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Reversal of Behavioral and Metabolic Abnormalities, and Insulin Resistance Syndrome, by Dietary Restriction in Mice Deficient in Brain-Derived Neurotrophic Factor

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Dietary restriction (DR) extends life span and improves glucose metabolism in mammals. Recent studies have shown that DR stimulates the production of brain-derived neurotrophic factor (BDNF) in brain cells, which may mediate neuroprotective and neurogenic actions of DR. Other studies have suggested a role for central BDNF signaling in the regulation of glucose metabolism and body weight. BDNF heterozygous knockout (BDNF+/−) mice are obese and exhibit features of insulin resistance. We now report that an intermittent fasting DR regimen reverses several abnormal phenotypes of BDNF(+/−) mice including obesity, hyperphagia, and increased locomotor activity. DR increases BDNF levels in the brains of BDNF(+/−) mice to the level of wild-type mice fed *ad libitum*. BDNF(+/−) mice exhibit an insulin-resistance syndrome phenotype characterized by elevated levels of circulating glucose, insulin, and leptin; DR reduces levels of each of these three factors. DR normalizes blood glucose responses in glucose tolerance and insulin tolerance tests in the BDNF(+/−) mice. These findings suggest that BDNF is a major regulator of energy metabolism and that beneficial effects of DR on glucose metabolism are mediated, in part, by BDNF signaling. Dietary and pharmacological manipulations of BDNF signaling may prove useful in the prevention and treatment of obesity and insulin resistance syndrome-related diseases. (*Endocrinology* 144: 2446–2453, 2003)

THE NEUROENDOCRINE MECHANISMS that regulate food intake, body weight, and energy metabolism are complex, involving both peripheral organs and the brain (1). The hypothalamus plays a major role in regulating food intake by integrating signals from higher brain regions with peripheral signals of the metabolic status of the body including the proteins leptin, insulin, and ghrelin. A metabolic syndrome X characterized by obesity, hyperglycemia, and increased levels of insulin and leptin is becoming increasingly common in industrialized countries, apparently as the result of a combination of increased caloric intake and decreased physical activity (2). Studies of animal models have demonstrated important roles for reduced leptin and insulin responsiveness in the pathogenesis of obesity and type 2 diabetes (3, 4) but have also led to the realization that there are additional, unknown neural and endocrine mechanisms that regulate food intake, energy metabolism, and body weight. Insight into additional mechanisms that regulate food intake and energy metabolism has come from studies of a protein called brain-derived neurotrophic factor (BDNF) that is widely expressed by neurons in the brain. BDNF is best known for its roles in development of the nervous system (5–7) and for its involvement in the processes of synaptic plasticity (8) and neurogenesis (9, 10) in the adult brain. Recent findings suggest that BDNF may also be an important regulator of food intake and energy metabolism. As evidence, BDNF+/− mice are obese (11), conditional deletion of BDNF in the brain results in obesity and hyperactivity (12), and BDNF administration reduces food intake and blood glucose concentrations in diabetic mice by a central nervous system (CNS)-mediated mechanism (13). Collectively, these findings suggest that BDNF signaling in the brain may play a major role in regulating energy metabolism throughout the body.

Dietary restriction (DR; reduced caloric intake or meal frequency with maintained nutrition) can reduce body weight and normalize blood glucose, insulin, and leptin levels in obese animals and humans (14). DR also increases both the mean and maximum lifespan of rodents, and this antiaging effect is associated with enhanced insulin sensitivity (15). DR affects cells throughout the body including the CNS, where improvements in motor and cognitive functions (16, 17) and increased resistance of neurons to insults in models of age-related neurodegenerative disorders (18–20) in rodents maintained on DR have been reported. Two different paradigms of DR have been widely employed because of their highly reproducible ability to increase lifespan in rats and mice. In one paradigm, the animals receive food daily but are limited to a specific amount, which is typically 30–40% less than the *ad libitum* (AL) consumption of the control group. The second paradigm involves intermittent fasting in which the animals are deprived of food for a full day, every other day, and are fed AL on the intervening days. Analyses of various physiological parameters in animals maintained on these two different DR regimens have revealed several similarities including decreases in body weight, temperature, heart rate, blood pressure, and glucose and insulin levels. These DR regimens have also been shown to have

Abbreviations: AL, *Ad libitum*; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; DR, dietary restriction; TBST, Tris-buffered saline with Tween 20.

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beneficial effects on the brain (9, 10, 14–21). There are several possible molecular mechanisms that might explain the beneficial effects of DR on aging and disease including a reduction in mitochondrial oxyradical production, induction of a cytoprotective cellular stress response, and stimulation of the production of growth factors (15, 21, 22). In the present study, we present evidence supporting a role for BDNF in mediating beneficial effects of one DR regimen, intermittent fasting, on body weight and energy metabolism in mice. Mice with BDNF haploinsufficiency exhibit obesity and elevated levels of glucose, insulin, and leptin; DR stimulates increased production in the brain and this is associated with normalization of the metabolic and neuroendocrine abnormalities.

