Brain-Derived Neurotrophic Factor Improves Blood Glucose Control and Alleviates Fasting Hyperglycemia in C57BLKS-Lepr^{db}/lepr^{db} Mice

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Systemic administration of brain-derived neurotrophic factor (BDNF) decreases nonfasted blood glucose in obese, non-insulin-dependent diabetic C57BLKS-Lepr^{db}/lepr^{db} (*db/db*) mice, with a concomitant decrease in body weight. By measuring percent HbA_{1c} in BDNF-treated and pair-fed animals, we show that the effects of BDNF on nonfasted blood glucose levels are not caused by decreased food intake but reflect a significant improvement in blood glucose control. Furthermore, once established, this effect can persist for weeks after cessation of BDNF treatment. Oral glucose tolerance tests were performed to examine the effects of BDNF on blood glucose control in the fasted state and after an oral glucose challenge. BDNF treatment normalized fasting blood glucose from initially hyperglycemic levels and also showed evidence for beneficial, although less marked, effects on the ability to remove exogenous glucose from blood. One means to lower fasting blood glucose is to reduce the glucose output of peripheral tissues that normally play a part in the maintenance of fasting hyperglycemia. Because the liver is the major endogenous source of glucose in blood during fasting, and because hepatic weight and glucose output are increased in type 2 diabetes, we evaluated the effects of BDNF on liver tissue. BDNF reduced the hepatomegaly present in *db/db* mice, in association with reduced liver glycogen and reduced liver enzyme activity in serum, supporting the possible involvement of liver tissue in the mechanism of action for BDNF. *Diabetes* 48:588–594, 1999

Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth factor–related family of neurotrophins, which also includes neurotrophin-3 and neurotrophin 4/5 (1). These proteins exert trophic effects on specific populations of neurons that express their respective high-affinity receptors (2). In addition to promoting the survival of responsive neurons, the neurotrophins can affect such basic cellular processes as protein expression, cellular differentiation, and neurite extension (3–5). Recently it was reported that systemic administration of BDNF lowered nonfasted blood glucose in hyperglycemic C57BLKS-Lepr^{db}/lepr^{db} (*db/db*) mice, a model for obesity and non-insulin-dependent diabetes (6), but only if given before the mice were 23 weeks of age (7). BDNF decreased food intake and body weight in *db/db* mice, but pair-feeding to the reduced food intake level of BDNF-treated mice did not cause the same effects on blood glucose, suggesting that the effects of BDNF on body weight and blood glucose are separable. BDNF was also effective in reducing nonfasted blood glucose in mildly hyperglycemic *ob/ob* mice but did not affect blood glucose in nondiabetic mice or insulin-dependent diabetic rats (7).

We expanded on those studies to demonstrate the potential of BDNF as a novel hypoglycemic agent for the treatment of non-insulin-dependent diabetes. Results show not only that BDNF reduces blood glucose levels during treatment, but also that the effect continues for weeks after treatment cessation, even when BDNF is no longer detected in blood. Measurements of the percent HbA_{1c} (8) were used to demonstrate that the effects of BDNF on nonfasted blood glucose reflect a dramatic improvement in blood glucose control that is not dependent on the timing of blood glucose measurements relative to food intake. To gain insights into the mechanism of action of BDNF, oral glucose tolerance tests (OGTTs) were performed. Results from those tests and additional studies show that BDNF can normalize fasting blood glucose in hyperglycemic mice, and this effect may be related to effects of BDNF on the liver.

RESEARCH DESIGN AND METHODS

Mice. Male *db/db* mice on a C57BLKS/J background were obtained from the Jackson Laboratory (Bar Harbor, ME) at 4–8 weeks of age. Animals were originally housed three per cage but were single-housed 1 week before the start of neurotrophin treatment. All treatments were started at 10 weeks of age. Mice were given food (Purina Rodent Laboratory Chow 5001; Purina Mills, Richmond, IN) and water ad libitum, except for mice that were pair-fed daily to a BDNF-treated group (see below). Male heterozygous *db*/+ and wild-type C57BL6 mice were used as nondiabetic, nonobese controls. All animal use in this study was conducted in compliance with approved institutional animal care and use protocols and according to NIH guidelines (*Guide for the Care and Use of Laboratory Animals*, NIH publication no. 86–23, 1985).

BDNF administration and pair-feeding. Mice were injected subcutaneously two (Monday and Thursday), three (Monday, Wednesday, and Friday), or five (Monday through Friday) times a week with recombinant human BDNF (Amgen, Thousand Oaks, CA, or Sumitomo, Osaka, Japan; no difference in efficacy with respect to nonfasted blood glucose, fasting blood glucose, and percent HbA_{1c}). A

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ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; AUC, area under the curve; BDNF, brain-derived neurotrophic factor; ELISA, enzyme-linked immunosorbent assay; OGTT, oral glucose tolerance test; PLSD, paired least-squares difference.

