Impact of a Glycogen Phosphorylase Inhibitor and Metformin on Basal and Glucagon-Stimulated Hepatic Glucose Flux in Conscious Dogs.

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Abbreviations: GPI, glycogen phosphorylase inhibitor; MT, metformin; NHGO, net hepatic glucose output; NHGU, net hepatic glucose uptake; M-HGR, unidirectional
hepatic glucose release; M-HGU, unidirectional hepatic glucose uptake; G-6-P, glucose-6-phosphate; GK, glucokinase; G-6-Pase, glucose-6-phosphatase.
ABSTRACT
The effects of a glycogen phosphorylase inhibitor (GPI) and metformin (MT) on hepatic glucose fluxes (µmol·kg⁻¹·min⁻¹) in the presence of basal and four-fold basal levels of plasma glucagon were investigated in 18-hour fasted conscious dogs. Compared to the vehicle treatment, GPI infusion suppressed net hepatic glucose output (NHGO) completely (-3.8 ± 1.3 vs 9.9 ± 2.8) despite increased glucose-6-phosphate (G-6-P)-neogenesis from gluconeogenic precursors (G-6-P-neogenesis; 8.1 ± 1.1 vs 5.5 ± 1.1). MT infusion did not alter those parameters. In response to a four-fold rise in plasma glucagon levels, in the vehicle group, plasma glucose levels were increased two folds, NHGO was increased (43.9 ± 5.7 at 10 min and 22.7 ± 3.4 at steady state) without altering G-6-P-neogenesis (3.7 ± 1.5 and 5.5 ± 0.5, respectively). In the GPI group, there was no increase in NHGO due to decreased glucose-6-phosphatase flux associated with reduced G-6-P concentration. A lower G-6-P concentration was due to increased net glycogenesis without altering G-6-P-neogenesis. In the MT group, the increment in NHGO (22.2 ± 4.4 at 10 min and 12.1 ± 3.6 at steady state) was about half that of the vehicle group. The lesser NHGO was associated with reduced glucose-6-phosphatase flux, but a rise in G-6-P concentration and only a small incorporation of plasma glucose into glycogen. Conclusions: 1) The inhibition of glycogen phosphorylase a activity decreases basal and glucagon-induced NHGO via redirecting glucose-6-phosphate flux from glucose toward glycogen. 2) MT decreases glucagon-induced NHGO by inhibiting glucose-6-phosphatase flux and thereby reducing glycogen breakdown.
INTRODUCTION

The liver produces glucose via glycogen breakdown and/or gluconeogenesis and the relative contribution of each to total glucose production changes with altered nutritional and metabolic states. Several studies in dogs and humans have shown that increased delivery of gluconeogenic precursors, such as alanine (Diamond et al., 1988; Wolfe et al., 1988), glycerol (Jahoor et al., 1990), or lactate (Jenssen et al., 1990; Connolly et al., 1993), to the liver has no acute effect on the amount of glucose produced by that organ. Gluconeogenic precursors can alter hepatic glycogen metabolism by exerting regulatory effects on glycogen phosphorylase and synthase in addition to serving as substrates for glycogen synthesis (Youn and Bergman, 1990). Of which glucose-6-phosphate (G-6-P), an intermediate at a central cross point between the metabolic pathways of glycogen metabolism and gluconeogenesis, has been shown in studies using isolated hepatocytes to regulate glycogen synthase (Ciudad et al., 1986) and phosphorylase activity within a physiological range (Aiston et al. 2003; 2004). The above data suggest the existence of autoregulatory control of glycogenolysis by gluconeogenesis within the liver such that the desired rate of hepatic glucose output can be maintained regardless of the gluconeogenic precursor supply. Conversely, Staehr et al. reported that a galactose-induced increase in hepatic glycogenolysis resulted in a concomitant decrease in hepatic gluconeogenesis in 44 h-fasted healthy humans (Staehr et al., 2007). In keeping with this we showed that a concomitant increase in hepatic gluconeogenesis resulted from an inhibition of hepatic glycogenolysis (Shiota et al., 1997) although this was not confirmed by others (Fosgerau et al., 2001). These findings suggest the existence of an autoregulatory mechanism between net glycogenolysis and gluconeogenesis within the liver to maintain the desired rate of hepatic glucose output. Furthermore, the flux from glucose to glycogen has two highly regulated steps, glucose phosphorylation by glucokinase and the formation of a glycoside bond between C1 of the activated glucose, uridine 5'-diphosphate (UDP)-glucose and C4 of a terminal glucose
residue of glycogen by glycogen synthase. It has been reported that increasing both glucokinase and glycogen synthase activity synergistically increases glycogen synthesis from glucose in cultured hepatocytes isolated from normal rats (Gomis et al., 2000; Hampson and Agius, 2005). Therefore, net glycogen flux may be tightly linked to fluxes in other pathways including gluconeogenesis, glucose phosphorylation and glucose-6-phosphate dephosphorylation. It is possible that alteration of net hepatic glucose output resulting from a modification in glycogenolytic flux involves a secondary change in other metabolic pathway(s).

In patients and animals with type 2 diabetes, the diabetic hyperglycemia is associated with inappropriately increased endogenous glucose production, a lesser suppression of endogenous glucose production and a blunted glucose disposal in response to increased plasma glucose and insulin (Firth et al., 1986; Consoli, 1992; Iozzo et al., 2003). The blunted response of hepatic glucose flux to raised insulin and glucose is associated with blunted response of net hepatic glycogen flux (Krssak et al., 2004). The normalization or reduction of net hepatic glycogenolysis has attracted attention as a potential therapeutic strategy. During the past decade, a specific inhibitor of glycogen phosphorylase that catalyzes glycogen breakdown to glucose-1-phosphate, a rate-limiting step of glycogenolysis, was generated to directly decrease glycogen breakdown. Treatment with the inhibitor has been shown to reduce hyperglycemia acutely in a model of type 2 diabetes (Treadway et al., 2001; Ogawa et al., 2003). Metformin \((N,N\text{-dimethylbiguanide})\) has been used for decades to improve glycemic control in diabetic patients and is thought to decrease blood glucose levels by reducing hepatic glucose output (Bailey and Turner, 1996; Kirpichnikov et al., 2002). The most widely accepted mechanism of metformin action is the inhibition of transcription of key gluconeogenic genes in the liver (Shaw et al., 2005; Viollet et al., 2009). On the other hand, it has been reported that metformin reduces net hepatic glucose production acutely by decreasing glycogenolysis in the normal dog (Chu et al., 2000).
To verify the mechanism by which glycogen phosphorylase inhibition decreases NHGO, we examined whether any alteration of glucose phosphorylation, gluconeogenesis or glycogen synthesis is associated with the inhibition of glycogen phosphorylase. Further, to assess the mechanism by which metformin decreases net glycogenolysis, we compared the alterations in liver glucose flux caused by metformin with those caused by a glycogen phosphorylase inhibitor.
MATERIALS AND METHODS

