Huntington's disease : hypothalamic, endocrine and metabolic aspects

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Chapter 2

Weight loss in Huntington's disease increases with higher CAG repeat number

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ABSTRACT

Objective: Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by an expanded number of CAG repeats in the *huntingtin* gene. A hallmark of HD is unintended weight loss, the cause of which is unknown. In order to elucidate the underlying mechanisms of weight loss in HD, we studied its relation to other disease characteristics including motor, cognitive and behavioral disturbances and CAG repeat number. *Methods:* In 517 early-stage HD patients, we applied mixed-effects model analyses to correlate weight changes over three years to CAG repeat number and various components of the Unified Huntington's Disease Rating Scale (UHDRS). We also assessed the relation between CAG repeat number and body weight and caloric intake in the R6/2 mouse model of HD. *Results:* In HD patients mean body mass index decreased with -0.15 units per year ($p<0.001$). However, no single UHDRS component, including motor, cognitive and behavioral scores, was independently associated with the rate of weight loss. HD patients with a higher CAG repeat number had a faster rate of weight loss. Similarly, R6/2 mice with a larger CAG repeat length had a lower body weight, whereas caloric intake increased with larger CAG repeat length. *Conclusions:* Weight loss in HD is directly linked to CAG repeat length and is likely to result from a hypermetabolic state. Other signs and symptoms of HD are unlikely to contribute to weight loss in early disease stages. Elucidation of the responsible mechanisms could lead to effective energy-based therapeutics.

Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disorder caused by an expanded number of CAG repeats in the *huntingtin* gene.[1] It is characterized by motor disturbances, cognitive decline and behavioral problems.[2] Unintended weight loss is also a hallmark of the disease, both in HD patients[3-6] and several transgenic mouse models of HD[7]. Weight loss frequently leads to general weakening and a decline in the quality of life of HD patients.[8] On the other hand, a higher Body Mass Index (BMI) has been associated with a slower rate of disease progression.[9]

The cause of weight loss in HD is unknown. It might result from decreased caloric intake, increased motor activity or a higher metabolic rate.[10] Previous studies in both HD patients and transgenic mouse models of HD have shown that loss of weight occurs despite adequate or even increased caloric intake.[11-13] Weight loss is already manifest in presymptomatic HD gene carriers[14] and is particularly marked in the final hypokinetic stages of the disease[5]. These observations and recent findings in HD transgenic mice suggest that weight loss might be due to an increased metabolic rate.[13,15,16] Other reports suggest, however, that loss of body weight is secondary to a higher sedentary energy expenditure due to unwanted movements.[17-20] Thus, studies on the mechanisms underlying weight loss in HD patients have yielded conflicting results and are inconclusive.[10] The different outcomes of these studies are likely to be due to small group sizes and their cross-sectional nature.

Interestingly, the direct relation between the number of CAG repeats in the mutant *huntingtin* gene and weight loss has not been assessed before. Mutant *huntingtin* could interfere with mitochondrial function in peripheral tissues in a CAG repeat length-dependent manner.[21,22] Consequently, CAG repeat length may predict the extent of systemic energy defects in HD patients.

In this study we therefore aimed to 1) specify the course of weight loss in a large, homogenous group of clinically well-characterized HD patients during a long-term follow-up, 2) determine which factors (including motor, cognitive and behavioral) are associated with weight loss, 3) assess whether CAG repeat length is directly related to the rate of weight loss, and 4) determine whether CAG repeat length is also associated with body weight and caloric intake in the most widely used transgenic mouse (R6/2) model of HD.

METHODS

HD patients. Participants were from the European Huntington's Disease Initiative (EHDI) study, a randomized placebo-controlled trial over three years to study the effects of riluzole on the progression of HD.[23] For inclusion, participants were required to be between 25 and 65 years of age, to carry a CAG-repeat expansion in the HD gene of ≥ 36, to manifest clinical signs of HD, and yet to be in an early stage of the disease (defined on the Unified Huntington's Disease Rating Scale (UHDRS) as a motor score ≥ 5 and Total Functional Capacity (TFC)-score ≥ 8). Patients on anti-choreatic (neuroleptic) treatment were not included and start of such medication was a predefined end point. In total, 537 HD patients were randomized of whom 379 completed three years of follow-up. One-hundred-fifty-eight (158) HD patients (29.4%) dropped out due to different reasons, e.g. adverse events, suicide and suicide attempts, start of anti-choreatic medication. Here,

we excluded 20 participants (3.7%) from our analyses due to erroneous data on body weight (n=6) or missing data on height (n=14).