Materials and Methods

Mice

Breeding pairs of BDNF heterozygous (+/−) mice were kindly provided by Dr. L. Tessarollo at the National Cancer Institute; details of their generation, genetic background and phenotypes can be found elsewhere (23). The haploinsufficiency of the BDNF+/− mice was confirmed in preliminary studies which showed that BDNF protein levels were decreased by approximately 50% in the cerebral cortex, hippocampus, and striatum of BDNF+/− mice (Fig. 1D). Three-month-old male wild-type and BDNF+/− littermate mice were divided into two groups (8–10 mice/group), an AL group that had continuous access to food, and a DR group which was fasted for a 24-h period on alternate days as described previously (19). Mice were maintained on the diets for 3 months; body weight and food intake were measured on a weekly basis. Mice were

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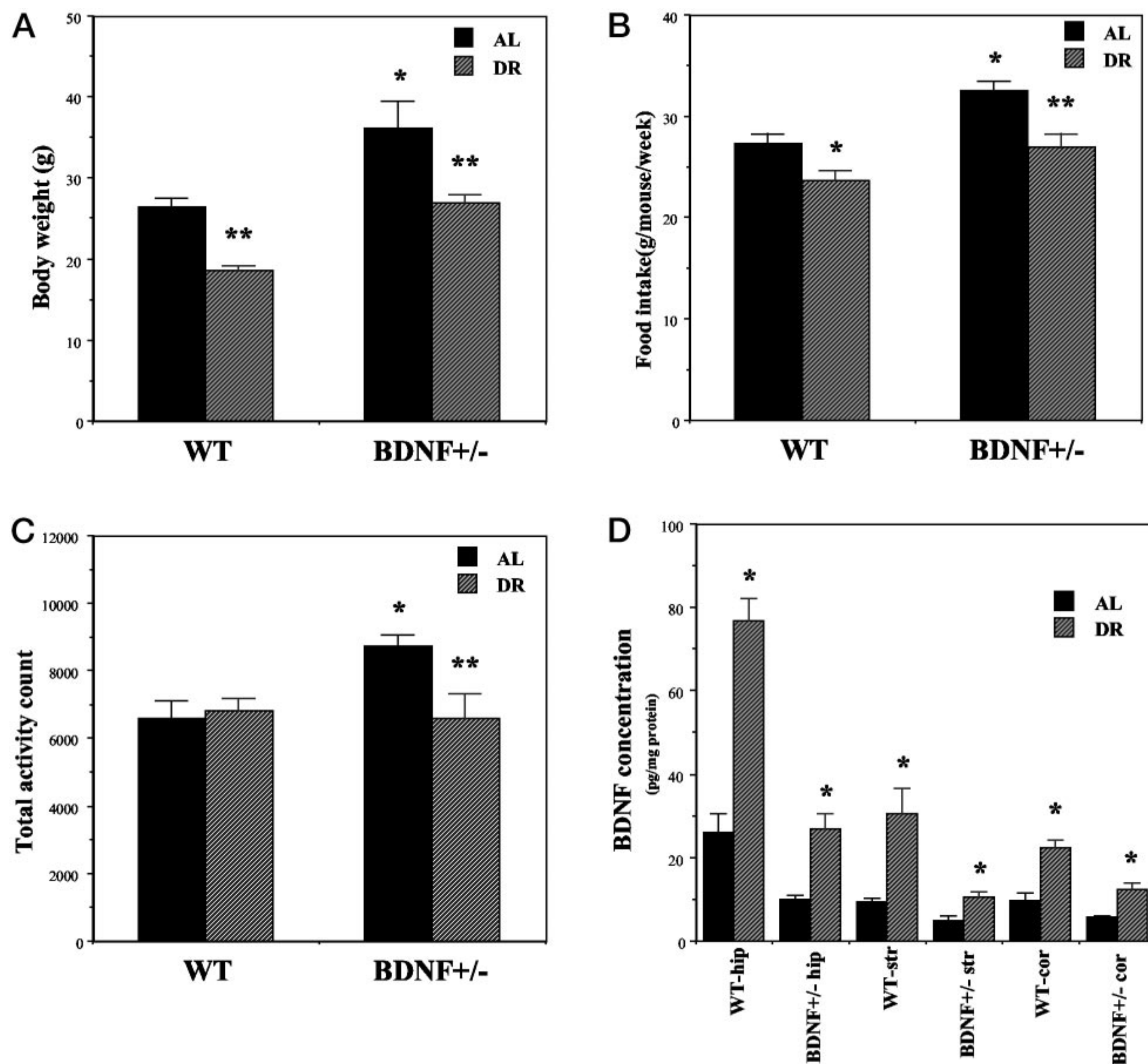

FIG. 1. DR reverses abnormal phenotypes of BDNF+/− mice. Wild-type (WT) and BDNF+/− mice were maintained for 3 months on AL or DR feeding regimens. A, Body weights: *, $P < 0.05$ compared with the WT-AL value; **, $P < 0.01$ compared with the corresponding value for AL-fed mice. B, Food intake: *, $P < 0.05$ compared with the WT-AL value; **, $P < 0.01$ compared with the BDNF+/− AL value. C, Spontaneous activity: *, $P < 0.05$ compared with the WT-AL value; **, $P < 0.01$ compared with the BDNF+/− AL value. D, BDNF concentration: *, $P < 0.01$ compared with the corresponding value for mice fed AL. All values are the mean and SEM of determinations made in 8–10 mice per group; statistical comparisons were made using ANOVA and Scheffé's *post hoc* tests.

provided free access to water and were maintained on a 12-h light, 12-h dark cycle. All procedures were approved by the NIA Animal Care and Use Committee in compliance with NIH guidelines.