Animals and surgical procedures. Experiments were performed on 15 overnight-fasted mongrel dogs (17.4-29.0 kg, mean 22.4 ± 1.1 kg) of either sex, which had been fed once daily a standard meat and chow diet (34% protein, 46% carbohydrate, 14% fat, and 6% fiber based on dry weight; Kal Kan, Vernon, CA and Purina Lab Canine Diet No. 5006, Purina Mills, St. Louis, MO). The dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. At least 16 days before an experiment, a laparotomy was performed under general endotracheal anesthesia (15 mg/kg body wt pentothal sodium presurgery and 1.0% isoflurane as an inhalation anesthetic during surgery), and catheters for blood sampling were placed into a femoral artery and the portal, a hepatic, a jejunal, and a splenic vein as previously described (Connolly et al., 1993; Diamond et al., 1988) A catheter for drug infusion was placed into the stomach as previously described (Moore et al., 1994). On the day of the experiment, the catheters were exteriorized under local anesthesia (2% lidocaine: Abbott, North Chicago, IL), their contents were aspirated, and they were flushed with saline. Angiocaths (20 gauge; Abbott) were inserted into both cephalic veins for infusion of indocyanine green, radioactive tracers and glucose, and a saphenous vein for the infusion of somatostatin.

On the day before an experiment the leukocyte count and hematocrit were determined. Dogs were used for an experiment only if they had 1) a leukocyte count <18000/mm³, 2) a hematocrit >38%, 3) a good appetite, and 4) normal stools.

Experimental design. Each experiment consisted of a 100-min tracer and dye equilibration period (-140 to -40 min), a 40-min control period (-40 to 0 min), and two test periods; Test Period I (0 to 90 min) and Test Period II (90 to 210 min). A priming dose of [3-3H]-glucose (41.7 µCi) was given at -140 min. Continuous infusions of [3-3H]-glucose (0.34 µCi/min) and indocyanine green (0.1 mg/m²-min⁻¹) were also started.
at -140 min and were continued throughout the experiment. At -140 min a peripheral infusion of somatostatin (0.8 µg·kg^{-1}·min^{-1}) was started to inhibit endogenous insulin and glucagon secretion. Intraportal replacement infusions of insulin (0.25 mU·kg^{-1}·min^{-1}) and glucagon (0.6 ng·kg^{-1}·min^{-1}) were started simultaneously with initiation of the somatostatin infusion. Plasma glucose was then monitored every 5 minutes and the rate of insulin infusion was adjusted until the level of plasma glucose was stabilized at a euglycemic value. Once stabilization was achieved, the insulin infusion rate was left unchanged. Three experimental protocols were used: 1) Vehicle (placebo) group, an intragastric bolus (0.5 ml·kg^{-1}) of polyethylene glycol (PEG)-500 (10%) was given at 0 min and saline was infused into the portal vein at 7 µl·kg^{-1}·min^{-1} during the test periods. 2) GPI group, an intragastric bolus (10 mg·kg^{-1}) of GPI (CP-316819, Pfizer, Inc., Groton, CT) (Treadway et al., 2001) with PEG500 (0.5 ml·kg^{-1}) was given at 0 min due to its low solubility in vehicle and then saline was infused into the portal vein at 7 µl·kg^{-1}·min^{-1} during the test periods. 3) Metformin (MT) group, an intragastric bolus of PEG500 (0.5 ml·kg^{-1}) was given at 0 min and metformin was infused into the portal vein at 0.15 mg·kg^{-1}·min^{-1} with 7 µl saline·kg^{-1}·min^{-1} during the test periods since metformin causes diarrhea if given orally. Based on hepatic blood flow rate (~30 ml·kg^{-1}·min^{-1}), hepatocrit (~40%) and the infusion rate of metformin (150 µg·kg^{-1}·min^{-1}), hepatic sinusoidal plasma concentrations of metformin were expected to be >8 µg/ml, which are 10 times higher than plasma concentrations of metformin (in range of 0.5 to 2.0 µg·ml^{-1}) seen when metformin was given at 1g·day^{-1} in patients with type 2 diabetes (Scheen, 1996). After a 90 min test period, the infusion rate of glucagon was increased fourfold in all groups for another 120 min.

**Analytical procedures.** Plasma glucose concentrations and plasma glucose radioactivity were determined as previously described (Fujimoto et al., 2006; Torres et al., 2009). Blood concentrations of gluconeogenic precursors (lactate, alanine and glycerol) and plasma concentration of non-esterified fatty acids were determined
according to the methods reported previously (Fujimoto et al., 2006; Torres et al., 2009). Individual blood amino acid (alanine, serine, threonine and glycine) levels were assessed by high-performance liquid chromatography methods with an interassay coefficient of variation (CV) of 4% (Venkatakrishnan et al., 1996). Plasma arterial and hepatic vein indocyanine green concentrations were determined spectrophotometrically at 805 nm (Leevy et al., 1962). Immunoreactive plasma insulin, glucagon, and cortisol as well as plasma epinephrine and norepinephrine were determined as previously described (Wada et al., 1995).

