

# Decreased cerebral perfusion in Duchenne muscular dystrophy patients

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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 ( $40.0 \pm 6.4$  and  $47.8 \pm 6.3$  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.

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**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<sub>A</sub>-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

Table 1  
Patient characteristics.

	DMD	DMD_Dp140+	DMD_Dp140–	Controls
Participants (n)	26	11	11	19
Age (years), mean ± sd	12.6 (3.1)	13.5 (3.7)	12.0 (2.8)	13.3 (2.0)
Age range (years)	8–18	9–18	8–16	8–16
Steroid treatment (n)	22	9	9	–
On/off 10 day treatment cycle (n)	20	9	8	–
Wheelchair bound (n)	13	6	6	–
Age of loss of ambulation (years), mean ± sd	10.6 (2.0)	11.5 (2.4)	9.9 (1.3)	–

maps were co-registered with the T1w scan to Montreal Neurological Institute (MNI) standard space using FSL fnirt.

#### 2.4. Statistics

SPSS v. 20 (IBM, Inc.) was used for all analyses of variance (ANOVAs), t-tests and Pearson's correlations, and significance was set at  $p < 0.05$  using the Bonferroni–Holmes method to correct for multiple comparisons. To test the group differences between DMD and control in age and mean CBF with and without PVC, Student's t-test was performed. Patients were also subdivided into ambulant and non-ambulant to see if these groups differed in CBF using Student's t-test. Pearson's correlations were calculated on CBF versus grey matter volume, age and the three neuropsychological composite scores. To test the differences between controls, DMD\_Dp140+ and DMD\_Dp140– patients, ANOVA was performed. Voxelwise group analyses were performed to locate regions with different PVC CBF between controls and all DMD patients, DMD\_Dp140+ or DMD\_Dp140– patients. FSL VBM and RANDOMISE were used, with age as a covariate and cluster-based multiple comparison correction (TCFE) was applied.

### 3. Results

#### 3.1. Participant characteristics

Three DMD participants were excluded because of withdrawal of informed consent ( $n = 2$ ) or technical problems ( $n = 1$ ). Seven pCASL scans were excluded due to motion artifacts or inefficient pCASL labeling defined as a signal void upon visual inspection of the data ( $n = 4$  for DMD and  $n = 3$  for control). In the remaining 26 patients and 19 controls there was no significant difference in age. Four DMD patients were steroid naïve, two were taking ACE-inhibitors (perindopril) and one was taking methylphenidate for ADHD. The DMD groups' performance on neuropsychological testing was representative of the Dutch DMD population, with DMD\_Dp140– performing worst, as previously described [9]. Participant characteristics are shown in Table 1.

#### 3.2. Cerebral blood flow

Fig. 1 shows representative net CBF and PVC CBF maps from a nine year old DMD patient and an age-matched control participant. Analysis of all subjects showed that DMD patients had a 17% lower CBF compared to healthy controls ( $p = 0.0002$ ) (Fig. 2A and Table 2). This difference remained

after PVC ( $p = 0.0002$ ). Grey matter volume and CBF were not correlated in either group (Fig. 3). Voxel-wise analysis showed that the reduced CBF was found throughout the grey matter (Fig. 4A).

#### 3.3. Dystrophin isoform Dp140

The lowest mean CBF was found in the DMD\_Dp140– group, which was significantly lower than in the control group ( $p = 0.0013$ ) (Fig. 2B and Table 2). The DMD\_Dp140+ group also differed significantly from control ( $p = 0.014$ ). The differences remained after PVC. Voxel-wise comparison showed a greater difference between DMD\_Dp140– and control than between DMD\_Dp140+ and control (Fig. 4B, C).

#### 3.4. Age, ambulation, cardiac function and blood viscosity

No correlations were found between CBF and age (Fig. 5). There was also no difference in CBF values between ambulant patients and non-ambulant patients (Table 2). In 24 out of 26 patients, cardiac function data were obtained. Three of these patients had low shortening fractions indicating compromised heart function. The other twenty-one had normal left ventricular function.

In the cerebral blood flow calculation, haematocrit values are assumed normal. The average haematocrit level in DMD patients was 0.43 L/L (range 0.36–0.49, SD 0.03 L/L), and all were within reference levels (0.35–0.50).

