Improvement of Glucose Homeostasis in Obese Diabetic *db/db* Mice Given *Plasmodium yoelii* Glycosylphosphatidylinositols

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We have previously reported that infection with *Plasmodium yoelii, Plasmodium chabaudi,* or injection of extracts from malaria-parasitized red blood cells induces hypoglycemia in normal mice and normalizes the hyperglycemia in streptozotocin (STZ)-diabetic mice. *P yoelii* glycosylphosphatidylinositols (GPIs) were extracted in chloroform:methanol:water (CMW) (10:10:3), purified by high-performance thin layer chromatography (HPTLC) and tested for their insulin-mimetic activities. The effects of *P yoelii* GPIs on blood glucose were investigated in insulin-resistant C57BL/ks-*db/db* diabetic mice. A single intravenous injection of GPIs (9 and 30 nmol/mouse) induced a significant dose-related decrease in blood glucose ($P < .001$), but insignificantly increased plasma insulin concentrations. A single oral dose of 2.7 μmol GPIs per db/db mouse significantly lowered blood glucose ($P < .01$). *P yoelii* GPIs in vitro (0.062 to 1 μmol/L) significantly stimulated lipogenesis in rat adipocytes in a dose-dependent manner both in the presence and absence of 10^{-8} mol/L insulin ($P < .01$). *P yoelii* GPIs stimulated pyruvate dehydrogenase phosphatase (PDH-Pase) and inhibited both cyclic adenosine monophosphate (cAMP)-dependent protein kinase A and glucose-6-phosphatase (G6Pase). *P yoelii* GPIs had no effect on the activity of the gluconeogenic enzymes fructose-1,6-bisphosphatase (FBPase) and phosphoenolpyruvate carboxykinase (PEPCK). This is the first report of the hypoglycemic effect of *P yoelii* GPIs in murine models of type 2 diabetes. In conclusion, *P yoelii* GPIs demonstrated acute antidiabetic effects in *db/db* mice and in vitro. We suggest that *P yoelii* GPIs, when fully characterized, may provide structural information for the synthesis of new drugs for the management of diabetes.

HYPOGLYCEMIA IS A recognized complication of falciparum malaria, particularly in children and pregnant women[1] and is associated with poor prognosis. The cause of hypoglycemia is not fully defined. Several hypotheses have been proposed including increased glucose consumption by both host and parasites, impaired gluconeogenesis, reduced hepatic glycogen, and increased lactate.[1,2] In some studies, hypoglycemia has been attributed to the hyperinsulinemic effect of treatment with quinine,[2,3] although hyperinsulinemia has also been shown to occur before treatment.[4]

Hypoglycemia also occurs in murine models of malaria where it is associated with hyperinsulinemia.[5,6] We have previously shown that injection of heat-stable, pronase-digested, water-soluble molecules derived from *Plasmodium yoelii* parasites induced a significant decrease in blood glucose of normal[7] and streptozotocin (STZ)-diabetic mice.[6] We subsequently showed that formalin-fixed *P yoelii* parasites[8] and inositol phosphoglycans (IPGs) obtained from them[9] induced a significant decrease in blood glucose in diabetic *ob/ob* and *db/db* mice. IPGs mimic several actions of insulin and have been suggested to be unique insulin mediators (reviewed in Varela-Nieto et al[10] and Rademacher et al[11]).

The C57BL/KsJ-*db/db*-diabetic mouse is a well-studied murine model of type 2 diabetes. It is characterized by obesity, hyperglycemia, and hyperinsulinemia.

Glycosylphosphatidylinositols (GPIs) are essential components of the plasma membrane of cells[12] including those of malaria parasites, both as membrane anchors for proteins and as free glycolipids.[13,14] The GPI precursors for the membrane anchors and those for the IPGs appear to be chemically distinct.[15] In their role as precursors of IPGs, GPIs play a role in growth factor and hormone signal transduction.[10,11] For example, the binding of insulin to its receptor leads to cleavage of GPIs by a glycosylphosphatidylinositol-specific phospholipase[16] and the generation of IPGs.[17-19] The pathogenesis of a number of human disorders has been linked to either the inappropriate release of IPG families, for example in obesity[20] and in obese type 2 diabetes,[21] or to the overproduction of specific IPGs, for example in pre-eclampsia.[22] A role for GPIs in the pathogenesis of malaria-associated hypoglycemia has been suggested.[23-25]

Plasmodium falciparum GPIs differ from those of humans with respect to both acyl substituents and the carbohydrate moiety.[14] It has been proposed that these structural differences may contribute to the observed naturally elicited immunologic responses against the parasite GPIs in humans.[14] GPIs isolated from malaria parasites have been tested in vivo only in normal mice pretreated with thioglycollate.[25] To our knowledge no GPIs from any source have been studied in murine models of type 2 diabetes. Therefore, we administered *P yoelii* GPIs to diabetic *db/db* mice and monitored changes in the concentration of their blood glucose and insulin levels. We have also evaluated the effect of *P yoelii* GPIs on adipocytes and key enzymes involved in glucose homeostasis and explored their other insulin mimetic activities in vitro. Here, we continue along the lines of previous studies[5-9] to highlight *P yoelii* GPIs as a paradigm of biologically active compounds and to demonstrate their antidiabetic activities both in vivo and in vitro.