ELISA analysis of BDNF protein levels

Freshly dissected hippocampal, cortical and striatal tissues were homogenized in lysis buffer (137 mM NaCl; 20 mM Tris; 1% Nonidet P-40 detergent; 10% glycerol; 1 mM phenylmethylsulfonylflouride; 10 mg/ml aprotinin; 1 mg/ml leupeptin; and 0.5 mM sodium orthovanadate, pH 7.2) at 4 C. Homogenates were centrifuged at 2000 × *g* for 20 min (4 C), and supernatants were used for ELISA analysis. BDNF protein levels were quantified using a commercially available kit (Promega Corp., Madison, WI) according to the manufacturer's protocol. Briefly, samples were processed by acidification and subsequent neutralization. Ninety-six-well plates were coated with mouse monoclonal BDNF antibody, incubated in the presence of block and sample buffer and washed in TBST (Tris-buffered saline with Tween 20). Samples were added in triplicate to wells in each plate, and serial dilutions of BDNF standard (0–500 pg/ml) were added in duplicate to wells in each plate to generate a standard curve. Plates were incubated for 2 h, washed five times in TBST, and incubated for 2 h in a solution containing rabbit polyclonal BDNF antibodies. Wells were washed five times with TBST, and a hydrogen peroxide solution was added together with a peroxidase substrate, and plates were incubated for 10 min. The intraassay and interassay variabilities for the BDNF ELISA were 6% and 13%. Reactions were stopped by adding 100 μl of 1 M phosphoric acid, and absorbance was measured at 450 nm using a plate reader. The concentrations of BDNF in each sample were determined in triplicate, and the average of the three values was used as the value for that mouse for the statistical analysis. Values were expressed as picograms BDNF per milligram of protein.

Measurements of glucose, insulin, IGF-I and leptin, and glucose and insulin tolerance tests

Fasting blood samples were taken from both the DR and AL groups 14 h after withdrawal of food. Blood samples designated as feeding were drawn 6 h after introducing food into the cages of both DR and AL mice that had been subjected to a preceding 14-h fast. Blood glucose concentrations were measured using a glucometer (Lifescan Inc., Milpitas, CA). Insulin levels were determined in duplicate using 10 μl of serum using an UltraSensitive Mouse Insulin ELISA kit (ALPCO Diagnostics, Windham, NH) according to manufacturer's protocol. Serum leptin levels and IGF-I levels were determined in duplicate in 50-μl serum samples using a leptin ELISA kit (Cayman Chemical Co., Ann Arbor, MI) and an IGF-I ELISA kit (Diagnostic Systems Laboratories, Inc., Webster, TX) according to the manufacturer's protocols. For the glucose tolerance test mice were given an oral bolus of D-glucose (2 g/kg body weight) and the blood glucose concentration was measured in samples taken at 0, 15, 30, 60, and 120 min after glucose administration. For the insulin tolerance test, mice were overnight fasted, and insulin (1 U/kg, Sigma, St. Louis, MO) was administered by ip injection and blood glucose concentrations were determined at 0, 15, 30, and 60 min after insulin administration.

Locomotor activity

An automated activity monitor (Digiscan Micro; Omnitech, Columbus, OH) was used to quantify spontaneous activity during a 2-h recording period. Locomotor activity was measured at feeding condition (6 h after addition of food) for both AL and DR groups to avoid the effect of the food searching behavior in the DR group. Each mouse was placed in a recording cage in which 16 infrared sensors monitored the mouse's movement. Data were automatically collected and transferred to a computer for later analysis of locomotor activity; the recording period was initiated 15 min after placing the cage in the recording area. The total number of sensors triggered during the 2-h test period was used as a measure of overall activity level of the mouse.

Statistical analyses

Data were analyzed using one-way ANOVA and *post hoc* comparisons of means were based on Scheffé's test. $P < 0.05$ was considered statistically significant. Analyses were performed using Statview software (SAS Institute, Cary, NC).

Results

DR reverses abnormal phenotypes of BDNF+/− mice including obesity, hyperphagia, and altered locomotor activity, and normalizes BDNF levels in the brain

Two-month-old wild-type and BDNF+/− mice were maintained on either an AL or an intermittent fasting DR feeding regimen for 3 months. The body weights and food intakes of wild-type mice maintained on the DR regimen were significantly decreased compared with mice fed AL (Fig. 1, A and B). Consistent with a previous study (24), BDNF+/− mice exhibited increased body weight and hyperphagia. The DR regimen decreased the body weights and food intake of BDNF+/− mice as well as wild-type mice to levels similar to those of wild-type mice on the AL diet (Fig. 1, A and B). We found that spontaneous locomotor activity was significantly increased in BDNF+/− mice on the AL diet, and that DR normalized this hyperactivity (Fig. 1C).

