Reversal of Type 2 Diabetes in Mice by Products of Malaria Parasites: I. Effect of Inactivated Parasites

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C57BL/KsJ-*db/db* and C57BL/KsJ-*ob/ob* mice are good models for studies on human obesity and type 2 diabetes. We have previously shown that infection with blood-stage malaria or injection of extracts from malaria-parasitized red blood cells induces hypoglycemia in normal mice and normalizes hyperglycemia in mice made moderately diabetic by streptozotocin. In the present study, we show that a single intravenous (IV) injection of Formalin-fixed *Plasmodium yoelii* YM (FFYM) preparation decreases blood glucose in *db/db* mice from an initial value of 19 mmol/L to a normal value of 7 mmol/L ($P < .0001$) for at least 24 hours and reduces food intake. Plasma insulin concentrations in *db/db* mice were not altered. FFYM was also active in normal and *ob/ob* mice, an effect associated with an increase in plasma insulin. Although the rate of weight gain in lean *ob/+* and lean *db/+* was not altered by this treatment, there was a significant reduction in weight gain in *db/db* and *ob/ob* mice ($P < .001$). We suggest that malaria-derived molecules, when fully characterized, may provide structural information for the development of new agents for the management of type 2 diabetes.

HYPOGLYCEMIA is a common complication of *Plasmodium falciparum* malaria, affecting primarily children and pregnant women.[1] Its mechanism of induction in malaria remains incompletely understood. Hypoglycemia can be reproduced in murine models of blood-stage malaria, and using these, we have shown that malaria infection or the injection of parasite extracts can induce hypoglycemia and hyperinsulinemia in normal mice,[2,3] while a parasite extract is also able to enhance insulin-mediated glucose uptake into adipocytes in vitro.[4] Furthermore, malaria-induced hypoglycemia can be prevented by agents that inhibit insulin secretion, namely diazoxide, somatostatin, and adrenaline.[5]

In a model of type 1 diabetes (streptozotocin-induced), malaria infection reduced blood glucose to normal levels, provided some residual islet function was present.[6] However, in mice made severely diabetic with streptozotocin, malaria parasite extracts did not affect hyperglycemia significantly.[6] It has also been reported that a European diabetic patient with non–insulin-dependent diabetes mellitus (NIDDM) developed hypoglycemia during an infection with *P falciparum* in Kenya (30% parasitemia). The patient received prophylactic doses of glucose for 3 days and the hypoglycemia resolved after eradication of the parasitemia.[7]

We therefore thought it of interest to determine whether malaria parasite–derived molecules also affect diabetic parameters in obese diabetic hyperglycemic hyperinsulinemic C57BL/Ks-*db/db* and C57BL/6J-*ob/ob* mice, widely regarded as good models for human NIDDM.[8-11]

MATERIALS AND METHODS

Mice

Obese diabetic C57BL/Ks-*db/db*, C57BL/6J-*ob/ob*, and lipopolysaccharide-unresponsive (C3H/HeJ) mice were obtained from Harlan Olac (Bicester, UK). We used female mice aged 8 to 12 weeks, when both blood glucose and insulin levels are markedly elevated.[11] Controls were lean age-matched, normoglycemic, heterozygous littermates (*db*/+ and *ob*/+). The normal (C57BL/6 × BALB/c) F1 mice were bred in our animal colony. Mice were allowed to acclimatize for at least 7 days before the study started. All animals had free access to water and were fed ad libitum with normal laboratory chow.

