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Creatine supplementation lowers brain glutamate levels in Huntington's disease

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Abstract There is evidence from in vitro and animal experiments that oral creatine (Cr) supplementation might prevent or slow down neurodegeneration in Huntington's disease (HD). However, this neuroprotective effect could not be replicated in clinical trials, possibly owing to treatment periods being too short to impact on clinical endpoints. We used proton magnetic resonance spectroscopy ($^1\text{H-MRS}$) as a surrogate marker to evaluate the effect of Cr supplementation on brain metabolite levels in HD.

Twenty patients (age 46 ± 7.3 years, mean duration of symptoms 4.0 ± 2.1 years, number of CAG repeats 44.5 ± 2.7) were included. The primary endpoint was metabolic alteration as measured by $^1\text{H-MRS}$ in the parieto-occipital cortex before (t1) and after 8–10 weeks (t2) of Cr administration. Secondary measures comprised the motor

section of the Unified Huntington's Disease Rating Scale and the Mini Mental State Examination.

$^1\text{H-MRS}$ showed a 15.6% decrease of unresolved glutamate (Glu) + glutamine (Gln; Glu + Gln = Glx; $p < 0.001$) and a 7.8% decrease of Glu ($p < 0.027$) after Cr treatment. N-acetylaspartate trended to fall ($p = 0.073$) whereas total Cr, choline-containing compounds, glucose, and lactate remained unchanged. There was no effect on clinical rating scales.

This cortical Glx and Glu decrease may be explained by Cr enhancing the energy-dependent conversion of Glu to Gln via the Glu-Gln cycle, a pathway known to be impaired in HD. Since Glu-mediated excitotoxicity is presumably pivotal in HD pathogenesis, these results indicate a therapeutic potential of Cr in HD. Thus, long-term clinical trials are warranted.

Key words Huntington's disease · creatine · proton magnetic resonance spectroscopy · glutamate · neuroprotection

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Introduction

Huntington's disease (HD) is an autosomal dominant neurodegenerative condition, clinically characterized

by chorea, dementia, and personality changes. At present, there is no effective treatment.

HD is caused by expansion of a CAG triplet repeat leading to polyglutamine expansions in huntingtin, a protein of still unknown function. Glutamate (Glu) exci-

toxicity has been implicated in HD pathogenesis for several reasons. For example, an elevated ratio of Glx (combined Glu and glutamine [Gln]) to creatine (Cr) was demonstrated by proton magnetic resonance spectroscopy (^1H -MRS) in the striatum of HD patients [49]. In addition to excitotoxicity, impaired energy metabolism seems to play an important role in HD pathogenesis [4]: (i) elevated brain lactate (Lac) was shown by ^1H -MRS in patients [24, 27, 28]; (ii) activity of respiratory chain complexes II and III is decreased in post-mortem brains [9, 22, 47]; (iii) systemic administration of the respiratory chain complex II inhibitor 3-nitropropionic acid leads to a HD-like phenotype and pathology in rats [23]; and (iv) energy production may be hampered by the interaction of mutant huntingtin with the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase [11, 38].

If mitochondrial impairment plays a key role in HD pathogenesis, then augmenting intracellular energy levels may ameliorate the neurodegenerative process. Cr and phosphocreatine (PCr) constitute an energy-buffering and transport system, connecting sites of energy production with sites of energy consumption. Previous studies have demonstrated neuroprotective effects of Cr in several in vitro and in vivo models of neurological diseases. In toxic and transgenic rodent models of HD, Cr improves motor and cognitive performance, pro-

longs survival, and ameliorates striatal lesions [1, 20, 44], even if started after the onset of clinical symptoms [17]. Unfortunately, this neuroprotective effect could not be replicated in recent clinical trials [46, 50]. This may be because the treatment period (maximum 1 year) is rather short for impact on clinical endpoints.

On these grounds, we employed ^1H -MRS as a surrogate marker and conducted an open clinical pilot study on the effect of 8–10 weeks of Cr supplementation on brain metabolite levels and clinical symptoms in 20 HD patients.

