Paroxetine Retards Disease Onset and Progression in Huntingtin Mutant Mice

Wenzhen Duan, MD, PhD,[1] Zhihong Guo, MD,[1] Haiyang Jiang, MD,[1] Bruce Ladenheim, MS,[2] Xiangru Xu, PhD,[1] Jean Lud Cadet, MD,[2] and Mark P. Mattson, PhD[1,3]

We report that administration of paroxetine, a widely prescribed antidepressant drug that acts by inhibiting reuptake of the neurotransmitter serotonin, suppresses the neurodegenerative process and increases the survival of huntingtin mutant mice, an animal model of Huntington's disease (HD). Paroxetine attenuated motor dysfunction and body weight loss and improved glucose metabolism in the HD mice. Paroxetine was beneficial when treatment was initiated before or after the onset of motor dysfunction, suggesting a potential for such antidepressant drugs in the treatment of presymptomatic and symptomatic HD patients.

Ann Neurol 2004;55:590–594

Huntington's disease (HD) is an inherited disorder characterized by progressive degeneration of neurons in the striatum and cerebral cortex resulting in abnormal involuntary movements, psychiatric and cognitive abnormalities, and death.[1] HD is caused by expansion of CAG trinucleotide repeats in exon 1 of the huntingtin gene resulting in polyglutamine expansions in the huntingtin protein.[2] Mutant huntingtin may cause neuronal degeneration by impairing energy metabolism and inducing oxidative stress and apoptosis.[3–5] A class of drugs that is widely used for the treatment of patients with depression and severe anxiety disorders is the serotonin selective reuptake inhibitors (SSRIs).[6] SSRIs can attenuate psychiatric abnormalities in HD patients,[7] but the possibility that SSRI also might affect the neurodegenerative process in HD has not been tested. Here, we show that when huntingtin mutant mice are treated with paroxetine, depleted serotonin levels are restored, neuronal degeneration and motor dysfunction are retarded, energy metabolism is improved, and the survival of the mice is extended.

From the [1]Laboratory of Neurosciences, National Institute on Aging Intramural Research Program; [2]Molecular Neuropsychiatry Branch, National Institute on Drug Abuse; and [3]Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD.

Received Nov 6, 2003, and in revised form Jan 15, 2004. Accepted for publication Jan 15, 2004.

Published online Mar 22, 2004, in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.20075

Address correspondence to Dr Duan, Laboratory of Neurosciences, National Institute on Aging Gerontology Research Center, 5600 Nathan Shock Drive, Baltimore, MD 21224.
E-mail: duanwe@grc.nia.nih.gov

Materials and Methods

Mice

Breeding pairs of HD-N171-82Q mice were kindly provided by D. R. Borchelt. These mice express a human N-terminal truncated huntingtin with 82 polyglutamine repeats driven by a mouse prion protein promoter[8] and were maintained as described previously.[9] HD and nontransgenic mice were given either paroxetine (5mg/kg/day) or vehicle subcutaneously. Disease progression and survival status were monitored daily. Mice were scored by a trained observer blind to the genotype of the mice. Body weight was recorded weekly. All procedures were approved by the Institutional Animal Care and Use Committee.

Behavioral Testing and Glucose Measurements

Motor performance was assessed with a rotarod apparatus using a protocol similar to that described previously.[9] The rotadrum was filled with water to a level just below the bottom of the rod; the mice were placed on the rotating rod and the time until they fell off was recorded. This was repeated (with a rest period that increased by 5 seconds with each fall) until the total time was 5 minutes. Both the total time spent on the rotating rod and the total number of falls for each mouse were recorded. Blood glucose concentrations were quantified as described previously.[9]

Neurochemical and Histological Analyses

Mice were killed and the brains were removed. Striatal tissue was removed and stored at −80°C. The tissue were weighed and sonicated in 0.1M perchloric acid containing 10ng/mg tissue weight of the internal standard dihydroxybenzilamine and centrifuged at 20,000*g* for 5 minutes. Concentrations of dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), serotonin (5-HT) and its metabolites 5-hydroxyindoleacetic acid (5-HIAA) in striatal tissue extracts were measured by high-performance liquid chromatography with electrochemical detection as we described previously.[10] Contents of DA, DOPAC, HVA, 5-HT, and 5-HIAA were calculated as picograms per milligrams of tissue. The methods for histological analyses were identical to those described previously.[9]

Statistics

Comparisons among different groups (genotypes and treatments) were made using ANOVA, and Scheffé post hoc test was used for pairwise comparisons.