Parasites

The lethal YM line of *P yoelii* strain 17× (from Dr A. Holder, National Institute for Medical Research, London, UK) was maintained in our mice by blood passage of parasitized red blood cells. Mice were bled 5 to 7 days after intravenous (IV) infection with 10^4 parasites. Parasitized red blood cells (>90% parasitemia) were lysed by incubation in 0.01% saponin for 5 minutes at 37°C. Freed parasites were washed 3 times with phosphate-buffered saline (PBS), fixed overnight in 0.06% Formalin (Formalin-fixed *P yoelii* YM [FFYM]), washed again with PBS, counted by phase-contrast microscopy, resuspended at 5×10^8/mL in PBS containing streptomycin (100 µg/mL) and penicillin (100 IU/mL), and stored at 4°C. FFYM was either injected IV or IP (10^7 to 10^8 parasites in 0.2 mL) or fed orally (1 to 2×10^8 parasites) in a 0.2-mL vol via a gastric tube. Control preparations containing the same number of normal red blood cell ghosts were made by treating blood from uninfected mice with saponin and Formalin in exactly the same way.

Blood Glucose

Glucose concentrations were determined using Glucostix and an Ames Glucometer (Miles, Stoke Poges, UK) on a drop of tail blood collected between 10 AM and midday, or at intervals thereafter as indicated. Results are expressed as the mean ± SEM.

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 concentrations were determined in 50-µL vol of plasma by a double-antibody radioimmunoassay (kit supplied by ICN Biomedical, Irvine, CA) using a crystalline rat insulin standard (Novo, Bagsvaerd, Denmark). The results are expressed as the geometric mean with 95% confidence limits.

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Food Intake

The mean food intake per day was estimated by subtracting the weight of food remaining from the initial weight of food in the cage, and dividing the result by the number of mice housed in the cage (2 to 4).

Statistical Analysis

Statistical significance was assessed using Student's *t* test or the Mann-Whitney *U* test for unpaired samples as appropriate. *P* values less than .02 were considered significant.

RESULTS

Hypoglycemic Effect of FFYM in Normal Mice

IV injection of FFYM into 9 normal (C57BL/6 × BALB/c) F1 mice induced a dose-related decrease in blood glucose. FFYM (2.5×10^9/kg body weight [BW]) produced a highly significant decrease in blood glucose ($P < .001$). A more rapid hypoglycemic effect (blood glucose <2 mmol/L in 4 hours, $P < .001$) was observed with 5×10^9/kg BW, whereas 1.25×10^9/kg BW caused only a slight decrease in blood glucose. The highest dose of Formalin-fixed normal red blood cells (5×10^9/kg BW) had no effect on blood glucose or plasma insulin (Fig 1 and Table 1).

Hypoglycemia induced by FFYM is associated with hyperinsulinemia ($P < .0001$). Oral administration of FFYM (8×10^9/kg BW) also induced a significant ($P < .0001$) decrease in blood glucose (from 6.7 ± 0.2 to 3.7 ± 0.1 mmol/L within 7 to 8 hours, n = 6), indicating that the active molecules were not destroyed in the gut. IV injection of FFYM (2.5×10^9/kg BW) into lipopolysaccharide-unresponsive C3H/HeJ mice induced a highly significant decrease in blood glucose from 6.72 ± 0.12 to 1.02 ± 0.27 within 4 to 5 hours ($P < .0001$, n = 8), excluding possible bacterial lipopolysaccharides as the cause (Table 1).

Table 1. Effect of FFYM on Blood Glucose and Plasma Insulin

Group	Blood Glucose (mmol/L)	Plasma Insulin (ng/mL)
db/db + PBS	19.36 ± 0.7	66.06 (50.1, 87.1)
db/db + FFYM (1.25×10^9/kg BW)	7.83 ± 0.9†	69.18 (53.7, 89.12)
ob/ob + PBS	18 ± 0.51	64.56 (44.6, 93.3)
ob/ob + FFYM (1.25×10^9/kg BW)	6.74 ± 0.37†	123 (93.3, 162.2)*
F_1 + PBS	7.12 ± 0.17	2.278 (1.77, 2.918)
F_1 + FFYM (2.5×10^9/kg BW)	2.11 ± 0.26†	15.13 (10.47, 21.87)†
F_1 + FFNRBCs (5×10^9/kg BW)	6.94 ± 0.31	1.78 (1.44, 2.2)

NOTE. Blood glucose and plasma insulin 4 hours after a single IV injection of FFYM, Formalin-fixed normal red blood cells (FFNRBCs), or PBS in obese diabetic and normal (F_1) mice. Glucose data (mmol/L) are expressed as the arithmetic mean ± SEM. Plasma insulin is expressed as the geometric mean with 95% confidence limits. Insulin values were converted to logarithms (base 10) before statistical analysis (n = 12-14).

