Original Article

Inhibition of Fructose 1,6-Bisphosphatase Reduces Excessive Endogenous Glucose Production and Attenuates Hyperglycemia in Zucker Diabetic Fatty Rats

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Gluconeogenesis is increased in type 2 diabetes and contributes significantly to fasting and postprandial hyperglycemia. We recently reported the discovery of the first potent and selective inhibitors of fructose 1,6-bisphosphatase (FBPase), a rate-controlling enzyme of gluconeogenesis. Herein we describe acute and chronic effects of the lead inhibitor, MB06322 (CS-917), in rodent models of type 2 diabetes. In fasting male ZDF rats with overt diabetes, a single dose of MB06322 inhibited gluconeogenesis by 70% and overall endogenous glucose production by 46%, leading to a reduction in blood glucose of >200 mg/dl. Chronic treatment of freely feeding 6-week-old male Zucker diabetic fatty (ZDF) rats delayed the development of hyperglycemia and preserved pancreatic function. Elevation of lactate (~1.5-fold) occurred after 4 weeks of treatment, as did the apparent shunting of precursors into triglycerides. Profound glucose lowering (~44%) and similar metabolic ramifications were associated with 2-week intervention therapy of 10-week-old male ZDF rats. In high-fat diet–fed female ZDF rats, MB06322 treatment for 2 weeks fully attenuated hyperglycemia without evidence of metabolic perturbation other than a modest reduction in glycogen stores (~20%). The studies confirm that excessive gluconeogenesis plays an integral role in the pathophysiology of type 2 diabetes and suggest that FBPase inhibitors may provide a future treatment option. *Diabetes* 55:1747–1754, 2006

Increased endogenous glucose production is a common abnormality associated with type 2 diabetes that, in conjunction with poor pancreatic function and reduced glucose disposal, contributes to the hyperglycemia characteristic of the disease (1–3). The liver is the primary site of endogenous glucose production and produces glucose either de novo from 3-carbon precursors (gluconeogenesis) or via the breakdown of glycogen stores (glycogenolysis). Recent studies using magnetic resonance spectroscopy and/or the deuterated water method suggest that gluconeogenesis is largely responsible for the overproduction of glucose in fasting type 2 diabetic patients, whereas glycogenolysis is either unchanged or even reduced (4–6). In the postprandial state, reduced suppression of endogenous glucose production contributes significantly to the impaired glucose tolerance of type 2 diabetes (7–9).

Because endogenous glucose production rates correlate well with the severity of fasting hyperglycemia in type 2 diabetic patients with mild or advanced disease (10–12), and because endogenous glucose production plays a predominant role in postprandial hyperglycemia, gluconeogenesis and glycogenolysis represent potential targets for pharmacological intervention. None of the currently marketed drugs for type 2 diabetes modulate endogenous glucose production directly, although indirect effects have been described. Metformin, for instance, is believed to partially inhibit gluconeogenesis (13), potentially by indirect effects on AMP-activated protein kinase (14). Exenatide's actions include insulin release and the attenuation of glucagon release (15), which are expected to result in partial inhibition of endogenous glucose production. Lowering of nonesterified free fatty acids (NEFAs) and/or amelioration of hepatic insulin resistance via a reduction in liver fat may contribute to reduced endogenous glucose production in patients treated with thiazolidinediones (16–18).

A variety of novel pharmacological approaches for the indirect and direct inhibition of endogenous glucose production are currently being explored. Indirect approaches include the dipeptidyl peptidase-IV inhibitors, which augment insulin and suppress glucagon secretion (19); glucagon and glucocorticoid antagonists, which block the stimulatory actions of the corresponding endogenous hormones on specific hepatic receptors (20,21); 11-β-hydroxysteroid dehydrogenase inhibitors, which reduce circulating glucocorticoids (22); and pyruvate dehydrogenase kinase inhibitors, which reduce gluconeogenesis precursor supply (23). Glucokinase activators have also been described that reduce the rate of endogenous glucose production by promoting hepatic glucose uptake and stimulating insulin release (24). Direct approaches target key enzymes in glycogenolysis or gluconeogenesis, such as glycogen phosphorylase (25), glucose-6-phosphatase translocase (26), or PEPCK (27). Of these direct inhibitors of endogenous glucose production, to date only glycogen phosphorylase inhibitors have been evaluated clinically.

We recently reported the rational design and character-

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FBPase, fructose 1,6-bisphosphatase; NEFA, nonesterified free fatty acid.

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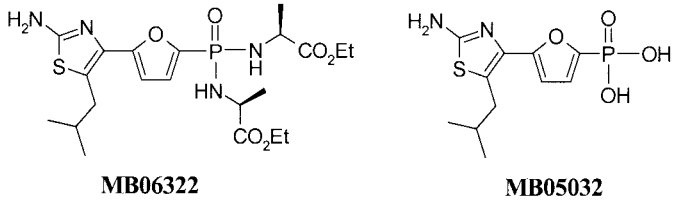

FIG. 1. Structures of MB06322 and MB05032. MB06322 is an oral prodrug of MB05032, a potent FBPase inhibitor (IC_{50} [half-maximal inhibitory concentration] 16 nmol/l) (26) that binds to the allosteric AMP-binding site of the enzyme. MB06322 is converted to MB05032 in vivo via the sequential actions of an esterase and phosphoramidase.

ization of the first potent, direct, and selective inhibitors of fructose 1,6-bisphosphatase (FBPase), a rate-controlling enzyme in the pathway of gluconeogenesis (28) that has been targeted by the pharmaceutical industry since the 1970s. MB06322 (CS-917), the lead compound, lowers both fasting and postprandial blood glucose after acute administration to male Zucker diabetic fatty (ZDF) rats without affecting insulin secretion. Herein we describe the acute effects of FBPase inhibition on gluconeogenesis and glycogenolysis rates in male ZDF rats and the metabolic consequences of long-term and profound FBPase inhibition in male and high-fat diet–fed female ZDF rats.