Clinical evaluations. Demographic data included age, gender and age of onset. Height, weight and the clinical scores on the UHDRS were recorded at baseline. Weight and UHDRS scores were also measured at subsequent visits at 2, 6, 12, 18, 24, 30, 36 and 37 months after the start of the study. Missing values at baseline observations were replaced by the corresponding value obtained at the screening visit wherever available.[23] The UHDRS is divided into four components assessing motor performance, cognition, behavior and functional capacity.[3,24,25] In addition, symptoms of depression were also assessed with the Beck Depression Inventory (BDI).

R6/2 transgenic mice. We used eight transgenic HD mice of the R6/2 line and eight wild-type littermates (Jackson Laboratories, Bar Harbor, ME, USA).[13] They were obtained by crossing heterozygous males with females of their background strain (C57BL/6). CAG repeat lengths were assessed using a polymerase chain reaction assay (Mangiarini et al., 1996). The mice were singly housed from five weeks of age and had *ad libitum* access to water and food under standard conditions (12 h light/dark cycle, 22 °C). They were fed a standard diet (15% fat on a caloric basis). As R6/2 mice develop progressive locomotor problems, food was placed in dishes on the bottom of the cage. We monitored food intake four times per week over 24 h by pre-weighing a portion of food and weighing it 24 h later. From week six to week 12, we measured body weight twice per week. At 12 weeks of age, mice were euthanized for ethical reasons. The experimental procedures were approved by the Regional Ethical Committee of Lund University, Sweden.

Statistical analyses. *HD patients:* We used linear mixed-effects models[26] to examine changes in BMI during the follow-up period. To account for the correlation between the repeated measurements on each individual, we used a model with both fixed and random terms for time passed since the start of the trial. Disease duration at the start of the trial was considered as a fixed covariate. We also added the quadratic term for time to investigate potential non-linear relations between BMI and time. However, this term was not significant and was therefore left out. The associations between BMI changes and other demographic and clinical variables such as gender, various UHDRS subscores and CAG repeat length were studied by adding these variables one by one as explanatory variables into the model. To assess whether a variable was significantly associated with the rate of BMI change, also the interaction of this variable with the time variable was added and tested. A significant interaction entails that the variable of concern influences the rate of BMI change. To identify which predictor variables from Table 2 were *independently* associated with the rate of BMI change, we also used a stepwise regression procedure based on forward selection (an independent predictor of BMI change is a variable that remains significantly associated with the rate of BMI change after adjustment for the effects of other significant predictor variables.) Models were validated both graphically and analytically.[26] The linear mixed-effects models procedure applied here is valid under the Missing at Random assumption. Under the Missing at Random assumption drop out of subjects is allowed to depend on both previous outcome measurements as well as predictor variables.[26] In addition, we also verified that drop out of subjects did not depend on any baseline characteristic using a Cox proportional-hazards regression model. Differences between the baseline characteristics of the placebo and the riluzole group were statistically evaluated by the unpaired Student's t-test

or the χ^2-test where appropriate. *R6/2 mice:* As R6/2 mice first display reduced growth and later (from nine weeks of age and onwards) lose weight, for each mouse we calculated the total area under the curve for both body weight and caloric intake (kCal per gram of body weight). Pearson's correlation coefficients were then computed to assess correlations.

All data are presented as means ± SEM. All tests were two-tailed and values of $p < 0.05$ were considered to be significant. Programming was performed in SPSS version 14.0 for Windows (SPSS Inc, Chicago, Ill, USA) and SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

HD patients

Baseline characteristics

All baseline characteristics of the HD patients who were included are summarized in Table 1. Except for small differences in age at the start of the trial, age of onset and the number of CAG repeats, there were no significant differences between the baseline characteristics of patients on either placebo or riluzole.