*$P < .01$, †$P < .0001$ *v* PBS-treated.

Hypoglycemic Effect of FFYM in Obese Diabetic ob/ob Mice

A group of 12 *ob/ob* mice and lean age-matched *ob*/+ mice were injected IP with FFYM (5×10^8/kg BW) daily for 2 weeks. Figure 2 shows that this dose of FFYM, which had no effect on blood glucose in lean mice (data not shown), prevented the significant increase ($P < .001$) in blood glucose in PBS-treated *ob/ob* mice. The same mice then received a single IV injection of a larger dose of FFYM (1.25×10^9/kg BW) and blood glucose concentrations were determined for a further 24 hours. IV administration of this dose of FFYM induced a slight decrease in blood glucose in *ob*/+ mice, an

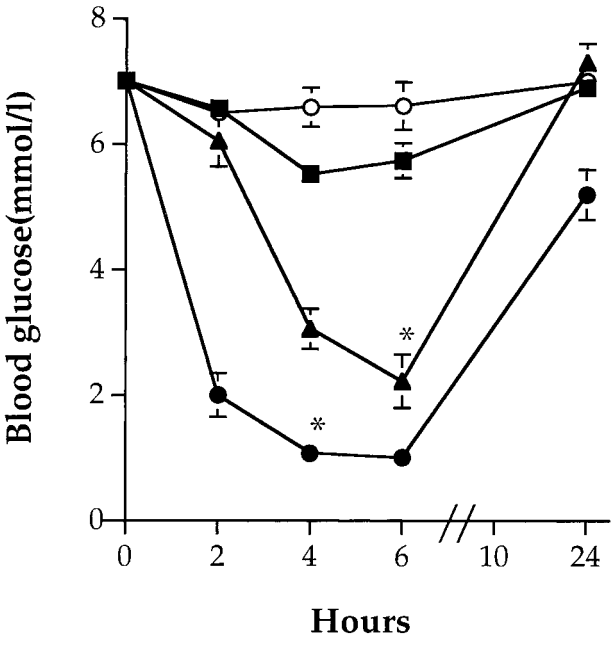

Fig 1. Effect of a single IV injection of FFYM (■, 1.25×10^9/kg; ▲, 2.5×10^9/kg; ●, 5×10^9/kg) and Formalin-fixed normal erythrocytes (○, 5×10^9/kg) on blood glucose in normal (C57BL/6 × BALB/c) F_1 mice (mean ± SEM, n = 9). *$P < .0001$ *v* PBS-treated controls.