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Submitted October 6, 2003; accepted March 30, 2004.

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0026-0495/04/5308-0026$30.00/0
doi:10.1016/j.metabol.2004.03.013

MATERIALS AND METHODS

Adenosine-5-monophosphate, glucose-6-phosphate, Trinder reagent, fructose 1,6 bisphosphate (FBPase), nicotinamide adenine dinucleotide phosphate ($NADP^+$), sodium pyruvate, thiamine pyrophosphate, dithiothreitol, ascorbic acid, ammonium molybdate, TRIZMA base, orcinol ferric chloride (Bial's reagent), and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (Poole, Dorset, UK). D-[3-^{3}H]glucose (18 Ci/mmol) was obtained from Amersham Pharmacia (Amersham, Bucks, UK). Collagenase D from *Clostridium histolyticum*, adenosine triphosphate (ATP), NAD^+, EGTA, phosphoglucose isomerase, glucose-6-phosphate dehydrogenase, and bovine insulin were obtained from Roche (Lewes, East Sussex, UK). Silica gel 60 high-performance thin layer chromatography (HPTLC) aluminium sheets were obtained from Merck (Darmstadt, Germany). BetaMax scintillation fluid and fatty acid-free bovine serum albumin were obtained from ICN Biomedicals (Thames, Oxfordshire, UK). C_8 Bond Elute cartridges were obtained from Phenomenex (Macclesfield, Cheshire, UK. All other materials were of high purity and were obtained from BDH (Poole, Dorset, England).

Animals

The normal (C57BL/6 × BALB/c) F_1 mice and obese diabetic (C57BL/Ks-*db/db*) mice were bred in house. We used male *db/db* diabetic mice 8- to 12-weeks-old. Wistar albino male rats (120 to 150 g body weight) were obtained from Harlan Olac (Bicester, UK) All animals had free access to water and were fed ad libitum with normal laboratory chow. All animal procedures were performed in accordance with regulations specified by the United Kingdom 'Animals (Scientific Procedures) Act 1986.

Measurement of Blood Glucose

Blood glucose concentrations were determined enzymatically on 10 μL vol of tail blood, collected between 10 AM and midday or at intervals thereafter as indicated below, using Glucotide strips and Glucometer 4 (Bayer, Newbury, UK) according to the manufacturer's instructions. Results (mmol/L) are expressed as mean ± SEM.

Measurement of Immunoreactive Insulin

Blood was collected into heparinized tubes from the trunk after decapitation. Plasma was separated by centrifugation and frozen at −20°C. Immunoreactive insulin (IRI) concentrations were determined in 50 μL aliquots of plasma by a double-antibody radioimmunoassay (kit supplied by Linco Research, St Charles, MO). Results (in ng/mL) are expressed as mean ± SEM.

Parasites

The lethal YM line of *P yoelii* strain 17X (from Dr A. Holder, National Institute of Medical Research, London, UK) was maintained in (C57BL/6 x BALB/c) F_1 mice by blood passage of parasitized red blood cells. Mice were bled 5 to 7 days after intravenous infection with 10^4 parasites and parasitemia was determined from blood films stained with Giemsa.

Extraction and Purification of GPIs

Parasitized blood (>90% parasitemia) was washed 3 times in sterile saline and centrifuged at 2,000 × *g* at 4°C for 15 minutes. The pellet was lysed by incubation in 0.01% saponin for 3 minutes at room temperature. Released parasites were washed 3 times with sterile saline by centrifugation at 2,000 × *g* at 4°C for 15 minutes. The pellet was suspended in saline, sonicated for 12 seconds, and freeze-dried. GPIs were extracted following a procedure described previously[26] with some modifications. Briefly, parasites (9×10^{10}) from 10 mice (0.1 g dry weight) were extracted twice with 10 mL chloroform:methanol:water (10:10:3) (CMW) and centrifuged for 15 minutes at 1,000 × *g*. Extracts were pooled, dried in a speed evaporator (Eyala, Tokyo, Rikakikai, Japan) and suspended in 5 mL water-saturated n-butanol. An equal volume of water was added, thoroughly mixed, and centrifuged for 10 minutes at 1,000 × *g*. The organic top layer containing GPIs was removed by aspiration and the bottom layer of water was back-extracted with an equal volume of water-saturated n-butanol. The organic phases were pooled and dried. Nonlipidic material was removed following a procedure described previously with some modifications.[27] Briefly GPIs were dried and suspended in 1 mL 5% 1-propanol containing 0.1 mol/L ammonium acetate and applied to a C_8 Bond Elute cartridge equilibrated with 5% 1-propanol containing 0.1 mol/L ammonium acetate. Each cartridge was then washed with 10 mL 5% 1-propanol containing 0.1 mol/L ammonium acetate. GPIs were eluted with 10 mL 40% 1-propanol and 10 mL 60% 1-propanol. Eluates containing GPI were pooled and concentrated in a Speed-Vac. GPIs were freeze-dried and stored at −20°C. Before use, the lipidic GPIs were sonicated in sterile saline for 12 seconds.