Table 1. Baseline characteristics of Huntington's patients that participated in the study

	Total cohort (n=517)
Group (n)	
Placebo	173 (33.5%)
Riluzole	344 (66.5%)
Men (%)	260 (50.3%)
Age (yrs) †	45.65 (0.43)
Age of onset (yrs) †	43.52 (0.44)
Disease duration (yrs)	2.13 (0.09)
BMI (kg/m²)	23.29 (0.16)
Years of education	11.33 (0.17)
CAG repeat number †	45.44 (0.19)
UHDRS motor score	28.21 (0.66)
UHDRS TFC score	10.88 (0.07)
UHDRS FAS score	22.07 (0.13)
UHDRS cognitive score	175.99 (2.79)
UHDRS behavioral score	11.85 (0.43)
BDI score	10.10 (0.39)

Data are presented as means (± SEM). Abbreviations: BMI = Body Mass Index; UHDRS = Unified Huntington's Disease Rating Scale; TFC = Total Functional Capacity; FAS = Functional Assessment; BDI = Beck Depression Inventory; † The placebo and the riluzole group differed significantly on these baseline characteristics ($p < 0.05$).

BMI and rate of BMI change

The rate of BMI change seemed to differ significantly between the placebo and the riluzole group (Table 2). However, since the two groups differed on a number of basal characteristics (Table 1), we corrected for these differences by including age, age of onset, CAG repeat number and baseline BMI and their interactions with time in the model. After correction for these confounders the two treatment groups ceased to differ significantly in their rate of BMI change (p-value of group × time interaction = 0.126). Moreover, stepwise regression did not identify treatment group as an independent predictor of BMI change (Table 2). Therefore, we based all our subsequent analyses on the pooled data.

At baseline the average BMI of the total study population (n = 517) was 23.29 (SEM = 0.16). The mean BMI decreased with -0.15 units per year (SEM = 0.038; 95% CI: -0.23 to -0.08; $p < 0.001$). A higher BMI at baseline was associated with a faster rate of body weight decline (adjusted p for baseline BMI × time interaction = 0.001; Table 2). On average women weighed significantly less than men by about -0.88 BMI units

(SEM = 0.322; 95% CI: -1.51 to -0.25; p = 0.007). The significant interaction between gender and time (p = 0.037; Table 2) disappeared when corrected for BMI at baseline (p = 0.078) indicating that men and woman do not differ in their rate of weight loss. Age of disease onset was not associated with BMI or the rate of BMI change (both p ≥ 0.061; Table 2).

Table 2. Correlates of BMI and the rate of BMI change in patients with Huntington's disease

	Predictor variable	*Effect on average BMI [a] (p-value)*	*Effect on rate of BMI change [b] (p-value)*	*Stepwise forward selection [c] (p-value)*
General variables	*Age of onset*	0.031 (0.061)	0.001 (0.805)	-
	Baseline BMI, kg/m²	0.997 (<0.001) **	-0.030 (0.005) *	-0.034 (0.001) **
	Gender [d]	-0.879 (0.007) **	0.158 (0.037) *	-
	CAG repeat number	-0.136 (<0.001) **	-0.022 (0.017) *	-0.027 (0.006) **
	Group (riluzole/ placebo) [e]	0.568 (0.098)	-0.202 (0.011)*	-
	Combined score	-0.005 (0.262)	-0.001 (0.698)	-
	UHDRS TFC score	0.012 (0.580)	0.003 (0.827)	-
	UHDRS FAS score	0.012 (0.425)	-0.001 (0.949)	-
Motor variables	*UHDRS total motor score*	-0.005 (0.172)	-0.002 (0.367)	-
	Chorea	-0.011 (0.242)	-0.005 (0.418)	-
	Dystonia	-0.021 (0.109)	0.005 (0.599)	-
	Rigidity	0.005 (0.853)	-0.030 (0.151)	-
	Bradykinesia	-0.075 (0.059)	-0.061 (0.038)*	-
Behavioral variables	*UHDRS total behavioral score*	-0.004 (0.292)	-0.002 (0.515)	-
	Total behavioral score frequency	-0.006 (0.330)	-0.004 (0.444)	-
	Total behavioral score severity	-0.009 (0.244)	-0.003 (0.638)	-
	Depression score	-0.020 (0.170)	-0.003 (0.638)	-
	Apathy score	0.003 (0.832)	-0.020 (0.079)	-
	BDI score	-0.008 (0.063)	-0.003 (0.365)	-
Cognitive variables	*UHDRS total cognitive score*	0.001 (0.291)	<0.001 (0.652)	-
	Verbal Fluency	0.007 (0.089)	0.008 (0.148)	-
	Symbol Digit Test	0.011 (0.028) *	<0.001 (0.956)	-
	Color naming	0.003 (0.430)	0.001 (0.569)	-
	Word reading	-0.001 (0.754)	0.002 (0.136)	-
	Interference	-0.004 (0.355)	-0.001 (0.577)	-