Liver samples were obtained at the end of each experiment by euthanizing the dog with pentobarbital sodium, exposing the liver by laparotomy, and freeze clamping approximately 5 g liver sections from each lobe. The time elapsed from euthanization to freeze clamping was less than 4 min. The entire liver was then removed from the dog and weighed. The frozen liver samples were stored at -70 °C for subsequent analysis. Glycogen and $^3$H in glycogen were determined as described previously (Fujimoto et al., 2006; Torres et al., 2009). Liver content of UDP-glucose and UDP-galactose were obtained through two sequential chromatographic separations and the amount of [3H] radioactivity in each fraction was measured as described previously (Fujimoto et al., 2006). Glycogen synthase and phosphorylase activities were measured as reported previously (Fujimoto et al., 2006; Torres et al., 2009). It has been reported that barbiturate derivatives do not have a direct effect on activity of liver phosphorylase in vitro (Brunner and Haugaard, 1965). However, Mikines et al. (Mikines et al., 1986) showed a time-dependent decrease in glycogen phosphorylase a activity in liver after anesthesia of rats with pentobarbital. Thus, there is a possibility that the activity of liver glycogen phosphorylase was altered to some extent during the time between euthanasia and freeze clamping the tissue.

**Materials.** [3-$^3$H]-glucose (New England Nuclear, Boston, MA) was used as the glucose tracer (500 µCi/0.005 mg). Indocyanine green (Hynson, Westcott, and Dunning,
Baltimore, MD) was prepared in sterile water. Insulin (Squibb-Novo, Princeton, NJ), and glucagon (Eli Lilly, Indianapolis, IN) and cyclic somatostatin (Bachem, Torrance, CA) were prepared with normal saline and contained 3% (vol/vol) of the dog's own plasma. Cortisol radioimmunoassay kits were obtained from Micromedic Systems (Horsham, PA). GPI, (3S,2R)-3-(5-chloroindole-2-carbonyl)amino-2-hydroxy-4-phenylbutyric acid N-methyl-N-methoxyamide (CP-316819; see Hoover et al., 2001), was synthesized at Pfizer Global Research and Development (Groton, CT).

Calculations. Hepatic blood flow was assessed by measuring hepatic extraction of indocyanine green (Leevy et al., 1962). Based on data from Greenway and Stark (Greenway and Stark, 1971), the proportions of the hepatic blood supply provided by the hepatic artery and portal vein were assumed to be 28 and 72%, respectively, which conforms to data we obtained with Doppler flow probes during pancreatic clamps (Myers et al., 1991). These proportions were assumed to remain constant throughout all experiments since treatment did not significantly affect hepatic blood flow. Net hepatic substrate balance was calculated using the formula \[ H - (0.28A + 0.72P) \] x HF, where A, P, and H are the arterial, portal vein, and hepatic vein substrate concentrations, respectively, and HF is the hepatic blood or plasma flow. When substrate levels in blood were measured then blood flow was used in the calculation whereas plasma flow was used when plasma substrate levels were measured.

For calculation of minimal estimates of unidirectional hepatic glucose uptake (M-HGU), which does not include glucose taken up by liver and then returned (glucose cycling), the net hepatic [3-^3^H]-glucose uptake was divided by average sinusoidal [3-^3^H] glucose specific activity (SA). The sinusoidal [3-^3^H]-glucose SA was calculated using the formula \[ \{(0.28 \times [3-^3^H]SA in artery) + (0.72 \times [3-^3^H]SA in portal vein)\}. Minimal estimates of unidirectional hepatic glucose release (M-HGR), which does not contain glucose released via glucose cycling, was determined by adding M-HGU to net hepatic glucose balance, which is net hepatic glucose output (NHGO) when the balance is
positive and net hepatic glucose uptake (NHGU) when the balance is negative. Assuming that there is 100% conversion of gluconeogenic precursors taken up by the liver into glucose-6-phosphate (G-6-P) (Goldstein et al., 2002), the gluconeogenic flux to G-6-P (G-6-P-neogenesis) was determined by summation of net hepatic uptake rates of the gluconeogenic precursors (alanine, glycine, serine, threonine, glycerol and lactate), converting the sum to glucose equivalents, and dividing by two to account for the incorporation of three carbon precursors into the six-carbon molecules. When lactate was released, net lactate uptake was calculated as zero. The fractional contribution of plasma glucose via the direct pathway to UDP-glucose flux was calculated as the ratio of [3-\(^{3}\)H]SA in hepatic UDP-glucose to the liver sinusoidal [3-\(^{3}\)H]-glucose SA. The remaining G-6-P was formed via other pathways, mainly glycogenolysis and G-6-P-neogenesis. Glucose-6-phosphatase (G-6-Pase) flux was calculated using the formula \([M-HGR ÷ (1-[3-\(^{3}\)H]SA in hepatic UDP-glucose/[3-\(^{3}\)H]SA in plasma glucose])\). Glucose cycling was calculated as the difference between G-6-Pase flux rate and M-HGR.

Hepatic glycogen content was determined by multiplying the glycogen concentration (mg·g\(^{-1}\) of liver) by liver weight. The amount of glycogen synthesized from glucose (the direct pathway) was calculated by dividing \(^{3}\)H radioactivity incorporated into liver glycogen by the average \([3-\(^{3}\)H]SA in arterial plasma glucose between 0 and 180 min. Net glycogenolysis was calculated as the difference between M-HGR and G-6-P-neogenesis, assuming all of the G-6-P derived from G-6-P-neogenesis were converted to glucose.

Statistical analysis. Data are expressed as means ± standard error (SE). Statistical comparisons were made using two-way analysis of variance with repeated-measures design. Post-hoc analysis was performed using either paired t-test or unpaired t-test.
RESULTS

Hematocrit, hepatic blood flow and Hormone levels. Arterial hematocrit (Fig. 1A) and hepatic arterial and portal vein blood flow (Fig. 1B) were not significantly different over time among the three groups. Portal and arterial insulin levels remained at basal levels throughout the study among all three groups (Fig. 1C). Arterial and portal glucagon levels were basal and unchanged in all protocols during the first two time periods, and then in the third period (Test Period II) rose approximately 2- and 3-fold, respectively, when the glucagon infusion rate was increased (Fig. 1D). Arterial cortisol, norepinephrine and epinephrine levels were not significantly changed with either GPI or MT treatment (Table 1).