GPIs were dissolved in CMW (10:10:3) and applied to silica gel HPTLC plates. Plates were developed twice in a solvent containing chloroform:acetone:methanol:glacial acetic acid:water (50:20:10:10:5). GPIs were detected using orcinol and ninhydrin reagents. GPIs that remained at 0.7 cm from the origin were scraped and eluted with 2 × 20 mL methanol. Eluates were filtered through 0.2 μm filters, concentrated in a Speed-Vac, and stored at −20°C. Control preparations derived from the same number of normal red cell ghosts were made in parallel.

Inorganic phosphate in *P yoelii* GPIs was determined following the standard procedure,[28] and the content was assumed to equal the GPI concentration.

Lipogenesis Assay

Adipocytes were obtained from the epididymal adipose tissue of male Wistar rats (120 to 150 g body weight) by digestion with collagenase. Stimulation of lipogenesis was measured as the increased incorporation of [3-^{3}H] glucose into toluene-extractable lipids. Briefly, into a 96-multiwell plate, 100 μL of adipocyte suspension (3.5×10^5/mL) was incubated for 30 minutes at 37°C in a CO_2 incubator with 2 μL of various concentrations of *P yoelii* GPIs and/or insulin (10^{-8} mol/L). Lipogenesis was initiated by the addition of 100 μL of Krebs-Ringer Hepes buffer (KRH) containing 0.2 μCi D-[3-^{3}H] glucose, and the incubation continued for 2 hours. Adipocytes were harvested onto glass-fiber filter mats using a cell harvester and rinsed with 5 mmol/L glucose in 0.154 mol/L NaCl. A 3-mL toluene-based scintillation cocktail (BetaMax) was added to each filter disc for counting the radioactivity incorporated into lipids.

Enzyme Assays

HPTLC-purified *P yoelii* GPIs were sonicated in 100 μL water and tested for stimulation of pyruvate dehydrogenase (PDH)-phosphatase by a procedure described earlier.[29]

The ability of GPIs to inhibit protein kinase A (PKA) activity was determined using a colourimetric assay kit and a standard PKA preparation (Pierce, Rockford, IL).

Glucose-6-phosphatase (G6Pase) activity was measured using a method described previously[30] with some modifications. The assay mixture contained 900 μL Trinder reagent, 200 μg/mL liver microsomes, 2 or 10 mmol/L G6Pase, in the presence and absence of *P yoelii* GPIs (0 to 7 μmol/L). The rate of production of quinoneimine was followed spectrophotometrically at 505 nm and 28°C (Jasco V560 spectrophotometer; Jasco, Tokyo, Japan).

The activity of FBPase in the liver obtained from *db/db* mice pretreated with *P yoelii* GPIs was compared with that of saline-treated

