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Decreased cerebral perfusion in Duchenne muscular dystrophy patients

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Abstract

Duchenne muscular dystrophy is caused by dystrophin gene mutations which lead to the absence of the protein dystrophin. A significant proportion of patients suffer from learning and behavioural disabilities, in addition to muscle weakness. We have previously shown that these patients have a smaller total brain and grey matter volume, and altered white matter microstructure compared to healthy controls. Patients with more distal gene mutations, predicted to affect dystrophin isoforms Dp140 and Dp427, showed greater grey matter reduction. Now, we studied if cerebral blood flow in Duchenne muscular dystrophy patients is altered, since cerebral expression of dystrophin also occurs in vascular endothelial cells and astrocytes associated with cerebral vasculature. T1-weighted anatomical and pseudo-continuous arterial spin labeling cerebral blood flow images were obtained from 26 patients and 19 age-matched controls (ages 8–18 years) on a 3 tesla MRI scanner. Group comparisons of cerebral blood flow were made with and without correcting for grey matter volume using partial volume correction. Results showed that patients had a lower cerebral blood flow than controls (40.0 ± 6.4 and 47.8 ± 6.3 mL/100 g/min respectively, $p = 0.0002$). This reduction was independent of grey matter volume, suggesting that they are two different aspects of the pathophysiology. Cerebral blood flow was lowest in patients lacking Dp140. There was no difference in CBF between ambulant and non-ambulant patients. Only three patients showed a reduced left ventricular ejection fraction. No correlation between cerebral blood flow and age was found. Our results indicate that cerebral perfusion is reduced in Duchenne muscular dystrophy patients independent of the reduced grey matter volume.

Keywords: Brain; CBF; Duchenne muscular dystrophy; Dystrophin; Imaging

1. Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive neuromuscular disorder caused by mutations in the *DMD*-gene that impair the expression of the full length dystrophin protein (Dp427) in muscle in all patients. In addition to skeletal muscle pathology, DMD is characterized by cognitive and behavioural problems. There is a one-standard deviation shift in IQ, which means that approximately one third of patients show (mild) cognitive impairment [1] and 40% have reading deficits similar to those observed in patients with phonological dyslexia [2–4]. Moreover, there is a higher incidence of attention-deficit/hyperactivity disorder (ADHD) (24–44%), anxiety disorder (27%), autism spectrum disorders (ASD) (15–21%), epilepsy (6.3%), and obsessive-compulsive disorder (OCD) (4.8%) in boys with DMD [5–8]. We previously reported reduced grey matter (GM) volume and altered white matter (WM) microstructure in DMD patients using magnetic resonance imaging (MRI) [9]. These differences were most

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profound in patients with mutations in the distal part of the *DMD* gene. Cognitive impairment was also more prominent in this subgroup.

Both full length dystrophin (Dp427) and the shorter isoforms Dp140, Dp71 and Dp40 are expressed in the central nervous system, but whether expression of these shorter isoforms is impaired depends on the location of the mutation within the *DMD* gene [10–13]. Dp427 is expressed in association with $GABA_A$-receptors in neurons in the cerebral cortex, cerebellar cortex and hippocampus, suggesting a role in neuronal signalling [14]. Dp140 is expressed in micro-vessels in the brain and Dp71 is expressed in astrocyte end-feet wrapped around cerebral microvasculature closely associated with pericytes, co-expressed with aquaporin4 receptors [15,16]. These factors indicate a possible role of dystrophin on cerebral microvasculature.

In DMD patients regional brain glucose hypometabolism was reported using positron emission tomography. The authors suggested this might indicate cytoarchitectural alterations, but it might also be a result of lower cerebral blood flow (CBF) [17]. A recent study in *mdx*-mice, the most commonly used animal model for DMD, showed an 18% reduction in CBF compared to wild type mice [18]. Additional results from *mdx*-mice showed a reduction in aquaporin4 expression in the brain and this reduction was associated with swollen perivascular astrocyte processes and coupled with impaired development of the blood–brain barrier [15].

In the current study, we investigated if cerebral haemodynamics is altered in DMD using pseudo-continuous arterial spin labeling (pCASL) MRI.