#### 3.5. Neuropsychological composite scores

The three composite scores were significantly different between DMD patients and controls. DMD patients scored lower for information processing and word reading as well as higher for behavioural problems [9]. However, no significant

Table 2  
Mean cerebral blood flow (CBF) with and without partial volume correction.

	CBF (mL/100 g/min) mean ± sd	PVC CBF (mL/100 g/min) mean ± sd
Control (n = 19)	47.8 ± 6.3	61.4 ± 9.0
DMD (n = 26)	40.0 ± 6.4***	51.1 ± 8.1***
DMD_Dp140+ (n = 11)	41.7 ± 6.0*	53.3 ± 7.1*
DMD_Dp140– (n = 11)	38.6 ± 6.9**	49.1 ± 8.7**
DMD ambulant (n = 13)	40.3 ± 8.8	50.3 ± 8.7
DMD non-ambulant (n = 13)	41.9 ± 6.2	51.7 ± 7.9

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  compared to control.

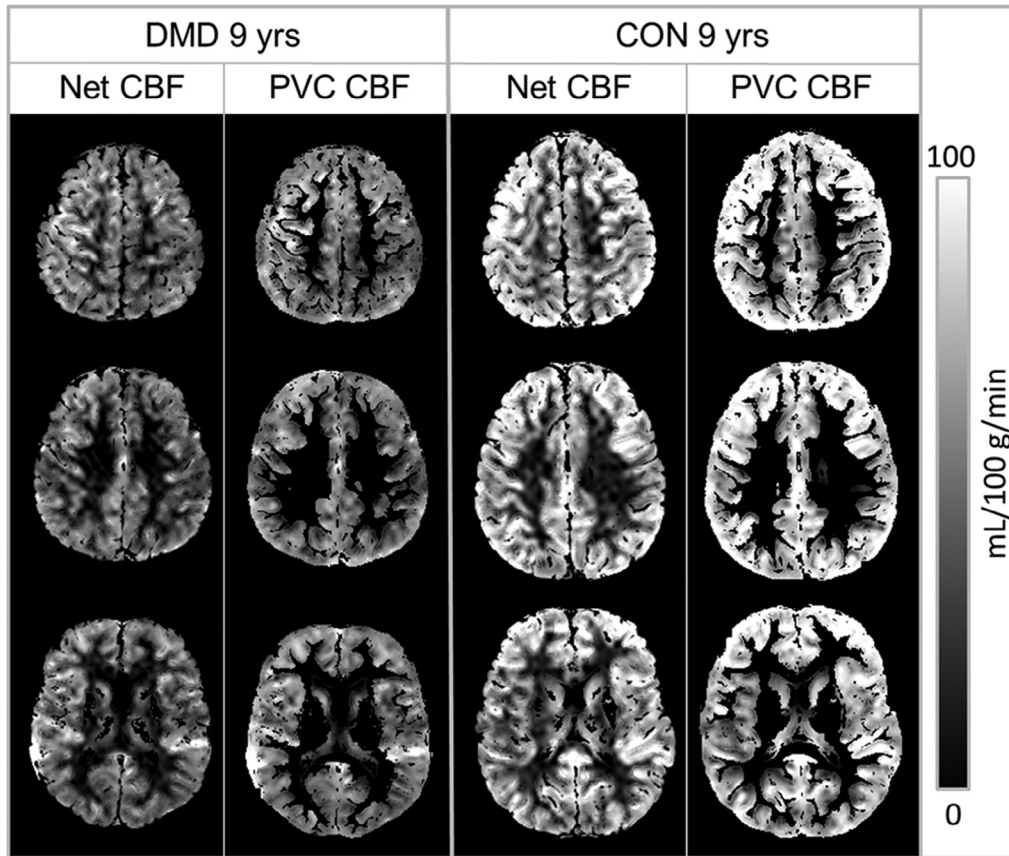


Fig. 1. Representative cerebral blood flow (CBF) maps. Three transverse slices of the same individual in native space. The left two columns show a nine year old participant with DMD with average CBF for the DMD group. The first column shows the net CBF and the second column shows the partial volume corrected (PVC) grey matter CBF. The right two columns show a nine year old control with average CBF for the control group, again with the first column showing net CBF and the second column showing the PVC CBF. The grey scale shows the perfusion in mL/100 g/min.

correlation was found between CBF and the composite scores in DMD patients or controls (Fig. 6).