RESEARCH DESIGN AND METHODS

MB06322 (CS-917) (Fig. 1) was synthesized at Metabasis Therapeutics and formulated in carboxymethylcellulose (0.1%) for acute oral administration.

Male ZDF rats were purchased from Genetic Models (Indianapolis, IN) at ~5 or ~10 weeks of age and allowed to acclimate 1–2 weeks before evaluation. They were maintained on Formulab 5008 rat chow (PMI Nutrition International, Brentwood, MO). Female ZDF rats (Charles River, Kingston, NY) were purchased at 6 weeks of age and placed on a diet containing 48% kcal fat (diet 13004; Research Diets, New Brunswick, NJ) for 30 days before evaluation. All animals were housed under a 12-h lighting cycle (7:00 A.M. to 7:00 P.M. light) in a temperature-controlled environment (21°C) and had access to drinking water ad libitum. All experiments were conducted in accordance with the *Guidelines for the Care and Use of Laboratory Animals* of the National Institutes of Health, and all protocols were approved by the institutional animal care and use committee.

Dose rationale. High doses of MB06322 (~100–400 mg/kg) were deliberately administered in the acute and chronic studies to accentuate the effects of gluconeogenesis inhibition on carbohydrates, lactate, lipids, and other aspects of metabolism. The minimum dose that results in significant suppression of de novo glucose synthesis, as assessed by a qualitative [^{14}C]bicarbonate tracer technique, is 30 mg/kg, whereas a maximal response is achieved at 300 mg/kg (28). Only partial suppression of de novo glucose synthesis (15–30%) is required for a significant glucose-lowering response. Because of species differences at the target enzyme level, MB06322 inhibits gluconeogenesis approximately sevenfold more potently in human versus rat hepatocytes (28).

Rate of endogenous glucose production in conscious male ZDF rats. The rate of endogenous glucose production was determined using standard tracer dilution techniques (29). Male ZDF rats (10–11 weeks old) were fasted at 5 A.M. and instrumented with tail vein and artery catheters under brief halothane anesthesia. Food was withheld for the remainder of the protocol. A 16-μCi priming dose of 6-[^{3}H]glucose (48 μCi/ml saline; Moravek, Brea, CA) was administered intravenously at 6:00 A.M. and was followed by a maintenance infusion at 8 μCi/h. To better simulate physiological conditions, neither blood glucose nor insulin was clamped. Baseline samples were taken from the arterial lines at 7:30 A.M. and 8:00 A.M., rats were divided into glucose-matched groups (n = 6 per group), and vehicle or MB06322 (300 mg/kg) was administered orally. To evaluate the specific activity of blood glucose, arterial blood samples were obtained at 1:00 and 3:00 P.M. The specific activity of glucose was determined as previously described (29). Liver samples were removed under halothane anesthesia from a subset of animals at the end of the procedure (n = 4 per group) and were snap frozen for determination of glycogen content.

Fractional contribution of gluconeogenesis to endogenous glucose production in conscious male ZDF rats. To avoid the stress and potential disruption of carbohydrate metabolism associated with multiple simultaneous procedures, this assessment was conducted in separate groups of animals that were matched closely in terms of age, weight, and glycemia to the groups used in the endogenous glucose production determinations. Male ZDF rats (~10 weeks old) were fasted at 6:00 A.M., and food was withheld for the remainder of the protocol. At 8:00 A.M., baseline blood glucose was determined in blood samples obtained from the tail vein. Rats were subsequently divided into glucose-matched groups (n = 6 per group) and gavaged with either vehicle or MB06322 (300 mg/kg). Deuterated water (16 ml/kg; Isotec, Miamisburg, OH) was administered intraperitoneally under light halothane anesthesia at 9:00 A.M. Blood samples (0.5 ml) were obtained via a tail vein catheter inserted under brief halothane anesthesia at 1:00 and 3:00 P.M. Deuterium enrichment at the C2 and C5 position of glucose was measured and the fractional contribution of gluconeogenesis to endogenous glucose production determined as previously described (30,31).

Rate calculations. The rate of endogenous glucose production was calculated by means of the Steele equation (32) to compensate for the delivery of tracer to a changing systemic glucose pool. The rates of gluconeogenesis and glycogenolysis were determined by multiplying the fractional contributions of each in individual rats by the average rate of endogenous glucose production in control and treated animals, as appropriate.

Prevention and intervention treatments, male ZDF rats. Male ZDF rats were divided into three blood glucose– and weight-matched groups (n = 8 per group) at ~6 weeks of age just before the onset of overt hyperglycemia and hyperinsulinemia. One group was provided powdered Formulab 5008 chow and a second the same chow mixed with MB06322 (0.4%, wt/wt). The third matched group received powdered chow for 4 weeks and was then switched to the MB06322 food admixture. Biochemical and physiological parameters were measured weekly, at minimum. Animals were housed singly in metabolic cages at prespecified times (see Table 2) for the assessment of glycosuria and measurement of food and water intake under controlled conditions. Oral glucose tolerance was tested by administration of a 2-g/kg oral bolus of glucose after a 6-h fast. Drug treatment was withheld after 6 weeks (prevention) or 2 weeks (intervention) of treatment, and blood glucose and lactate were monitored weekly for 3 weeks thereafter.