Methods

■ Patients

Twenty patients with symptomatic HD (9 male, 11 female, age 46 ± 7.3 years [average \pm standard deviation], mean duration of symptoms 4.0 ± 2.1 years) were enrolled after giving informed written consent. The average CAG repeat number was 44.5 ± 2.7 (Table).

■ Study protocol

The study protocol was approved by the Ethics Committee of the University of Munich. The primary endpoint was metabolic alteration as measured by ^1H -MRS of the parieto-occipital cortex before (t1) and after 8 to 10 weeks (t2) of oral Cr treatment (20 g/d for five days, 6 g/d thereafter, pausing on Sundays in order to avoid downregulation of

Table Patient characteristics

No	Age (years)	Sex	Disease duration (y)	CAG	UHDRS motor section	MMSE	TFC	$\Delta\text{Glx t2-t1}$ [%]
1	61	m	6.5	41	32	30	3	-17.8
2	50	f	4	43	42	24	3	-12.2
3*	44	m	2	43	6	30	2	n. a.
4	55	m	7	46	27	23	3	-18.4
5	51	m	6	44	34	27	1	-31.3
6*	55	m	6	43	26	28	3	n. a.
7	35	m	3	47	27	26	2	-19.3
8	38	m	6	47	n. a.	n. a.	3	-10.4
9	35	f	5	44	21	29	3	-6.1
10	46	f	5	49	41	26	3	-2.5
11	46	f	1.5	46	16	27	1	-29.7
12	44	f	2	47	54	28	1	-27.4
13	48	f	5	43	38	29	3	-22.4
14	39	f	7	46	22	29	3	-10
15	38	f	4	46	46	28	2	-15.5
16**	38	f	2	48	14	28	3	n. a.
17	47	m	0.5	42	21	n. a.	1	-3.0
18	55	f	4	40	48	24	3	+17.7
19	50	f	2	40	27	26	3	-25.5
20***	45	m	1.5	n. a.	49	n. a.	3	n. a.
Mean	46		4.0	44.5	31.1	27.2	2.45	-14.6
SD	7.3		2.1	2.7	13.2	2.1	0.8	12.5

All clinical scores at t1 (n. a. = not available; * drop out; ** clinical data only; *** MRS data discarded due to FWHM > 0.1 ppm; UHDRS = Unified Huntington's Disease Rating Scale; MMSE = Mini Mental State Examination; TFC = total functional capacity score according to Shoulson [45]; SD = Standard deviation; FWHM = full width at half maximum)

the Cr transporter). This dosing regimen is commonly used in clinical studies [3, 29]. Secondary measures comprised the motor section of the Unified Huntington's Disease Rating Scale (UHDRS in the 1996 version [25]: 31 items with motor scoring range from 0 to 124 with higher scores indicating poorer motor function) and the Mini Mental State Examination (MMSE). For additional characterization of disease severity at t1, we employed the total functional capacity score according to Shoulson [45], a five-point disease scale with stage 1 representing functional independence and stage 5 the need for total care.

Patients were asked at the end of the trial, whether they rated their general well-being as unchanged, improved, or worsened.

■ ¹H-MRS protocol

Cortical ¹H-MRS was acquired at 1.5 T using an automated PRESS acquisition (probe-p, GE Medical systems) with TR = 4000 ms, TE = 35 ms, 128 averages from a 15 ml large parieto-occipital voxel. Basal ganglia spectra were recorded from the anterior striatum including parts of the putamen and caudate head using PRESS (TR = 2000 ms, TE = 35 ms, 128 averages) with a cubic 8 ml voxel.

Metabolite quantification was done using an automated fitting program (LCModel). Absolute concentration estimates were expressed as institutional units (IU), allowing for direct intra- and interindividual comparison. No attempt was made to apply correction factors for conversion in mmol/l. Data quality and accuracy were analysed qualitatively by visual inspection and quantitatively by the linewidth (full width at half maximum, FWHM) and individual fit accuracy. A measure for the latter is automatically provided by the LCModel software as standard deviation [SD %]. To be included in the final data analysis, spectra were required to have a FWHM < 0.1 ppm and concentration estimates to have a mean SD of < 20% (mean comprised of the concentration estimates of both visits from all patients) and an individual SD of < 50%.