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pair-fed group of vehicle (phosphate-buffered saline)-treated *db/db* mice was included in some of the studies because BDNF treatment decreases food intake by about 50% in *db/db* mice (results not shown) compared with vehicle-treated mice (7). Mice in the pair-fed group were fed the average amount of food consumed by BDNF-treated mice over the previous 24 h. Food intake was estimated by weighing the food remaining from the previous day. Pair-fed animals received their daily ration of food between 0900 and 1100, always after nonfasted blood glucose measurements. At death (see below), trunk blood and tissues were collected in the morning before pair-feeding for that day. Measurements of fasted blood glucose and glycated hemoglobin were made to overcome the possible dependence of certain measures of blood glucose control (that is, nonfasted blood glucose) on the timing of the measurements relative to food consumption.

Blood and tissue assays. Nonfasted blood glucose was measured in blood collected from the tip of the tail between 0800 and 1200 using a Glucometer 3 Blood Glucose Meter (Bayer, Elkhart, IN) (glucose oxidase method). Percent HbA_{1c} (8) was measured with a Diastat Analyzer (Biorad, Hercules, CA). Insulin, glucagon (Linco, St. Charles, MO), and corticosterone (ICN, Costa Mesa, CA) levels were assayed in serum by radioimmunoassay. To minimize the effects of euthanasia on levels of the stress hormone corticosterone, mice were killed by decapitation (9) after acclimation in an undisturbed room for 1–2 h. No effect of BDNF treatment on nonfasted corticosterone was detected (results not shown). Alanine aminotransferase (ALT/SGPT, coupled ALT/lDH method [10]), aspartate aminotransferase (AST/SGOT, coupled AST/MDH method [10]) and glucose (hexokinase method) were also assayed in serum using the Monarch blood chemistry analyzer (Instrumentation Laboratory, Lexington, MA). BDNF was assayed by enzyme-linked immunosorbent assay (ELISA) (Promega, Madison, WI) in plasma. Liver (left anterior lobe), heart, and fat (white adipose tissue; left and right epididymal fat pads combined) were dissected at death and weighed.

Oral glucose tolerance test. After an 18- to 20-h fast starting at 1430, animals were bled from the tail for a baseline (time 0) measurement. Afterward, animals were administered 89 mg D-glucose (Sigma, St. Louis, MO) dissolved in 0.2 ml distilled water (~2.2 g/kg body wt) through a feeding needle (VWR, Plainfield, NJ). Blood was drawn from the tail 20, 60, and 210 min after glucose administration. The area under the blood glucose curve (AUC) was calculated after subtracting the fasting blood glucose value from all glucose measurements. AUC is reported for calculations from 0 to 60 min after glucose administration, but results were the same for calculations from 0 to 210 min.

Glycogen determination. Liver glycogen was determined using a protocol similar to that previously described (11). Glycogen was isolated from liver (stored at –80°C) by ethanol precipitation after the tissue was homogenized in 20 mmol/l Tris-HCl with 0.1% Triton-X100, pH 8.6. Glycogen was hydrolyzed enzymatically with amyloglucosidase (Sigma) in 0.4 mol/l sodium acetate buffer, pH 4.8, followed by glucose determination with a Sigma glucose diagnostic kit (Glucose HK 20, hexokinase method).

Data analysis. Differences in means were tested using analysis of variance (ANOVA) with Fisher's paired least-squares difference (PLSD) as a post hoc test (Statview; Abacus, Berkeley, CA). Differences in blood glucose during the OGTTs were evaluated with a repeated-measures ANOVA followed by Fisher's PLSD as a post hoc test (Statview). $P < 0.05$ was considered statistically significant.

RESULTS

Lasting effects of BDNF on nonfasted blood glucose. As shown previously (7), BDNF decreased nonfasted blood glucose in *db/db* mice (Fig. 1*A*). BDNF administered five times a week, starting at 10 weeks of age, normalized nonfasted blood glucose (nondiabetic ~130 mg/dl) (Table 1) after 1 week of treatment, and glucose remained at this level through 4 weeks of treatment (Fig. 1*A*, black bar). Pair-feeding did not affect nonfasted blood glucose. This result differs from a previous result that reported a small but significant decrease in nonfasted blood glucose after 8 weeks of pair-feeding (7), possibly due to the use of females rather than males or the different source of *db/db* mice (Clea Japan) by Ono et al. (7).

After treatment was stopped, blood glucose in BDNF-treated animals remained below pretreatment levels for an additional 4 weeks, and lower than that of vehicle-treated and pair-fed *db/db* controls for 12 weeks (Fig. 1*A*). In contrast, body weights for BDNF-treated *db/db* mice were depressed during treatment but increased rapidly after treatment was stopped, surpassing those of vehicle-treated *db/db* mice 8 weeks before blood glucose values were equivalent in the two groups (Fig. 1*B*).