To confirm that BDNF levels were decreased in the brains of BDNF+/− mice, and to determine whether DR affected BDNF levels, we performed ELISA analysis to quantify BDNF protein levels in three different brain regions (hippocampus, striatum, and cerebral cortex) of wild-type and BDNF+/− mice that had been maintained on AL and DR diets for 3 months. As expected, BDNF levels were decreased in all three brain regions of BDNF+/− mice on the AL diet when compared with wild-type mice on the AL diet; the magnitude of the decreases ranged from 45–65% (Fig. 1D). BDNF levels were increased by 2- to 3-fold in each brain region of wild-type mice maintained on the DR regimen compared with wild-type mice fed AL. BDNF levels were also increased by 2- to 3-fold in each brain region of BDNF+/− mice that had been maintained on the DR diet compared with BDNF+/− mice fed AL (Fig. 1B). Thus, the single copy of the BDNF gene in BDNF+/− mice appears to be as responsive to DR as are the BDNF genes in wild-type mice.

DR normalizes glucose regulation in BDNF+/− mice

It was previously reported that DR can reduce blood glucose levels and increase insulin sensitivity in rats, mice and nonhuman primates (15, 25, 26). Because BDNF+/− mice are hyperphagic and obese, and because DR increases BDNF production, we sought a link between BDNF levels and peripheral glucose metabolism. Measurements of blood glucose in wild-type and BDNF+/− mice that had been maintained on an AL diet revealed a significant increase in fasting glucose levels in BDNF+/− mice (Fig. 2A). Glucose levels were also significantly increased in BDNF+/− mice, compared with wild-type mice, under feeding conditions (Fig. 2B). BDNF+/− mice that had been maintained on DR exhibited significant decreases in blood glucose levels under both fasting and feeding conditions compared with BDNF+/− mice that had been maintained on an AL diet (Fig. 2, A and B). Diabetic rodents and humans exhibit an abnormal blood glucose response to feeding, with glucose levels typically rising to a higher level and remaining elevated for a prolonged time period compared with nondiabetic subjects. We performed glucose tolerance tests in wild-

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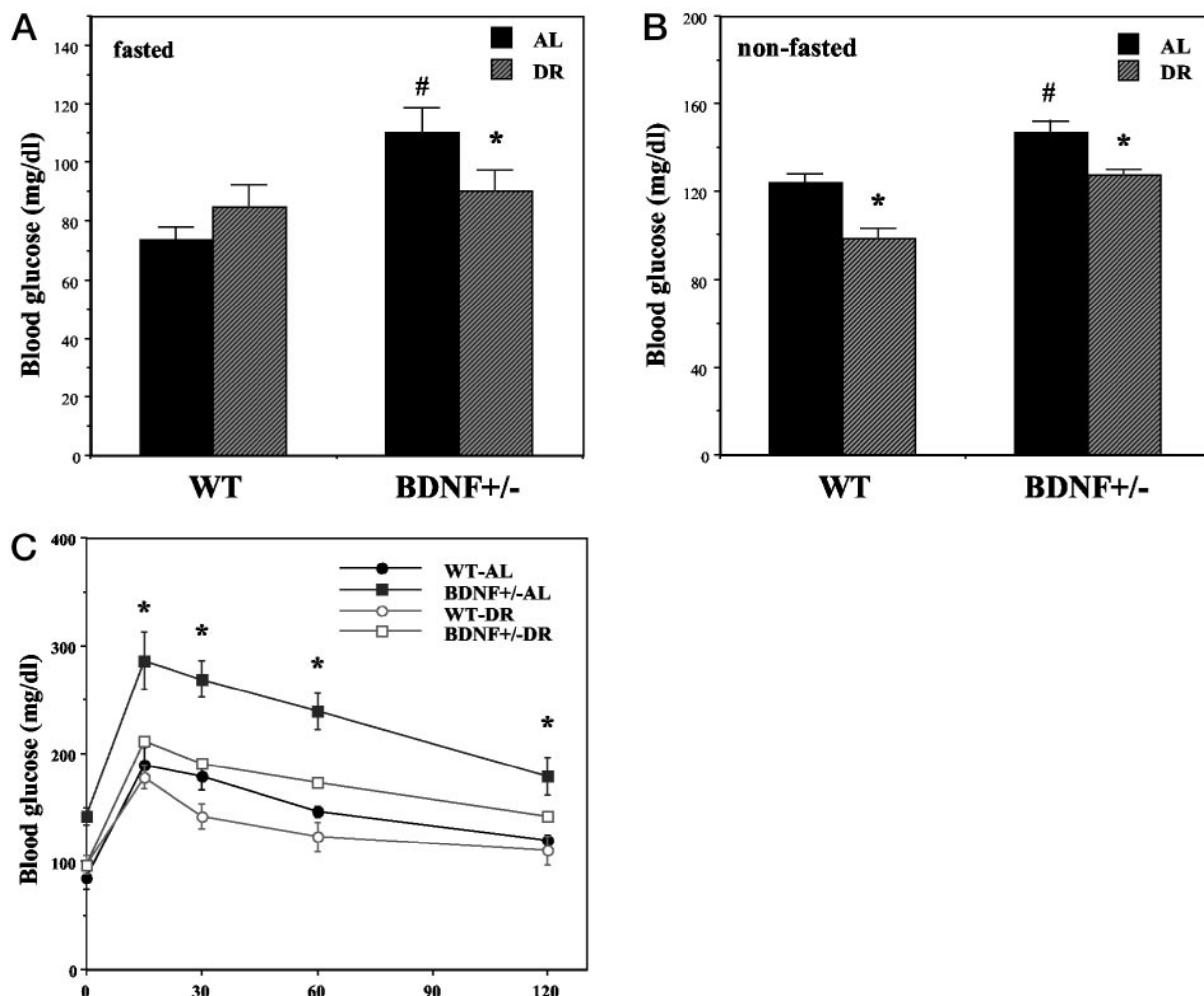

