± 0.3 to 1.4 ± 0.3 μmol · kg^{-1} · min^{-1}) or Con dogs (112 ± 28 to 97 ± 30 μmol/L and 2.2 ± 0.3 to 1.7 ± 0.4 μmol · kg^{-1} · min^{-1}; Fig 4 and Table 2). Likewise, the arterial plasma level and net hepatic uptake of FFAs decreased but did not change significantly in either group (981 ± 73 to 639 ± 117 μmol/L and 3.0 ± 0.5 to 2.6 ± 0.5 μmol · kg^{-1} · min^{-1} in Met dogs and 1,018 ± 206 to 745 ± 155 μmol/L and 4.4 ± 1.3 to 3.5 ± 1.5 μmol · kg^{-1} · min^{-1} in Con dogs). The findings for ketones paralleled those for FFAs. That is, the blood level and net hepatic output of βOHB (65 ± 22 to 36 ± 6 μmol/L and 1.9 ± 0.4 to 1.1 ± 0.3 μmol · kg^{-1} · min^{-1} in Met dogs and 87 ± 19 to 50 ± 17 μmol/L and 3.1 ± 0.8 to 1.9 ± 0.6 μmol · kg^{-1} · min^{-1} in Con dogs) and AcAc (103 ± 20 to 82 ± 17 μmol/L and 1.9 ± 0.4 to 2.4 ± 0.6 μmol · kg^{-1} · min^{-1} in Met dogs and 101 ± 6 to 100 ± 13 μmol/L and 2.0 ± 0.6 to 1.5 ± 0.4 μmol · kg^{-1} · min^{-1} in Con dogs) did not change significantly (Fig 4 and Table 2).

Arterial Blood or Plasma Level and Net Hepatic Balance of Alanine and Lactate

The blood level and net hepatic uptake of alanine did not change significantly in Met dogs (254 ± 25 to 287 ± 60 μmol/L and 1.9 ± 0.3 to 2.0 ± 0.4 μmol · kg^{-1} · min^{-1}) or Con dogs (229 ± 10 to 247 ± 20 μmol/L and 2.0 ± 0.3 to 1.9 ± 0.5 μmol · kg^{-1} · min^{-1}). Likewise, the blood level and net hepatic uptake of lactate did not change significantly in either group (369 ± 79 to 446 ± 84 μmol/L and 7.6 ± 1.0 to 6.3 ± 1.3 μmol · kg^{-1} · min^{-1} in Met dogs and 451 ± 167 to 439 ± 98 μmol/L and 8.8 ± 2.1 to 6.2 ± 2.2 μmol · kg^{-1} · min^{-1} in Con dogs) (Fig 5 and Table 2).

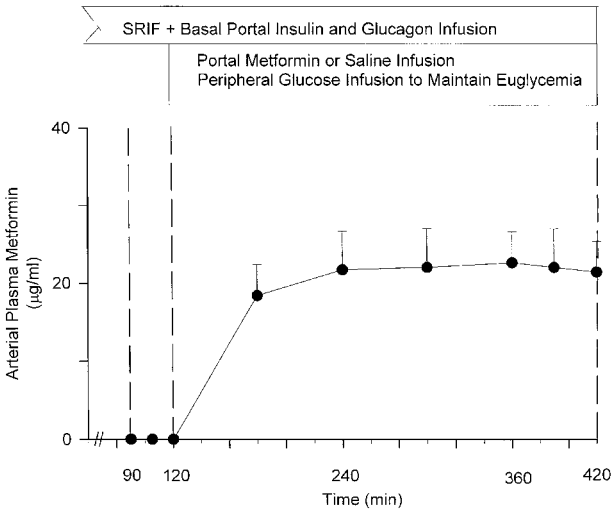

Fig 2. Arterial plasma metformin in the Met group during the experimental period in the presence of a pancreatic clamp in conscious 42-hour-fasted dogs (n = 5).