[a]) This column indicates the increase or decrease in average BMI (calculated over the whole study period) per unit increase of the predictor variable. [b]) This column indicates the increase or decrease in the rate of BMI change [BMI units/year] per unit increase of the predictor variable. [c]) This column indicates the increase or decrease in the rate of BMI change [BMI units/year] per unit increase of those predictor variables that were selected by stepwise forward selection; these predictor variables are independently associated with the rate of BMI change. [d]) Gender was coded as: male = 0, female = 1. [e]) Group was coded as: placebo = 0, riluzole = 1. *Abbreviations:* BMI = Body Mass Index; UHDRS = Unified Huntington's Disease Rating Scale; TFC = Total Functional Capacity; FAS = Functional Assessment; BDI = Beck Depression Inventory; * $p < 0.05$; ** $p < 0.01$.

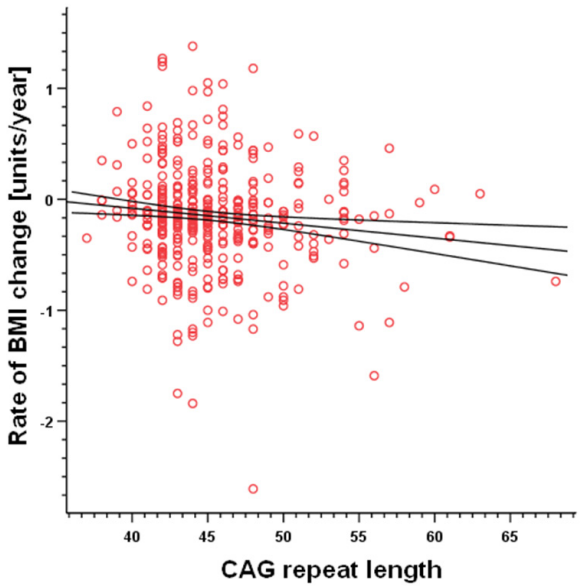
Effects of motor scores on the rate of weight loss

Of all the motor signs of HD only bradykinesia was significantly associated with the rate of BMI change (Table 2). Patients who became more bradykinetic during their illness had a significantly accelerated rate of body weight loss compared to others ($p = 0.038$). However, stepwise regression did not identify bradykinesia as an independent predictor of BMI change (Table 2). As the average bradykinesia score increased with 0.023 units for each unit increase in CAG repeat length ($p = 0.003$), CAG repeat length is likely to have confounded the relation between bradykinesia and weight loss.

No effect of cognitive and behavioral scores on weight change

Except for the Symbol Digit Modalities test score, which very weakly correlated with BMI, other cognitive and behavioral variables did not correlate with BMI. Moreover, no single cognitive or behavioral variable was associated with the rate of BMI change in the HD cohort (Table 2).

Figure 1. *Huntington's disease patients with larger CAG repeat lengths have a faster rate of weight loss. The straight line represents the regression line, while the outer lines delineate the 95% confidence intervals.*