#### 4. Discussion

In DMD patients, a substantial reduction in CBF (17%) was found compared to healthy age-matched control participants. Because GM has a 2–4-fold higher perfusion than WM and GM volume is reduced in DMD patients [9], correcting for this volume is essential in order to be able to discriminate between lower ASL signal due to GM hypo-perfusion or due to a lesser amount of GM in the volume-of-interest. As the difference between DMD patients and controls remained significant after PVC and no correlation between GM volume and CBF was observed, our results show that the lower CBF was independent of GM volume.

Compared to literature data of typically developing children and young adults, CBF values in DMD were at the lower end of the scale although within normal limits [24]. Similar to our results in DMD patients, a reduction of 18% in CBF is also seen in *mdx* mice, the most common animal model for DMD [18]. In the same mice, blood–brain barrier disruption was detected, as well as increased arteriogenesis in the cerebrum. There are also reports of vessel alterations in the *mdx* mouse brain with

thickening of the perivascular basement membrane and absence of laminin and agrin protein content and expression [16]. It is unknown if these vessel alterations also occur in DMD patients, but the lower CBF values reported in our study indicate that this may well be the case.

The CBF reduction was slightly larger in DMD patients missing Dp140. Dp140 is expressed at the astrocyte end-feet that are associated with pericytes as well as at microvessels [15,16,25] whereas Dp427 is located at the post-synaptic membrane in neurons [12,26]. Local demands for oxygen and glucose from the brain are regulated by pericytes, astrocytes and neurons, and active relaxation of pericytes is proposed to contribute to 84% of the blood flow increase upon sensory stimulation [27]. Even though the scan was performed at rest, without sensory stimulation, the close association between Dp140 and the cerebral vasculature may indicate a role in the regulation of vessel dilation and relaxation through pericytes, and its absence may therefore contribute to the greater reduction in CBF.

In neurons, the dystrophin–glycoprotein complex containing Dp427 is involved in the organization of  $\gamma$ -aminobutyric acid type A (GABA<sub>A</sub>) receptors which mediate a component of the vasodilation produced in the cortex [25]. However, neuronal signalling to blood vessels generally occurs through synaptic