One possible explanation for the lasting effect of BDNF on blood glucose is that BDNF has a prolonged plasma half-life. To test this possibility, BDNF was injected three times a week for 2 weeks at 30 or 50 mg/kg and then treatment was stopped. Plasma BDNF was measured by a sensitive two-site ELISA at 3, 6, 10, and 17 days after the final treatment. BDNF was detectable in plasma for up to 6 days after the last injection, but was not detectable by 10 days (Fig. 2*B*). In contrast, the effect on blood glucose was maintained through 17 days after the last BDNF injection (Fig. 2*A*). These data indicate that BDNF treatment results in a lasting improvement in the ability of *db/db* mice to maintain blood glucose near nondiabetic levels that, once established, can persist for weeks without additional BDNF (Fig. 1*A*).

Percent HbA_{1c}. Although BDNF treatment decreases nonfasting blood glucose, the decrease in food intake observed during BDNF treatment suggested that the effect may depend on the timing of glucose measurements relative to food consumption. To address this issue, we evaluated the percent of hemoglobin nonenzymatically glycated (percent HbA_{1c}), which is an integrated measure of blood glucose regulation during the weeks before measurement (8). We found that after 4 weeks treatment, starting at 10 weeks of age, percent HbA_{1c} in BDNF-treated *db/db* mice was 45% below that of vehicle-treated *db/db* mice and similar to percent HbA_{1c} in nondiabetic controls (Fig. 3). Pair-feeding to the reduced food intake of BDNF-treated mice had no effect on percent HbA_{1c}. In addition, treatment of *db/db* mice for 4 weeks with a control protein, cytochrome c (12) (30 mg/kg), did not alter percent HbA_{1c} (results not shown). Thus, BDNF treatment dramatically improved blood glucose control, and this effect was specific and not caused by alterations in food intake.

Effects of BDNF are maintained when treatment frequency is reduced. The lasting effects of BDNF on blood glucose control (Fig. 1*A*) and the presence of BDNF in blood for days after the last injection (Fig. 2*B*) suggested that efficacy might be maintained with less frequent administration of BDNF. Since decreasing treatment frequency would be both experimentally and clinically relevant, we examined the efficacy of BDNF when administered two, three, or five times a week. We found that less frequent dosing of BDNF (two and three times a week) had effects on percent HbA_{1c} that were similar, if not identical, to dosing at five times a week (Table 1). Pair-feeding caused an equivalent reduction in body weight compared to the three times a week BDNF treatment, unlike pair-feeding to the five times a week BDNF treatment (Fig. 1*B*), but percent HbA_{1c} was not affected by pair-feeding.

Oral glucose tolerance test. To gain insights into the mechanism of action of BDNF, we examined the effects of BDNF on blood glucose control in the fasted state and after administration of a standard oral glucose load by utilizing an OGTT. Ten untreated *db/db* mice were randomly selected from a larger group of mice and an OGTT was performed at 10 weeks of age (Fig. 4). Treatment of the remaining mice in the group was started 1 day before the test and continued for 4.5 weeks (three times a week), at which time an OGTT was performed (Fig. 4).

Blood glucose levels during the OGTTs (Fig. 4*A*) were significantly different in the different groups tested ($P < 0.0001$;

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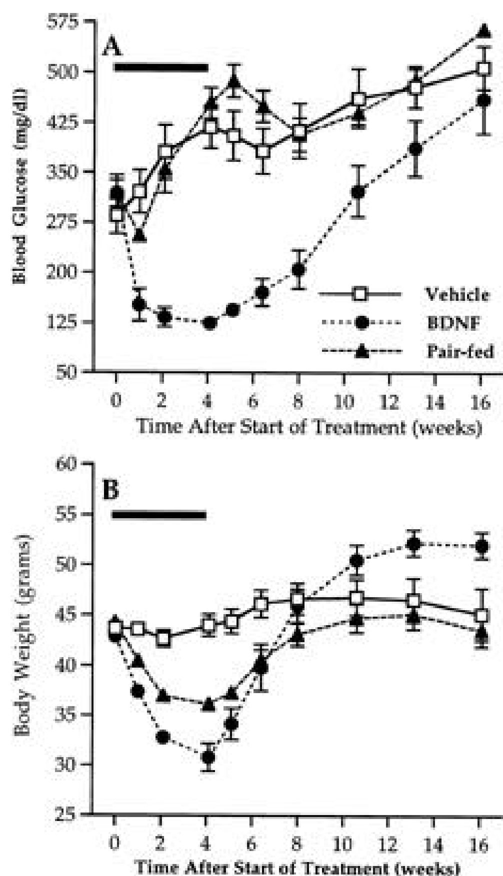

FIG. 1. Recovery after BDNF treatment cessation. Time course of non-fasted blood glucose (*A*) and body weight during and after BDNF treatment (five times a week, 30 mg/kg) (*B*) in *db/db* mice. The black bar represents the duration of BDNF treatment (4 weeks). Mean ± SE is plotted for n = 9–10 per group.

effect of treatment group). As reported previously (7), BDNF-treated mice had lower blood glucose levels during an OGTT compared with pair-fed mice (P < 0.0001). In addition, BDNF improved the OGTT results when compared with the pretreatment test performed at 10 weeks of age (P < 0.0001). This effect is observed to be due to a lowering of fasting blood glucose (time 0) from the hyperglycemic levels observed at 10 weeks of age to the normal level observed in nondiabetic heterozygous mice (P < 0.0001 for BDNF-treated *db/db* vs. *db/db* at 10 weeks; one-way ANOVA).