Hepatic glucose balance. Plasma glucose levels were maintained at basal during the control period and Test Period I (Fig. 2A). During the control period, there were no significant differences in NHGO and M-HGU, among the groups (Fig. 2C and 2D). During Test Period I, in the vehicle group, glucose infusion (5.6 ± 1.6 µmol·kg⁻¹·min⁻¹) was necessary to maintain euglycemia (Fig. 2B). Net hepatic balance of glucose and hepatic glucose uptake changed minimally (Fig. 2C and 2D). In the GPI group, glucose needed to be infused at 13.3 ± 2.6 µmol·kg⁻¹·min⁻¹ to maintain euglycemia in Test Period I. NHGO was completely suppressed by 60 min (-1.5 ± 2.3 µmol·kg⁻¹·min⁻¹) and thereafter switched to net hepatic glucose uptake (NHGU; 3.8 ± 1.3 µmol·kg⁻¹·min⁻¹) by 90 min (Fig. 2C). Hepatic glucose uptake more than doubled in response to the GPI. In the MT group, small amount of glucose (2.3 ± 0.6 µmol·kg⁻¹·min⁻¹) needed to be infused to maintain euglycemia. NHGO tended to decrease from basal (8.9 ± 0.9 µmol·kg⁻¹·min⁻¹) to 7.6 ± 1.3 µmol·kg⁻¹·min⁻¹ and was not significantly different from that of the vehicle group (Fig. 2C). Hepatic glucose uptake was not altered by MT treatment.

In response to the rise in plasma glucagon levels, in the vehicle group, the plasma glucose level rose from 5.8 ± 0.2 to 11.1 ± 1.0 mM by the end of the study (Fig. 1A). NHGO increased by 42 ± 6 µmol·kg⁻¹·min⁻¹ in 10 min and then fell but remained
elevated relative to the previous period (Fig. 2C) and there was no change in hepatic glucose uptake (Fig. 2D).

In the GPI group, the plasma glucose levels were completely matched to the levels seen in the vehicle group by infusing glucose at $35 \pm 5 \, \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (Fig. 2A and 2B). NHGO was not observed in response to the rise in plasma glucagon (Fig. 2C and Table 3). Net and absolute hepatic uptake of glucose were increased in parallel (Fig. 2C and 2D). In the MT group NHGO was increased by $21 \pm 5 \, \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ within 10 min, after which it returned close to the rate evident in the previous period (Fig. 2C), although the increment of NHGO was significantly smaller than that in the vehicle group (Fig. 2C). Hepatic glucose balance uptake did not change significantly in response to MT treatment.

**Net hepatic balances of gluconeogenic precursors.** As shown in Table 2 and 3, the arterial level and net hepatic balance of all of measured gluconeogenic precursors were not significantly changed from control values by treatment with vehicle or by the rise in glucagon in the presence of vehicle (Test Period I). On the other hand, treatment with GPI during Test Period I caused markedly decreased net hepatic lactate release and brought about a switch to hepatic lactate uptake (Table 3). The sum of net hepatic gluconeogenic precursors uptake increased significantly (Fig. 3). The rise in glucagon in the presence of GPI treatment brought about an immediate decrease in the sum of net hepatic gluconeogenic precursor uptake, which was due to a sudden decrease in net hepatic lactate uptake. Treatment with metformin tended to decrease net hepatic gluconeogenic precursor uptake (Fig. 3 and Table 3). In response to the rise in plasma glucagon and glucose, in all of the three groups, arterial amino acids levels tended to decrease (Table 2) with a tendency toward an increase in net hepatic uptake and fractional extraction (data not shown) of these precursors (Table 3 and Fig. 3).

**Arterial NEFA and net hepatic balance.** Compared with the control period, arterial levels and net hepatic uptake of NEFA did not change significantly during Test
Period I with either GPI or MT treatment. Likewise the rise in plasma glucagon levels that occurred during Test Period II (Table 2 and 3) had no effect. Likewise there were no changes in lipolysis as indicated by the stability in plasma glycerol levels.

**Intermediary metabolism in the liver.** At the end of Test Period II, compared to the vehicle group, the MT group had 2 fold higher G-6-P content but 50% lower UDP-glucose content (Fig. 4A and 4B). The ratio of $[^3]H$ specific activity of UDP-glucose to that of plasma glucose, reflecting the percent contribution of plasma glucose to UDP-glucose flux, was similar (Fig. 4C). Hepatic glycogen content was similar and the amounts of $[^3]H$ in hepatic glycogen were similarly very low although slightly elevated with metformin (Fig. 4D and 4E). In the GPI group compared with the vehicle group, both G-6-P and UDP-glucose contents were markedly lower. The percent contribution of plasma glucose to UDP-glucose flux was increased by 40% (55 ± 6% in GPI vs 42 ± 2% in vehicle). The hepatic glycogen content was higher by 30% and the incorporated amount of [3-$^3$H]-glucose into hepatic glycogen was 100-times greater (Fig. 4D and 4E).

**Glycogen phosphorylase and synthase activity (Fig. 5).** Compared to the vehicle group, in the GPI group, glycogen phosphorylase a activity was markedly lower and in contrast, glycogen synthase I activity and the ratio of active form (I) to total activity were higher. In the MT group, glycogen phosphorylase a activity was similar to that in the vehicle group. However, the active form of glycogen synthase and the ratio of active to total activity were slightly but significantly higher.

**Estimated hepatic glucose fluxes (Table 4).** During the control period, there were no significant differences in NHGO, M-HGU, M-HGR, G-6-P-neogenesis, net glycogenolytic rate and percent contribution of gluconeogenesis and glycogenolysis to M-HGR among the groups. During Test Period I, in the vehicle group, NHGO and M-HGR tended to reduce with significant decrease in the rate and the contribution of glycogenolysis to M-HGR from that in the control period. On the other hand, G-6-P-neogenesis was not changed and the percent contribution of gluconeogenesis to M-HGR
rose relative to that in the control period. In the GPI group, GPI treatment switched NHGB from NHGO to NHGU with a marked decrease in M-HGR and net glycogenolytic rate and an increase in the gluconeogenic rate. In the MT group, NHGO, M-HGR, net glycogenolysis and G-6-P-neogenesis tended to decrease from that in the control period. The percent contribution of glycogenolysis and gluconeogenesis to M-HGR remained.