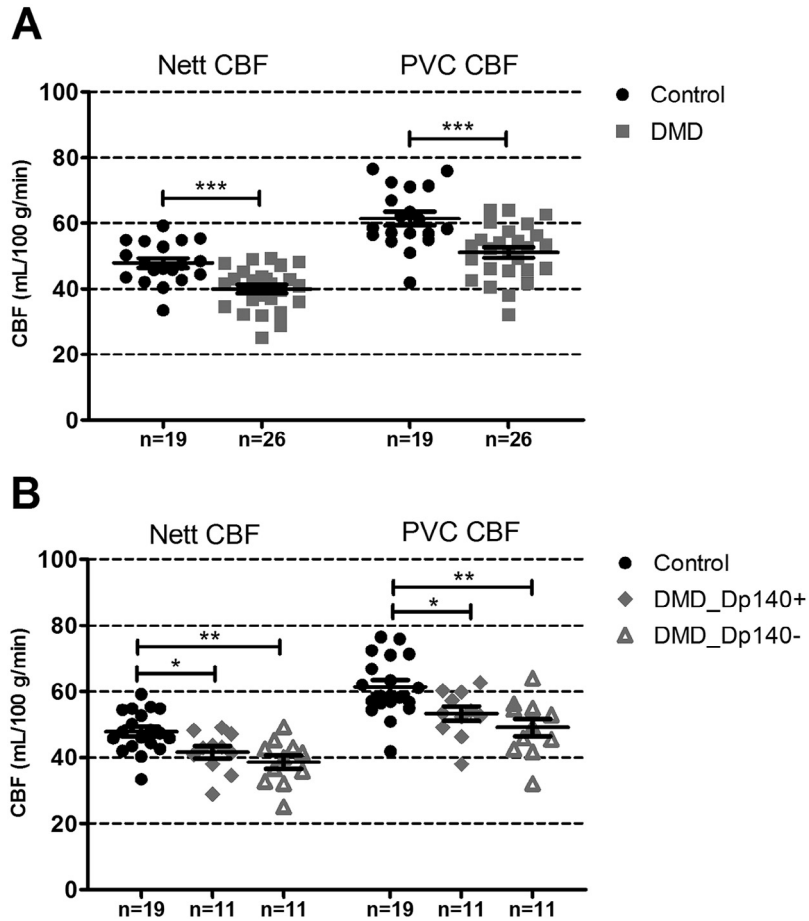


Fig. 2. Quantified cerebral blood flow (CBF) with partial volume correction (PVC CBF) and without (Net CBF). The quantified CBF in milliliter per 100 gram brain tissue per minute is shown. (A) depicts control and the whole patient group with a significantly lower CBF in patients. (B) shows control versus DMD\_Dp140+ and DMD\_Dp140- with a significantly lower CBF in both patient groups compared to control (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

glutamate release which activates *N*-methyl-D-aspartate (NMDA) receptors. The resulting entry of  $Ca^{2+}$  into neurons activates neuronal nitric oxide synthase (nNOS) which releases NO which dilates vessels. nNOS is implicated in the reduced perfusion of muscle in DMD as well as Becker muscular

dystrophy (BMD) patients in whom dystrophin is partially functional, but little is known about the brain in this respect [28–31]. In BMD patients, only the event-related response was affected by a drug that potentiates NO responses [32]. As no differences in CBF were found at rest between drug and

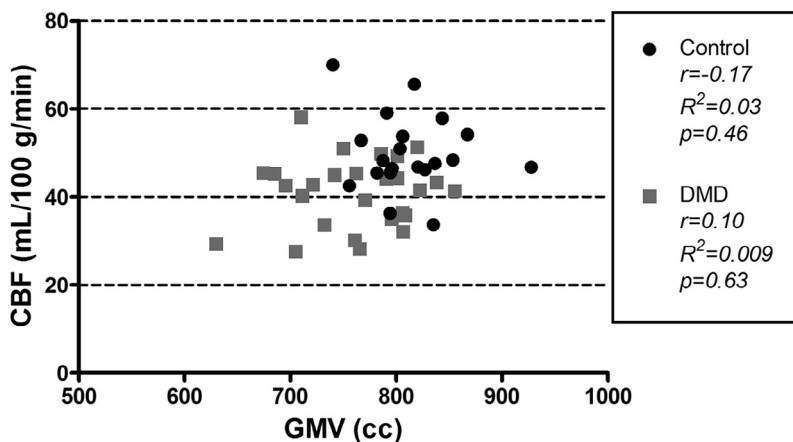


Fig. 3. Grey matter volume (GMV) versus cerebral blood flow (CBF). No significant correlations were found within the DMD or control groups between CBF and GMV.