FIG. 2. Hyperglycemia and impaired glucose tolerance in BDNF+/− mice are normalized by DR. Wild-type (WT) and BDNF+/− mice were maintained for 3 months on AL or DR feeding regimens. A and B, Glucose concentrations were measured in blood samples taken after an overnight fast (A) or during feeding conditions (B). Note that the scales for the glucose concentrations in the two graphs are different. *, $P < 0.01$ compared with the value for the same genotype of mice fed AL; #, $P < 0.05$ cmpared to the WT-AL value. C, Mice were administered an oral bolus of glucose (2 g/kg) and the glucose concentration in blood samples taken at the indicated times was determined. *, $P < 0.01$ compared with the value for each of the other three groups at that time point. Values are the mean and SEM of measurements made in 8–10 mice per group. Statistical comparisons were made using ANOVA and Scheffé's *post hoc* tests.

type and BDNF+/− mice that had been maintained on AL or DR diets; mice were given an oral bolus of glucose, and glucose concentrations were determined in blood samples taken 15, 30, 60, and 120 min later. BDNF+/− mice that had been fed AL exhibited a much greater elevation of blood glucose levels, which remained elevated much longer, than did wild-type mice fed AL (Fig. 2C). In contrast, BDNF+/− mice that had been maintained on DR exhibited a blood glucose response to the oral glucose challenge that was similar to that of wild-type AL mice, although greater than that of wild-type mice on DR. These findings demonstrate a profound abnormality in glucose regulation in BDNF+/− mice that can be normalized by DR.

DR reverses hyperinsulinemia and improves insulin sensitivity in BDNF+/− mice

Because hyperglycemia and an abnormal glucose tolerance test are often associated with hyperinsulinemia and reduced insulin sensitivity, we measured serum insulin levels and evaluated insulin sensitivity in wild-type and BDNF+/− mice that had been maintained on AL and DR diets. AL-fed BDNF+/− mice exhibited a dramatic 9-fold increase in fasting serum insulin levels compared with AL-fed wild-type mice (Fig. 3A). Under feeding conditions the BDNF+/− mice exhibited a 20-fold greater serum insulin concentration compared with wild-type mice (Fig. 3B). DR resulted in highly significant decreases in serum insulin levels in both wild-type and BDNF+/− mice under fasting and nonfasting conditions (Fig. 3, A and B). To provide insight into the effects of decreased BDNF levels and DR on insulin sensitivity, wild-type, and BDNF+/− mice that had been maintained on AL or DR diets were administered insulin and glucose concentrations were measured in blood samples taken 15, 30, and 60 min later. Wild-type mice exhibited a marked and sustained reduction in blood glucose levels following insulin administration, with no appreciable difference observed between