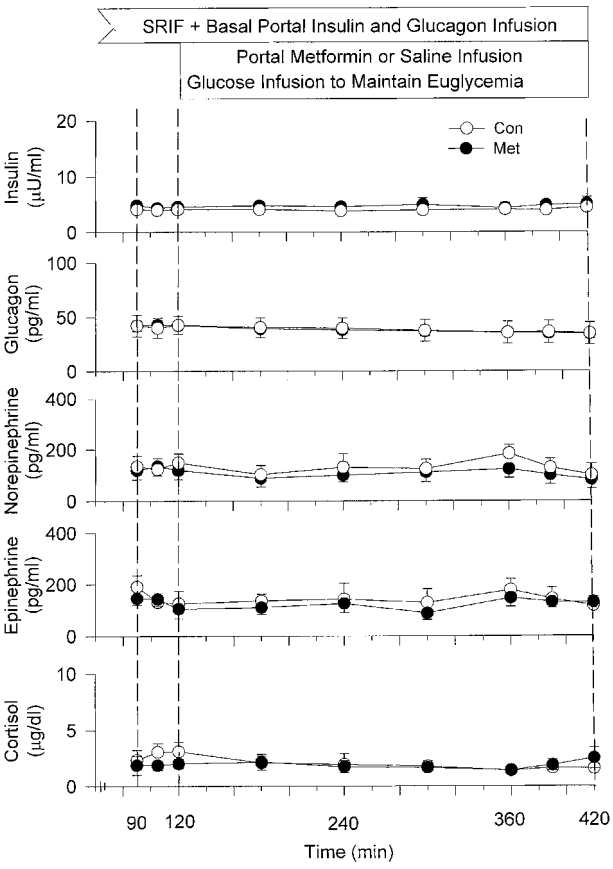

Fig 3. Arterial plasma insulin, glucagon, norepinephrine, epinephrine, and cortisol during the basal and experimental periods in the presence of a pancreatic clamp in conscious 42-hour-fasted dogs (n = 5 per group).

Glucose Kinetics

The arterial blood glucose concentration was clamped at euglycemia throughout the study in both groups. In response to the portal infusion of metformin, NHGO decreased from 1.9 ± 0.2 to 0.7 ± 0.1 mg · kg^{-1} · min^{-1} ($P < .05$) by the end of the

Table 1. Hepatic Blood Flow (mL · kg^{-1} · min^{-1}) in the Presence of a Pancreatic Clamp in Conscious 42-Hour-Fasted Dogs During the Basal Period and During Portal Infusion of Metformin or Saline

Group	Basal Period	Portal Infusion of Metformin or Saline (min)					
		180	240	300	360	390	420
Con	30 ± 2	30 ± 2	30 ± 3	31 ± 2	30 ± 2	30 ± 2	30 ± 2
Met	24 ± 2	26 ± 2	25 ± 2	26 ± 2	26 ± 3	25 ± 2	26 ± 2

NOTE. Data are the mean ± SE (n = 5 per group). The basal period is the mean of the 90- and 120-minute sampling times.