Results and Discussion

Eight-week-old HD mice and nontransgenic mice were divided into two groups, a vehicle-treated control group and a paroxetine-treated group (5mg parox-

Table. Effect of Chronic Administration of Paroxetine on Levels of Serotonin, Dopamine, Norepinephrine and Their Metabolites in Striatal Tissues of HD Mice and Nontransgenic Control Mice

Group	5-HT	5-HIAA	Dopamine	DOPAC	HVA	NE
Nontg-vehicle	1,118 ± 34	986 ± 95	4,377 ± 1,519	3,818 ± 1,722	2,457 ± 651	156 ± 35
Nontg-paroxetine	1,433 ± 76[a]	1,309 ± 389[a]	4,580 ± 1,247	2,827 ± 1,557	2,098 ± 752	266 ± 49
HD-vehicle	424 ± 47[b]	398 ± 37[b]	3,202 ± 1,059	2,222 ± 283	1,575 ± 374	117 ± 14
HD-paroxetine	1,083 ± 33[c]	969 ± 33[c]	3,832 ± 708	1,436 ± 249	1,187 ± 78	163 ± 14

Eight-week-old HD mice and age-matched nontransgenic control mice were given paroxetine (5 mg/kg, once a day) or vehicle; 6 weeks later, mice were killed, and striatal tissue samples were removed and levels of serotonin (5-HT), 5-HIAA, dopamine, DOPAC, HVA, and norepinephrine were quantified. Values (pg/mg tissue weight) are the mean ± SE of determinations made in five to six mice/group.

[a] $p < 0.05$; [b] $p < 0.01$ compared with value of Nontg-vehicle group; [c] $p < 0.01$ compared with the value of HD-vehicle group. ANOVA with Scheffé post hoc tests.

etine/kg body weight/day). In 14-week-old HD mice, there was a significant depletion of both serotonin and 5-HIAA in the striatum compared with nontransgenic control mice (Table). Administration of paroxetine increased serotonin and 5-HIAA levels in the striata of HD mice and in nontransgenic mice (see Table). In contrast, levels of dopamine and norepinephrine were not changed significantly in the striatum of HD mice compared with nontransgenic mice, and there was no significant effect of paroxetine on levels of dopamine and norepinephrine in striatum of nontransgenic or HD mice (see Table).

The onset of behavioral symptoms was significantly delayed by an average of 14 days in both male and female HD mice treated with paroxetine (Fig 1A). Paroxetine significantly increased the maximum life span of both male and female HD mice an average of 15 days (see Fig 1B). We previously reported that the HD mice exhibit progressive weight loss.[9] HD mice given paroxetine lost weight at a significantly slower rate than did vehicle-treated HD mice (see Fig 1C).

We next evaluated motor function in 16-week-old mice that had been treated with paroxetine or vehicle beginning at 8 weeks of age. There was a significant impairment in performance of HD mice on the rotarod apparatus at 16 weeks of age compared with nontransgenic mice (see Fig 1D). HD mice treated with paroxetine exhibited superior motor performance compared to vehicle-treated HD mice (see Fig 1D).

To determine whether the improved motor function and increased survival of HD mice treated with paroxetine resulted from a slowed progression of the neurodegerative process, we performed histological analyses of the brains. As disease progressed, brain atrophy occurred in the HD mice as indicated by an increase in the size of the lateral ventricles and a thinning of the cerebral cortex compared with nontransgenic mice (Fig 2A). The magnitude of ventricular enlargement was decreased in HD mice that had been treated with paroxetine compared with HD mice treated with vehicle (see Fig 2A). Caspase 1 cleavage, an indicator of apoptosis, also was decreased in paroxetine-treated HD mice compared with vehicle-treated HD mice (data not shown).