The ability to respond to an oral glucose challenge and clear the exogenous glucose load from blood was evaluated by comparing the areas under the OGTT curves. To control for effects of treatment on fasting blood glucose, AUC was calculated after shifting the OGTT curve of each animal so that all mice started from a common baseline at time 0. AUC (Fig. 4*B*) was not significantly reduced by BDNF treatment compared with the 10-week-old *db/db* mice (P = 0.26) but was significantly improved compared with vehicle-treated and pair-fed controls after 4.5 weeks of treatment (P < 0.006 for both). Compared with nondiabetic heterozygous mice, AUC in BDNF-treated *db/db* mice was elevated (P < 0.01). Thus the ability to respond to an oral glucose load showed signs of improvement with BDNF treatment. Compared with the pretreatment condition, however, BDNF did not significantly improve the ability to remove exogenous glucose from blood, in spite of a normalization of fasting blood glucose (Fig. 4*A*, time 0). Furthermore, in a separate study, BDNF (30 mg/kg, three times a week for 4.5 weeks) normalized fasting blood glucose but did not significantly improve the response to an oral glucose load (results not shown). The cause of the differential effects of BDNF on the response to a glucose load is unknown. However, results demonstrate that the normalizing effects of BDNF on fasting blood glucose do not require an improvement in the ability to remove exogenous glucose from blood.

Insulin and glucagon are potent regulators of blood glucose, so BDNF could affect fasting blood glucose by altering their release. Two weeks of daily BDNF treatment (20 mg/kg body wt) starting at 7 weeks of age has been reported to decrease nonfasted plasma insulin levels in *db/db* mice (7). Our results agree with this finding, in that 4 days of BDNF treatment lowered nonfasted serum insulin compared with vehicle-treated *db/db* mice (Table 2). To control for differential food intake before measurement, we evaluated the effects of 4.5 weeks of BDNF treatment (30 mg/kg, three times a week) on insulin and glucagon release in the fasted state, when blood glu-

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TABLE 1
Effects of BDNF treatment on percent HbA_{1c} and tissue weights

Treatment	Strain	n	% HbA_{1c}	Blood glucose (mg/dl)	Body weight (g)	Heart (mg)	Fat (g)	Liver (mg)
None	*db/+*	5	3.3 ± 0.1	131 ± 4	30.1 ± 0.5	147 ± 14	0.76 ± 0.06	443 ± 17
Vehicle	*db/db*	7	8.4 ± 0.2*	476 ± 44*	42.9 ± 1.9*	151 ± 11	2.37 ± 0.04*	848 ± 58*
Two times per week BDNF (30 mg/kg)	*db/db*	7	5.2 ± 0.4 *†	140 ± 15†	35.7 ± 1.3*†	123 ± 5*†	1.99 ± 0.06*†	563 ± 20*†
Three times per week BDNF(30 mg/kg)	*db/db*	7	4.3 ± 0.2*†	116 ± 4.8†	34.2 ± 1.1*†	121 ± 5*†	1.99 ± 0.05*†	482 ± 29†
Five times per week BDNF (30 mg/kg)	*db/db*	7	4.3 ± 0.2*†	115 ± 6.6†	28.8 ± 1.3*†	115 ± 5*†	1.59 ± 0.09*†	410 ± 11†
Pair-fed to three times per week BDNF	*db/db*	6	8.5 ± 0.3*	545 ± 17*†	32.1 ± 1.4*†	125 ± 4†	1.75 ± 0.1*†	600 ± 23*†

Data are means ± SE. Percent HbA_{1c} and glucose were measured on the last day of a 5-week treatment. Animals were killed 3 days after the final treatment, and tissue weights were obtained. *P < 0.05 vs. heterozygous mice; †P < 0.05 vs. vehicle-treated *db/db* mice, data analysis by ANOVA followed by Fisher's PLSD.