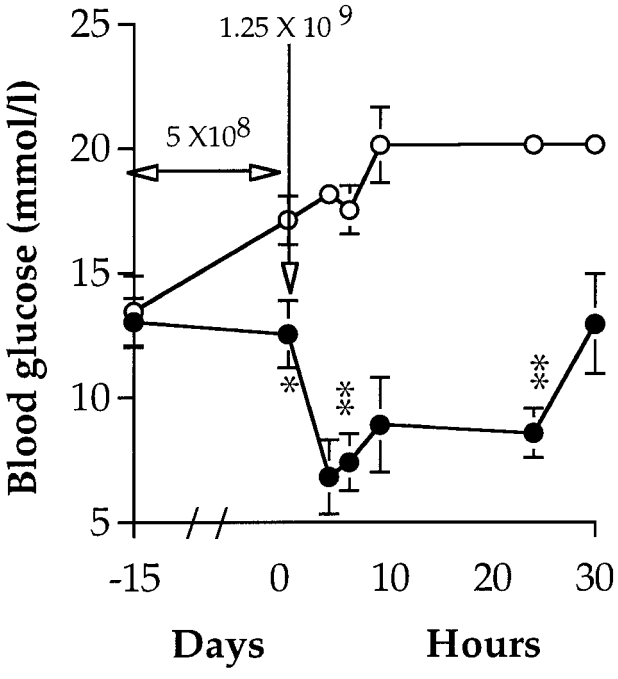

Fig 2. Effect of 5×10^8 FFYM/kg injected IP daily for 14 days followed by 1.25×10^9/kg injected IV on day 15 on blood glucose in *ob/ob* diabetic mice (●) as indicated by arrows compared with PBS-treated mice (○) (mean ± SEM, n = 6-8). *$P < .001$, **$P < .0001$.

effect that lasted for 2 hours (data not shown). However, the same dose induced a highly significant ($P < .0001$) decrease in blood glucose in *ob/ob* mice and normalized the hyperglycemia for at least 24 hours (Fig 2).

In a separate experiment, a single IV injection of FFYM (1.25×10^9/kg BW) in *ob/ob* mice induced a highly significant decrease in blood glucose within 4 hours ($P < .0001$) and a significant increase in plasma insulin ($P < .01$; Table 1).

Food Intake and BW

FFYM (5×10^8/kg BW) was given IP to *ob/ob* diabetic mice and lean-matched *ob*/+ littermates daily for 14 days, followed by 1.25×10^9/kg BW IV on day 15 and IP on days 16 and 17 (Fig 3A and B). BW and food intake were measured every 24 hours. Administration of FFYM resulted in a dose-dependent decrease in food intake, which was highly significant compared with PBS-treated controls ($P < .001$; Fig 3A). These results suggest that *ob/ob* mice, like *db/db* mice, were more sensitive to FFYM than the lean controls (*ob*/+) whose food intake was not altered by a similar treatment (data not shown).

There was also a significant difference in BW between FFYM-treated *ob/ob* mice and PBS-control *ob/ob* mice ($P < .001$). The rate of weight gain was not altered in lean *ob*/+ mice (Fig 3B).

Hypoglycemic Effect of FFYM in Obese Diabetic db/db Mice

A single IV injection of FFYM (1.25×10^9/kg BW) into 12 obese diabetic (C57BL/KsJ *db/db*) mice induced a highly significant decrease in blood glucose from a basal level of 19.4 ± 0.7 mmol/L to a normal level of 7.1 ± 0.8 mmol/L, an effect that lasted at least 24 hours ($P < .0001$; Fig 4). This dose of FFYM had no significant effect in matched lean *db*/+ mice, while 2.5×10^9/kg BW IV caused a decrease in blood glucose in 4 hours from 6.5 ± 0.32 to 3.24 ± 0.17 mmol/L. In a separate experiment (Table 1), a single injection of FFYM (1.25×10^9/kg BW IV) in 12 *db/db* mice induced a highly significant decrease in blood glucose within 4 hours ($P < .001$), with no change in the already very high plasma insulin concentration.

Oral Treatment With FFYM

When FFYM (4×10^9/kg BW) was given daily for 1 week as a single oral bolus to 6 *db/db* mice, there was a significant decrease in blood glucose compared with 6 controls fed with PBS ($P < .001$; Fig 5A). This treatment led to suppression of food intake (at day 7, there was a 30% reduction in food consumption *v* day 0, while there was an 8% increase with PBS; Fig 5B).