Because huntingtin mutant mice[9,11] are hyperglycemic, we determined whether this metabolic abnormality could be ameliorated by paroxetine treatment in the HD mice. Blood glucose concentrations in vehicle-treated HD mice were two- to threefold greater than in nontransgenic mice (see Fig 2B, C). Blood glucose concentrations in HD mice that had been treated with paroxetine were significantly lower than blood glucose levels in vehicle-treated HD mice.

To determine whether SSRI might modify the course of the symptomatic phase of the disease, we performed a study in which a cohort of HD mice (15 mice of each sex per group) were given paroxetine or vehicle at the time of onset of motor dysfunction The survival of paroxetine-treated symptomatic mice was significantly increased compared with the survival of vehicle-treated symptomatic mice (see Fig 2D).

These findings provide evidence that enhanced serotonergic signaling can counteract the neurodegenerative process and improve glucose metabolism in HD. We found that levels of serotonin and its metabolite 5-HIAA are decreased in brain tissue of HD mutant mice, consistent with a previous study of a different (R6/2) transgenic mouse model of HD.[12] SSRI can increase extracellular serotonin levels and serotonin synthesis resulting in increased serotonergic signaling and increased brain-derived neurotrophic factor (BDNF) expression.[13–15] Serotonin might have direct neuroprotective effects on striatal and cortical neurons because other signals that activate cyclic AMP and CREB have been shown to protect neurons against insults relevant to HD. In addition to BDNF, there are several other target genes of cyclic AMP-CREB signaling that might play roles in the neuroprotective effects of SSRI including Bcl-2 and NF-κB.[16,17]

The hyperglycemic state of the huntingtin mutant mice was normalized by paroxetine, suggesting that serotonergic signaling improves the ability of the mice to regulate glucose metabolism. Based on previous findings,[9,18] it is likely that BDNF mediates the effect of