At the end Test Period II, in the vehicle group, NHGO was increased three-fold which was associated with a three-fold increase in M-HGR that was accompanied by increased net glycogenolysis without an alteration of G-6-P-neogenesis. GPI treatment markedly decreased glucagon-induced increase in NHGO, M-HGR and net glycogenolysis. G-6-Pase flux and glucose cycling rate were markedly lower in the GPI group compared to that in the vehicle group. On the other hand, M-HGU was markedly increased. G-6-P-neogenesis and GK flux in the GPI group were not significantly different from that in the vehicle group. MT treatment reduced glucagon-induced increase in NHGO, M-HGR and net glycogenolysis by about half (by 58%, 44% and 52%, respectively). G-6-Pase flux, GK flux and glucose cycling in the MT group were lower by 40% compared to that in the vehicle group. On the other hand, the percent contribution of net glycogenolysis and gluconeogenesis to M-HGR and the percent contribution of net glycogenolysis, gluconeogenesis and glucose cycling to G-6-Pase flux were similar with that of the vehicle group.
DISCUSSION

The present study shows that decreased basal and glucagon-induced NHGO by inhibiting glycogen phosphorylase is associated with decreased G-6-Pase flux due to lower G-6-P concentrations which in turn resulted from an activation of net glycogenesis. On the other hand, metformin decreases G-6-Pase activity, promoting glycogen sparing via increased G-6-P content, leading up to decreased NHGO.

Effect of GPI and metformin on basal hepatic glucose flux.

GPI-induced suppression of NHGO was associated with a marked decrease in M-HGR (from 12.3 to 0.9 µmol·kg⁻¹·min⁻¹). Yet, the increased rate of G-6-P-neogenesis (4 to 8 µmol C6 unit·kg⁻¹·min⁻¹) exceeded M-HGR (0.9 µmol·kg⁻¹·min⁻¹), which indicated the suppression of M-HGR by GPI treatment was associated with not only abolished net glycogenolysis but also redirected G-6-P flux from glucose release toward other pathway(s), such as glycogen synthesis and glycolysis. In addition, with GPI treatment, there was a shift of net hepatic lactate balance from production to uptake and with a rapid equilibration between the plasma lactate pool and the intracellular lactate and pyruvate pools (Wolfe et al., 1988), this shift in lactate balance may have reflected a shift from pyruvate generation to pyruvate consumption in the net sense. Intracellular pyruvate is generated by glycolysis and deamination of amino acids mediated by transaminases. Since net hepatic amino acid uptake was not decreased by GPI, the switch of hepatic lactate flux from production to uptake probably resulted from decreased net glycolytic flux. This would support that GPI treatment redirects G-6-P from glucose release and glycolysis towards glycogen synthesis.

An inhibition of hepatic glycogenolysis using GPI resulted in a concomitant increase in hepatic gluconeogenesis in agreement with our previous study in dogs (Shiota...
et al., 1997). In contrast, this was not observed by Fosgerau et al. (Fosgerau et al., 2001) using 1,4-diderexy-1,4-imino-D-arabinitol (DAB), which can induce a small degree of phosphorylation of phosphorylase in hepatocytes, thus leading to inhibition of glycogen synthase (Latsis et al., 2002). However, DAB had no effect on glycogen synthesis in hepatocytes (Andersen et al., 1999; Latsis et al., 2002). On the other hand, a 1,4-dihydroxy-pyridine-2,3-dicarboxylate derivative (BAY R3401) and an indole 2-carboxamide (CP-316819), which were used in the previous (Shiota et al., 1997) and present study, respectively, cause conversion of phosphorylase a into phosphorylase b and activation of glycogen synthase, and as a result, promote net glycogenesis (Bergans et al., 2000; Shiota et al., 1997). Thereby, BAY R3401 and CP-316819 might pull gluconeogenic flux by increasing G-6-P flux toward glycogen via stimulation of net glycogenesis. Therefore, the observed differences in the effects of GPI on gluconeogenesis between Fosgerau et al. (Fosgerau et al., 2001) and us (Bergans et al., 2000; Shiota et al., 1997) may be explained as a difference in the mechanism of action among the compound used.

Metformin tended to decrease NHGO and M-HGR that was associated with decrease in both net glycogenolysis and gluconeogenesis. The percent contribution of gluconeogenesis and glycogenolysis to M-HGR was not changed by metformin. It is likely, therefore, that decreased NHGO by metformin result from an inhibition of common site in the metabolic pathway where glucose is produced via glycogenolysis and gluconeogenesis.

**Effect of glucagon on hepatic glucose flux in the presence of GPI.**

The increment of NHGO brought about by glucagon was associated exclusively with increased net glycogenolysis in the placebo group. Glucagon failed to increase NHGO in
the presence of GPI. Net hepatic glucose flux is a balance between glucose phosphorylation rate mediated by glucokinase and G-6-P dephosphorylation rate mediated by G-6-Pase. A failure of glucagon to increase NHGO in the presence of GPI was associated with a failure to increase in G-6-Pase flux, since glucokinase flux was not altered. Hepatic content of G-6-P, a substrate for G-6-Pase, was less than half of that in the vehicle group, implying that the failure of glucagon to increase G-6-Pase flux arose from the failure of the hormone to maintain or increase the G-6-P pool. The rate of glucose phosphorylation (GK flux) or G-6-P-neogenesis were not significantly altered, therefore, the decreased G-6-P content resulted from greater conversion of G-6-P into glycogen.