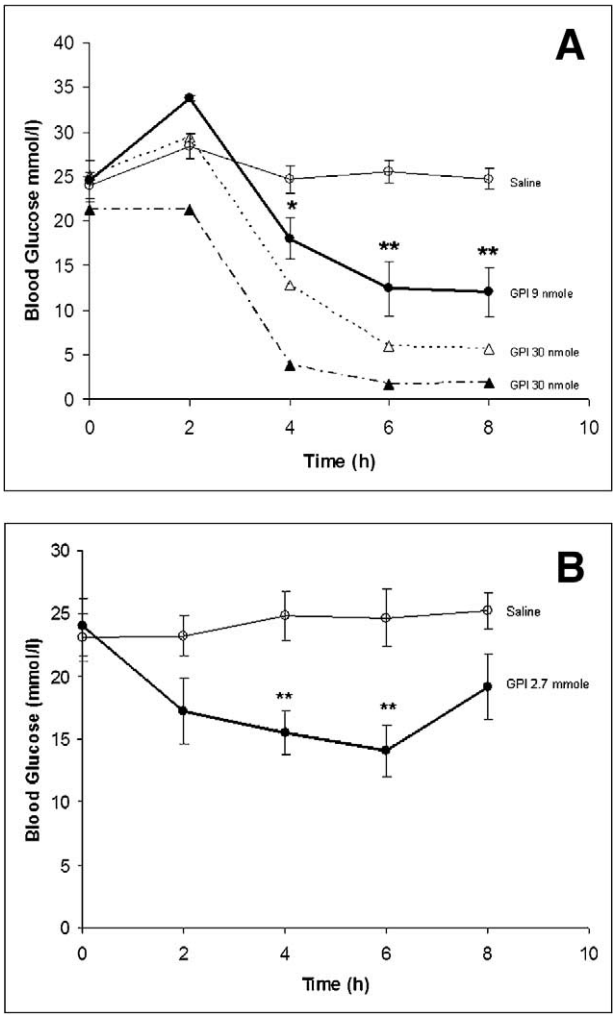

Fig 1. (A) Blood glucose concentrations in *db/db* diabetic mice given a single IV injection of *P yoelii* GPIs (9 nmol/mouse, n = 5) (●); *P yoelii* GPIs (30 nmol/mouse, n = 2) (△▲);or saline (○), n = 7. Values are mean ± SEM; **P* < .001, ***P* < .0001 *v* saline. (B) Blood glucose concentrations in *db/db* diabetic mice given a single oral dose of *P yoelii* GPIs (2.7 μmol/mouse, n = 6) (●); or saline (○), n = 8. Values are means ± SEM; ***P* < .01 *v* saline.

animals. FBPase activity was measured using a method described previously.[31]

Phosphoenolpyruvate carboxykinase (PEPCK) activity was measured using a reverse reaction of the standard method coupled with malate dehydrogenase (MDH).[32]

Statistical Analysis

Statistical significance was assessed using Student's *t* test. Values of $P < .05$ were considered statistically significant.

RESULTS

Effect of GPIs on Blood Glucose of db/db Diabetic Mice

P yoelii GPI (9 nmol/mouse, intravenous [IV]) induced a significant decrease in blood glucose from 26.85 ± 1.5 to 15.1 ± 0.5 mmol/L within 4 hours ($P < .001$, n = 5, Fig 1A) and lasted for at least another 4 hours. A higher dose of GPIs (30 nmol/mouse, IV in 2 mice) induced a larger decrease in blood glucose from 21.3 and 25.1 mmol/L to 1.9 and 5.7 mmol/L, respectively (Fig 1A). The blood glucose in saline-treated mice remained between 25 and 30 mmol/L during the course of the experiment. In another experiment, the decrease in blood glucose at 8 hours in a group of 6 db/db mice treated with 8 nmol GPI was associated with a nonsignificant increase in plasma insulin concentration (Table. 1). In a separate experiment *P yoelii* GPIs administered orally (2.7 μmol/mouse) induced a significant decrease in blood glucose at 4 and 6 hours in C57 Bl/Ks-*db/db* diabetic mice (Fig 1B, $P < .01$, n = 6). GPIs extracted from normal red blood cells prepared in parallel (10 to 20 nmol/mouse, IV) had no effect on blood glucose of *db/db*-diabetic mice (data not shown). In moderately diabetic STZ-treated mice with residual functioning pancreatic islets, *P yoelii* GPIs significantly increased plasma insulin secretion after 6 hours and caused a decrease in blood glucose (manuscript in preparation).

Effects of GPIs on Lipogenesis

Two batches of *P yoelii* GPIs stimulated basal lipogenesis in a dose-related manner, and batch B at a concentration as low as 1 μmol/L stimulated lipogenesis by more than 5-fold (Fig 2A). Insulin-stimulated lipogenesis was enhanced by *P yoelii* GPIs 0.062 to 1 μmol/L (Fig 2B).

Effect of GPIs on Enzymes of Gluconeogenesis and Glucose Oxidation

P yoelii GPIs (0.5 to 4.5 × 10^{-7} mol/L) stimulated PDH-Pase in a dose-dependent manner with an ED_{50} of 0.16 μmol/L. It modestly inhibited PKA by 23% and 40% at 2 and 6.25 × 10^{-6}mol/L. It did not inhibit hepatic PEPCK and FBPase (Table 1), but competitively inhibited hepatic microsomal G6Pase (Fig 3).

DISCUSSION

There is still a need for new antidiabetic agents, because long-term treatment with some thiazolidenediones and biguanides may result in secondary failure of efficacy with enhancement of obesity in 50% of patients.[33]

Hypoglycemia can be induced in normal mice by injection of water-soluble extracts of parasitized, but not of nonparasitized red blood cells.[7-9] These extracts are capable of synergizing with insulin in enhancing lipogenesis by adipocytes in vitro,[7] suggesting that *P yoelii* GPIs either sensitize cells to insulin action or act directly through a pathway different from that

Table 1. Effect of *P yoelii* GPIs on Blood Glucose, Plasma Insulin, and FBPase in *db/db* Mice

Treatment	No.	Blood Glucose (mmol/L)	Insulin (ng/mL)	Hepatic FBPase (mU/mg tissue)
GPI	6	17.57 ± 2.6*	13.6 ± 5.5 (NS)	74 ± 3 (NS)
Saline	9	30.71 ± 0.89	4.27 ± 1.2	76 ± 1

NOTE. Blood and livers were collected 8 hours after injection of *P yoelii* GPI (8 nmol/mouse IV). Data are mean ± SEM of the number of observations (n).