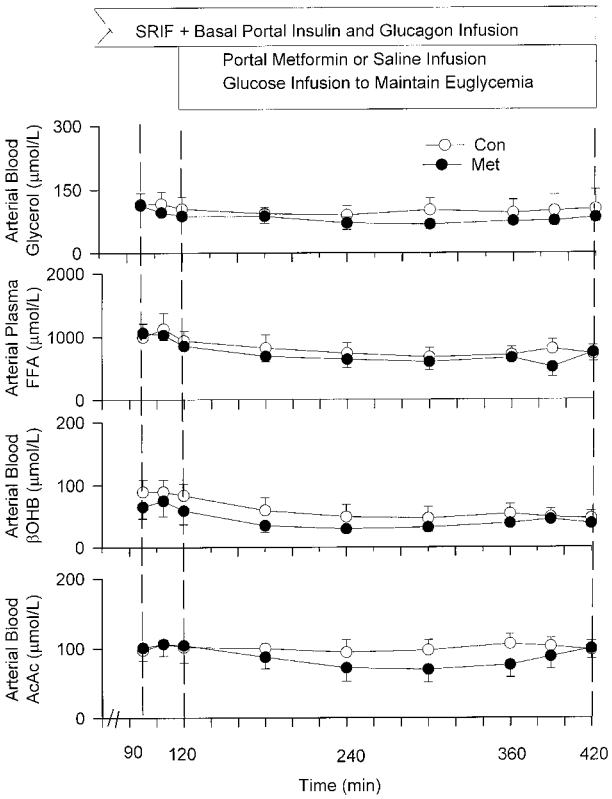

Fig 4. Arterial blood or plasma glycerol, FFA, βOHB, and AcAc during the basal and experimental periods in the presence of a pancreatic clamp in conscious 42-hour-fasted dogs (n = 5 per group).

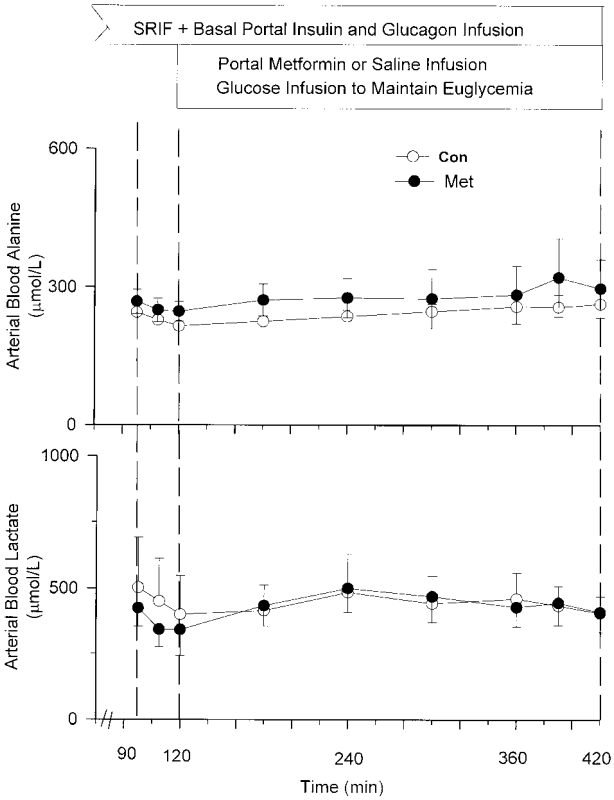

Fig 5. Arterial blood alanine and lactate during the basal and experimental periods in the presence of a pancreatic clamp in conscious 42-hour-fasted dogs (n = 5 per group).

study. NHGO did not change significantly in the Con group (1.7 ± 0.3 to 1.5 ± 0.3 mg · kg^{-1} · min^{-1}). The tracer-determined EGP data paralleled the NHGO data in both groups (Fig 6). The rate of glucose infusion required to maintain euglycemia was 1.0 ± 0.2 and 0.5 ± 0.3 mg · kg^{-1} · min^{-1} ($P < .05$) in the Met and Con groups, respectively, by the end of the study. Tracer-determined glucose utilization did not change significantly in either group (2.5 ± 0.2 to 2.4 ± 0.2 and 2.5 ± 0.3 to

Table 2. Net Hepatic Balance (μmol · kg^{-1} · min^{-1}) of Glycerol, FFA, βOHB, AcAc, Alanine, and Lactate in the Presence of a Pancreatic Clamp in Conscious 42-Hour-Fasted Dogs During the Basal Period and During Portal Infusion of Metformin or Saline