Glucagon increases net glycogenolysis via activation of glycogen phosphorylase and by inhibition of glycogen synthesis via inactivation of glycogen synthase. Compared with the vehicle group, glycogen phosphorylase a activity was significantly lower and glycogen synthase activity was higher with GPI treatment. Activation of glycogen phosphorylase via protein kinase A and phosphorylase kinase, results in enhanced phosphorylation of the inactive form (glycogen phosphorylase b), which promotes the formation of an active enzyme complex (glycogen phosphorylase a) (Bollen et al., 1998). The GPI, CP-316819, has been reported to bind the indole inhibitor site of glycogen phosphorylase, by enhancing the dephosphorylation of the glycogen phosphorylase a complex, converting it to an inactive form (glycogen phosphorylase b) (Treadway et al., 2001). This is thought to occur as a result of a binding-induced conformational change that makes glycogen phosphorylase a better substrate for the regulatory phosphatase (protein phosphatase 1) (Kasvinsky et al., 1981) and/or interference with the binding of phosphorylase a to the C-terminal domain of the glycogen-targeting subunit of protein...
phosphatase 1 (Pautsch et al., 2008; Kelsall et al., 2007). The indole-2-carboxamide (CP-91149, Pfizer) was shown to counteract the phosphorylation caused by glucagon (Latsis et al., 2002). Therefore the treatment with GPI may interfere with the action of glucagon by activation of protein phosphatase 1.

The inactivation of glycogen synthase is mediated by enhancing the phosphorylation of the active form (glycogen synthase I) and converting it to an inactive form (glycogen synthase D) via activation of protein kinase A and mediated by protein phosphatase 1 (Bollen et al., 1998). Glycogen phosphorylase a may inhibit protein phosphatase 1 allosterically through the binding of the enzyme to the C-terminal domain of the glycogen-targeting subunit of protein phosphatase 1 (Armstrong et al., 1998). The failure of glucagon to inactivate glycogen synthase in the presence of GPI likely results from a defective activation of glycogen phosphorylase by this hormone.

Insulin inactivates glycogen phosphorylase a by activation of protein phosphatase 1 via activation of PKB/Akt (Aiston et al., 2006). It has been reported that inhibition of phosphorylase activity is critical to activate glycogen synthase (glycogen synthesis) by elevation of glucose and insulin in cultured hepatocytes isolated from normal rats (Aiston et al., 2003). It is likely, therefore, that acute alteration of net glycogen flux in liver by glucagon and insulin is primarily mediated by regulating glycogen phosphorylase activity.

**Effect of glucagon on hepatic glucose flux in the presence of metformin.**

Partial reduction of the glucagon-induced increase in NHGO in the presence of metformin was also associated with decreased G-6-Pase flux without an alteration of GK flux. In contrast to the decreased G-6-Pase flux in the GPI group with a marked reduction in metabolites in the GPI group, the decreased G-6-Pase flux with metformin treatment
was accompanied by a markedly greater content of G-6-P compared to the vehicle group. This increase in the G-6-P pool occurred without an increase in glucose phosphorylation (GK flux), G-6-P-neogenesis or glycogenolysis. Furthermore the lower G-6-Pase flux was associated with equally lower rates of gluconeogenesis, glucose cycling and glycogenolysis without changes in the percent contribution of glycogenolysis, gluconeogenesis and glucose cycling to G-6-Pase flux. These results suggest that the reduced glucagon-induced NHGO brought about by treatment with metformin resulted primarily from an inhibition of G-6-Pase activity by metformin.

Decreased glucagon-induced glycogenolysis in the presence of metformin was brought about without decreased phosphorylase a activity. On the other hand, glycogen synthase activity and incorporation of glucose into glycogen were slightly but significantly higher in the metformin group compared to that in the vehicle group. The greater glycogen synthase activity in the metformin group might have arised due to the increased G-6-P content, since the latter is a strong activator of glycogen synthase. Our results are consistent with the report by Mithieux et al. (Mithieux et al., 2002) that the suppression of hepatic glucose production by chronic treatment with metformin in insulin-resistant high fat diet fed rats is accompanied by increased content of G-6-P in liver. It is likely, therefore, that inhibition of G-6-Pase activity by metformin decreased net glycogenolysis by promoting glycogen sparing through increased G-6-P content.

The glucose-lowering effects of metformin are mainly a consequence of reduced hepatic glucose output through inhibition of both gluconeogenesis and glycogenolysis in patients with type 2 diabetes (Hundal et al., 2000; Kirpichnikov et al., 2002). The in vitro studies using isolated hepatocytes (Argaud et al., 1993) or perfused liver (Radziuk et al., 1997) demonstrated the acute effect of metformin to inhibit gluconeogenesis.
Although its primary action has been proposed to be mitochondrial function, the exact mechanism through which metformin reduces hepatic gluconeogenesis remains unclear (Kirpichnikov et al., 2002). The present study demonstrates that the primary action of metformin to reduce net glycogenolysis may not be through glycogen phosphorylase inhibition. Glucose production via glycogenolysis and gluconeogenesis share one common step in their metabolic pathway, dephosphorylation of G-6-P by G-6-Pase. The present data suggest therefore that G-6-Pase is one of the primary sites of metformin’s action.
Authorship Contributions.

Participated research design: Shiota and Treadway.

Conducted experiments: Torres, Sasaki, Lacy, Donahue and Shiota.

Contributed to new reagents: Treadway

Performed data analysis: Shiota and Cherrington

Wrote or contributed to the writing of the manuscript: Shiota, Printz and Cherrington
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glycogen-targeting subunit interaction with phosphorylase a can be blocked by C-


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FOOTNOTES

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LEGENDS TO FIGURES

Figure 1. Arterial blood hematocrit (Panel A), hepatic arterial and portal blood flows (Panel B), arterial and portal plasma levels of insulin (Panel C) and glucagon (Panel D) before (control) and after drug treatment (Test Period I) and subsequent increase of plasma glucagon (Test Period II) in 18 h-fasted conscious dogs. The animals received intragastric bolus injection of vehicle or GPI (10 mg·kg\(^{-1}\)) at 0 min, continuous infusion of metformin or vehicle into the portal vein (0.15 mg·kg\(^{-1}\)·min\(^{-1}\)) from 0 min, and a four fold increase in intraportal glucagon brought about from 90 min. Data are means ± SE for 5 experiments.