■ Statistics

A MANOVA repeated measure design was applied to test for significant treatment effects on cerebral metabolites, followed by univariate F-tests if applicable. Metabolites not consistently reaching sufficient fitting accuracy such as Lac and Gln, clinical scores and body weight were tested for treatment effects using the non-parametric Wilcoxon test.

Results

Three patients did not complete the spectroscopic protocol and were excluded from further analysis. One additional cortical ¹H-MRS was discarded because of a FWHM of 0.11 ppm. Striatal spectra did not result in reliably high spectral quality and were thus excluded from further analysis as well.

N-acetyl-aspartate (NAA), choline-containing compounds (Cho), Cr, Glu, Glx and glucose fulfilled our accuracy criteria (i.e. SD < 20%, for details see Fig. 1). Here, Wilks multivariate test revealed an effect of 8–10 weeks of Cr supplementation on cortical ¹H-MRS metabolites ($F[6, 10] = 4.82, p = 0.015$). Univariate tests assigned this effect mainly to a decrease in Glx from 4.31 ± 0.78 IU to 3.64 ± 0.60 IU ($F = 22.6, p < 0.001$) and a drop in Glu from 3.17 ± 0.48 IU to 2.93 ± 0.34 IU ($F = 6.01, p = 0.027$). NAA trended to decrease ($F = 3.72,$

$p = 0.073$). No changes were seen for Cr, Cho and glucose (Fig. 1).

Lac and Gln estimates on the other hand did not fulfill our accuracy criteria (mean SD = 51% and 77% respectively). In this case, we explored a treatment effect using the nonparametric Wilcoxon test, which revealed no change in Lac ($p = 0.86$), but a decrease in Gln ($Z = -2.70, p = 0.007$).

The qualitative group comparison of averaged spectra without any further exclusion criteria visually confirms these results (Fig. 2).

Cr had no clinical effect on UHDRS ($p = 0.73$) and MMSE ($p = 0.19$) performance. Body weight increased on average by $+1.96 \pm 2.88$ kg ($Z = -1.97, p = 0.049$). Five patients reported an improvement of general well-being, while 14 patients reported no change. Cr was well tolerated by all patients.

Discussion

In this pilot study, ¹H-MRS showed a significant decrease in Glx in the parieto-occipital cortex of HD patients after 8–10 weeks of oral Cr treatment. Glx is used to describe the combined and thus more reliable concentration estimates of both Glu and Gln to account for the known difficulty in separating Glu from Gln and other resonances at 1.5 T. Despite this field strength-related limitation, a reduction was observed also for the individual estimates Glu and Gln.

Glu, the main excitatory neurotransmitter, and Gln are closely linked in the Glu-Gln cycle: during neurotransmission, Glu is released into the synaptic cleft, where its levels must be kept low in order to minimize excitotoxicity [13]. This is achieved by glial Glu uptake

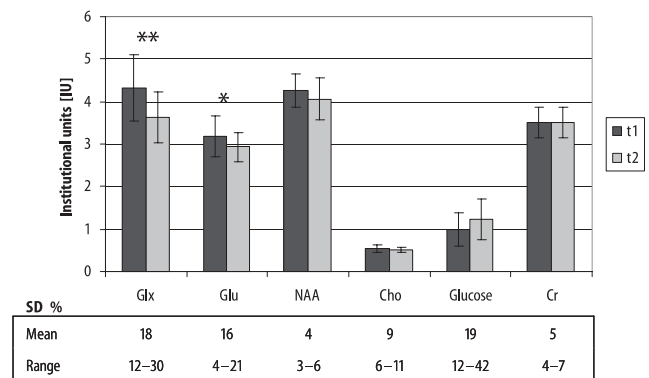


Fig. 1 Metabolite levels in the occipital cortex at baseline (t1) and after 8–10 weeks of Cr treatment (t2): Note a marked decrease of Glx and a lesser decrease of Glu, while NAA, Cho, glucose, and Cr remain unchanged. (* marks significant changes at $p < 0.05$, ** at $p < 0.001$). The table below the metabolites gives means and ranges (mean of all patients from all visits) for data accuracy values (standard deviation as provided by the analysis software; required to be < 20% for sufficient accuracy)