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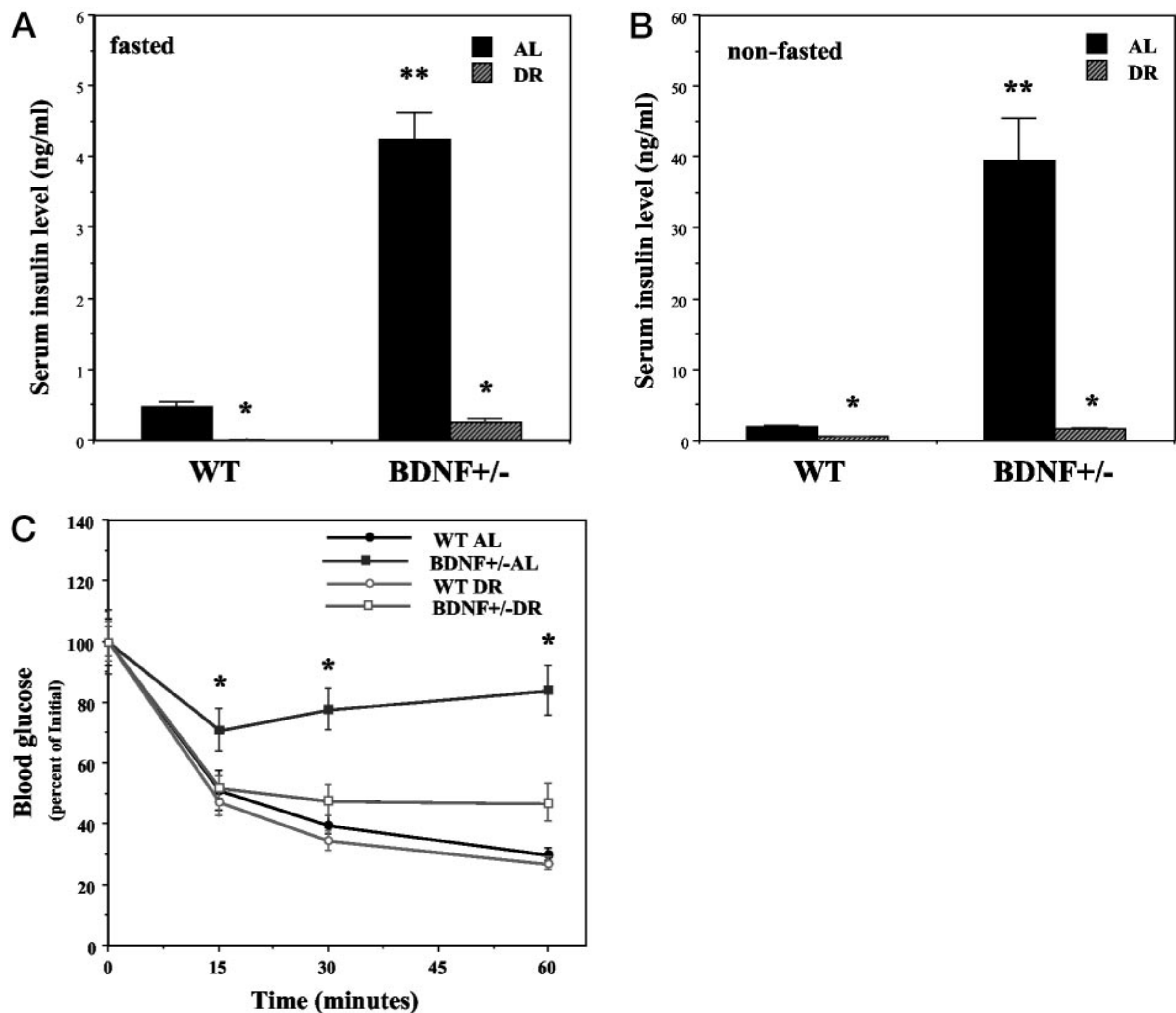

FIG. 3. Mice with reduced BDNF levels exhibit insulin insensitivity that is normalized by DR. Wild-type (WT) and BDNF+/− mice were maintained for 3 months on AL or DR feeding regimens. A and B, Insulin concentrations were measured in blood samples taken after an overnight fast (A) or during feeding conditions (B). *, $P < 0.001$ compared with the value for the same genotype of mice fed AL; **, $P < 0.001$ compared with the WT-AL value. C, Mice were administered insulin (1 U/kg) and the glucose concentration in blood samples taken at the indicated times was determined. *, $P < 0.01$ compared with the value for each of the other three groups at that time point. Values are the mean and SEM of measurements made in 8–10 mice per group. Statistical comparisons were made using ANOVA and Scheffé's *post hoc* tests.

mice that had been maintained on AL or DR diets (Fig. 3C). BDNF+/− that had been fed AL exhibited a striking insensitivity to insulin whereas, in contrast, BDNF+/− mice on DR exhibited a decrease in blood glucose concentration in response to insulin that was similar to that of wild-type mice (Fig. 3C). These results demonstrate that mice with reduced BDNF levels are relatively insensitive to insulin, and that this abnormality can be corrected by DR.

In some studies, increased levels of IGF-1 have been associated with diabetes (27) and decreased levels of IGF-1 may occur in animals maintained on low calorie diets (28). However, the role of such changes in glucose metabolism and physiological actions of DR are unknown. We therefore measured levels of IGF-1 in serum samples from wild-type and BDNF+/− mice that had been maintained on AL or alternate day fasting DR diets. There were no significant effects of genotype or diet on fasting IGF-1 levels, although IGF-1 levels tended to be somewhat lower in mice on DR (Table 1). Similarly, in nonfasted mice there were no differences in IGF-1 levels between wild-type and BDNF+/− mice on either diet, nor did diet affect IGF-1 levels in blood samples drawn in nonfasted mice (Table 1). The lack of effects of BDNF levels and diet on IGF-1 levels suggest that the beneficial effects of DR on the abnormal pheno-

TABLE 1. IGF-I levels are similar in BDNF+/− mice and wild-type (WT) mice, and DR has no significant effect on IGF-I levels in either BDNF+/− mice or WT mice

	Fasted		Nonfasted	
	AL	DR	AL	DR
WT	2657 ± 22	2136 ± 162	2812 ± 197	2645 ± 101
BDNF+/−	2708 ± 91	2480 ± 139	2781 ± 130	2472 ± 73

The values for IGF-1 levels are nanograms per milliliter of serum.