Parameter	Basal Period	Portal Infusion of Metformin or Saline (min)					
		180	240	300	360	390	420
Glycerol							
Con	−2.4 ± 0.3	−1.7 ± 0.4	−1.6 ± 0.2	−2.0 ± 0.5	−1.9 ± 0.4	−1.9 ± 0.5	−1.8 ± 0.5
Met	−2.0 ± 0.3	−1.6 ± 0.4	−1.3 ± 0.3	−1.4 ± 0.4	−1.5 ± 0.3	−1.2 ± 0.3	−1.6 ± 0.2
FFA							
Con	−4.7 ± 1.3	−2.5 ± 1.4	−3.9 ± 1.6	−3.5 ± 1.6	−4.0 ± 1.3	−4.2 ± 1.3	−2.8 ± 1.9
Met	−2.9 ± 0.5	−2.3 ± 0.3	−2.1 ± 0.5	−2.1 ± 0.8	−2.6 ± 0.3	−2.6 ± 0.3	−3.7 ± 0.9
βOHB							
Con	3.2 ± 0.9	1.9 ± 0.6	1.8 ± 0.6	1.6 ± 0.6	2.1 ± 0.8	1.9 ± 0.6	2.0 ± 0.5
Met	2.0 ± 0.4	1.2 ± 0.4	0.8 ± 0.2	0.9 ± 0.3	1.2 ± 0.2	1.2 ± 0.2	1.4 ± 0.4
AcAc							
Con	1.7 ± 0.6	1.7 ± 0.2	1.5 ± 0.4	1.5 ± 0.3	1.1 ± 0.6	1.4 ± 0.6	1.8 ± 0.4
Met	1.8 ± 0.4	2.3 ± 0.8	2.0 ± 0.5	2.3 ± 0.6	2.9 ± 0.7	2.5 ± 0.5	2.1 ± 0.5
Alanine							
Con	−2.1 ± 0.3	−1.9 ± 0.7	−2.3 ± 0.7	−1.9 ± 0.5	−2.2 ± 0.4	−1.7 ± 0.3	−1.4 ± 0.1
Met	−2.0 ± 0.3	−2.4 ± 0.3	−1.7 ± 0.4	−2.0 ± 0.4	−2.3 ± 0.5	−1.6 ± 0.5	−1.9 ± 0.5
Lactate							
Con	−9.9 ± 2.1	−5.5 ± 3.5	−5.9 ± 2.9	−5.5 ± 2.1	−6.8 ± 1.6	−6.9 ± 1.4	−6.8 ± 1.9
Met	−8.5 ± 1.0	−6.3 ± 1.0	−5.5 ± 2.0	−5.6 ± 1.3	−5.8 ± 0.2	−6.6 ± 1.1	−7.9 ± 2.4

NOTE. Data are the mean ± SE (n = 5 per group). The basal period is the mean of the 90- and 120-minute sampling times.