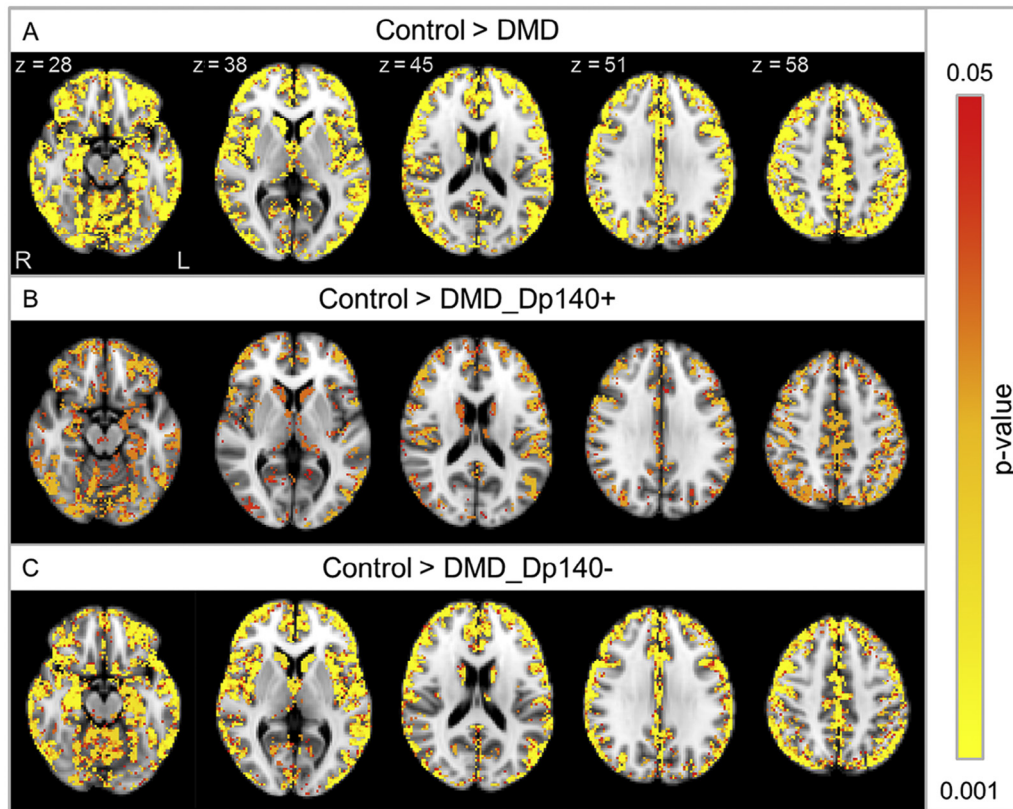


Fig. 4. Localization of significant differences within the grey matter of the brain. Statistical maps, overlaid on MNI\_152\_2 mm\_brain from FSL, localizing regions that differ significantly between groups. Image dimension are shown in the radiological convention with the right hemisphere on the left side of the image and with the z-values indicating the cross-section positioning. There were no regions in which controls showed lower CBF than patients. (A) depicts the results for control versus the whole patient group showing widespread reduced CBF. (B) shows the results for controls versus patients who have isoform Dp140 where the regions with significantly reduced CBF are slightly smaller and more diffuse. (C) depicts the results for controls versus patients missing Dp140 showing widespread reduced CBF.

placebo, this seems very similar to the functional ischaemia seen in muscle in BMD and DMD. Therefore, it may be that nNOS regulation of perfusion is affected in the brain as well as muscle and, as such, contributes to the lower CBF in DMD.

Limitations of our study include that CBF may be influenced by cardiac dysfunction. Cardiomyopathy is a common clinical

symptom affecting 95% of patients by the age of twenty [33,34]. Cardiac impairment has previously been associated with reduced CBF and cognitive impairment in candidates for heart transplantation surgery, although this was a substantially different patient population than DMD [35]. Nonetheless, after surgery, the CBF values in those patients returned to baseline

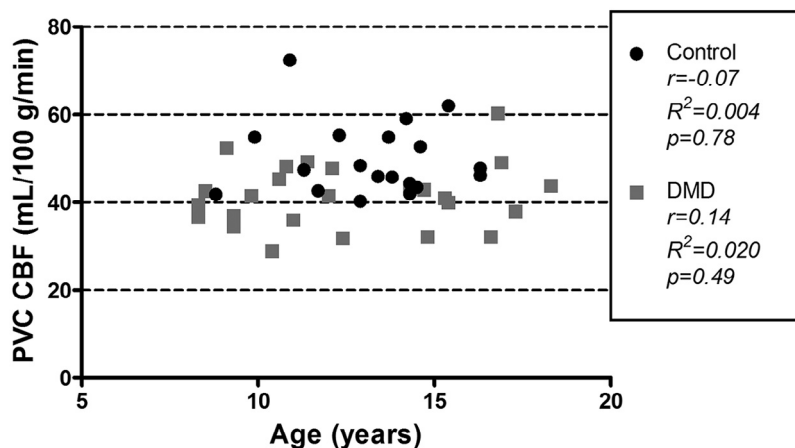


Fig. 5. Age versus partial volume corrected cerebral blood flow (PVC CBF). No significant correlations were found within the DMD or control groups between PVC CBF and age.