Intervention treatment, high-fat diet–fed female ZDF rats. Rats were divided into three glucose- and weight-matched groups (n = 8 per group) and treated for 14 days either with powdered control diet or diet mixed with an appropriate amount of MB06322 to achieve daily doses of 100 or 300 mg/kg. Blood samples were taken at 8:00 A.M. and/or 4:00 P.M. via a tail vein nick for analysis of biochemical parameters. At the end of treatment, livers were harvested and snap frozen for analysis of glycogen and triglyceride content, and plasma samples were prepared for clinical chemistry analysis. Food intake and body weight were monitored on a regular basis throughout the study.

Biochemical analyses. Blood glucose was measured in blood samples obtained by nicking the tail vein, using a glucose analyzer (HemoCue, Mission Viejo, CA) or OneTouch blood glucose meter (Lifescan, Milpitas, CA). Blood lactate, plasma triglycerides, and plasma cholesterol were measured, using standard kits from ThermoDMA (Arlington, TX). Plasma NEFAs and β-hydroxybutyrate were analyzed, using a kit obtained from Wako Diagnostics (Richmond, VA). Plasma insulin and glucagon were determined by enzyme-linked immunosorbent assay (Alpco Diagnostics, Windham, NH) and radioimmunoassay (Linco, St. Charles, MO), respectively. Liver triglycerides and glycogen content were determined as previously described (33,34). Clinical chemistry analysis of plasma samples was performed at BTS (San Diego, CA).

Statistical analysis. Results are expressed as the means ± SE. Statistical significance was determined with Student's t test or repeated-measures ANOVA followed by Dunnett's post hoc test as appropriate. Statview software (SAS Institute, Cary, NC) was used.

RESULTS

Acute inhibition of endogenous glucose production and gluconeogenesis in fasting conscious male ZDF rats. Baseline endogenous glucose production was 24.1 ± 2.8 and 23.0 ± 2.5 mg · kg^{-1} · min^{-1} for the vehicle- and MB06322-treated groups, respectively (Fig. 2*A*). A maximal dose of MB06322 (300 mg/kg) reduced endogenous glucose production by 57%, to 9.8 ± 1.0 mg · kg^{-1} · min^{-1}, whereas endogenous glucose production in vehicle-treated animals declined ~25%, to 18.2 ± 3.5 mg · kg^{-1} · min^{-1}. Endogenous glucose production was thus reduced ~46% in the MB06322-treated group relative to the vehicle-treated group. Consistent with the inhibition of endogenous glucose production, blood glucose was lowered from 427 ± 15 to 213 ± 15 mg/dl ($P < 0.05$) in the MB06322-

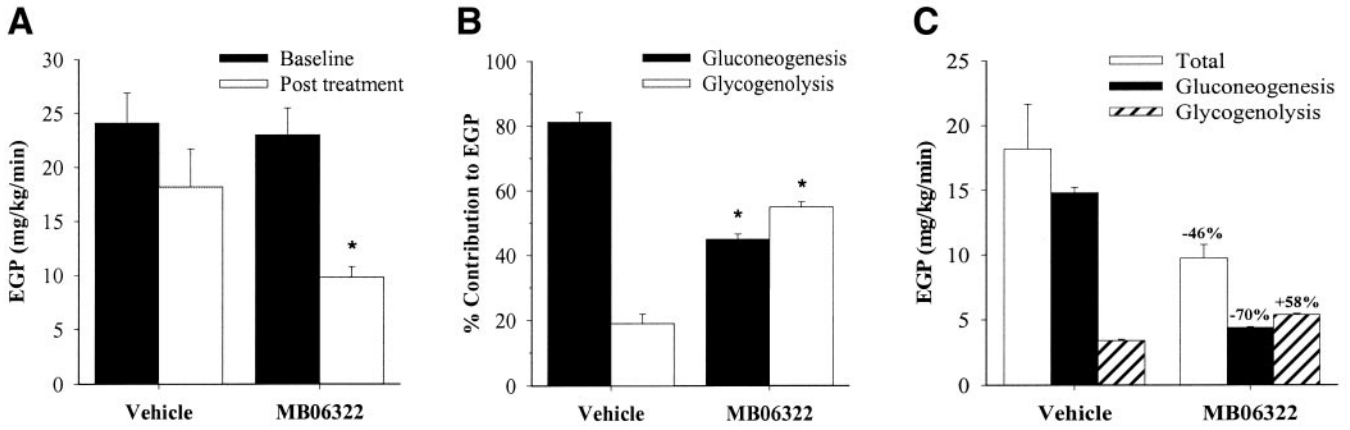

FIG. 2. Effect of acute MB06322 administration (300 mg/kg) on endogenous glucose production (EGP) rates (*A*), the fractional contribution of gluconeogenesis and glycogenolysis to endogenous glucose production (*B*), and gluconeogenesis and glycogenolysis rates (*C*) in fasting conscious ~10-week-old male ZDF rats. Endogenous glucose production was determined by standard tracer methodology, whereas the fractional contribution of gluconeogenesis and glycogenolysis to endogenous glucose production was determined by the deuterated water method, as described in RESEARCH DESIGN AND METHODS. Statistical analysis was not applied to the gluconeogenesis and glycogenolysis rates because they are the products of parameters (endogenous glucose production and fractional contributions of gluconeogenesis and glycogenolysis at 1:00 P.M.) measured in two different, but carefully matched, sets of animals ($n = 6$ per group). *$P < 0.05$ compared with vehicle (Student's t test).

treated group (a ~50% reduction), whereas blood glucose remained largely unchanged in the vehicle-treated group. Glucose lowering by MB06322 was associated with glycogen mobilization; liver glycogen content was ~30% lower in the treated versus the control group at the end of the protocol (87.3 ± 3.2 vs. 124.5 ± 14.4 μmol glucosyl units/g; $P < 0.05$).