Abbreviation: NS, not significant.

**P* < .01 *v* saline.

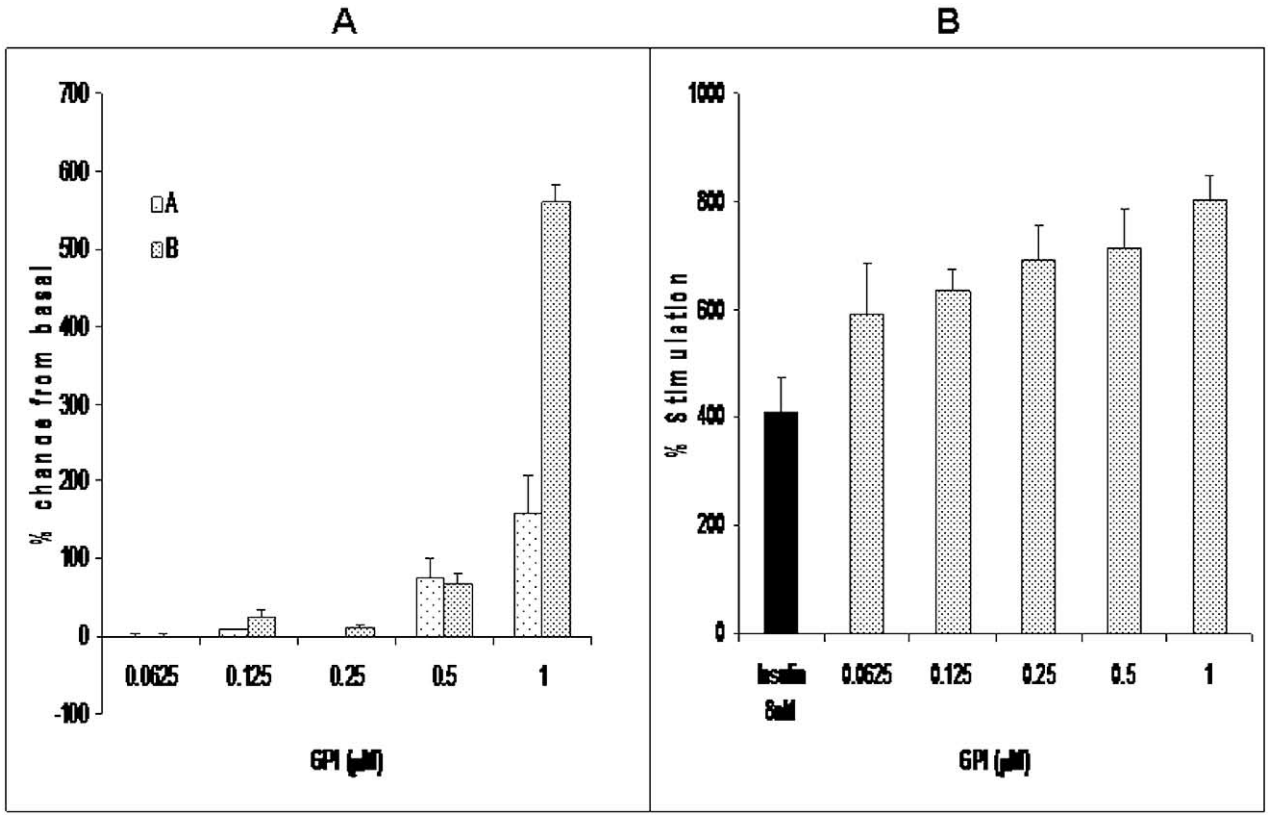

Fig 2. Dose-response of 2 different batches of *P yoelii* GPIs on (A) basal lipogenesis by rat and on (B) lipogenesis maximally stimulated with insulin (10^{-8} mol/L); (B). Values are mean ±SEM of 2 experiments each performed in quadruplicate. Basal lipogenesis and insulin-stimulated lipogenesis were 752 ± 31and 4,119 ± 84 cpm/3.5 × 10^4 cells/2 h, respectively.

activated by insulin. Based on inorganic phosphate content, *P yoelii* GPIs appear to be 5 to 10 times more potent stimulators of lipogenesis than IPGs.[9]