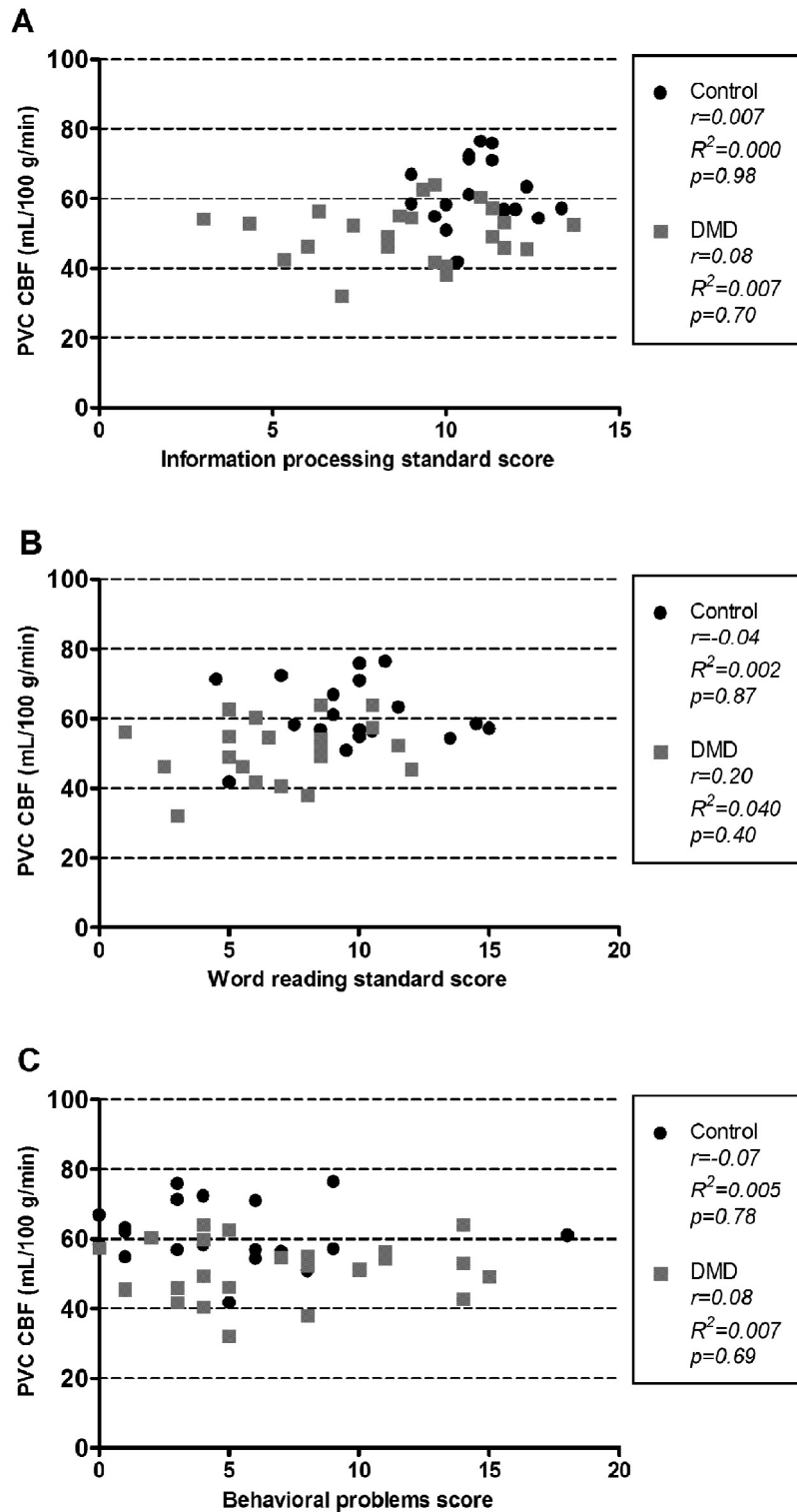


Fig. 6. Neuropsychological composite scores versus CBF. No significant correlations were found between information processing (A), word reading (B) or behavioural problems (C) composite scores obtained from the neuropsychological test battery to PVC CBF in controls and DMD patients.

and cognitive function was restored, suggesting an important link between cardiac function and CBF. However, CBF is tightly controlled to accommodate fluctuations in cardiac output. Although we do not have cardiac data from the control group, which precludes direct comparison of cardiac output

between patients and controls, we were able to review cardiac function in the DMD group and the vast majority (21 out of 24) had normal left ventricular function. The fact that CBF was reduced in both younger and older patients and the absence of any correlation between CBF and age further support our



hypothesis that cardiac dysfunction is not the major contributor for the reduced CBF.