Gluconeogenesis accounted for 81.3 ± 2.9 and 88.4 ± 1.9% of endogenous glucose production in vehicle-treated rats at the 1:00 and 3:00 P.M. time points, respectively (Fig. 2*B*). MB06322 reduced the contribution of gluconeogenesis to endogenous glucose production to 45.0 ± 1.6 and 61.2 ± 3.9% at 1:00 and 3:00 P.M. (or after 5 and 7 h of drug treatment), respectively. Blood glucose was reduced by MB06322 to a similar extent as in the endogenous glucose production study described above (~42% relative to vehicle). When endogenous glucose production results are combined with the fractional contribution of gluconeogenesis to endogenous glucose production at 1:00 P.M., MB06322 elicited a ~70% reduction of gluconeogenesis-derived glucose, which is partially countered by a 58% increase in glycogenolysis, thus resulting in an overall 46% reduction of endogenous glucose production (Fig. 2*C*). A similar effect of drug treatment on the rates of gluconeogenesis (−63%) and glycogenolysis (+79%) was evident at 3:00 P.M. (not shown).

Prevention, male ZDF rats

Glucose and insulin levels. MB06322 markedly attenuated the development of hyperglycemia throughout the 6-week treatment period (Fig. 3*A*). In five of eight MB06322-treated animals (full responders), blood glucose levels were indistinguishable from baseline at the end of treatment, whereas hyperglycemia was attenuated in the remaining three animals (694 ± 43, control, vs. 306 ± 57 mg/dl, partial responders). The partial response in some animals may reflect subtle differences in baseline characteristics, such as peripheral insulin resistance. MB06322-treated animals also showed attenuated hyperinsulinemia up until the age of ~8 weeks (Fig. 3*B*). At 10 weeks of age, insulin levels increased in the treated group, and, in contrast to control animals, which showed a characteristic deterioration of pancreatic function, they remained elevated throughout the remainder of the treatment period. Oral glucose tolerance was improved in MB06322-treated

TABLE 1
Physiological and metabolic parameters in control and MB06322-treated pre-diabetic male ZDF rats (nonfasted)*

	Control			MB06322 (0.4%) prevention			MB06322 (0.4%) intervention	
Parameter	Age 6 weeks	Age 10 weeks	Age 12 weeks	Age 6 weeks	Age 10 weeks	Age 12 weeks	Age 10 weeks	Age 12 weeks
Length of treatment (weeks)	0	4	6	0	4	6	0	2
Lactate (mmol/l)†	1.85 ± 0.11	1.9 ± 0.09	1.73 ± 0.05	1.76 ± 0.09	1.89 ± 0.21	2.66 ± 0.62	1.83 ± 0.15	3.11 ± 0.27‡
NEFA (mmol/l)	0.9 ± 0.04	2.1 ± 0.1	4.9 ± 0.2	1.0 ± 0.05	2.8 ± 0.2‡	6.1 ± 0.22‡	1.8 ± 0.2	5.23 ± 3
Triglycerides (mg/dl)	191 ± 11	598 ± 45	609 ± 53	210 ± 17	713 ± 57	1,016 ± 97‡	640 ± 60	945 ± 88‡
Cholesterol (mg/dl)	72 ± 3	137 ± 4	144 ± 5	83 ± 3‡	152.±8	175 ± 20	153 ± 6	236 ± 8‡
β-OH-butyrate (μmol/l)	—	—	223 ± 14	—	—	158 ± 17‡	—	182 ± 12‡
Water intake (ml/day)	28 ± 3	111 ± 5	144 ± 4	30 ± 1	44 ± 5‡	35 ± 4‡	116 ± 2	42 ± 2‡
Food intake (g/day)	28 ± 1	33 ± 0	36 ± 0	27 ± 0	33 ± 1	36 ± 2	32 ± 0	33 ± 0‡
Body weight (g)	184 ± 3	337 ± 5	365 ± 6	191 ± 2	361 ± 5‡	423 ± 11‡	335 ± 8	358 ± 8

Data are means ± SE. *Weekly measurements were made. Parameters measured after 2 or 4 and 6 weeks of treatment are shown to highlight metabolic changes that occurred during these time periods. †The lactate elevations observed in the prevention and intervention groups were resolved within 3 days after the cessation of drug treatment; ‡$P < 0.05$ vs. control, Student's t test.