Figure 2. Arterial plasma glucose levels (Panel A), glucose infusion rates (Panel B) and changes in net hepatic glucose (Panel C) and [3\(^{-3}\)H]-glucose balance (Panel D) before (control) and after drug treatment (Test Period I) and subsequent increase of plasma glucagon (Test Period II) in 18 h-fasted conscious dogs. The animals received intragastric bolus injection of vehicle or GPI (10 mg·kg\(^{-1}\)) at 0 min, continuous infusion of metformin or vehicle into the portal vein (0.15 mg·kg\(^{-1}\)·min\(^{-1}\)) from 0 min, and a four fold increase in intraportal glucagon brought about from 90 min. Values are means ± SE for 5 experiments. *Significantly different from the corresponding value in vehicle group (P<0.05). †Significantly different from the control period with the same group (P<0.05). ‡ Significantly different from the corresponding value at 90 min in the Test Period I with the same group (P<0.05).

Figure 3. Net hepatic gluconeogenic precursor uptake before (control) and after drug treatment (Test Period I) and subsequent increase of plasma glucagon (Test Period II) in 18 h-fasted conscious dogs. The animals received intragastric bolus injection of vehicle or GPI (10 mg·kg\(^{-1}\)) at 0 min, continuous infusion of metformin or vehicle into the portal vein (0.15 mg·kg\(^{-1}\)·min\(^{-1}\)) from 0 min, and a four fold increase in intraportal glucagon brought about from 90 min. The rate was obtained by summation of net hepatic uptake rates of lactate, glycerol, pyruvate, and all gluconeogenic amino acids. Lactate
uptake was considered as zero when lactate was released by the liver. Values are means ± SE for 5 experiments. *Significantly different from the corresponding value in the vehicle group (P<0.05). † Significantly different from the control period (P<0.05). ‡ Significantly different from the corresponding value at 90 min in the Test Period I with the same group (P<0.05).

Figure 4. Hepatic content of G-6-P (Panel A), UDP-glucose (Panel B) and glycogen (Panel C) and amount of [3-3H] glucose incorporated into glycogen (Panel D) at the end of the Test Period II. Data are means ± SE for 5 experiments. *Significantly different from the corresponding value in the vehicle group (P<0.05). † Significantly different from the control period (P<0.05).

Figure 5. Hepatic glycogen synthase and phosphorylase activities in the liver at the end of the Test Period II. Data are means ± SE for 5 experiments. *Significantly different from the corresponding value in the vehicle group (P<0.05). † Significantly different from the control period (P<0.05).
Table 1. Arterial plasma levels of norepinephrine, epinephrine and cortisol before (control) and after drug treatment (Test Period I) and subsequent increase of plasma glucagon (Test Period II) in 18 h-fasted conscious dogs.