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types of BDNF+/− mice are unlikely to be mediated by IGF-1.

DR reverses hyperleptinaemia in BDNF+/− mice

Leptin is a hormone released from adipose cells that is transported to brain where it binds to its receptor in hypothalamus to regulate food intake and energy expenditure (29). The findings to this point suggested that BDNF exerts some actions similar to leptin, namely, reduced food intake and body weight and improved insulin sensitivity. We therefore measured the concentration of leptin in blood samples from wild-type and BDNF+/− mice that had been maintained on AL or DR diets. Fasting leptin levels were significantly increased, by approximately 2-fold, in BDNF+/− mice compared with wild-type mice (Fig. 4A). Both wild-type and BDNF+/− mice that had been maintained on DR exhibited large and highly significant decreases in fasting leptin levels (Fig 4A). In nonfasted mice that had been maintained on DR, leptin levels were significantly decreased in both wild-type and BDNF+/− mice compared with AL-fed mice, with the effect of DR being greater in wild-type mice compared with BDNF+/− mice (Fig. 4B).

Discussion

The present findings demonstrate that a reduction in BDNF levels in the brain resulting from BDNF gene haploinsufficiency causes abnormalities in glucose metabolism and body weight regulation in mice that are provided free access to food. The abnormalities include hyperphagia, obesity, hyperglycemia, hyperinsulinemia, hyperleptinemia, and decreased insulin sensitivity. We found that when the food intake of the BDNF+/− mice was restricted by maintaining them on an intermittent fasting regimen, their glucose regulation abnormalities and obesity were ameliorated. The correction of the behavioral and metabolic abnormalities of BDNF+/− mice by intermittent fasting was associated with an increase in brain BDNF levels to levels present in wild-type mice fed AL.

The mechanism(s) whereby BDNF regulates food intake, body weight, and glucose metabolism are not known. Conditional deletion of BDNF in the brains of mice resulted in obesity (12), suggesting that the metabolic phenotype of BDNF+/− mice documented in the present study is the result of the decrease in BDNF levels in the brain rather than in peripheral sites. Moreover, it was recently reported that infusion of BDNF into the lateral ventricles results in a reduction in blood glucose levels, demonstrating that BDNF signaling in the CNS can modify peripheral glucose regulation (13). BDNF is widely expressed by neurons in multiple brain regions and which of these brain regions is involved in the antiobesity and antidiabetic actions of BDNF remains to be determined. Previous studies have established important roles for BDNF signaling in synaptic plasticity in the hippocampus and cerebral cortex (30, 31), and these brain regions do influence a variety of behaviors including food intake and body weight (32, 33). BDNF is also produced by hypothalamic cells (34) and could, in principle, act locally in the hypothalamus to suppress food intake. Interestingly, BDNF signaling can induce the growth of serotonergic fibers and may enhance serotonergic signaling (23, 24). Studies of antidepressant drugs and other serotonin-modulating drugs have provided evidence that serotonin can suppress appetite and induce weight loss (35). Therefore, enhanced serotonergic signaling might mediate the antiobesity and antidiabetic actions of BDNF.

The normalization of brain BDNF levels in BDNF+/− mice maintained on an intermittent fasting DR regimen may account for its ability to reverse behavioral and metabolic abnormalities in these mice. It was previously reported that DR can increase BDNF levels in the hippocampus and cortex of mice, and it was proposed that this up-regulation of BDNF plays an important role in the neuroprotective and neurogenesis-promoting actions of DR (9, 22). In addition, it was recently reported that a high-fat, refined sugar diet decreases levels of BDNF in the hippocampus of rats (36), and that physical exercise and enriched environments up-regulate BDNF levels in the brain (37, 38). Interestingly, patients with Huntington's disease and transgenic mice expressing mutant huntingtin proteins exhibit reduced levels of BDNF in their brains and are hyperglycemic (39–42). Therefore, multiple lines of evidence support an important role for BDNF signaling in the brain in the regulation of energy metabolism and body weight in various physiological and pathological states. Although the present data do not provide conclusive evidence that the beneficial effects of DR on peripheral glucose metabolism are mediated by BDNF signaling in the brain, they are consistent with such a possibility.