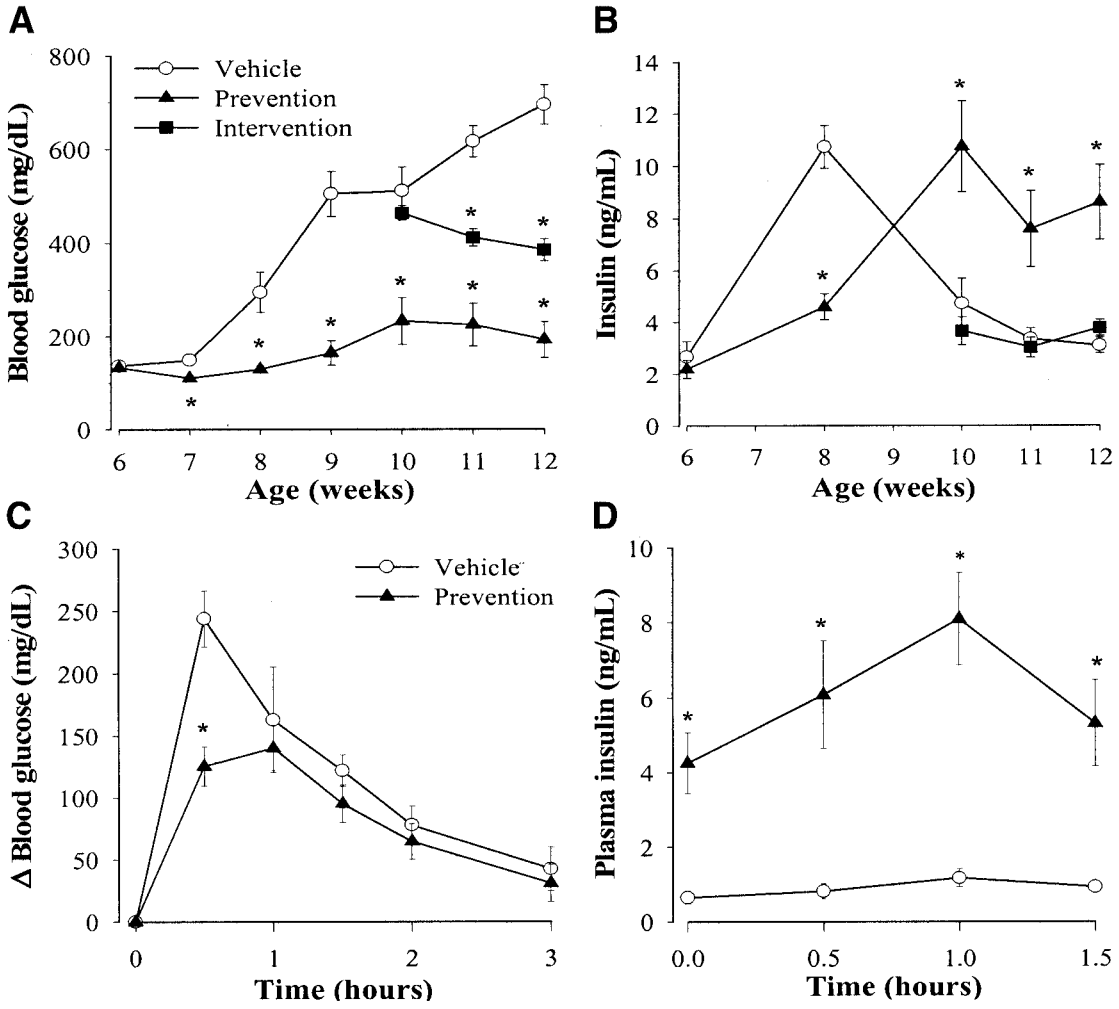

FIG. 3. Effects of MB06322 prevention and intervention therapy in male ZDF rats. *A*: Blood glucose (nonfasted). *B*: Plasma insulin (nonfasted). *C*: Blood glucose levels during an oral glucose tolerance test administered after 5 weeks of treatment in the prevention study. To reflect metabolic changes independent of acute gluconeogenesis inhibition, the food/drug admixture was withdrawn for 6 h before administration of a 2-g/kg oral glucose load. Baseline blood glucose levels were 540 ± 19 and 201 ± 49 mg/dl for control and drug-treated animals, respectively. *D*: Insulin levels during the oral glucose tolerance test in the prevention study. Drug was administered as a food admixture (0.4%, wt/wt) to 6-week-old (prevention) and 10-week-old (intervention) rats as described in RESEARCH DESIGN AND METHODS. Based on food intake, the average dose of MB06322 administered was 400 mg · kg^{-1} · day^{-1}, which is well in excess of the 30-mg/kg dose required for a profound, acute glucose-lowering effect in this model (28). When drug therapy was withdrawn at 12 weeks of age, blood glucose gradually rebounded to levels approximating those of the control animals in both treated groups (not shown), $n = 8$ per group. *$P < 0.05$ compared with controls (ANOVA).

rats, as was glucose-stimulated insulin secretion (Figs. 3*C* and *D*). Polydipsia, polyuria, and glycosuria were also reduced by drug treatment (Tables 1 and 2).

Lactate, NEFAs, triglycerides, cholesterol, and ketone bodies. Lactate levels were largely unaffected by MB06322 during the first 5 weeks of treatment, but they were elevated ~1.5-fold thereafter (Table 1). Elevated lactate was evident only in the partial responders (1.74 ± 0.05, 1.67 ± 0.1, and 4.3 ± 1.2 mmol/l for control, full, and partial responders, respectively). NEFAs were slightly increased at the end of treatment, whereas a more marked increase in triglycerides manifested itself during the last 2

TABLE 2
Physiological and metabolic parameters in control and MB06322-treated pre-diabetic male ZDF rats (nonfasted): metabolic cage measurements

	Control			MB06322 (0.4%) prevention			MB06322 (0.4%) intervention	
Parameter	0.5 weeks of treatment	2.5 weeks of treatment	4.5 weeks of treatment	0.5 weeks of treatment	2.5 weeks of treatment	4.5 weeks of treatment	0 weeks of treatment	0.5 weeks of treatment
Age (weeks)	6.5	8.5	10.5	6.5	8.5	10.5	10	10.5
Water intake (ml/day)	27 ± 2.3	79 ± 8	151 ± 5	24 ± 2	37 ± 3*	44 ± 7*	—	79 ± 4*
Urine output (ml/day)	15 ± 1	62 ± 7	121 ± 5	14 ± 1	21 ± 2*	29 ± 1*	—	55 ± 3*
Glucose excretion (g/day)	0.38 ± 0.18	7.87 ± 1.67	16.74 ± 1.97	0.026 ± 0.07	0.99 ± 0.21*	3.28 ± 1.59*	—	5.25 ± 0.67*

Data are means ± SE. *$P < 0.05$ vs. control for corresponding timepoint, unpaired Student's t test.