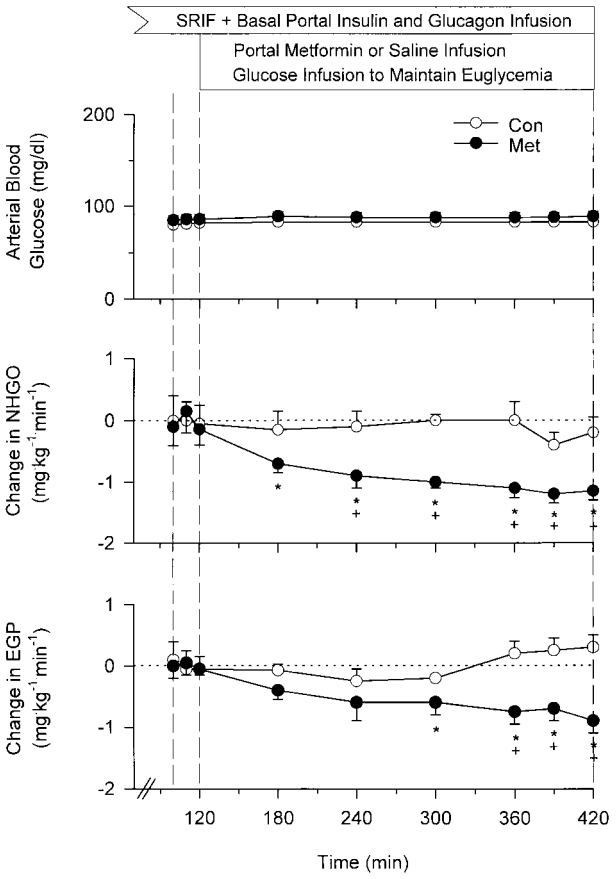

Fig 6. Arterial blood glucose and changes in NHGO and tracer-determined EGP during the basal and experimental periods in the presence of a pancreatic clamp in conscious 42-hour-fasted dogs (n = 5 per group). *$P < .05$ *v* corresponding basal period. +$P < .05$, Con *v* Met.

Table 4. Arterial Blood Level and Net Hepatic Balance of Glutamate, Glutamine, Glycine, Serine, and Threonine in the Presence of a Pancreatic Clamp in Conscious 18-Hour-Fasted Dogs During the Basal Period and During Portal Infusion of Metformin or Saline

Amino Acid	Arterial Blood Level (μmol/L)		Net Hepatic Balance (μmol · kg⁻¹ · min⁻¹)	
	Basal Period	Test Period	Basal Period	Test Period
Glutamate				
Con	61 ± 17	49 ± 11	0.2 ± 0.3	0.1 ± 0.2
Met	60 ± 7	53 ± 10	0.2 ± 0.2	0.0 ± 0.3
Glutamine				
Con	1,006 ± 48	1,007 ± 56	1.7 ± 1.7	1.0 ± 1.7
Met	1,117 ± 75	1,067 ± 110	0.4 ± 0.7	−0.2 ± 0.5
Glycine				
Con	176 ± 40	169 ± 20	−0.5 ± 0.3	−0.7 ± 0.3
Met	197 ± 16	179 ± 20	−0.7 ± 0.5	−0.6 ± 0.4
Serine				
Con	118 ± 7	114 ± 13	−0.3 ± 0.1	−0.4 ± 0.2
Met	135 ± 16	120 ± 18	−0.7 ± 0.4	−0.6 ± 0.3
Threonine				
Con	227 ± 28	215 ± 38	−0.2 ± 0.3	−0.2 ± 0.3
Met	232 ± 30	206 ± 21	−0.4 ± 0.5	−0.4 ± 0.4

NOTE. Data are the mean ± SE (n = 5 per group). The basal period is the mean of 2 sampling times; the test period is the mean of 6 sampling times between 180 and 420 minutes.

2.7 ± 0.2 mg · kg^{-1} · min^{-1}) (Table 3). Tracer-determined glucose clearance also remained unchanged in both groups.

Arterial Blood Level and Net Hepatic Uptake of Gluconeogenetic Amino Acids

Neither the arterial blood level nor the net hepatic balance of glutamate, glutamine, glycine, serine, or threonine were significantly changed in either group throughout the study (Table 4).

Gluconeogenesis and Glycogenolysis

The hepatic gluconeogenetic rate did not change significantly in either Met dogs (1.2 ± 0.3 to 1.3 ± 0.3 mg · kg^{-1} · min^{-1}) or Con dogs (1.3 ± 0.4 to 1.0 ± 0.3 mg · kg^{-1} · min^{-1}) during the test period. On the other hand, the net hepatic glycogenolytic

Table 3. Tracer-Determined Glucose Utilization and Clearance, and the Exogenous Glucose Infusion Rate in the Presence of a Pancreatic Clamp in Conscious 18-Hour-Fasted Dogs During the Basal Period and During Portal Infusion of Metformin or Saline