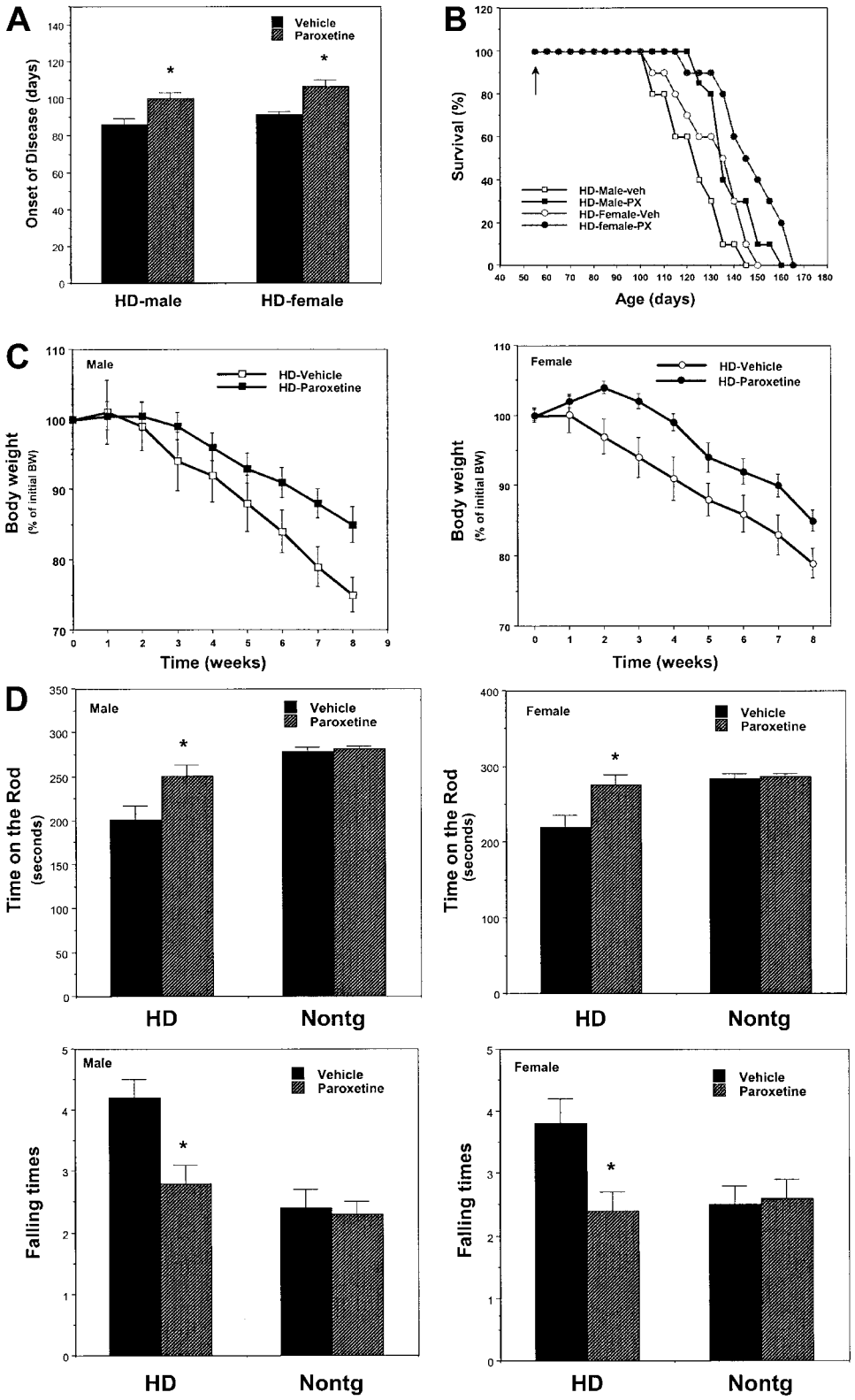

*Fig 1. Paroxetine delays the onset of motor dysfunction, attenuates weight loss, improves motor function, and increases the survival of huntingtin mutant mice. Eight-week-old male and female huntingtin mutant mice (HD) and nontransgenic control mice (Nontg) were given paroxetine (5mg/kg, subcutaneous injection daily) or vehicle . (A–C) The onset of motor dysfunction (A), survival (B), and body weights (C) were determined. The arrow in panel B indicates the time at which paroxetine treatment was initiated. (D) Motor behavioral performance was evaluated by using a rotarod apparatus in 16-week-old mice. Values are the mean and SE (12–15 mice per group). *$p < 0.05$ compared with the value of HD vehicle group. BW = body weight.*