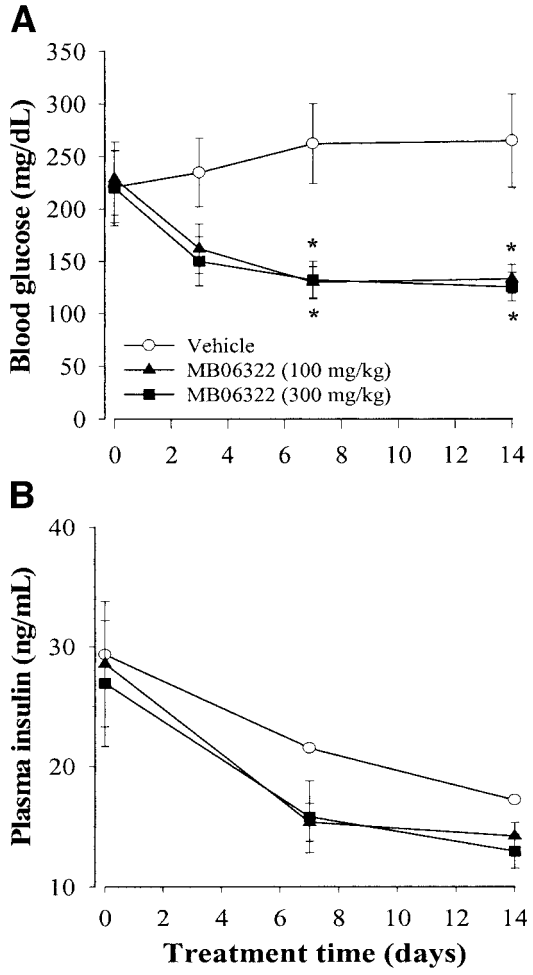

FIG. 4. MB06322 treatment of high-fat diet–fed female ZDF rats. *A*: Blood glucose (nonfasted). *B*: Plasma insulin (nonfasted). Rats were fed a high-fat diet (48% kcal fat) for 30 days before administration of MB06322 as a high-fat chow admixture, as described in RESEARCH DESIGN AND METHODS. The 100- and 300-mg/kg doses are 3- and 10-fold multiples, respectively, of the minimum dose that elicits 30% blood glucose lowering in this model (50), $n = 8$ per group. *$P < 0.05$ compared with controls (repeated-measures ANOVA).

treatment weeks. The changes in triglyceride homeostasis were more pronounced in the partial than the full responders in the treated group (609 ± 54, 938 ± 123, and 1,145 ± 152 mg/dl for control, full, and partial responders, respectively). Cholesterol levels were unchanged and β-hydroxybutyrate levels reduced (~30%) by drug treatment.

Food intake and body weight. MB06322 treatment did not alter food intake (Table 1). Although treatment was not associated with weight gain during the first 4 treatment weeks, MB06322-treated animals gained more weight than controls during weeks 5 and 6.

Intervention, male ZDF rats

Glucose and insulin levels. The response to drug treatment in 10-week-old rats was rapid, as evident from the ~69% reduction in glycosuria on days 3–4 (Table 2). All animals responded to treatment; blood glucose was lowered on average by ~33 and ~44% relative to controls after 1 and 2 weeks of therapy, respectively (Fig. 3*A*). Polydipsia and polyuria were also markedly reduced (Tables 1 and 2). Insulin levels decreased gradually in parallel with those in control animals (Fig. 3*B*). Oral glucose tolerance, measured after 10 days of treatment, was not improved relative to control animals (not shown).

Lactate, NEFAs, triglycerides, cholesterol, and ketone bodies. Lactate, triglycerides, and cholesterol levels were elevated ~1.8-, ~1.5-, and ~1.6-fold relative to controls at the end of treatment (Table 1), whereas there was no difference in NEFA levels between the groups. β-Hydroxybutyrate levels were reduced by ~18% in the MB06322-treated group.

Food intake and body weight. Food intake was slightly reduced by treatment (Table 1). In contrast to the prevention group, body weight gain was similar to that of control animals.

Intervention, high-fat diet–fed female ZDF rats

Glucose and insulin levels. Profound blood glucose lowering was evident within 3 days of MB06322 treatment (100 and 300 mg/kg), and normalization of morning (Fig. 4) and evening (not shown) blood glucose occurred within 7 days. The full potential of glucose lowering by MB06322 was clearly achieved at the lower dose (100 mg/kg). Although insulin levels declined in parallel in control and treated animals, the latter tended to have lower insulin

TABLE 3
Physiological and metabolic parameters in control and MB06322-treated high-fat diet–fed female ZDF rats (nonfasted)