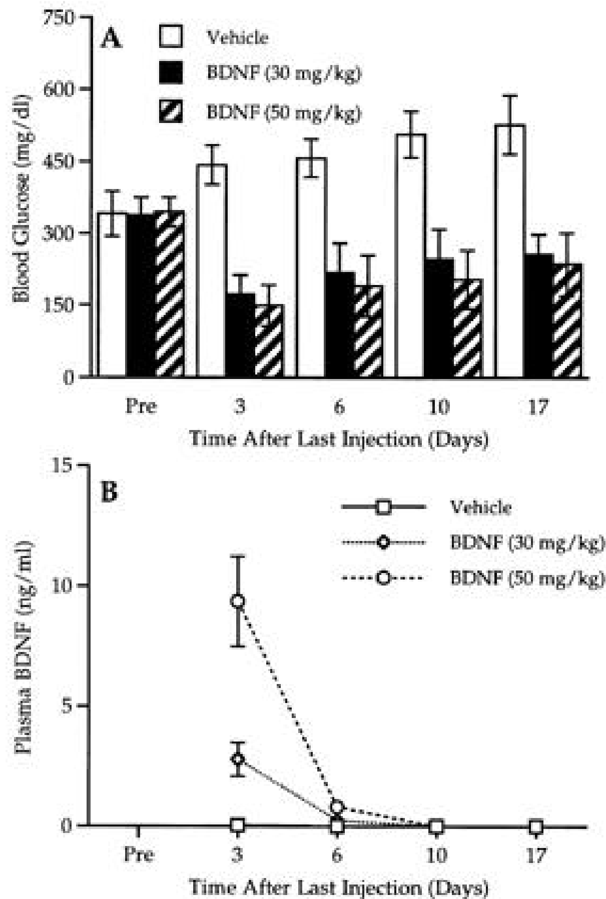

FIG. 2. Lack of a correlation between blood glucose and plasma BDNF after treatment cessation. Nonfasted blood glucose (*A*) and plasma BDNF (*B*) in *db/db* mice after 2 weeks of treatment three times a week at the BDNF dosage indicated. Measurements were made 3, 6, 10, and 17 days after the final injection. Blood glucose was also measured 3 days before the start of treatment (Pre). Mean ± SE is plotted for n = 5–6 per group.

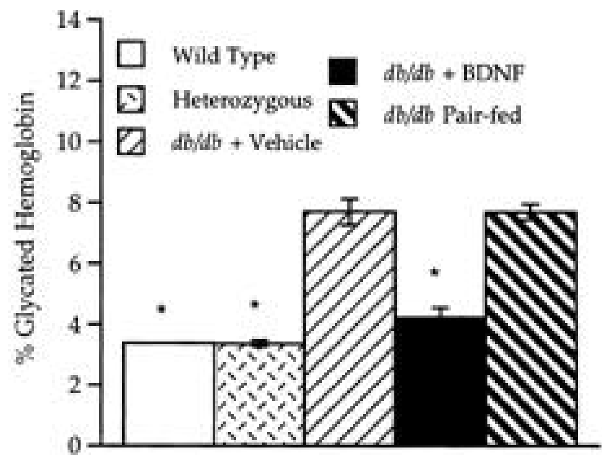

FIG. 3. Effect of BDNF on % HbA_{1c}. Mice were treated five times a week for 4 weeks (BDNF 30 mg/kg), and % HbA_{1c} was measured 1 week after treatment cessation. Wild-type refers to C57BL6 age- and sex-matched mice. Pair-fed animals were fed daily the average amount of food consumed by the BDNF-treated mice the previous day. Mean ± SE is plotted for n = 5–10 mice per group. *P < 0.0001 vs. vehicle-treated *db/db* mice by ANOVA followed by Fisher's PLSD.

cose was shown to be lower in BDNF-treated *db/db* mice (Fig. 4, time 0). Fasting insulin and glucagon were elevated by 42 and 53% (P < 0.0003 for both), respectively, in vehicle-treated *db/db* mice compared with heterozygous mice. BDNF treatment or pair-feeding did not affect fasting insulin or glucagon levels (P > 0.25 for both versus vehicle, results not shown). Thus BDNF does not affect fasting blood glucose by altering pancreatic insulin or glucagon secretion, although changes in insulin responsiveness may be involved (see DISCUSSION).

Liver weight and glycogen. Through gluconeogenesis and glycogenolysis, the liver is the major source of glucose during fasting (11,13–15). The finding that BDNF normalizes fasting blood glucose compared with pretreatment levels, without a commensurate marked improvement in the ability to remove exogenous glucose from blood (Fig. 4), suggests that decreased glucose output by the liver might be part of the mechanism of action for BDNF. Liver weight is disproportionately increased in *db/db* mice, and this weight increase is associated with elevated hepatic glucose output (6,11). Consistent with previous studies, we found that the wet weights of liver and epididymal fat pad (6,11,16), but not heart (16), were elevated in *db/db* mice compared with age-matched heterozygous mice (Table 1). Five weeks of BDNF treatment or pair-feeding lowered total body weight as well as the wet weight of liver, heart, and fat. Of significance from this study is the finding that, although the BDNF-induced decrease in the weight of heart and fat could be mimicked by pair-feeding, the decrease in liver weight was significantly greater in BDNF-treated animals than in pair-fed animals. In fact, BDNF treatment at three or five times a week (30 mg/kg) resulted in near normal (heterozygous) liver weights. When liver weight is expressed as a percentage of body weight (Fig. 5), it is seen that the excess weight of the liver (hepatomegaly) in *db/db* mice, in relation to body weight (6), was not observed in BDNF-treated animals but was still present after pair-feeding.