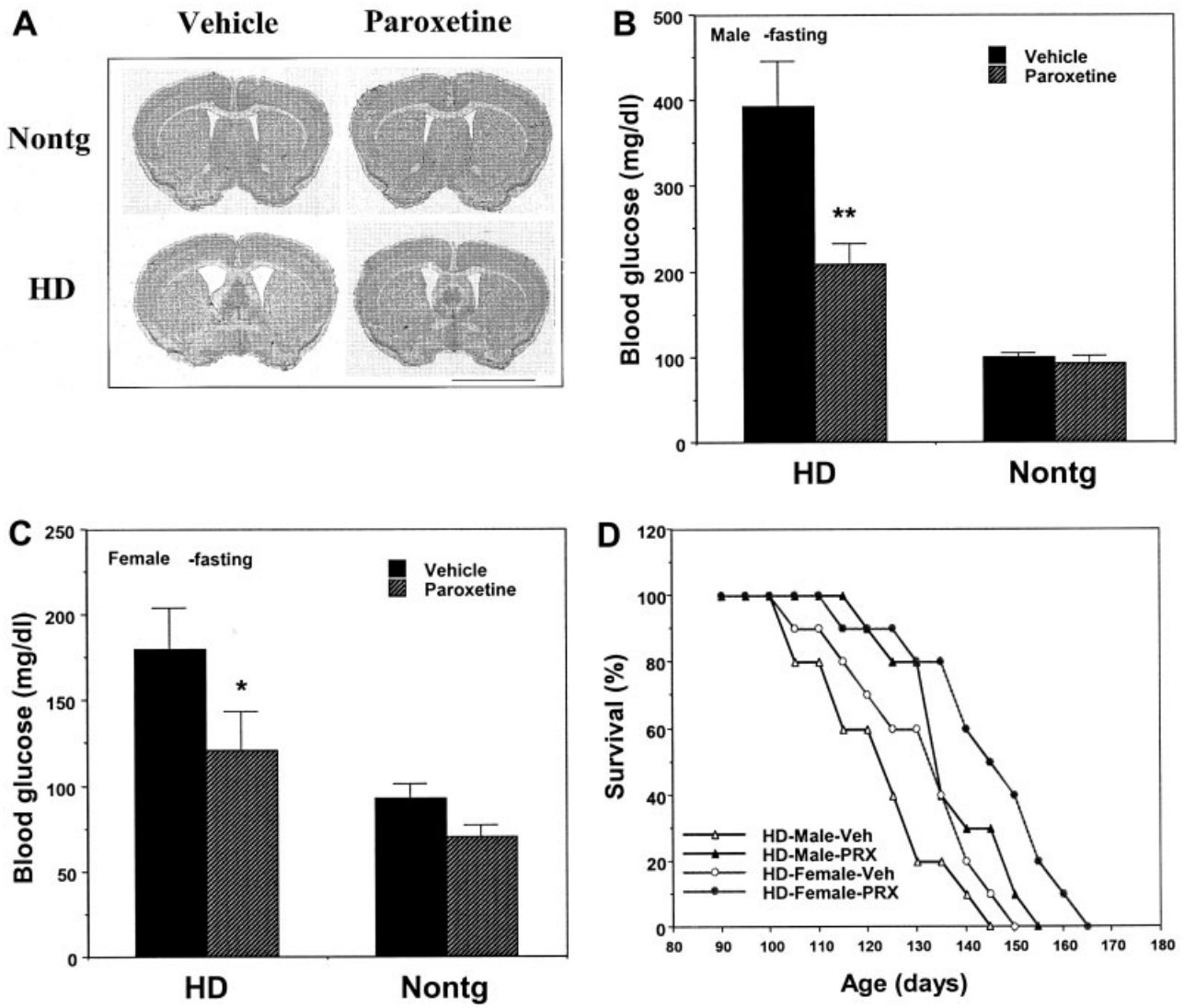

*Fig 2. Paroxetine reduces brain atrophy and ameliorates hyperglycemia in Huntington's disease (HD) mice when administrated before onset of motor dysfunction and also extends survival of HD mice when administered after the onset of motor dysfunction. (A) Photomicrographs of cresyl violet–stained brain sections from HD mice and nontransgenic mice (Nontg) that had been given paroxetine or vehicle for 8 weeks. The micrographs are representative of four to five mice evaluated in each treatment group. (B, C) Eight-week-old male and female HD mice and nontransgenic control mice were given paroxetine (5mg/kg) or vehicle for 2 months. Concentrations of glucose in blood samples were measured after an overnight fast. The values are the mean and SE ($n = 12–15$ mice). *$p < 0.05$, **$p < 0.01$ compared with the value of HD-vehicle group. (D) HD mice were evaluated daily until the first date when limb tremors were detected. Each symptomatic mouse then was treated with either paroxetine or vehicle until they reached the end stage of their life. Values are the mean and SE (15 mice per group), *$p < 0.05$ compared with the value of HD vehicle group.*

paroxetine on glucose regulation. The ability of paroxetine to attenuate the progressive weight loss that occurs in HD mice suggests that this weight loss is secondary to the central nervous system abnormalities caused by mutant huntingtin. Suppressing the neurodegenerative process by the enhancement of serotonergic signaling may delay development of the hypermetabolic state and tissue wasting characteristic of HD.[19,20]

Because patients can be treated with SSRI for extended time periods of many years with few or no side effects, this class of antidepressant drugs might be given to individuals who harbor HD-causing polyglutamine expansions in huntingtin before their becoming symptomatic. However, we also found that paroxetine increases the survival of HD mice even when administered after the onset of motor dysfunction, suggesting the possibility that HD patients might benefit from SSRI even after they become symptomatic. Our preclinical findings provide a rationale for further investigating the effects of SSRI in human HD patients.