	Control		MB06322 (100 mg · kg^{-1} · day^{-1})		MB06322 (300 mg · kg^{-1} · day^{-1})	
Parameter	0 days of treatment	14 days of treatment	0 days of treatment	14 days of treatment	0 days of treatment	14 days of treatment
Insulin (ng/ml)	29.3 ± 4.7	17.2 ± 3.4	28.6 ± 5	14.2 ± 1	26.9 ± 5.3	12.9 ± 1.4
Glucagon (pg/ml)	—	122 ± 6	—	114 ± 6	—	108 ± 8
Lactate (mmol/l)	1.22 ± 0.18	1.94 ± 0.46	1.12 ± 0.08	1.63 ± 0.51	1.58 ± 0.16	1.55 ± 0.26
NEFA (mmol/l)	—	1.2 ± 0.3	—	1.1 ± 0.1	—	1.2 ± 0.1
Triglycerides (mg/dl)	1,291 ± 120	1,706 ± 229	1,110 ± 103	1,145 ± 164	1,094 ± 154	1,154 ± 60
Cholesterol (mg/dl)	—	133 ± 8	—	120 ± 5	—	158 ± 4
β-Hydroxybutyrate (μmol/l)	—	133 ± 13	—	132 ± 16	—	117 ± 12
Liver triglycerides (mg/g)	—	15.3 ± 2.5	—	13 ± 3	—	12 ± 1
Liver glycogen (μmol/g)	—	276 ± 11	—	244 ± 13	—	222 ± 13*
Alkaline phosphatase (IU/ml)	—	196 ± 28	—	110 ± 8*	—	118 ± 14*
Water intake (ml/day)	27 ± 5	31 ± 6	29 ± 5	15 ± 2	30 ± 6	16 ± 1
Food intake (g/day)	16 ± 1	17 ± 1	15 ± 1	15 ± 1	15 ± 1	13 ± 2
Body weight (g)	339 ± 5	388 ± 7	326 ± 4	370 ± 4	323 ± 7	360 ± 9

Data are means ± SE. *$P < 0.05$ vs. control, ANOVA with Dunnett's post hoc test.

levels, consistent with their attenuated degree of hyperglycemia (Table 3).

Lactate, NEFAs, triglycerides, cholesterol, and ketone bodies. There were no significant differences in blood lactate or plasma NEFAs, triglycerides, cholesterol, or β-hydroxybutyrate between the groups at the end of treatment (Table 3).

Liver enzymes, triglycerides, glycogen, and weight. A reduction in alkaline phosphatase was observed in both MB06322-treated groups (Table 3). Other standard clinical chemistry parameters, including markers of hepatic and renal function, were unchanged (not shown). Liver weight and triglyceride content were similar in control and treated animals, whereas glycogen content was ~20% reduced at the higher dose of MB06322 tested.

Food intake, water intake, and body weight. Food intake was not statistically different between the control and treated groups (Table 3). A trend toward lower food intake was observed in the 300-mg/kg dose group, but this did not provide additional glycemic control (Fig. 4). As expected from its antihyperglycemic effects, water intake tended to be reduced by MB06322 treatment. Body weight was similar at baseline and at the end of treatment between all groups.

DISCUSSION

Overproduction of glucose via gluconeogenesis is a major contributor to fasting and postprandial hyperglycemia in type 2 diabetes and is an abnormality that is addressed only partially and indirectly by current medications. We focused our drug discovery efforts on FBPase, a regulatory enzyme of gluconeogenesis that catalyzes the second-to-last step in the pathway and controls glucose production from all common substrates. The lead compound, MB06322, represents the first potent and selective inhibitor of FBPase described (28) and is currently undergoing clinical evaluation.

Although reported to inhibit de novo glucose synthesis in vivo (28), the effects of MB06322 on the rates of gluconeogenesis and glycogenolysis were not previously defined. As in type 2 diabetic patients, endogenous glucose production was found to be significantly increased in fasting 10-week-old male ZDF rats in the current studies, with rates elevated more than twofold relative to those described in normal rats in a similar nutritional state (29). The contribution of gluconeogenesis to overall endogenous glucose production was high but not dissimilar to that in human patients, where the contribution of gluconeogenesis has been reported to be ~68 and 88% after a 15- and 23-h fast, respectively (6,35). Acute MB06322 treatment inhibited gluconeogenesis by ~70%. The lack of complete inhibition may be related to the pharmacokinetic properties of the drug in liver or to residual activity associated with the FBPase-inhibitor complex in vivo (28). Increased contribution of renal gluconeogenesis to endogenous glucose production is an unlikely explanation because MB06322 effectively inhibits glucose production in perfused rat kidneys (36). Partial inhibition of gluconeogenesis may potentially provide a reserve capacity during periods of high glucose demand.

Acute inhibition of gluconeogenesis in male ZDF rats by MB06322 was accompanied by increased glycogenolysis. Perhaps not coincidentally, the net effect of FBPase inhibition and compensatory glycogenolysis was an overall reduction of endogenous glucose production to a rate similar to that of normal rats in a similar nutritional state (29). Reciprocal regulation of gluconeogenesis and glycogenolysis has also been described in humans, and it is termed "hepatic autoregulation" (37,38). In type 2 diabetic patients, one aspect of hepatic autoregulation is believed to be partially impaired; increased gluconeogenesis is not generally accompanied by a reciprocal decrease in glycogenolysis (39). Whether inhibition of gluconeogenesis can be countered by a reciprocal increase in glycogenolysis has been explored in type 2 diabetic patients administered alcohol, a nonspecific inhibitor of gluconeogenesis, with disparate results (40,41). Our studies with a specific inhibitor of gluconeogenesis suggest that hepatic autoregulation via increased glycogenolysis remains intact in experimental diabetes to defend a normal rate of endogenous glucose production but not the excessive rate evident in untreated animals. This counterregulatory phenomenon could potentially provide an important mechanism for prevention of hypoglycemia when gluconeogenesis is inhibited during an extended fast.

