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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}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}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}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-

totoxicity 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 (^{1}H-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 ^{1}H-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 ^{1}H-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 ^{1}H-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	ΔGlx 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 as 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 as 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 ± 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
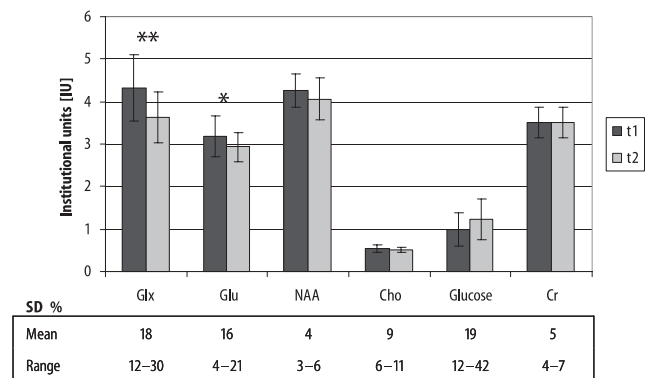

Fig. 1 Metabolite levels in the occipital cortex at baseline (t1) and after 8–10 weeks of Cr treatment (t2): Note a marked decrease of Glx and a lesser decrease of Glu, while NAA, Cho, glucose, and Cr remain unchanged. (* marks significant changes at p < 0.05, ** at p < 0.001). The table below the metabolites 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)
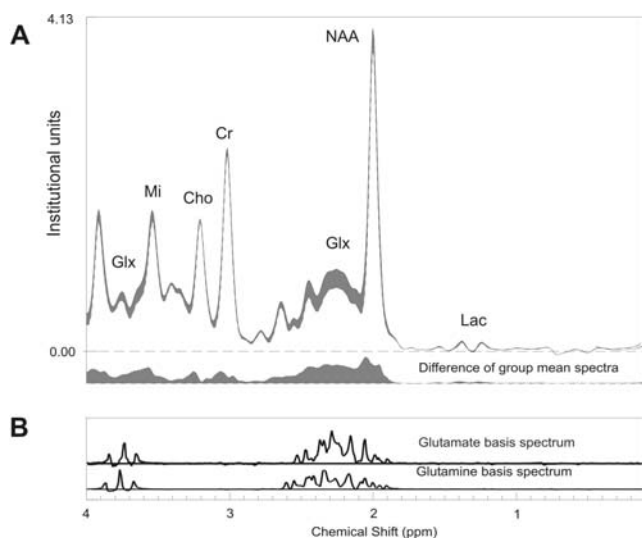
Fig. 2 (**A**) Upper part of the figure: averaged ¹H-MRS spectra before (t1 = upper limit of grey area) and after (t2 = lower limit of grey area) Cr treatment in 16 patients who completed both scans. Lower part of the figure: metabolite deltas (t2-t1). All spectra fits were normalized to NAA. Note the marked difference in signals at the Glx region (Mi = myo-inositol, for other abbreviations see text). (**B**) Basis spectra of Glu und 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 synthethase, 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 ^{1}H-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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