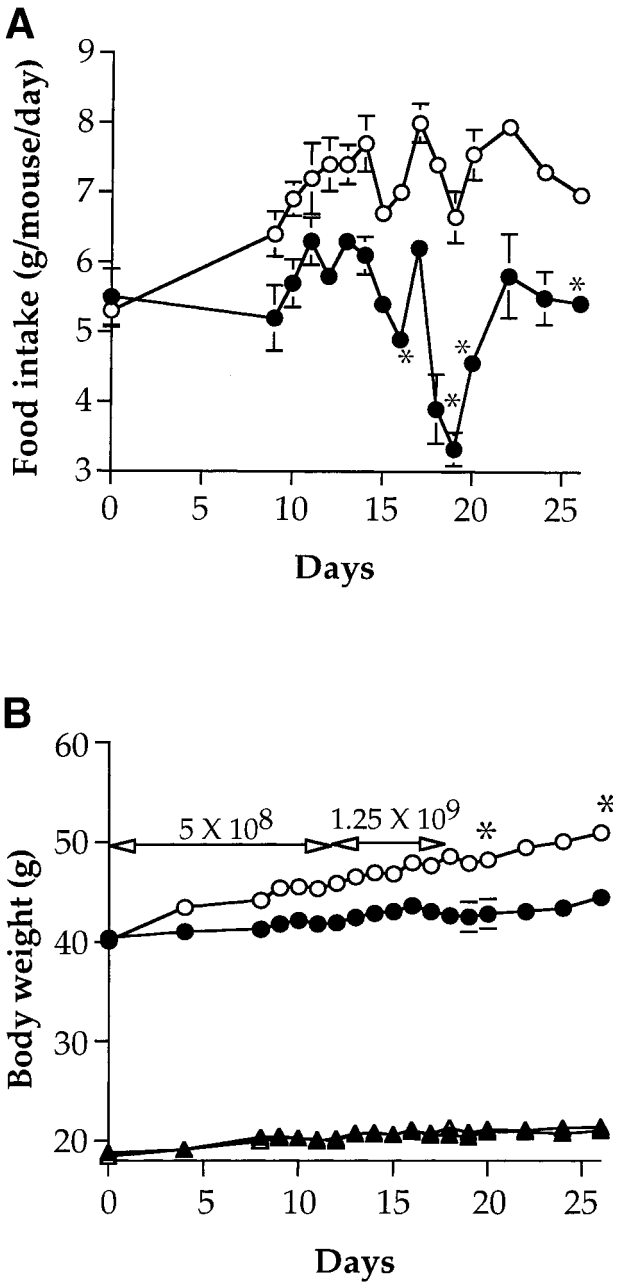

Fig 3. Effect on food intake (A) and BW (B) of *ob/ob* diabetic mice (●) and lean age-matched *ob*/+ heterozygotes (▲) of 5×10^8 FFYM/kg injected IP daily for 14 days, followed by 1.25×10^9/kg IV on day 15 and IP on days 16 and 17 as indicated by arrows, compared with PBS-treated controls (○), respectively (mean ± SEM, n = 6-8). *$P < .001$.

DISCUSSION

Type 2 diabetes (NIDDM) is a common and complex disorder which results from a variable combination of defects in insulin secretion and impaired insulin sensitivity in peripheral tissues. NIDDM is characterized by hyperglycemia in both the fasted and fed states, variable degrees of hyperinsulinemia, and obesity. Current therapy includes diet and exercise, sulfonylureas to enhance insulin secretion, insulin itself, and biguanides and thiazolidinediones to reduce insulin resistance. There is a need for new antidiabetic agents, since biguanides are toxic and sulfonylureas are ineffective in patients with severely impaired islet cell function. Long-term treatment with these agents may result in a secondary failure of efficacy with an enhancement of obesity in 50% of patients.[12]

Hypoglycemia is a recognized complication of infection with the malaria parasite, *P falciparum.* Although often attributed to starvation and quinine therapy[1] or to overproduction of tumor necrosis factor (TNF), it can be reproduced in animal models of