At the age of ≥10 weeks, the male ZDF rat is a model characterized by severe hyperglycemia, dyslipidemia, and rapid pancreatic deterioration. Few of the currently prescribed drugs, particularly those dependent on pancreatic function, are active as an intervention in this model; insulin secretagogues, for instance, fail to lower blood glucose (42), and insulin sensitizers are either ineffective or show a high nonresponder rate (43). Importantly, MB06322 profoundly and rapidly lowered blood glucose in 10-week-old animals, both in the acute endogenous glucose production studies and in a progressive manner during 2 weeks of intervention treatment. The relative independence of the antihyperglycemic effects from pancreatic function may provide a treatment advantage for FBPase inhibitors in type 2 diabetic patients with advanced disease.

The delayed development of hyperglycemia and glycosuria in pre-diabetic male ZDF rats treated chronically with MB06322 was somewhat unexpected. The observations imply that gluconeogenesis, the rate of which is expected to double by the age of 10 weeks in control animals, plays a major role in the development of overt type 2 diabetes in this animal model. Consistent with the pronounced attenuation of hyperglycemia and reduced insulin demand, treated animals had lower insulin levels during the initial weeks of therapy. The hyperinsulinemia that developed with prolonged therapy appears counterintuitive, but it may be a response to the natural progression of insulin resistance caused by lipotoxicity (44). This response is potentially triggered by increased circulating levels of NEFAs or other mediators associated with insulin resistance (45). Hypersecretion of insulin has also been noted in male ZDF rats treated with peroxisome proliferator–activated receptor agonists (46), or the nonspecific AMP-activated protein kinase activator 5-aminoimidazole-4-carboxamide-1-β-D-riboruranoside (47), despite normalization of blood glucose by these agents

As suggested by improved glucose-stimulated insulin secretion during the oral glucose tolerance test and the extended hyperinsulinemic phase in ZDF rats in the prevention group, early treatment with an FBPase inhibitor prevented, or at minimum delayed, the deterioration in β-cell function observed in animals in both the control and intervention groups. The combined effects of gluconeogenesis inhibition and high circulating insulin levels are a potential explanation for the improved glycemic control in

the prevention group relative to the intervention group. Neither prevention nor intervention therapy led to a permanent correction of hyperglycemia; withdrawal from MB06322 therapy resulted in a gradual transition of all animals to a fully hyperglycemic phenotype.

The major nongluconeogenic fate of lactate, the predominant gluconeogenesis precursor, is metabolism to acetyl CoA, which is oxidized by the tricarboxylic acid cycle or is diverted into ketogenesis, lipogenesis, or sterologenesis. Remarkably, inhibition of gluconeogenesis in pre-diabetic ZDF rats was not associated with increased lactate, β-hydroxybutyrate, lipids, or cholesterol during the first 4 treatment weeks. In 10-week-old male ZDF rats in both the prevention and intervention groups, gluconeogenesis inhibition resulted in increased lactate and potential downstream products of lactate metabolism. These metabolic perturbations may be a consequence of reduced oxidative capacity and increased lactate supply (48), which are often associated with the progression of diabetes. In control animals, substrate excess was handled prominently by urinary excretion of chow- and gluconeogenesis-derived glucose, but even in this group, an aggravation of hyperlipidemia was noted that coincided with a reduction in plasma insulin levels. Hyperinsulinemia in a setting of substrate excess (i.e., lactate and lactate-derived products that are not typically cleared renally) may be responsible for the increased body weight gain observed in the prevention group. Neither hyperinsulinemia nor excess weight gain were apparent in the intervention group.

The metabolic perturbations noted in male ZDF rats after MB06322 treatment were largely absent in the more moderately diabetic high-fat diet–fed female ZDF rats; normalization of blood glucose without changes in lactate, β-hydroxybutyrate, lipids, cholesterol, or body weight was observed. The only notable difference was a ~20% reduction in hepatic glycogen stores at the higher dose of MB06322 evaluated (~300 $mg \cdot kg^{-1} \cdot day^{-1}$). As in male ZDF rats, this was probably caused by compensatory glycogenolysis, although reduced glycogen synthesis via the indirect (gluconeogenesis) pathway cannot be ruled out. The modest reduction in glycogen stores suggests that in this model, as in humans (49), glycogen synthesis via the direct pathway (from glucose) predominates or, alternatively, can compensate for inhibition of gluconeogenesis. The lack of other metabolic or body weight changes related to MB06322 treatment suggests that the female ZDF rat is readily able to compensate for lactate and lactate-derived substrate excess. Insulin levels tended to be lower as a consequence of MB06322 treatment but were not normalized; as in male ZDF rats, alleviation of glucotoxicity in this hyperphagic, high-fat–fed model may not be sufficient to overcome insulin resistance due to lipotoxicity.

In summary, inhibition of FBPase represents an effective approach for controlling excessive glucose production in experimental type 2 diabetes. Acute treatment of fasting male ZDF rats markedly inhibited gluconeogenesis and endogenous glucose production and lowered blood glucose. In freely feeding, pre-diabetic male ZDF rats, MB06322 at minimum delayed the development of hyperglycemia and deterioration of β-cell function, whereas in male ZDF rats and high-fat diet–fed female ZDF rats with established hyperglycemia, drug treatment lowered blood glucose. These observations suggest that excessive gluconeogenesis is central to the pathophysiology of type 2 diabetes. MB06322 was well tolerated; metabolic perturbation in the form of modest lactate elevation and increased lipids (and sterols) was evident only in male ZDF rats with advanced disease. Hypoglycemia, a major potential concern with any therapeutic approach that modulates endogenous glucose production, was not evident in these long-term evaluations, perhaps as a result of partial inhibition of gluconeogenesis and counterregulation via glycogenolysis. Inhibitors of FBPase, either as a monotherapy or in combination with drugs that ameliorate the dyslipidemia that accompanies type 2 diabetes, may provide a future option for combating both postprandial and fasting hyperglycemia.

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