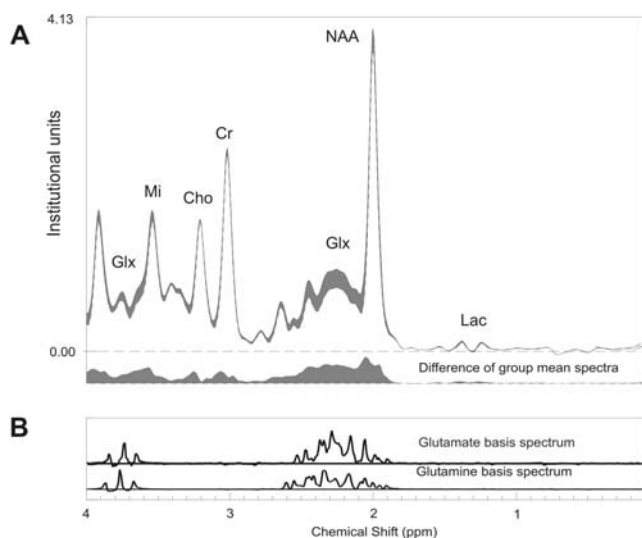


Fig. 2 (A) Upper part of the figure: averaged ^1H -MRS spectra before (t_1 = upper limit of grey area) and after (t_2 = lower limit of grey area) Cr treatment in 16 patients who completed both scans. Lower part of the figure: metabolite deltas ($t_2 - t_1$). All spectra fits were normalized to NAA. Note the marked difference in signals at the Glx region (Mi = myo-inositol, for other abbreviations see text). (B) Basis spectra of Glu and Gln to point out that changes in the spectrum around 2.3 and 3.7 ppm are consistent with reductions in Glx

through the Glu transporter GLT-1 [30]. Further ATP-dependent enzymes (Gln synthetase, glutaminase) are involved in the process of transporting Glu back to the presynaptic neuron, where it can either be stored in vesicles [7] or oxidized to 2-oxo-glutarate, which enters the citric acid cycle [18, 53]. Thus, the Glu-Gln system provides not only a major neurotransmitter, but is also linked to neuronal energy requirements [15, 19]. In the brain, between 60 and 80% of the energy derived from glucose oxidation is used to support events associated with Glu neurotransmission [42].

There is increasing evidence that perturbation of the Glu-Gln cycle and Glu-mediated excitotoxicity play an important role in HD pathogenesis: (i) intrastriatal injections of Glu agonists in mice produce striatal lesions and clinical symptoms closely resembling HD [5, 14]; (ii) treatment with the Glu antagonist riluzole increases survival in HD mice [43]; (iii) in HD transgenic mice and post mortem human brain, there is a reduction of GLT-1 mRNA and a subsequent reduction of glial Glu uptake [6, 35]; (iv) Gln synthetase mRNA and glutaminase activity are decreased in HD transgenic mice and human HD caudate nucleus respectively [12, 35]; (v) in vitro, N-terminal huntingtin fragments bind to presynaptic vesicles and inhibit Glu uptake [34]; (vi) mutant huntingtin sensitizes striatal neurons to elevated extracellular Glu levels, which may contribute to their selective degeneration in HD [54]; (vii) a ^1H -MRS study in early-stage HD patients showed an increase in Glx/Cr ratios in the striatum, but not in the occipital cortex [49]. Our own pre-

liminary data show as a trend ($p = 0.058$) elevated Glx concentrations in HD as compared with age-matched controls (data not shown). Some post mortem studies, however, show diminished Glu and normal Gln in HD basal ganglia [21, 40, 41].