DR reduced the serum levels of both leptin and insulin in

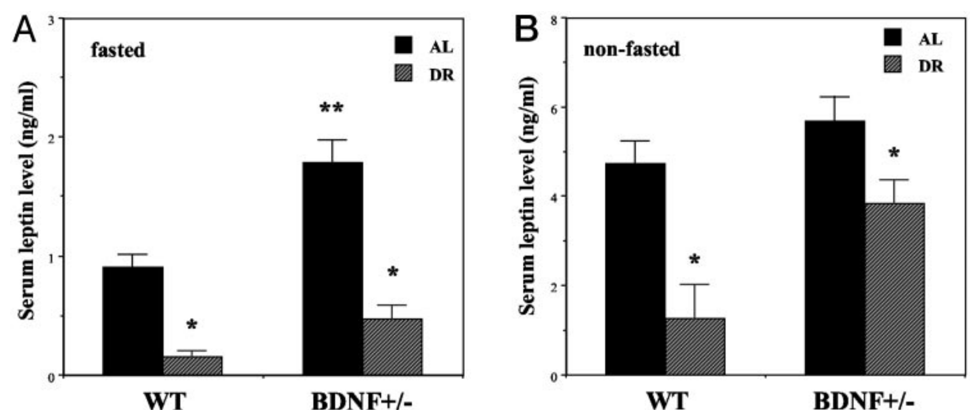

FIG. 4. DR reverses hyperleptinemia in BDNF+/− mice. Two-month-old mice were maintained on AL or DR regimens for 3 months. Serum leptin levels were measured in mice that had been fasted overnight (A) and in nonfasted mice (B). Values are the mean and SEM (n = 8–10 mice per group). *, $P < 0.01$ compared with the corresponding value for group AL. **, $P < 0.01$ compared with the value for WT mice on the AL regimen (ANOVA with Scheffé's *post hoc* tests).

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BDNF+/− mice. Insulin and leptin are released from peripheral tissues and are transported to brain through similar saturable transport mechanisms (43). Microdialysis has confirmed that insulin levels in the extracellular fluid of hypothalamic nuclei are regulated during meal absorption (44). Because circulating insulin and leptin can gain access to the CNS, the behavioral and metabolic effects of central insulin and leptin have important roles in the regulation of energy balance and peripheral action of these hormones (45, 46). The receptors for both hormones are expressed at particularly high levels in cells of the hypothalamus known to regulate energy homeostasis. The effects of DR on peripheral insulin and leptin levels might therefore result from peripheral and/or central effects of DR. BDNF might regulate energy metabolism by directly activating receptors in hypothalamus in neurons regulate food intake and energy balance, or it might act indirectly by enhancing leptin and/or insulin signaling in the CNS. In any case, the present findings suggest that the abilities of DR to increase insulin sensitivity and to reduce circulating glucose and leptin levels are associated with increased BDNF levels.

The phenotypes of BDNF+/− mice are very similar to those of humans with metabolic syndrome X, a condition that places them at increased risk of cardiovascular disease, stroke and type 2 diabetes (2). Studies of rodents, monkeys, and humans have clearly shown that DR can prevent and reverse the abnormalities in glucose metabolism associated with metabolic syndrome X, and can also enhance insulin sensitivity in subjects with normal glucose metabolism (14, 25, 26). In agreement with the latter findings, we found that DR reduces glucose, insulin, and leptin levels not only in BDNF+/− mice, but also in wild-type mice. Because DR induced marked increases in BDNF levels of the brains of wild-type mice, and because central administration of BDNF is sufficient to improve insulin sensitivity in animals fed AL (13), it seems likely that the increase in brain BDNF levels contributes to the beneficial effects of DR on glucose metabolism and body weight in normal subjects. Collectively, the available data therefore suggest that dietary and pharmacological manipulations of BDNF signaling may prove useful as therapeutic approaches for preventing and treating a range of disorders that involve abnormalities in body weight regulation and energy metabolism.

Our study employed only one DR regimen, intermittent fasting. It is therefore important to consider possible differences between calorie-restricted diets in which the animals are provided food every day, but with fewer calories, and the intermittent fasting regimen. In the present study, the mice in the DR group consumed only 10–15% less food over time compared with the mice on the control AL diet. However, previous studies have shown that this intermittent DR regimen extends lifespan by approximately 30% in the same strain of mice (47). We have previously shown that the alternate day fasting DR regimen increases neuronal resistance to dysfunction and death in several different rodent models of neurodegenerative diseases (18–22). Moreover, we have recently made a direct comparison of the effects of a 30% calorie restriction daily feeding to the alternate day fasting regimen in C57BL/6 mice. Despite only a 15% reduction in overall calorie intake in the mice maintained on the alternate day fasting regimen, the mice exhibited greater resistance to excitotoxin-induced damage to hippocampal neurons compared with the mice that had been maintained on the 30% caloric restriction diet (Guo, Z., and M. P. Mattson, unpublished data). We also found that BDNF levels were increased by a greater amount in the hippocampus and cerebral cortex of mice maintained on the alternate day fasting regimen compared with those on the 30% caloric restriction diet (Duan, W., and M. P. Mattson, unpublished data). When taken together with our previous data documenting that intermittent fasting DR regimens induce the expression of stress proteins (HSP-70 and GRP-78; Refs. 19, 20, 22), we believe this cellular stress response is key to the beneficial effects of DR on the brain. The possibility that the beneficial effects of intermittent fasting can, in part, be dissociated from caloric intake is supported by a very recent study that showed that targeted deletion of the insulin receptor in adipose cells results in increased longevity without a reduction in caloric intake (48). Although our findings suggest an important role for BDNF signaling in the regulation of glucose metabolism and brain aging, further studies will be required to determine whether BDNF signaling plays a key role in the life span-extending effects of DR.

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