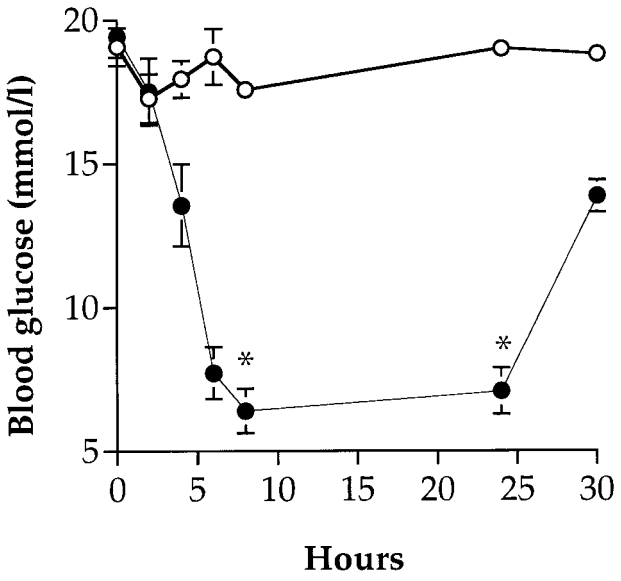

Fig 4. Effect of a single IV injection of 1.25 × 10⁹ FFYM/kg on blood glucose in *db/db* obese diabetic mice (●) compared with controls given PBS (○) (mean ± SEM, n = 6-8). *$P < .0001$.

malaria in the absence of any of these factors.[13] Infection with the lethal murine parasite *P yoelii YM* or the nonlethal parasite *P chabaudi* induces severe hypoglycemia at the time of high parasitemia and is associated with marked hyperinsulinemia.[3] Moreover, hypoglycemia can be induced in normal mice by injection of soluble extracts of parasitized (but not nonparasitized) red blood cells.[2] Similar extracts are capable of synergizing with insulin in enhancing glucose uptake in adipocytes in vitro,[4] and of inducing insulin secretion from isolated pancreatic islets (K.M. Elased, unpublished data, December 1996). We have therefore investigated the potential of malaria parasites and extracts as a source for new molecules for the management of diabetes. As a model of type 1 diabetes, we treated mice with streptozotocin to destroy islet tissue and showed that malaria infection could reduce the resulting hyperglycemia to normal levels, provided some residual islet function is present.[6]

The present results are the first to establish that FFYM has antidiabetic actions in murine models of type 2 diabetes. We used the diabetic mouse strains C57BL/KsJ-*db/db* and C57BL/6J-*ob/ob*, which exhibit many of the classic metabolic disturbances of human type 2 diabetes including hyperglycemia, obesity, and early hyperinsulinemia.[8-11] The dose of FFYM had no significant effect on plasma insulin levels in *db/db* mice. Since these diabetic mice were used at the time of peak hyperinsulinemia, it may be speculated that the major effect of FFYM is to amplify insulin action, possibly through sensitization. This agrees with an earlier study in vitro which demonstrated synergy for a malaria extract with insulin in rat adipocytes.[4]

The primary defective gene of *ob/ob* mice which produces the diabetic/obese syndrome has been cloned.[14] The ob gene product, leptin, is an adipocyte-derived protein that regulates BW.[15,16] Although recombinant leptin corrects hyperphagia and obesity in *ob/ob* mice, which do not produce endogenous leptin, it has no effect in obese diabetic *db/db* mice, which have an increased level of circulating leptin and do not respond to treatment with recombinant leptin.[15,16] It is thought that the defect in these mice may be at the level of the leptin receptor.[8-10,17] The effect of the malaria-derived product on food