Taken together, restoration of the disturbed Glu-Gln cycle may be a promising approach for the treatment of HD. We have shown here a decline in brain Glx after supplementation of Cr. Unexpectedly, we found no concomitant rise in brain Cr. This may be because the focus of our ^1H -MRS measurements was the parieto-occipital cortex, while the striatal spectra were of insufficient quality. However, in a human Cr supplementation trial, the increase of Cr was most pronounced in the thalamus (14.6%) and least in the cortex (4.7%) [16]. Moreover, for safety reasons our patients received a relatively low dose of Cr which may preclude a spectroscopic rise in total Cr.

Cr/PCr can exert neuroprotective and antiexcitotoxic effects wherever processes demand energy. Possible targets include the glial GLT-1 and Gln synthetase, as well as the neuronal glutaminase and the vesicular Glu reuptake. Xu et al. have shown that PCr can reduce extracellular Glu in cell culture by stimulating Glu uptake in synaptic vesicles [52]. An improved Glu uptake into the cellular compartment has two potentially protective effects: (i) abundant extracellular Glu can no longer promote excitotoxicity, and [2] Glu and Gln can be deducted from the astrocytal and neuronal pool via degradation to 2-oxo-glutarate. Indeed, the fact that Cr can protect neurons from Glu-mediated toxicity has been shown in cell culture [8, 10].

Apart from directly influencing the Glu-Gln cycle, Cr probably exerts its neuroprotective effects by several other pathways. The phosphorylation of Cr to PCr supplies the mitochondrial respiratory chain with additional ADP, thereby optimizing the process of oxidative phosphorylation [31] and reducing the production of free radicals [33]. PCr can provide additional ATP in periods of high energy demand and functions as an energy transporter between the sites of energy production (mitochondria) and consumption (cytosol) [48]. Cr helps to maintain ATP levels used by the Na^+/K^+ -ATPase and the Ca^{++} -ATPase, thus stabilizing the membrane potential [32]. Cr also prevents apoptotic cell death by stabilizing the mitochondrial CK, which inhibits opening of the mitochondrial transition pore [39].

The beneficial effects of Cr are not limited to in vitro systems, but have also been shown in animal models of neurodegenerative diseases. In the MPTP model of Parkinson's disease, Cr exerted highly significant neuroprotection [36]. Administration of Cr in a mouse model of amyotrophic lateral sclerosis (ALS) resulted in increased longevity and motor performance as well as reduced neurodegeneration [32]. Similar to our findings in HD, MRS demonstrated a significant reduction of the

Glx/Cho ratio in ALS mice [2] and patients [51] after Cr supplementation. Unfortunately, however, a recent trial did not find a beneficial effect on survival or disease progression in patients with ALS [26]. In transgenic and toxic mouse models for HD, Cr led to an increase in survival of up to 20% improved motor and cognitive performance, delay or decrease of striatal lesions, reduced brain atrophy, and diminished pathological changes [1, 20, 44]. In another Cr intervention study in HD mice, Matthews et al. found a reduction of the Lac/NAA ratio and an increase in brain ATP [37].

Unfortunately, clinical trials have been less successful in demonstrating a Cr benefit in HD patients. An open-label trial (13 patients, 10 g per day of Cr for 12 months) reported unchanged UHDRS motor scores and neuropsychological test results after 12 months of therapy [46]. This may indicate stabilization of the disease, and

follow-up at 24 months is planned. Verbessem et al. (41 HD patients, 5 g Cr per day for 12 months) found high safety and tolerability of Cr, but no significant differences in motor and cognitive function in a placebo-controlled double-blind trial [50].

To provide evidence for a neuroprotective effect of Cr on clinical endpoints, trial durations of more than five years may be necessary. Therefore, we employed ¹H-MRS as a surrogate marker and found a significant reduction of Glx and Glu after 8–10 weeks of Cr supplementation in 20 HD patients. This indicates that Cr has an impact on HD brain metabolism and may exert its effect by lowering Glu-mediated excitotoxicity. In the light of these results, long-term clinical trials are warranted.