Since glycogen is a stored source of glucose used by the liver during periods of fasting, decreased glycogen stores in BDNF-treated mice during periods of free food access could result in decreased glucose output during periods of fasting, resulting in lower fasting blood glucose (Fig. 4). In support of this, total glycogen content of the liver in the nonfasted condition was 36% lower in BDNF-treated mice compared with vehicle-treated controls (Table 3). Glycogen concentration was not affected by BDNF (33.6 ± 2.7 for BDNF vs. 34.4 ± 2 mg glycogen/g tissue for vehicle). These values at 14 weeks of age are comparable to those previously reported for 8-week-old *db/db* mice (51 and 64 mg glycogen/g liver weight) (6,11). Glycogen concentration in the liver of pair-fed mice is reduced by 42% compared with vehicle- and BDNF-treated mice (Table 3). Since pair-fed animals are hyperglycemic at the time of tissue sampling (data not shown; see Fig. 1*A*), this finding suggests that pair-fed animals are actively maintaining hyperglycemia when food access is restricted (see DISCUSSION).

Liver enzyme activities in serum. We also examined the serum activities of two enzymes released into blood by the liver, ALT and AST. In mice, ALT is found predominantly in liver, whereas AST is found in cardiac muscle, liver, blood vessels, and brain (17). Serum ALT activity was elevated in *db/db* mice compared with heterozygous mice (Table 2). BDNF

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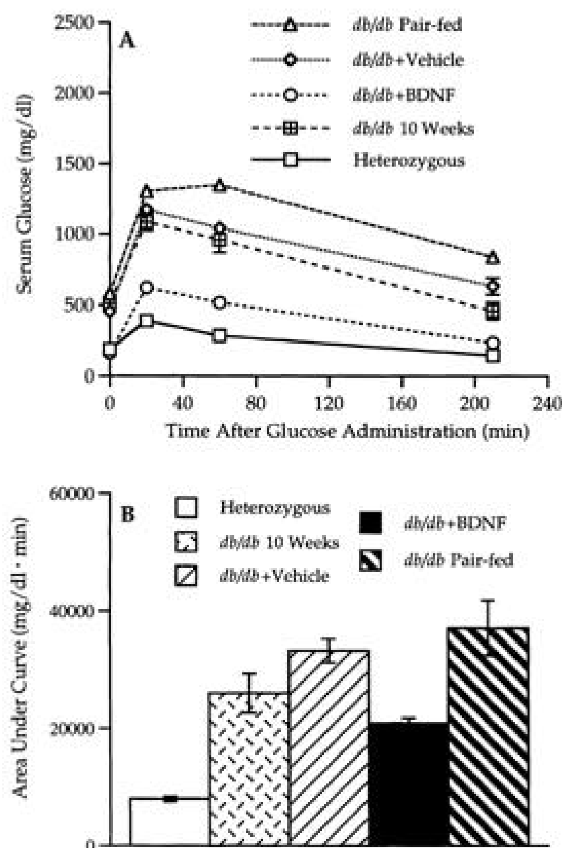

FIG. 4. Effect of BDNF on OGTTs. Mice were randomized into groups by body weight and nonfasted blood glucose at 9 weeks of age so that group means were not significantly different and were within 1 g of each other for body weight and 45 mg/dl for blood glucose. The oral glucose tolerance of one group from this lot was tested at 10 weeks of age (*db/db* 10 weeks). The remaining mice, including a group of age-matched heterozygous mice, were treated three times a week for 4.5 weeks (BDNF 30 mg/kg), at which time an OGTT was administered. Animals were fasted for 18 h before blood collection for fasting blood glucose (reported at Time 0). A standard glucose load (123 mg) was subsequently gavaged into the stomach, and capillary blood was sampled from the tail at 20, 60, and 210 min after gavage. *A*: Blood glucose values during the OGTT. *B*: Area under the OGTT curve (AUC) from 0 to 60 min after glucose administration. Before calculation of AUC, all curves were adjusted to a common baseline at time 0 by subtracting the initial glucose from all glucose measurements. Mean ± SE is plotted for $n = 5$ heterozygous mice and $n = 9$–10 *db/db* mice per group.

treatment for 4 consecutive days, but not pair-feeding, lowered ALT in both mutant and heterozygous mice. These changes in ALT appeared to parallel changes in liver weights. No difference in AST activity was observed between *db/db* and heterozygous mice, and no effect of BDNF treatment was detected, although a trend for increased AST was observed in BDNF-treated *db/db* mice (Table 2).