Parameter	Basal Period	Portal Infusion of Metformin or Saline (min)					
		180	240	300	360	390	420
TDGU (mg · kg^{-1} · min^{-1})							
Con	2.5 ± 0.2	2.4 ± 0.1	2.4 ± 0.2	2.4 ± 0.1	2.8 ± 0.2	2.9 ± 0.2	3.0 ± 0.2
Met	2.5 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	2.4 ± 0.2	2.4 ± 0.2	2.3 ± 0.3	2.5 ± 0.1
TDCL (mL · kg^{-1} · min^{-1})							
Con	2.2 ± 0.2	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.7 ± 0.2	2.6 ± 0.2	2.8 ± 0.2
Met	2.1 ± 0.1	2.0 ± 0.1	1.9 ± 0.2	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1	2.2 ± 0.1
EGI (mg · kg^{-1} · min^{-1})							
Con	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.2	0.4 ± 0.2	0.5 ± 0.3
Met	0.0 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.8 ± 0.1*	0.8 ± 0.2*	1.0 ± 0.2*
EHGU (EGI + NHGO) (mg · kg^{-1} · min^{-1})							
Con	1.7 ± 0.5	1.7 ± 0.3	1.7 ± 0.3	1.7 ± 0.2	1.5 ± 0.5	1.3 ± 0.3	1.6 ± 0.4
Met	1.9 ± 0.2	1.5 ± 0.2	1.2 ± 0.2	1.3 ± 0.1	1.7 ± 0.2	1.5 ± 0.2	1.8 ± 0.3

NOTE. Data are the mean ± SE (n = 5 per group). The basal period is the mean of the 90- and 120-minute sampling times.
Abbreviations: TDGU, tracer-determined glucose utilization; TDCL, tracer-determined glucose clearance; EGI, exogenous glucose infusion rate.
*$P < .05$ *v* Con group.

rate decreased in response to portal infusion of metformin from 1.0 ± 0.2 to 0.3 ± 0.2 mg · kg^{-1} · min^{-1} within 60 minutes ($P < .05$) and then further to −0.3 ± 0.2 mg · kg^{-1} · min^{-1} (net glycogen synthesis) by the end of the study. The net hepatic glycogenolytic rate did not change significantly in the Con group (0.8 ± 0.2 to 0.8 ± 0.3 mg · kg^{-1} · min^{-1}) during the study (Fig 7).

DISCUSSION

The aim of the present study was to determine the mechanism of acute metformin action on glucose metabolism in vivo in the absence of the influence of changes in pancreatic hormone or glucose levels. Arterial insulin, glucagon, epinephrine, norepinephrine, and cortisol remained at basal values in both groups throughout the study. Arterial blood glucose was maintained at a similar euglycemic level in both groups, and hepatic blood flow remained unchanged in both groups. Therefore, we were able to examine the acute action of metformin per se on glucose production (hepatic glycogenolysis and gluconeogenesis) and glucose utilization.

In response to the portal infusion of metformin, NHGO decreased from 1.9 ± 0.2 to 0.7 ± 0.1 mg · kg^{-1} · min^{-1} ($P < .05$) by the end of the 5-hour test period, while no significant change in NHGO was found in the Con group (1.7 ± 0.3 to 1.5 ± 0.3 mg · kg^{-1} · min^{-1}). Since there was no significant change in hepatic gluconeogenesis in either group, the decrease of NHGO in the Met group must have been caused by a decrease in hepatic glycogenolysis. In fact, net hepatic glycogenolysis decreased from 1.0 ± 0.2 to −0.3 ± 0.2 mg · kg^{-1} · min^{-1} during the metformin infusion in the present study. The negative value suggests that glycogen synthesis actually occurred. These data are consistent with our observation that the hepatic glycogen content at the end of the study was significantly greater in Met animals (35 ± 2 *v* 26 ± 3 mg/g liver). No significant change was found in glycogenolysis in the Con group (0.8 ± 0.2 to 0.8 ± 0.3 mg · kg^{-1} · min^{-1}). The data generated by the tracer method paralleled the above-mentioned results obtained using the arteriovenous-difference technique. No significant change was found in whole-body glucose utilization or clearance in either group. Considering these data together, one can therefore conclude that the primary acute effect of metformin on glucose metabolism in the conscious dog was a decrease in hepatic glucose production mediated by an inhibition of hepatic glycogenolysis.