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References

1. Andreassen OA, Dedeoglu A, Ferrante RJ, Jenkins BG, Ferrante KL, Thomas M, Friedlich A, Browne SE, Schilling G, Borchelt DR, Hersch SM, Ross CA, Beal MF (2001) Creatine increases survival and delays motor symptoms in a transgenic animal model of Huntington's disease. *Neurobiol Dis* 8:479–491
2. Andreassen OA, Jenkins BG, Dedeoglu A, Ferrante KL, Bogdanov MB, Kaddurah-Daouk R, Beal MF (2001) Increases in cortical glutamate concentrations in transgenic amyotrophic lateral sclerosis mice are attenuated by creatine supplementation. *J Neurochem* 77:383–390
3. Balsom PD, Soderlund K, Ekblom B (1994) Creatine in humans with special reference to creatine supplementation. *Sports Med* 18:268–280
4. Beal MF (1992) Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? *Ann Neurol* 31:119–130
5. Beal MF, Ferrante RJ, Swartz KJ, Kowall NW (1991) Chronic quinolinic acid lesions in rats closely resemble Huntington's disease. *J Neurosci* 11:1649–1659
6. Behrens PF, Franz P, Woodman B, Lindenberg KS, Landwehrmeyer GB (2002) Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation. *Brain* 125:1908–1922
7. Bellocchio EE, Reimer RJ, Fremereau RT Jr, Edwards RH (2000) Uptake of glutamate into synaptic vesicles by an inorganic phosphate transporter. *Science* 289:957–960
8. Brewer GJ, Wallimann TW (2000) Protective effect of the energy precursor creatine against toxicity of glutamate and beta-amyloid in rat hippocampal neurons. *J Neurochem* 74:1968–1978
9. Browne SE, Bowling AC, MacGarvey U, Baik MJ, Berger SC, Muqit MM, Bird ED, Beal MF (1997) Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. *Ann Neurol* 41:646–653
10. Brustovetsky N, Brustovetsky T, Dubinsky JM (2001) On the mechanisms of neuroprotection by creatine and phosphocreatine. *J Neurochem* 76:425–434
11. Burke JR, Enghild JJ, Martin ME, Jou YS, Myers RM, Roses AD, Vance JM, Strittmatter WJ (1996) Huntingtin and DRPLA proteins selectively interact with the enzyme GAPDH. *Nat Med* 2:347–350
12. Butterworth J, Yates CM, Reynolds GP (1985) Distribution of phosphate-activated glutaminase, succinic dehydrogenase, pyruvate dehydrogenase and gamma-glutamyl transpeptidase in post-mortem brain from Huntington's disease and agonal cases. *J Neurol Sci* 67:161–171
13. Coyle JT, Puttfarcken P (1993) Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 262:689–695
14. Coyle JT, Schwarcz R (1976) Lesion of striatal neurones with kainic acid provides a model for Huntington's chorea. *Nature* 263:244–246
15. Daikhin Y, Yudkoff M (2000) Compartmentation of brain glutamate metabolism in neurons and glia. *J Nutr* 130:1026S–1031S
16. Dechent P, Pouwels PJ, Wilken B, Hanelfeld F, Frahm J (1999) Increase of total creatine in human brain after oral supplementation of creatine-monohydrate. *Am J Physiol* 277:R698–R704
17. Dedeoglu A, Kubilus JK, Yang L, Ferrante RJ (2003) Creatine therapy provides neuroprotection after onset of clinical symptoms in Huntington's disease transgenic mice. *J Neurochem* 85:1359–1367
18. Erecinska M, Silver IA (1990) Metabolism and role of glutamate in mammalian brain. *Prog Neurobiol* 35:245–296
19. Erecinska M, Zaleska MM, Nissim I, Nelson D, Dagani F, Yudkoff M (1988) Glucose and synaptosomal glutamate metabolism: studies with [¹⁵N]glutamate. *J Neurochem* 51:892–902
20. Ferrante RJ, Andreassen OA, Jenkins BG, Dedeoglu A, Kuemmerle S, Kubilus JK, Kaddurah-Daouk R, Hersch SM, Beal MF (2000) Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. *J Neurosci* 20:4389–4397
21. Gramsbergen JB, Veenma-Van der Duin L, Venema K, Korf J (1986) Cerebral cation shifts and amino acids in Huntington's disease. *Arch Neurol* 43:1276–1281
22. Gu M, Gash MT, Mann VM, Javoy-Agid F, Cooper JM, Schapira AH (1996) Mitochondrial defect in Huntington's disease caudate nucleus. *Ann Neurol* 39:385–389