We thank D. R. Borchelt for providing breeding pairs of huntingtin mutant mice, and T. Kiruki and E. Dabney of the National Institute on Aging animal facility for technical assistance.

References

1. Sieradzan KA, Mann DM. The selective vulnerability of nerve cells in Huntington's disease. Neuropathol Appl Neurobiol 2001;27:1–21.
2. Andrew SE, Goldberg YP, Kremer B, et al. The relationship between trinucleotide repeat (CAG) length and clinical features of Huntington disease. Nat Genet 1993;7:513–519.
3. Gu M, Gash MT, Mann VM, et al. Mitochondrial defect in Huntington's disease caudate nucleus. Ann Neurol 1996;39: 385–389.
4. Beal MF, Kowall NW, Ellison DW, et al. Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. Nature 1986;321:168–171.
5. Schilling G, Coonfield ML, Ross CA, Borchelt DR. Coenzyme Q10 and remacemide hydrochloride ameliorate motor deficits in a Huntington's disease transgenic mouse model. Neurosci Lett 2001;315:149–153.
6. Blier P, Montigny C. Current advances and trends in the treatment of depression. Trends Pharmacol Sci 1994;15:220–226.
7. De Marchi N, Daniele F, Ragone MA. Fluoxetine in the treatment of Huntington's disease. Psychopharmacology (Berl) 2001;153:264–266.
8. Schilling G, Becher MW, Sharp AH, et al. Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. Hum Mol Genet 1999;8:397–407.
9. Duan W, Guo Z, Jiang H, et al. Dietary restriction normalizes glucose metabolism and BDNF levels, slows disease progression and increases survival in Huntingtin mutant mice. Proc Natl Acad Sci USA 2003;100:2911–2916.
10. Duan W, Zhu X, Ladenheim B, et al. p53 inhibitors preserve dopamine neurons and motor function in experimental parkinsonism. Ann Neurol 2002;52:597–606.
11. Hurlbert MS, Zhou W, Wasmeier C, et al. Mice transgenic for an expanded CAG repeat in the Huntington's disease gene develop diabetes. Diabetes 1999;48:649–51.
12. Reynolds GP, Dalton CF, Tillery CL, et al. Brain neurotransmitter deficits in mice transgenic for the Huntington's disease mutation. J Neurochem 1999;72:1773–1776.
13. Badawy AA, Morgan CJ. Effects of acute paroxetine administration on tryptophan metabolism and disposition in the rat. Br J Pharmacol 1991;102:429–433.
14. Nibuya M, Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci 1996; 16:2365–2372.
15. Chen B, Dowlatshahi D, MacQueen GM, et al. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 2001;50: 260–265.
16. Freeland K, Boxer LM, Latchman DS. The cyclic AMP response element in the Bcl-2 promoter confers inducibility by hypoxia in neuronal cells. Mol Brain Res 2001;92:98–106.
17. Yalcin A, Koulich E, Mohamed S, et al. Apoptosis in cerebellar granule neurons is associated with reduced interaction between CREB-binding protein and NF-kappaB. J Neurochem 2003; 84:397–408.
18. Duan W, Guo Z, Jiang H, et al. Reversal of behavioral and metabolic abnormalities, and insulin resistance syndrome, by dietary restriction in mice deficient in brain-derived neurotrophic factor. Endocrinology 2003;144:2446–2453.
19. Pratley RE, Salbe AD, Ravussin E, Caviness JN. Higher sedentary energy expenditure in patients with Huntington's disease. Ann Neurol 2000;47:64–70.
20. Morales LM, Estevez J, Suarez H, et al. Nutritional evaluation of Huntington disease patients. Am J Clin Nutr 1989;50: 145–150.