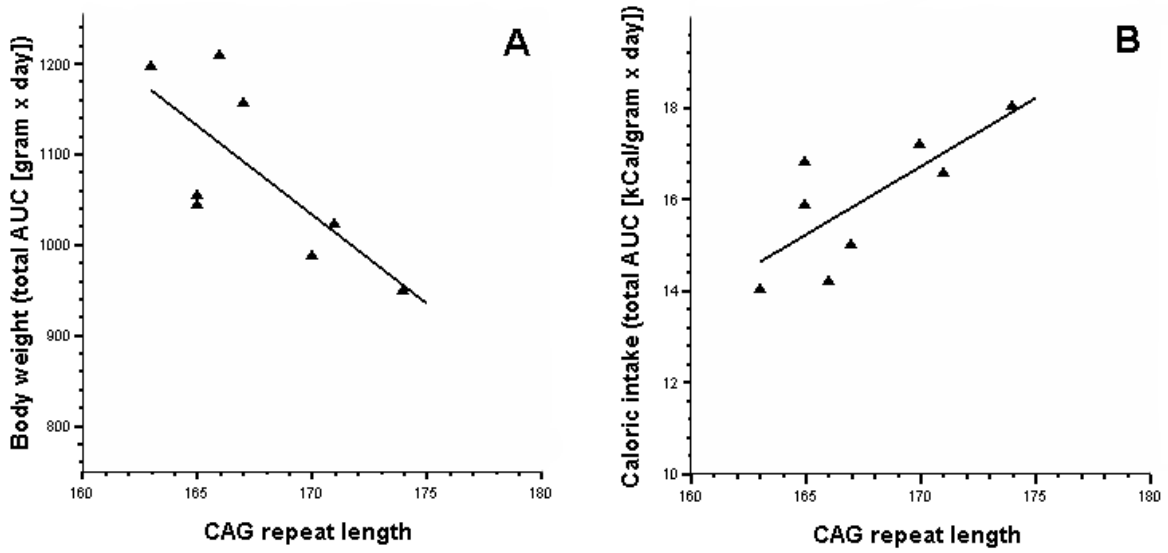
Lower mean BMI and faster rate of BMI decline with larger CAG repeat number

Interestingly, the BMI averaged over the follow-up period decreased with 0.136 units for every CAG codon increase in the mutant *huntingtin* gene ($p < 0.001$). Moreover, the number of CAG repeats in the mutant *huntingtin* gene significantly interacted with the time variable ($p = 0.017$), indicating that the rate of body weight decline also increases for each unit increase in CAG repeat length (Table 2 and Figure 1). Stepwise regression identified CAG repeat length also as an independent predictor of weight loss (adjusted p for CAG × time interaction = 0.006).

R6/2 transgenic mice

Similar to their wild-type littermates, R6/2 mice gained weight from the start of the study at six weeks of age until week nine.[13] However, from nine weeks of age and onwards, they progressively lost weight. The length of the CAG repeat in the transgene in our cohort of R6/2 mice varied between 163 and 175. The area under the body weight curve decreased with larger CAG repeat length ($r = -0.742$; $p = 0.035$), indicating that mice with a higher number of CAG repeats have a lower body weight. Conversely, the area under the caloric intake curve increased with larger CAG repeat length ($r = 0.763$; $p = 0.028$); i.e. R6/2 mice with higher repeat lengths consume more energy per gram of body weight.

Figure 2. *In R6/2 mice, a greater number of CAG repeats correlates with lower body weight (r = -0.742; p = 0.035) (A), and higher caloric intake (r = 0.763; p = 0.028) (B). AUC = area under the curve.*



DISCUSSION

We conducted a long-term follow-up study of body weight changes in a large group of HD patients who were at an early stage of the disease and not on neuroleptic treatment. We found a significant decrease in body weight; however, no single motor, cognitive or behavioral score was independently associated with weight loss. As weight loss in R6/2 mice is also not related to motor activity[13], our findings suggest that loss of body weight in HD is not secondary to hyperactivity or other symptoms, but rather results from a hypermetabolic state. As both HD patients and transgenic mice showed a higher rate of weight loss with greater CAG repeat number, this hypermetabolic state is likely to stem directly from interference of the mutant protein with cellular energy homeostasis. Weight loss could therefore reflect fundamental pathological mechanisms underlying HD and may serve as a biomarker to monitor disease progression. Moreover, patients with a higher number of CAG repeats are at increased risk of unintended weight loss. Therefore, their body weight should be monitored more closely.