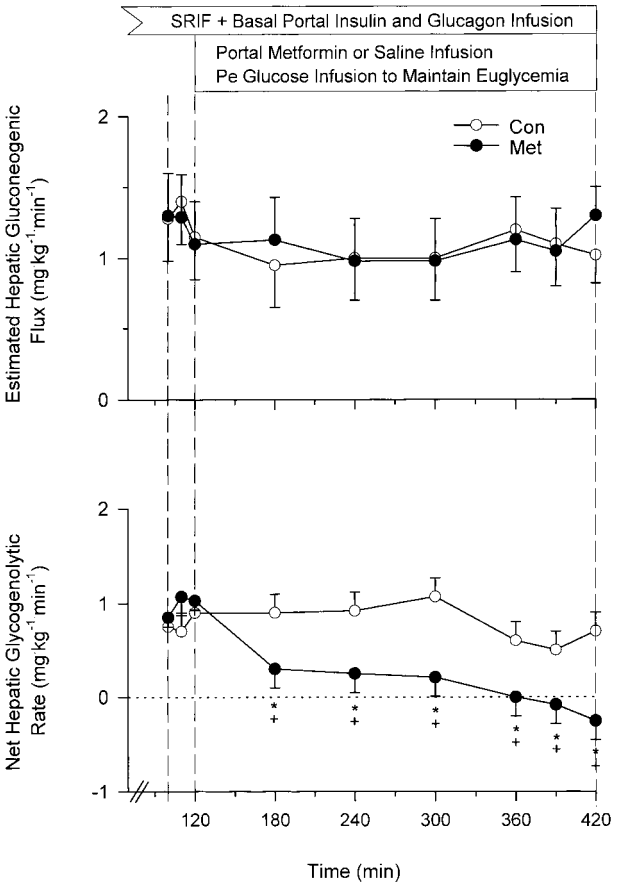

Fig 7. Net hepatic gluconeogenetic and glycogenolytic rates during the basal and experimental periods in the presence of a pancreatic clamp in conscious 42-hour-fasted dogs (n = 5 per group). *$P < .05$ *v* corresponding basal period. $^{+}P < .05$, Con *v* Met.

Recent studies in the human by DeFronzo et al,[3] Cusi et al,[5] and Christiansen et al[16] indicated that long-term treatment with metformin significantly decreases fasting plasma glucose in type 2 diabetic patients and that it significantly decreases basal EGP. The percentage of gluconeogenesis arising from lactate and the rate of lactate-derived gluconeogenesis, based on the incorporation of [^{14}C]lactate into [^{14}C]glucose, were not altered by metformin. In addition, they found no significant change in glucose utilization in response to metformin therapy.[3,5] Their data thus suggest an action of metformin on glycogenolysis rather than gluconeogenesis.

Recently, Stumvoll et al[4] reported that long-term metformin treatment decreases glucose production and the incorporation of [^{14}C]lactate into [^{14}C]glucose in obese type 2 diabetic patients. They concluded that the primary effect of metformin on hepatic glucose production is attributable to an inhibition of gluconeogenesis. Our study differs from theirs in several respects. It is well known[3-5,7] that fasting hyperglycemia in the type 2 patient is at least in part due to an elevated basal hepatic glucose production, which is primarily attributable to an increase in gluconeogenesis. The presence of a substantially increased gluconeogenetic rate might therefore allow the effect of metformin on hepatic gluconeogenesis to be manifest. Secondly, their study examined the effect of long-term metformin treatment in the absence of an insulin and glucagon clamp and under hyperglycemic conditions, whereas our study tested the acute effect of metformin in the absence of changes in insulin and glucagon and in the presence of euglycemia. Thirdly, they estimated gluconeogenesis using the incorporation of the administered [^{14}C]lactate into [^{14}C]glucose. Therefore, they had to assume that lactate accounts for 60% of overall gluconeogenesis,[4] that the incorporation of labeled lactate underestimates lactate conversion to glucose by 40%,[4] and that no other sources of carbon replaced the apparent decrease in lactate-derived gluconeogenesis. Interestingly, using the same approach, Cusi et al[5] reported no significant change in gluconeogenesis in type