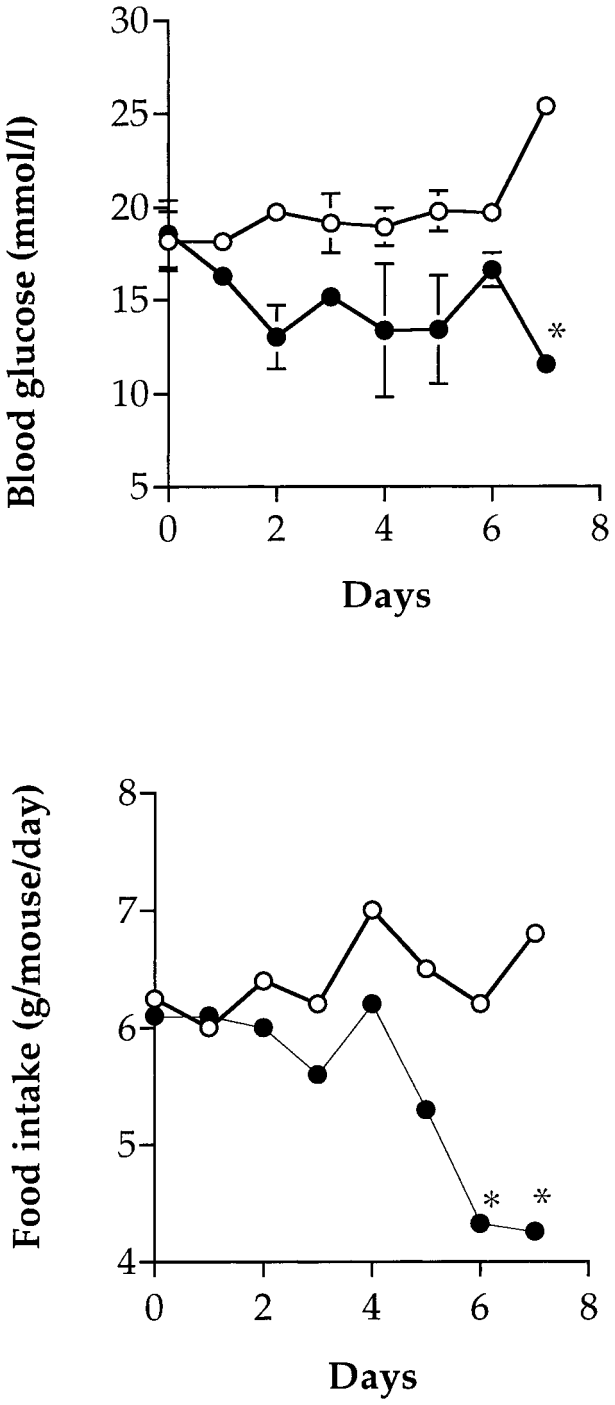

Fig 5. Effect on blood glucose (A) and food intake (B) of *db/db* obese diabetic mice given 5 × 10⁹ FFYM/kg orally daily for 7 days (●) compared with controls given PBS (○) (mean ± SEM, n = 6-8). *$P < .001$.

intake and BW suggests that it may directly or indirectly inhibit appetite, as shown in other infections.[18,19] However, this is unlikely due to the induction of leptin, since *db/db* mice do not respond to this protein.[15,16] Expression of leptin is increased in response to endotoxin, TNF, and interleukin-1, suggesting a role for leptin in the anorexia of infection.[18] However, malaria products can induce hypoglycemia in the presence of pentoxyfylline or antibody against TNF,[2,13] which seems to exclude TNF as a major cause of the hypoglycemic effect of FFYM.

Although the hypoglycemic effect of FFYM in normal mice appears to be secondary to the hyperinsulinemia, decreased caloric intake may contribute to the hypoglycemic effect after chronic treatment, since a reduction in BW decreases the need for insulin and improves insulin sensitivity. Although our malaria preparation caused a slight increase in plasma insulin in *db/db* mice, this is unlikely to account for the hypoglycemic effect, since pretreatment insulin levels in these mice were already high in presence of substantial hyperglycemia (Table 1). Although injection of insulin controls hyperglycemia in young *db/db* mice, once blood glucose exceeds 14 mmol/L, injection of insulin greater than 100 U/100 g no longer normalizes blood glucose.[8] It seems likely that the main effect of the malaria preparation was to reduce insulin resistance, with an additional effect in reducing appetite after chronic treatment.