Secondly, CBF was measured at an age where the brain is still developing and the relationship between age and CBF in healthy brain development is well documented [24,36–38]. By contrast, we found no significant correlation between age and CBF in any group, which likely reflects the large standard deviation in the correlation together with our relatively small group size. In addition, in the current study we looked at mean whole brain CBF, whereas different regions of the brain show different relations with age. The groups were age matched, and group statistics were corrected for age. The lower CBF is therefore unlikely to be due to age effects, but longitudinal studies would be required to truly assess this relationship.

Thirdly, the reduced CBF could be influenced by corticosteroids and body mass index (BMI). Despite conflicting reports on a BMI increase in DMD, it has been suggested that DMD patients have higher BMI which increases as the mobility decreases [39,40]. An increase in BMI is also a potential side effect of corticosteroids and the majority of patients in our study were receiving corticosteroid treatment. While studies are limited, there is a report linking high BMI to lower CBF values but it is unknown to what extent this study can be translated to the DMD population [41]. In addition, corticosteroids might also have a direct effect on cerebral perfusion [42]. To limit these effects, patients who were on a 10-day on, 10-day off regime were scanned in the off-period. As no effect of the chronic corticosteroid treatment on cerebral blood flow or cerebral blood volume has been reported in neuro-psychiatric systemic lupus erythematosus patients [43], and steroid naïve *mdx* mice showed a similar CBF reduction as in our cohort of DMD boys [18], we assume that corticosteroids are not the primary cause of the CBF reduction. Nevertheless, a contribution of corticosteroids and/or BMI to the reduced CBF cannot be excluded without a larger steroid naïve DMD control group.

Finally, the limited mobility of the patients may have an effect as studies have shown CBF elevations in healthy individuals who participate in competitive sports, training more than four times per week, compared to people with no regular physical activity [44]. There was no difference in CBF between ambulant and non-ambulant patients, but patients will be less active even before losing ambulation. Therefore it is difficult to assess the extent of this effect without a control group that has similar mobility.

The quantification of CBF requires a correction for the T1 value of blood. For this, literature haematocrit values are used [20]. We checked blood haematocrit levels taken from routine clinical practice from 40 patients, including seven patients in this study, which were all within normal limits.

DMD is clearly more than a muscle disorder. Patients suffer from cognitive and behavioural problems with varying degrees of severity [1,45]. Although cerebral GM volume, WM microstructure, and CBF are independently changed in DMD, it is difficult to assess the influence of each factor on cognition. There are indications of a relationship between vascular reactivity in boys with DMD and cognitive functioning from

a study that looked at hemispheric specialization using SPECT [46]. In this study boys with DMD were a control group for boys with dysphasia. In frontal, sensorimotor, auditive, temporo-parietal and temporo-occipital regions, the CBF ratio showed no left dominant function asymmetry in boys with dysphasia and DMD in contrast to control. In Broca's area only boys with DMD differed significantly from control. Unfortunately the groups in this study were too small to assess a relation between the lack of functional asymmetry and the degree of reading or motor disabilities. On a more general level, a significant correlation between IQ and CBF was reported in children [36], but in people over 60 years of age, correlations between cognition and CBF disappeared after correcting the CBF for brain volume [47]. We did not find a correlation between the composite scores available in this study and CBF. However, our study was not powered to find a correlation between cognition and CBF, but aimed at finding differences in CBF between patients and controls.

In conclusion, our results show that cerebral perfusion is reduced in DMD independent of the reduction in grey matter volume. Future studies should investigate the influence of cerebral autoregulation, the effect of corticosteroid treatment, the relationship with cognition and determine if this reduction is progressive within patients.

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