2 diabetics after long-term metformin treatment. It is of interest to note that a change in glycogen metabolism as in the present study would result in some gluconeogenetic carbon deposition in liver glycogen. Thus, although there was no acute decrease in gluconeogenetic flux, the diversion of gluconeogenetically derived glucose-6-phosphate to glycogen would reduce the contribution of gluconeogenesis to NHGO in the present study.

Argaud et al[10] reported that metformin enhanced the activity of pyruvate kinase in hepatocytes in vitro. Metformin at a concentration of 10^{-2} mol/L decreased glucose production from lactate, alanine, and glutamine compared with untreated cells. Minassian et al[17] have shown in the rat that metformin decreases the activity of glucose-6-phosphatase, thus inhibiting hepatic gluconeogenesis and glycogenolysis. It should be noted that in the current study all of the basal neural and hormonal signals impacting the liver were present and fixed, whereas they were absent in the in vitro studies. Secondly, the doses of metformin that caused a decrease in gluconeogenesis in vitro[10] were several orders of magnitude higher than the levels used in the present study and in human studies. Thirdly, all of the in vitro studies[7,10,17,18] used the 24- to 48-hour-fasted rat that was devoid of liver glycogen, thus perhaps allowing the effect of metformin on hepatic gluconeogenesis to be manifest.

Some previous human studies[19-21] showed that metformin can increase glucose utilization in type 2 diabetic patients. Fery et al[19] reported that long-term metformin treatment increased glucose disposal but did not decrease EGP in type 2 diabetic patients subjected to oral glucose tolerance tests. McIntyre et al[20] showed that long-term metformin treatment increased glucose utilization but did not decrease EGP in type 2 diabetic subjects during hyperinsulinemic euglycemia. In agreement with this, Hother-Nielsen et al[21] showed that long-term metformin treatment enhanced insulin-mediated glucose utilization in the presence of hyperinsulinemic and hyperglycemic conditions. In contrast, we did not detect an effect of metformin on glucose utilization but did observe a decrease in EGP. However, it should be noted that all of the above mentioned studies[19-21] examined the effect of metformin on glucose utilization under hyperinsulinemic (4- to 10-fold of basal)-hyperglycemic or hyperinsulinemic-euglycemic conditions. Since it is well known that both hyperinsulinemia and hyperglycemia suppress hepatic glucose production, any ability of metformin to further reduce hepatic glucose output would be difficult to detect. Therefore, the experimental design predisposed these studies to find an effect of metformin on glucose utilization. Secondly, since it has been reported that metformin decreases body weight and hyperlipidemia,[3,4] it is likely that plasma FFAs were decreased in these type 2 diabetic patients after long-term treatment with metformin. Therefore, the improvement in insulin sensitivity may well have been secondary to the decrease in body weight and lipids caused by long-term metformin treatment. In fact, it has been reported[3,22] that studies in lean type 2 subjects failed to show any increase in insulin sensitivity after 3 months of metformin treatment. Finally, the increase in insulin sensitivity found in the above-mentioned studies may also be attributable to reduced glucose toxicity[23] caused by long-term metformin treatment. No significant changes in plasma FFA or blood glycerol concentrations were found with metformin in the present study. This indicates the lack of an acute effect of metformin on lipolysis and/or FFA oxidation. This is further supported by the ketogenic data that showed no response to metformin.

In conclusion, in the conscious dog, (1) the primary acute effect of metformin on glucose metabolism was an inhibition of hepatic glucose production and not a stimulation of glucose utilization, and (2) the inhibition of glucose production was attributable to a decrease in hepatic glycogenolysis and not to an alteration in gluconeogenesis.

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