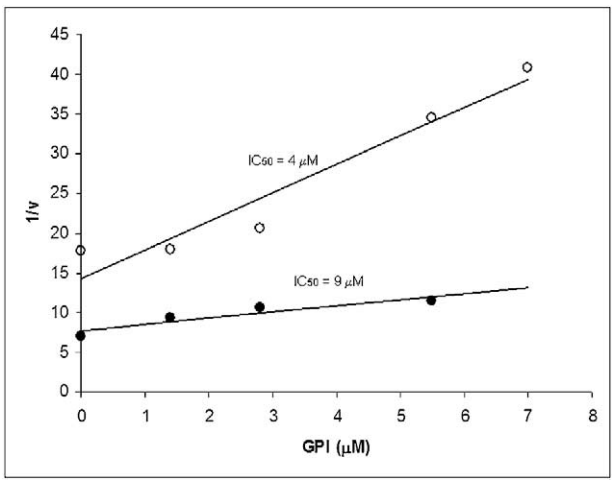

Fig 3. Dixon plot of *P yoelii* GPIs (1.4 to 7 μmol/L) on the activity of G6Pase using 2 concentrations of glucose-6-phosphate 10 mmol/L (●) or 2 mmol/L (○). Each point represents the mean from 2 experiments each performed in duplicate.

We administered *P yoelii* GPIs to genetically obese, insulin-resistant *db/db* mice and evaluated the changes in their blood glucose to confirm our previous findings with crude extracts of *P yoelii*.[8] The average blood glucose concentration of fed *db/db* mice was about 15 to 30 mmol/L being 3 to 4 times higher than those of non-obese littermates. Administration of a single dose of *P yoelii* GPIs induced a dose-dependent decrease in blood glucose and the improvement of hyperglycemia was observed 2 to 6 hours after treatment (Fig 1A). In the present study, *P yoelii* GPIs were found to be active orally after a single dose (Fig 1B). The hypoglycemic effect of *P yoelii* GPIs in *db/db* mice after 8 hours was associated with a nonsignificant increase in plasma insulin concentration (Table 1). Because *db/db* mice were used at the time of peak hyperinsulinemia, it may be speculated that the major effect of *P yoelii* GPIs is to amplify insulin action possibly through sensitization. This contrasts with the insulin-sensitive, moderately hypoinsulinemic STZ-diabetic mouse in which the glucose-lowering activity of *P yoelii* GPIs may be attributed to the increased insulin secretion from the residual islets (manuscript in preparation). In that study, GPIs extracted from normal red blood cells and from *Trypanosoma brucei* treated similarly to parasitized red blood cells had no effect on blood glucose in STZ-diabetic mice.

PDH-Pase has been shown to be activated by mammalian IPGs in vitro,[11,29] and the present study demonstrated that like

the IPGs, *P yoelii* GPIs stimulated PDH-Pase in a dose-related manner with an ED_{50} of 0.16 μmol/L. Like insulin, this stimulation would activate PDH, which would translate in vivo into increased glucose oxidation that may contribute to the lowering of blood glucose. Another insulin-like activity of *P yoelii* GPIs is inhibition of PKA and therefore of lipolysis in adipose tissue.[34]

The final step of gluconeogenesis and glycogenolysis is catalyzed by G6Pase located in the endoplasmic reticulum; this is a logical target for antidiabetic agents to suppress hepatic glucose production and ameliorate hyperglycemia. The rate of gluconeogenesis increases in the livers of *db/db* diabetic mice, and the increased activity of G6Pase was shown to be important for the development of hyperglycemia in *db/db* and STZ-diabetic mice[35] and in type 2 diabetic patients. In the present study, we measured the effect of *P yoelii* GPIs on the activity of the gluconeogenic enzyme G6Pase in vitro as a first step in elucidating the mechanism of the hypoglycemic effect. *P yoelii* GPIs showed significant G6Pase inhibition at concentrations as low as 0.1 μmol/L, with an IC_{50} of about 4 μmol/L. At this stage, it is not clear whether *P yoelii* GPIs affect glucose-6-phosphate translocase (T1), the endoplasmic glucose transporter (T2), or the inorganic phosphate transporter (T3). The present study is the first to demonstrate that *P yoelii* GPIs potently inhibit G6Pase in vitro. Because G6Pase is a crucial enzyme for the regulation of blood glucose homeostasis, this inhibition may at least contribute, but does not fully explain, the hypoglycemic activities of *P yoelii* GPIs. The insulin-sensitizing drug, troglitazone, was shown to inhibit the activity of hepatic G6Pase in vivo,[30] while the antidiabetic agent, tungstate, inhibited the activity of hepatic G6Pase in vitro.[36]

P yoelii GPIs had no effect on the activitiy of hepatic PEPCK in vitro. In addition, *P yoelii* GPIs appeared to have no effect on hepatic FBPase activity both in vitro and when given to *db/db* mice (8 nmol/mouse) (Table 1). This suggests that the modulation of gluconeogenesis by *P yoelii* GPIs is at the level of GPase.