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Groups</th>
<th>Study Periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>V</td>
<td>673±59</td>
</tr>
<tr>
<td>(pmol/l)</td>
<td>GPI</td>
<td>523±138</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>761±227</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>V</td>
<td>345±144</td>
</tr>
<tr>
<td>(pmol/l)</td>
<td>GPI</td>
<td>332±152</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>824±277</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>V</td>
<td>62±12</td>
</tr>
<tr>
<td></td>
<td>GPI</td>
<td>79±16</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>69±6</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 experiments.
Table 2. Arterial levels of lactate, alanine, serine, threonine, glycine, glycerol and free fatty acids before (control) and after drug treatment (Test Period I) and subsequent increase of plasma glucagon (Test Period II) in 18 h-fasted conscious dogs.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Group</th>
<th>Study Period</th>
<th>Control</th>
<th>Test period I</th>
<th>Test period II</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>Lactate</td>
<td>V</td>
<td>BL</td>
<td>495±30</td>
<td>504±64</td>
<td>448±26</td>
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<tr>
<td></td>
<td>GPI</td>
<td>BL</td>
<td>810±150</td>
<td>608±93</td>
<td>578±112</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>BL</td>
<td>830±111</td>
<td>822±125</td>
<td>849±115</td>
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<tr>
<td>Alanine</td>
<td>V</td>
<td>BL</td>
<td>507±47</td>
<td>553±28</td>
<td>503±39</td>
</tr>
<tr>
<td></td>
<td>GPI</td>
<td>BL</td>
<td>509±74</td>
<td>457±73</td>
<td>398±71</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>BL</td>
<td>556±26</td>
<td>577±36</td>
<td>540±23</td>
</tr>
<tr>
<td>Serine</td>
<td>V</td>
<td>BL</td>
<td>198±19</td>
<td>187±10</td>
<td>194±19</td>
</tr>
<tr>
<td></td>
<td>GPI</td>
<td>BL</td>
<td>174±14</td>
<td>174±22</td>
<td>184±29</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>BL</td>
<td>188±26</td>
<td>174±26</td>
<td>176±27</td>
</tr>
<tr>
<td>Threonin</td>
<td>V</td>
<td>BL</td>
<td>276±32</td>
<td>264±36</td>
<td>287±39</td>
</tr>
<tr>
<td></td>
<td>GPI</td>
<td>BL</td>
<td>277±36</td>
<td>270±51</td>
<td>288±58</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>BL</td>
<td>303±47</td>
<td>287±36</td>
<td>296±29</td>
</tr>
<tr>
<td>Glycine</td>
<td>V</td>
<td>BL</td>
<td>267±27</td>
<td>253±21</td>
<td>263±26</td>
</tr>
<tr>
<td></td>
<td>GPI</td>
<td>BL</td>
<td>253±32</td>
<td>257±39</td>
<td>264±48</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>BL</td>
<td>285±30</td>
<td>278±30</td>
<td>281±24</td>
</tr>
<tr>
<td>Glycerol</td>
<td>V</td>
<td>PL</td>
<td>124±14</td>
<td>112±12</td>
<td>104±10</td>
</tr>
<tr>
<td></td>
<td>GPI</td>
<td>PL</td>
<td>164±14</td>
<td>195±24</td>
<td>155±13</td>
</tr>
<tr>
<td></td>
<td>MT</td>
<td>PL</td>
<td>171±29</td>
<td>152±19</td>
<td>156±25</td>
</tr>
<tr>
<td>FFAs</td>
<td>V</td>
<td>PL</td>
<td>285±75</td>
<td>244±62</td>
<td>297±51</td>
</tr>
<tr>
<td></td>
<td>GPI</td>
<td>PL</td>
<td>315±82</td>
<td>304±73</td>
<td>311±61</td>
</tr>
</tbody>
</table>
Blood (BL) and plasma concentration (PL) of metabolites are described as µmol/l. Values are means ± SE for 5 experiments.
Table 3. Net hepatic balance of lactate, alanine, serine, threonine, glycine, glycerol and free fatty acids before (control) and after drug treatment (Test Period I) and subsequent increase of plasma glucagon (Test Period II) in 18 h-fasted conscious dogs.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Group</th>
<th>Study Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Lactate V</td>
<td></td>
<td>7.64±2.81</td>
</tr>
<tr>
<td>GPI V</td>
<td></td>
<td>9.39±3.99</td>
</tr>
<tr>
<td>MT V</td>
<td></td>
<td>7.00±2.31</td>
</tr>
<tr>
<td>Alanine V</td>
<td></td>
<td>-2.87±0.71</td>
</tr>
<tr>
<td>GPI V</td>
<td></td>
<td>-2.59±0.77</td>
</tr>
<tr>
<td>MT V</td>
<td></td>
<td>-3.11±0.48</td>
</tr>
<tr>
<td>Serine V</td>
<td></td>
<td>-1.48±0.27</td>
</tr>
<tr>
<td>GPI V</td>
<td></td>
<td>-0.79±0.35</td>
</tr>
<tr>
<td>MT V</td>
<td></td>
<td>-0.85±0.24</td>
</tr>
<tr>
<td>Threonin V</td>
<td></td>
<td>-1.13±0.4</td>
</tr>
<tr>
<td>GPI V</td>
<td></td>
<td>-0.72±0.33</td>
</tr>
<tr>
<td>MT V</td>
<td></td>
<td>-0.63±0.26</td>
</tr>
<tr>
<td>Glycine V</td>
<td></td>
<td>-1.69±0.39</td>
</tr>
<tr>
<td>GPI V</td>
<td></td>
<td>-0.88±0.41</td>
</tr>
<tr>
<td>MT V</td>
<td></td>
<td>-1.21±0.5</td>
</tr>
<tr>
<td>Glycerol V</td>
<td></td>
<td>-0.83±0.49</td>
</tr>
<tr>
<td>GPI V</td>
<td></td>
<td>-1.80±0.38</td>
</tr>
<tr>
<td>MT V</td>
<td></td>
<td>-1.59±0.49</td>
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<tr>
<td>FFAs V</td>
<td></td>
<td>-3.13±0.77</td>
</tr>
<tr>
<td>GPI V</td>
<td></td>
<td>-3.08±1.23</td>
</tr>
</tbody>
</table>
Net hepatic balance of metabolites are described as μmol·kg$^{-1}$·min$^{-1}$. Values are means ± SE for 5 experiments. Negative values indicate net hepatic uptake. *Significantly different from the corresponding value in vehicle group (P<0.05).
Table 4. Hepatic glucose and intermediate fluxes before (control) and after drug treatment (Test Period I) and subsequent increase of plasma glucagon (Test Period II) in 18 h-fasted conscious dogs.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Test Period I</th>
<th>Test Period II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V</td>
<td>GPI</td>
<td>MT</td>
</tr>
<tr>
<td>NHGO</td>
<td>8.8±1.2</td>
<td>8.9±2.8</td>
<td>8.9±1.3</td>
</tr>
<tr>
<td>M-HGU</td>
<td>3.3±0.8</td>
<td>2.4±1.0</td>
<td>2.4±0.9</td>
</tr>
<tr>
<td>G-6-P-neogenesis</td>
<td>4.2±0.9</td>
<td>3.9±0.8</td>
<td>3.6±0.6</td>
</tr>
<tr>
<td>Net Glycogenolysis</td>
<td>7.9±1.8</td>
<td>7.4±2.1</td>
<td>7.7±1.7</td>
</tr>
<tr>
<td>M-HGR</td>
<td>12.1±1.8</td>
<td>11.3±2.3</td>
<td>11.3±2.2</td>
</tr>
<tr>
<td>Percent contribution of GNG</td>
<td>35±2</td>
<td>35±4</td>
<td>32±2</td>
</tr>
<tr>
<td>Percent contribution of Glycogenolysis</td>
<td>65±4</td>
<td>65±5</td>
<td>68±5</td>
</tr>
<tr>
<td>GK flux</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose cycle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-6-Pase flux</td>
<td>Total</td>
<td>43.6±3.7</td>
<td>7.7±3.6*</td>
</tr>
<tr>
<td>Percent contribution of Glycogenolysis</td>
<td>45±3</td>
<td>-</td>
<td>47±4</td>
</tr>
<tr>
<td>Percent contribution of GNG</td>
<td>13±2</td>
<td>-</td>
<td>14±2</td>
</tr>
<tr>
<td>Percent contribution of Glucose cycling</td>
<td>42±4</td>
<td>-</td>
<td>39±3</td>
</tr>
</tbody>
</table>

Data are mean ± SE; n = 5 dogs for each group. NHGO, net hepatic glucose output (µmol·kg⁻¹·min⁻¹); M-HGU, minimal estimate of hepatic unidirectional glucose uptake (µmol·kg⁻¹·min⁻¹); GNG, conversion of G-6-P derived from gluconeogenic precursors to glucose; M-HGR, minimal estimate of hepatic unidirectional glucose release (µmol·kg⁻¹·min⁻¹).
G-6-Pase flux and GK flux are described as (µmol·kg⁻¹·min⁻¹). A negative values for NHGO represent net uptake. *Significant difference from the corresponding values of the placebo group in the identical test period (P<0.05). †Significant difference from the corresponding values of the GPI group in the identical test period (P<0.05). ‡ Significant difference from the corresponding values of the control period in the identical group (P<0.05). # Significant difference from the corresponding values of the test period I in the identical group (P<0.05).
Figure 2

A  Plasma Glucose

B  Glucose Infusion Rate

C  Net Hepatic Glucose Balance

D  Hepatic Glucose Uptake

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Figure 3
Figure 4
Figure 5