2. Materials and methods

2.1. Participants

Thirty-three participants (ages 8–18 years) with a diagnosis for DMD, previously confirmed by genetic testing, were recruited from the Dutch Dystrophinopathy Database. Twenty-two healthy age-matched control participants (ages 8–16 years) were recruited from local schools and leisure clubs using posters and flyers [9]. Recruitment was random. Exclusion criteria were the presence of MRI contraindications and the inability to lie supine for at least 30 minutes. In the DMD group, two subgroups were distinguished with mutations predicted to affect only Dp427 (n = 11) or both Dp427 and Dp140 (n = 11) expression. All except four patients received corticosteroid medication, of whom twenty were on a ten days on, ten days off regime. Data on cardiac function were obtained from routine follow-up with echocardiography. Left ventricular function was classified as normal using a cut-off for left ventricular shortening fraction of 28%, assessed after the MRI or at most three months before. Haematocrit levels were assessed from samples taken in routine clinical practice from seven patients in this study, as well as from 33 additional patients (age range 5–18 years). The protocol for this cross-sectional observational study was approved by the local Medical Ethical Committee. All participants and legal representatives provided written informed consent.

2.1.1. Neuropsychology

A neuropsychological examination (NPE) was performed in all participants yielding three composite scores. The reading score (standardized for age with a range of 1–19, mean 10 and standard deviation 3 in healthy controls) was based on the mono-syllabic word reading test and the one minute reading test derived from CB&WL: “continu benoemen en woorden lezen” (Bos & Lutje Spelberg, Boom test uitgevers, Amsterdam, The Netherlands). The information processing score (standardized as the reading score) utilized two subtests – number recall for auditory working memory and block counting for conceptual thinking – from the Kaufman Assessment Battery for Children and one subtest – symbol search – from the Wechsler Intelligence Scale for Children. The score for emotional and behavioural problems can be constructed on the basis of four problem based subscales from the Dutch version of the Strengths and Difficulties Questionnaire for parents [19]. General intellectual level was assessed by the Peabody Picture Vocabulary test (PPVT-III-NL).

2.2. MR acquisition

MR images were acquired without sedation or general anaesthetic. For patients who were on a ten day on/ten day off corticosteroid treatment regime, MR acquisition was performed in the off-period of corticosteroids. A 3D T1-weighted scan (T1w; echo time (TE) and repetition time (TR) 4.6/9.8 ms; spatial resolution $1.17 \times 0.92 \times 1.20$ mm; 4:55 min) was acquired for anatomical reference. A pseudo continuous arterial spin labeling scan (pCASL; TE/TR 14 ms/4020 ms; label duration 1650 ms; post-label delay 1525 ms; background suppression pulses (BGS) at 1680 ms and 2760 ms; voxel-size $3.0 \times 3.0 \times 7.0$ mm; 4:49 min) was acquired for cerebral perfusion measurements. An M_0 scan (TE/TR 14/10,000 ms; spatial resolution $3.0 \times 3.0 \times 7.0$ mm; NSA 4; 0:50 min) was acquired for CBF quantification. Images were obtained on a 3 T scanner (Philips Achieva, Philips Healthcare, Best, The Netherlands) using an 8 channel receive-only head coil.

2.3. Processing

Quantification of CBF was performed in accordance with recent white paper recommendations [20]. As grey matter volume is reduced in DMD patients, and the ASL signal in GM is much higher than in WM, we first calculated the net GM CBF, and then performed partial volume correction (PVC) to account for different amounts of WM and GM in those voxels located on the boundary between the two tissue types [21]. To this end, statistical parametric mapping software v.8 [22] and custom-written programs (MATLAB, Mathworks, Natick, USA) were used for motion correction, brain extraction, subtraction of label and control conditions, and segmentation into GM, WM and cerebral spinal fluid. Next, GM and WM voxel fractions were used to compute tissue-specific CBF maps for each subject [21]. From these tissue-specific CBF maps, partial GM, partial WM and net CBF were computed. FSL v.5 [23] was then used to compute the individual mean net CBF and mean PVC grey matter CBF. For voxel-wise group comparisons the CBF