P. falciparum GPIs activate protein tyrosine kinase $p59^{hck}$ and calcium-independent ϵ isoform of protein kinase C.[37] Both signals regulate gene expression of interleukin (IL)-1, tumor necrosis factor (TNF)-α and inducible nitric oxide (NO) synthase. However, *P yoelii* IPGs isolated by the method previously reported retain the insulin mimetic activity, but do not induce TNF-α in vitro.[38]

In view of the hyperinsulinemia observed during infection with *P yoelii* parasites,[5,6] *P yoelii* GPIs could be the mediators of this insulin-releasing activity. In the insulin-resistant *db/db* mice the lowering of blood glucose by *P yoelii* GPIs is unlikely to be due to increase in plasma insulin concentration. However, the lowering of blood glucose seen in STZ-diabetic mice treated with *P yoelii* GPIs could be partially explained by an increase in insulin secretion and by inhibition of gluconeogenic enzymes.

Partial characterization of *P yoelii* GPIs revealed that they are ninhydrin and orcinol positive, indicating the presence of amino groups and sugars, respectively. These molecules contain phosphate and, from Dionex analysis, they contain myoinositol, glycerol, glucosamine, galactosamine, galactose, and mannose (Elased et al. unpublished data).

In conclusion, acute administration of *P yoelii* GPIs demonstrated antidiabetic activities in animal models of type 2 diabetes. These data are consistent with GPIs playing a central role in glucose homeostasis and being a direct contributing factor in the modulation of lipogenesis, PDH-Pase, and PKA. When fully characterized, GPIs may provide useful information for the synthesis of drugs for the management of diabetes.

REFERENCES

1. World Health Organisation: Severe and complicated malaria. Trans R Soc Trop Med 84:1-65, 1990 (suppl 2)
2. White NJ, Warrell DA, Chanthavanich P, et al: Severe hypoglycemia and hyperinsulinemia in falciparum malaria. N EnglJ Med 309: 61-66, 1983
3. Phillips RE, Looareesuwan S, Molyneux ME, et al: Hypoglycaemia and counter regulatory hormone responses in severe falciparum malaria: Teatment with Sandostatin. QJM 86:233-240, 1993
4. Looareesuwan S, Phillips RE, White NJ, et al: Quinine and severe falciparum malaria in late pregnancy. Lancet 2:4-8, 1985
5. Elased K, Playfair JHL: Hypoglycemia and hyperinsulinemia in rodent models of severe malaria infection. Infect Immun 62:5157-5160, 1994
6. Elased K, de Souza JB, Playfair JHL: Blood-stage malaria infection in diabetic mice. Clin Exp Immunol 99:440-444, 1995
7. Taylor K, Bate CAW, Carr RE, et al: Phospholipid-containing toxic malaria antigens induce hypoglycaemia. Clin Exp Immunol 90: 1-5, 1992
8. Elased KM, de Souza JB, Playfair JHL: Reversal of tpe 2 diabetes in mice by products of malaria parasites.1. Effect of inactivated parasites. Metabolism 49:937-941, 2000
9. Elased KM, Gumaa KA, de Souza JB, et al: Reversal of type 2 diabetes in mice by products of malaria parasites. II. Role of inositol phosphoglycans (IPGs). Mol Genet Metab 49:937-941, 2001
10. Varela-Nieto I, Leon Y, Caro HN: Cell signaling by inositol phosphoglycans from different species. Comp Biochem Physiol 115B: 223-241, 1996
11. Rademacher TW, Caro HN, Kunjara S, et al: Inositolphosphoglycan second messengers. Braz J Med Biol Res 27:327-341, 1994
12. Thomas JR, Dwek RA, Rademacher TW: Structure, biosynthesis and function of glycosylphosphatidylinositols. Biochemistry 29: 5413-5422, 1990
13. Gerold P, Vivas L, Ogun SA, et al: Glycosylphosphatidylinositols of *Plasmodium chabaudi*: A basis for the study of malarial glycolipid toxins in a rodent model. Biochem J 328:905-911, 1997
14. Naik RS, Branch OH, Woods AS, et al: Glycosylphosphatidylinositol anchors of *Plasmodium falciparum*: Molecular characterization and naturally elicited antibody response that may provide immunity to malaria pathogenesis. J Exp Med 192:1563-1576, 2000
15. Martin-Lomas M, Khiar N, Garcia S, et al: Inositolphosphoglycan mediators structurally related to glycosylphosphatidylinositol anchors: Synthesis, structure and biological activity. Chemistry 6:3608-3621, 2000
16. Schofield JN, Rademacher TW: Structure and expression of the human glycosylphosphatidylinositol phospholipase D1 (*GPLD1*) gene. Biochim Biophys Acta 1494:189-194, 2000
17. Saltiel AR, Cuatrecasas P: Insulin mediates the generation from hepatic plasma membranes of modulators derived from an inositol glycolipid. Proc Natl Acad Sci USA 83:5793-5797, 1986
18. Mato JM, Kelly KL, Abler A, et al: Identification of a novel

insulin-sensitive glycophospholipid from H35 hepatoma cells. J Biol Chem 262:2131-2137, 1987