In humans, the ratio ALT/AST (DeRitis ratio), or its reciprocal, has been reported to differentiate between certain pathologies (18); for example, ALT/AST is elevated in morbidly obese humans with excess accumulation of triglycerides in hepatocytes (fatty liver) (19,20). In our study, ALT/AST was higher in *db/db* mice than in heterozygous mice (Table 2), consistent with the presence of fatty liver in *db/db* mice (21). After only 4 days of treatment, BDNF lowered the ALT/AST ratio in *db/db* mice. BDNF had a similar but less dramatic effect on the ALT/AST ratio in heterozygous mice

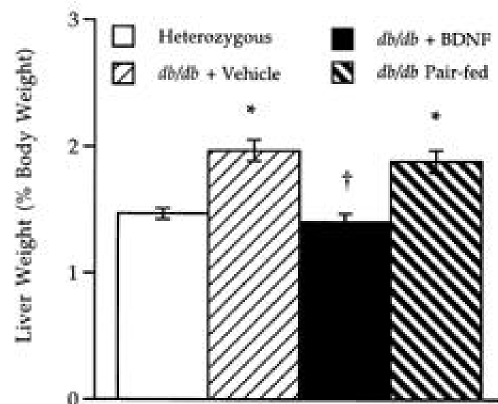

FIG. 5. Effect of BDNF on liver weight when expressed as a percentage of body weight. Mice were treated three times a week for 4 weeks (BDNF 30 mg/kg). Values are 100 × (liver wt/body wt). Means ± SE are plotted for $n = 5$–7 per group. *$P < 0.05$ vs. untreated heterozygous mice; †$P < 0.05$ vs. vehicle-treated and pair-fed *db/db* mice by ANOVA followed by Fisher's PLSD.

(Table 2). The same effect on ALT/AST in *db/db* mice was observed after 4 weeks of BDNF treatment (30 mg/kg, three times a week, results not shown).

DISCUSSION

The present study demonstrates that BDNF may have significant potential as a hypoglycemic agent for the treatment of non-insulin-dependent diabetes. BDNF treatment of *db/db* mice resulted in percent HbA_{1c} and fasting blood glucose values that were near those of nondiabetic mice. Since pair-feeding *db/db* mice to BDNF-treated mice had no effect on these measurements, our results show that the effects of BDNF on blood glucose are not caused by alterations in food intake. Of potential clinical importance for increasing patient compliance is the finding that BDNF is effective when administered as infrequently as two times a week. Furthermore, the improvement in blood glucose control compared with the pretreatment condition can last for weeks after treatment cessation, even when BDNF is no longer present in blood. Therefore lasting physiologic changes induced by BDNF, rather than pharmacokinetic properties of BDNF, result in maintained improvement in blood glucose control.

Improved blood glucose control in BDNF-treated animals is related to a dramatic reduction in fasting blood glucose. Beneficial effects of BDNF on the response to an oral glucose challenge probably contribute to improved blood glucose control, but the dramatic effect of BDNF on fasting blood glucose did not require an improvement in the ability to remove exogenous glucose from blood. This indicates that BDNF can reduce the glucose output of peripheral tissues that maintain fasting hyperglycemia. Thus the effect of BDNF on fasting hyperglycemia, as well as the effects on liver weight, liver glycogen, and liver enzyme activities in serum, suggests that reduced glucose output by the liver may be part of the mechanism of action for BDNF.

Reducing hepatic glucose production has previously been recognized as a means to treat diabetes, because hepatic glucose output is markedly elevated in type 2 diabetic patients (22), as well as *db/db* mice (11). This increase in hepatic glu-

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TABLE 2
Effects of BDNF on serum enzyme activities and insulin

Treatment	Strain	n	ALT/(IU/l)*†	AST/(IU/l)	ALT/AST*†‡	Insulin (ng/ml)†
Vehicle	*db/db*	11	80.3 ± 4.3	126.5 ± 7.4	0.65 ± 0.04	9.4 ± 2.6
BDNF (30 mg/kg)	*db/db*	11	61.2 ± 2.1	164.5 ± 15.6	0.40 ± 0.03	3.7 ± 0.4
Pair-fed to BDNF	*db/db*	10	70.9 ± 7.2	106.3 ± 14.3	0.71 ± 0.06	12.2 ± 2.8
Vehicle	Heterozygous	7	40.7 ± 1.0	141.3 ± 6.7	0.29 ± 0.01	0.7 ± 0.2
BDNF (30 mg/kg)	Heterozygous	7	34 ± 1.1	138.6 ± 11.1	0.26 ± 0.02	1.3 ± 0.5
Pair-fed to BDNF	Heterozygous	6	45.8 ± 1.7	140.8 ± 14.3	0.34 ± 0.03	2.0 ± 1.1