23. Guyot MC, Hantraye P, Dolan R, Palfi S, Maziere M, Brouillet E (1997) Quantifiable bradykinesia, gait abnormalities and Huntington's disease-like striatal lesions in rats chronically treated with 3-nitropropionic acid. *Neuroscience* 79:45–56
24. Harms L, Meierkord H, Timm G, Pfeiffer L, Ludolph AC (1997) Decreased N-acetyl-aspartate/choline ratio and increased lactate in the frontal lobe of patients with Huntington's disease: a proton magnetic resonance spectroscopy study. *J Neurol Neurosurg Psychiatry* 62:27–30
25. Huntington Study Group (1996) Unified Huntington's Disease Rating Scale: reliability and consistency. *Mov Disord* 11:136–142
26. Jan GG, Veldink JH, van dT, I, Kalmijn S, Beijer C, de Visser M, Wokke JH, Franssen H, van den Berg LH (2003) A randomized sequential trial of creatine in amyotrophic lateral sclerosis. *Ann Neurol* 53:437–445
27. Jenkins BG, Koroshetz WJ, Beal MF, Rosen BR (1993) Evidence for impairment of energy metabolism in vivo in Huntington's disease using localized 1H NMR spectroscopy. *Neurology* 43:2689–2695
28. Jenkins BG, Rosas HD, Chen YC, Makabe T, Myers R, MacDonald M, Rosen BR, Beal MF, Koroshetz WJ (1998) 1H NMR spectroscopy studies of Huntington's disease: correlations with CAG repeat numbers. *Neurology* 50:1357–1365
29. Juhn MS, Tarnopolsky M (1998) Oral creatine supplementation and athletic performance: a critical review. *Clin J Sport Med* 8:286–297
30. Kanner BI, Schuldiner S (1987) Mechanism of transport and storage of neurotransmitters. *CRC Crit Rev Biochem* 22:1–38
31. Kay L, Nicolay K, Wieringa B, Saks V, Wallimann T (2000) Direct evidence for the control of mitochondrial respiration by mitochondrial creatine kinase in oxidative muscle cells in situ. *J Biol Chem* 275:6937–6944
32. Klivenyi P, Ferrante RJ, Matthews RT, Bogdanov MB, Klein AM, Andreassen OA, Mueller G, Wermer M, Kaddurah-Daouk R, Beal MF (1999) Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat Med* 5:347–350
33. Lawler JM, Barnes WS, Wu G, Song W, Demaree S (2002) Direct antioxidant properties of creatine. *Biochem Biophys Res Commun* 290:47–52
34. Li H, Li SH, Johnston H, Shelbourne PF, Li XJ (2000) Amino-terminal fragments of mutant huntingtin show selective accumulation in striatal neurons and synaptic toxicity. *Nat Genet* 25:385–389
35. Lievens JC, Woodman B, Mahal A, Spasic-Bosovic O, Samuel D, Kerkerian-Le Goff L, Bates GP (2001) Impaired glutamate uptake in the R6 Huntington's disease transgenic mice. *Neurobiol Dis* 8:807–821
36. Matthews RT, Ferrante RJ, Klivenyi P, Yang L, Klein AM, Mueller G, Kaddurah-Daouk R, Beal MF (1999) Creatine and cyclocreatine attenuate MPTP neurotoxicity. *Exp Neurol* 157:142–149
37. Matthews RT, Yang L, Jenkins BG, Ferrante RJ, Rosen BR, Kaddurah-Daouk R, Beal MF (1998) Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington's disease. *J Neurosci* 18:156–163
38. Mazzola JL, Sirover MA (2002) Alteration of nuclear glyceraldehyde-3-phosphate dehydrogenase structure in Huntington's disease fibroblasts. *Brain Res Mol Brain Res* 100:95–101