19. Romero G, Luttrell L, Rogol A, et al: Phosphatidylinositol-glycan anchors of membrane proteins: Potential precursors of insulin mediators. Science 240:509-511, 1988

20. Kunjara S, Wang DY, McLean P, et al: Inositol phosphoglycans and the regulation of the secretion of leptin: In vitro effects on leptin release from adipocytes and the relationship to obesity. Mol Genet Metab 70:61-68, 2000

21. Kunjara S, Wang D, Greenbaum AL, et al: Inositol phosphoglycans in diabetes and obesity: Urinary levels of IPG A-type and IPG P-type, and relationship to pathophysiological changes. Mol Genet Metab 68:488-502, 1999

22. Kunjara S, Greenbaum AL, Wang DY, et al: Inositol phosphoglycans and signal transduction systems in pre-eclampsia and diabetes. Evidence for a significant regulatory role in pre-eclampsia at placental and systemic levels. Mol Genet Metab 69:144-158, 2000

23. Tachado SD, Gerold P, McConville MJ, et al: Glycosylphosphatidylionsitol toxin of plasmodium induces nitric oxide synthase expression in macrophages and vascular endothelial cells by a protein tyrosine kinase-dependent and protein kinase C-dependent signalling pathway. J Immunol 156:1897-1907, 1996

24. Vijaykumar M, Naik RS, Gowda DC: *Plasmodium falciparum* glycosylphosphatidylinositol-induced TNF-α secretion by macrophages is mediated without membrane insertion or endocytosis. J Biol Chem 276:6909-6912, 2001

25. Schofield L, Hackett F: Signal transduction in host cells by a glycosylphosphatidylinositol toxin of malaria parasites. J Exp Med 177:145-153, 1993

26. Gerold P, Dieckmann-Schuppert A, Schwarz RT: Glycosylphosphatidylinositols synthesized by asexual erythrocytic stages of the malaria parasite, *Plasmodium falciparum.* Candidates for plasmodial glycosylphosphatidylinositol membrane anchor precursors and pathogenicity factors. J Biol Chem 269:2597-2606, 1994

27. Zawadzki J, Scholz C, Currie G, et al: The glycoinositolphospholipids from *Leishmania panamensis* contain unusual glycan and lipid moieties. J Mol Biol 282:287-299, 1998

28. Bartlett GR: Phosphorous assay in column chromatography. J Biol Chem 234:466-468, 1958

29. Caro HN, Kunjara S, Rademacher TW, et al: Isolation and partial characterisation of insulin-mimetic inositol phosphoglycans from human liver. Biochem Mol Med 61:214-228, 1997

30. Aoki K, Saito T, Satoh S, et al: Dehydroepiandrosterone suppresses the elevated hepatic glucose-6-phosphatase and fructose-1,6-bisphosphatase activities in C57BL/Ksj-*db/db* mice: Comparison with troglitazone. Diabetes 48:1579-1585, 1999

31. Ulm EH, Pogell BM, De Maine MM, et al: Fructose-1,6-diphosphatase from rabbit liver. Methods Enzymol 42:369-374, 1975

32. Pogson CI, Smith SA: The activity of phosphoenolpyruvate carboxykinase in rat tissues: Assay techniques and effects of dietary and hormonal changes. Biochem J 152:401-408, 1975

33. Turner R and the UKPDS Group: Effect of intensive blood-glucose control with Metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet 352:854-865, 1998

34. Castan I, Wijkander J, Manganiello V, et al: Mechanisms of inhibition of lipolysis by insulin, vanadate and peroxovanadate in rat adipocytes. Biochem J 339:281-289, 1999

35. Kodama H, Fujita M, Yamaguchi I: Differential hypoglycaemic effect of 2,5-anhydro-D-mannitol, a putative gluconeogenic inhibitor, in genetically diabetic (db/db) and streptozotocin-induced diabetic mice. Jpn J Pharmacol 66:331-336, 1994

36. Foster JD, Young SE, Brandt TD, et al: Tungstate: A potent inhibitor of multifunctional glucose-6-phosphatase. Arch Biochem Biophys 354:125-132, 1998

37. Tachado SD, Gerold P, Schwarz R, et al: Signal transduction in macrophages by glycosylphosphatidylinositols of *Plasmodium, Trypanosoma,* and *Leishmania*: Activation of protein tyrosine kinases and protein kinase C by inositolglycan and diacylglycerol moieties. Proc Natl Acad Sci USA 94:4022-4027, 1997

38. Caro HN, Sheikh NA, Taverne J, et al: Structural similarities among malaria toxins, insulin second messengers, and bacterial endotoxin. Infect Immun 64:3438-3441, 1996