Data are means ± SE. Mice were treated daily for 4 days and killed by decapitation on the 5th day, 1 h after food removal. *P < 0.02 effect of treatment; †P < 0.0001 effect of strain; ‡P < 0.03 interaction between treatment and strain (two-way ANOVA).

cose production is crucial to the maintenance of fasting hyperglycemia (15,22) and is observed in spite of high serum insulin levels in *db/db* mice (6), indicating relative insensitivity of the liver to insulin. Such conditions, also reported in non-insulin-dependent diabetic patients (22), are thought to reflect an impaired feedback loop between the pancreatic β-cell and the liver (15). Both impaired β-cell glucose sensitivity and impaired hepatic insulin sensitivity have been postulated to result in increased glucose output by liver and hyperinsulinemia in type 2 diabetic patients (15,22). Fasting hyperinsulinemia was unaffected by BDNF in spite of a normalization in fasting blood glucose, indicating that in fasted condition the β-cell was still relatively glucose insensitive after BDNF treatment, while the insulin sensitivity of the liver may have been increased in BDNF-treated mice.

The involvement of insulin in the mechanism of action of BDNF is supported by lack of effect of BDNF on blood glucose in older *db/db* mice that have reduced insulin levels (6) and in hypoinsulinemic streptozotocin-treated diabetic rats (7). However, BDNF treatment resulted in significantly lower fasting blood glucose in younger hyperglycemic, hyperinsulinemic *db/db* mice without affecting fasting hyperinsulinemia. This finding suggests that in the fasted condition, if insulin is involved in the mechanism of action of BDNF, it is through an increase in insulin sensitivity rather than increased release (7). The finding that the ALT/AST ratio is decreased by BDNF may support this possibility because hepatic steatosis in patients with hypertriglyceridemia is linked to a decrease in insulin sensitivity (23). Alleviation of fatty liver, indicated by a reduction in the ALT/AST ratio (19,20), could therefore be associated with increased insulin sensitivity.

It is interesting that reduction of food intake in *db/db* mice pair-fed to BDNF-treated mice did not improve blood glucose levels through a decrease in the amount of ingested glucose. An explanation for this result is that pair-fed animals compensate for decreased food intake with increased glucose output by peripheral tissues to maintain hyperglycemic conditions and elevated HbA_{1c}, but BDNF-treated animals consuming equivalent amounts of food do not. This is supported by the decreased glycogen concentration in the liver of pair-fed animals, which suggests active glycogenolysis to compensate for the effects of reduced food intake on blood glucose. Thus simply reducing the food intake of *db/db* mice (pair-feeding) does not improve blood glucose levels when started at 10 weeks of age because of compensating mechanisms to maintain elevated blood glucose levels. In this regard, when *db/db* mouse liver is perfused with recirculating medium containing various initial concentrations of glucose, hepatic glucose uptake and output are adjusted to change the perfusate glucose concentration toward the physiologic, hyperglycemic level normally observed in *db/db* mice (11). This is in contrast to liver in wild-type mice, which adjusts hepatic glucose uptake and output to change the perfusate glucose concentration toward normoglycemic levels.

Based on the predominantly neural localization of the high-affinity BDNF tyrosine kinase receptor, trk B, effects of BDNF probably involve the nervous system to some extent, although non-neuronal tissue (such as spleen) can also express trk B mRNA (24,25). As previously suggested (7), BDNF may act on trk B–expressing neurons in the hypothalamus (26,27), an area implicated in the control of body weight, food intake, and metabolism (28). Additionally, BDNF could act on the autonomic nervous system, since anatomic changes in preganglionic and postganglionic sympathetic neurons have been reported in mice overexpressing BDNF in the superior cervical ganglion (29). Neural effects of BDNF could be transmitted to peripheral tissues, such as the liver, through the autonomic nervous system or endocrine pathways (13,14).

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TABLE 3
Effects of BDNF on liver glycogen

Treatment	Total glycogen (mg)	Glycogen concentration (mg/g wet wt)
Vehicle	25.0 ± 1.9	34.4 ± 2.0
BDNF (30 mg/kg)	15.0 ± 1.9*	33.6 ± 2.7
Pair-fed to BDNF	11.6 ± 2.1*	20.1 ± 3.3†

Data are means ± SE. n = 9 per group. Mice were treated three times a week for 4.5 weeks. The left anterior lobe of the liver was removed and stored frozen at −80°C until assayed for glycogen. *P < 0.001 vs. vehicle, †P < 0.001 vs. BDNF and vehicle (ANOVA).

In conclusion, the present results demonstrate that BDNF treatment results in dramatically improved blood glucose control in non-insulin-dependent diabetic mice, and this effect is not caused by decreased food intake. Our results indicate that BDNF can alleviate the fasting hyperglycemia in *db/db* mice that contributes to the development of diabetes. In this regard, effects on the liver suggest that this tissue may be involved in the mechanism of action for BDNF.

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