The molecular nature of the malaria-derived factors (MDFs) active in our experiments may be related to inositol phosphoglycans, since preliminary structural evidence suggests that the active MDFs are nonprotein, can be labeled with radioactive inositol, and contain phosphate and hexoses. A parallel study in which malaria extracts induced hypersecretion of TNF by macrophages also indicated that the active component was a phospholipid containing inositol,[2] which copurified with P-type inositol phosphoglycan–like insulin second messengers.[20] Experiments with glucose uptake into adipocytes suggested similar but probably not identical molecules with several effects on glucose metabolism; the components of ''malaria toxin'' preparations that induced TNF were different from those that induced hypoglycemia and synergized with insulin.[21,22] We propose that when fully characterized, the latter might form the basis for treatment of both type 1 and type 2 diabetes. Indeed, one case has been reported in which *P falciparum* infection induced hypoglycemia not related to quinine therapy in a patient with NIDDM.[7]

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REFERENCES

1. White NJ, Warrell DA, Chanthavanich P, et al: Severe hypoglycemia and hyperinsulinemia in falciparum malaria. N Engl J Med 309:61-66, 1983

2. Taylor K, Bate CAW, Carr RE, et al: Phospholipid-containing toxic malaria antigens induce hypoglycaemia. Clin Exp Immunol 90:1-5, 1992

3. Elased K, Playfair JHL: Hypoglycemia and hyperinsulinemia in rodent models of severe malaria. Infect Immun 62:5157-5160, 1994

4. Taylor K, Carr R, Playfair JHL, et al: Malarial toxic antigens synergistically enhance insulin signalling. FEBS Lett 311:231-234, 1992

5. Elased KM, Playfair JHL: Reversal of hypoglycaemia in murine malaria by drugs that inhibit insulin secretion. Parasitology 112:515-521, 1996

6. Elased K, de Souza JB, Playfair JHL: Blood-stage malaria infection in diabetic mice. Clin Exp Immunol 99:440-444, 1995

7. Shalev O, Tsur A, Rahav G: Falciparum malaria–induced hypoglycaemia in a diabetic patient. Postgrad Med J 68:281-282, 1992

8. Coleman DL, Hummel KP: Studies with the mutation, diabetes, in the mouse. Diabetologia 3:238-248, 1967

9. Coleman DL: Lessons from studies with genetic forms of diabetes in the mouse. Metabolism 32:162-164, 1982

10. Coleman D: Obese and diabetes: Two mutant genes causing diabetes-obesity syndrome in mice. Diabetologia 14:141-148, 1978

11. Shafrir E: Development and consequences of insulin resistance: Lessons from animals with hyperinsulinaemia. Diabetes Metab 22:122-131, 1995

12. Rachman J, Turner RC: Drugs on the horizon for treatment of type 2 diabetes. Diabet Med 12:467-478, 1995

13. Elased KM, Taverne J, Playfair JHL: Malaria, blood glucose, and the role of tumour necrosis factor (TNF) in mice. Clin Exp Immunol 105:443-449, 1996

14. Zhang Y, Proenca R, Maffei M, et al: Positional cloning of the mouse *obese* gene and its human homologue. Nature 372:425-432, 1994

15. Halaas JL, Gajiwala KS, Maffei M, et al: Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543-546, 1995

16. Pelleymounter MA, Cullen MJ, Baker MB, et al: Effects of the obese gene product on body weight regulation in *ob/ob* mice. Science 269:540-543, 1995

17. Chen H, Charlat O, Tartaglia LA, et al: Evidence that the diabetes gene encodes the leptin receptor: Identification of a mutation in the leptin receptor gene in *db/db* mice. Cell 84:491-495, 1996

18. Grunfeld C, Zhao C, Fuller J, et al: Endotoxin and cytokines induce expression of leptin, the *ob* gene product, in hamsters. J Clin Invest 97:2152-2157, 1996

19. Nabel GJ, Grunfeld C: Calories lost—Another mediator of cancer cachexia? Nat Med 2:397-398, 1996

20. 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

21. Taverne J, Sheikh N, Elased K, et al: Malaria toxins: Hypoglycaemia and TNF production are induced by different components. Immunol Today 11:462-463, 1995

22. Taverne J, Sheikh N, Elased K, et al: Malaria toxins: TNF-mediated phenomena. Parasitol Today 12:290, 1996 (letter)