39. O'Gorman E, Beutner G, Dolder M, Koretsky AP, Brdiczka D, Wallimann T (1997) The role of creatine kinase in inhibition of mitochondrial permeability transition. *FEBS Lett* 414:253–257
40. Perry TL, Hansen S (1990) What excitotoxin kills striatal neurons in Huntington's disease? Clues from neurochemical studies. *Neurology* 40:20–24
41. Reynolds GP, Pearson SJ (1987) Decreased glutamic acid and increased 5-hydroxytryptamine in Huntington's disease brain. *Neurosci Lett* 78:233–238
42. Rothman DL, Sibson NR, Hyder F, Shen J, Behar KL, Shulman RG (1999) In vivo nuclear magnetic resonance spectroscopy studies of the relationship between the glutamate-glutamine neurotransmitter cycle and functional neuroenergetics. *Philos Trans R Soc Lond B Biol Sci* 354:1165–1177
43. Schiefer J, Landwehrmeyer GB, Luesse HG, Sprunken A, Puls C, Milkereit A, Milkereit E, Kosinski CM (2002) Riluzole prolongs survival time and alters nuclear inclusion formation in a transgenic mouse model of Huntington's disease. *Mov Disord* 17:748–757
44. Shear DA, Haik KL, Dunbar GL (2000) Creatine reduces 3-nitropropionic acid-induced cognitive and motor abnormalities in rats. *Neuroreport* 11:1833–1837
45. Shoulson I (1981) Huntington disease: functional capacities in patients treated with neuroleptic and antidepressant drugs. *Neurology* 31:1333–1335
46. Tabrizi SJ, Blamire AM, Manners DN, Rajagopalan B, Styles P, Schapira AH, Warner TT (2003) Creatine therapy for Huntington's disease: Clinical and MRS findings in a 1-year pilot study. *Neurology* 61:141–142
47. Tabrizi SJ, Cleeter MW, Xuereb J, Taanman JW, Cooper JM, Schapira AH (1999) Biochemical abnormalities and excitotoxicity in Huntington's disease brain. *Ann Neurol* 45:25–32
48. Tarnopolsky MA, Beal MF (2001) Potential for creatine and other therapies targeting cellular energy dysfunction in neurological disorders. *Ann Neurol* 49:561–574
49. Taylor-Robinson SD, Weeks RA, Bryant DJ, Sargentoni J, Marcus CD, Harding AE, Brooks DJ (1996) Proton magnetic resonance spectroscopy in Huntington's disease: evidence in favour of the glutamate excitotoxic theory. *Mov Disord* 11:167–173
50. Verbessem P, Lemièr J, Eijnde BO, Swinnen S, Vanhees L, Van Leemputte M, Hespel P, Dom R (2003) Creatine supplementation in Huntington's disease: A placebo-controlled pilot trial. *Neurology* 61:925–930
51. Vielhaber S, Kaufmann J, Kanowski M, Sailer M, Feistner H, Tempelmann C, Elger CE, Heinze HJ, Kunz WS (2001) Effect of creatine supplementation on metabolite levels in ALS motor cortices. *Exp Neurol* 172:377–382
52. Xu CJ, Klunk WE, Kanfer JN, Xiong Q, Miller G, Pettegrew JW (1996) Phosphocreatine-dependent glutamate uptake by synaptic vesicles. A comparison with atp-dependent glutamate uptake. *J Biol Chem* 271:13435–13440
53. Yudkoff M, Nissim I, Daikhin Y, Lin ZP, Nelson D, Pleasure D, Erecinska M (1993) Brain glutamate metabolism: neuronal-astroglial relationships. *Dev Neurosci* 15:343–350
54. Zeron MM, Chen N, Moshaver A, Lee AT, Wellington CL, Hayden MR, Raymond LA (2001) Mutant huntingtin enhances excitotoxic cell death. *Mol Cell Neurosci* 17:41–53