Our findings indicate that weight loss is an inherent feature of HD and are in agreement with many other observations.[3,4,6,14] Indices of increased motor activity, such as chorea and dystonia, did not correlate with the rate of weight loss, neither did the total motor score of the UHDRS. Weight loss is therefore unlikely to result from hyperactivity. Although weight loss correlated with bradykinesia, this relation is likely confounded by CAG repeat number as bradykinesia increased with higher CAG repeat number and did not correlate with weight loss when adjusted for baseline BMI and CAG repeat number. Furthermore, cognitive impairment, as measured by the Symbol Digit Modalities test, weakly correlated with mean body weight. Cognitive impairment might cause more disability.[20] However, total functional capacity and ratings on the independence scale, both measures of disability, were not associated with weight loss. Therefore, it seems unlikely that there is a causative link between specifically bradykinesia or cognitive impairment and body weight loss. Although

we did not collect data regarding caloric intake, a decrease in energy intake is also unlikely to account for the weight loss. This is because all patients were at an early stage of the disease which is generally associated with increased rather than decreased caloric intake.[6,11,14] Similarly, R6/2 mice also do not exhibit decreased caloric intake until two weeks after the commencement of weight loss, indicating that decreased caloric intake is not the cause of weight loss.[13]

Here we demonstrate that CAG repeat number in mutant *huntingtin* is directly related to both the average body weight during follow-up and the rate of weight decline in HD patients. This extends upon earlier studies that have found associations between CAG repeat length and several other clinical features of HD, particularly age of disease onset.[27] Moreover, CAG repeat length has also been shown to correlate with the rate of disease progression as assessed by the extent of post mortem[28,29] or *in vivo*[30-32] striatal pathology. Interestingly, R6/2 mice with larger CAG repeat lengths had also lower body weights, despite relatively small differences in CAG repeat number between individual mice. When comparing different HD transgenic mouse models, CAG repeat length correlates with a number of biochemical abnormalities, such as decreases in brain N-acetyl aspartate levels.[33] However, the effect of small variations in CAG repeat number within the same mouse model is not known. Our findings suggest that even relatively small differences (up to 12) in the number of CAG repeats within the same transgenic strain may lead to phenotypic dissimilarities in, e.g., body weight.

Several mechanisms could account for the negative association between CAG repeat length and body weight. Mitochondrial dysfunction has long been implicated in HD pathogenesis as markers of energy metabolism are altered in HD brain, muscle[34,35] and lymphoblastoid cells[21]. The extent of mitochondrial dysfunction may critically depend on the length of the polyglutamine tract, as CAG repeat size has been shown to affect both mitochondrial depolarization and ATP/ADP ratio in lymphoblastoid cells.[21,22] Consequently, longer CAG expansions may cause both more central and peripheral pathology. Larger CAG repeat size has indeed been associated with more severe pathology in the striatum and cortex[28,29,36] and might also be related to more pathology in other brain structures, such as the hypothalamus, which are directly involved in energy homeostasis.[10,37] Interestingly, hypothalamic pathology occurs in both HD patients and transgenic mice.[10,13,38] Longer CAG repeats may cause more extensive changes in peripheral tissues as well. A recent study found reduced levels of branched chain amino acids in HD patients the levels of which were lower with increasing CAG repeat number.[14] Importantly, the levels of these amino acids were also associated with weight loss.[14] Similarly, we found that R6/2 mice with longer CAG repeats had lower body weights, whereas caloric intake was higher in mice with longer repeat lengths. Finally, mutant huntingtin with longer polyglutamine stretches might interfere more strongly with the function of the wild-type protein[39], which has been shown to influence body weight in some transgenic HD mice.[40]

Only two prior studies have investigated body weight changes in large groups of HD patients. [19,20] As weight loss is commonly considered a feature of HD[10], it is surprising that weight loss was not observed in these cohorts. In all likelihood, the heterogeneity of these cohorts combined with lack of clinical information on e.g. the use of nutritional supplements and drugs (notably neuroleptics) could account for the unexpected findings.[19,20] Moreover, CAG repeat length data were not available for these studies. In contrast, our cohort

of HD patients was very homogenous, consisting of patients at an early stage of the disease. Importantly, participants were also required not to be on neuroleptic treatment during the trial. Since neuroleptics are applied frequently in HD and often substantially influence systemic energy homeostasis, the EHDI cohort is the first large group of HD patients in which body weight changes could be investigated without confounding neuroleptic medication. Although our data were derived from a clinical trial with riluzole, riluzole treatment was reported not to affect any clinical outcome measure in this cohort[23]. We confirmed this and also showed that riluzole was not an independent predictor of weight change. Therefore, it is highly unlikely that riluzole treatment might have influenced our findings.

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