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Influence of a two-year steroid treatment on body composition as measured by Dual X-Ray Absorptiometry in boys with Duchenne Muscular Dystrophy

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Introduction

In Duchenne Muscular Dystrophy (DMD), body composition is altered by a progressive destruction of skeletal muscles and their replacement by adipose and fibrous tissue [1, 2]. A particular change in fat mass distribution is also seen because of an intramuscular fat accumulation [3].

In the nineties, steroids started to be used in DMD in several countries. Later, several studies with high levels of evidence demonstrated the benefits this treatment [4-7]. In the short term (6-24 months), steroids maintain or improve muscle strength, respiratory functions, and time-based test of motor function capacities [4, 6]. In the long term, steroids delay the loss of ambulation (by 2 to 5 years), prevent scoliosis and preserve the respiratory function [5-7].

However, the most commonly reported side-effects of steroids in DMD are short stature, delayed puberty, difficult behaviour, weight gain, and the development of a cushingoid facial appearance. Regarding weight, Griggs et al. shown in 1993 that, after 18 months of treatment, 75% of patients receiving 0.75 mg/kg/day prednisone presented a 20% increase in weight vs. only 43% of patients receiving placebo [8]. This weight gain is apparently more common with prednisone than with deflazacort [9], occurs more frequently in non-ambulant patients, and leads sometimes to steroid stoppage [10].

In fact, weight gain through fat mass (FM) is a well-known adverse effect of steroid treatments in children and adults. Children receiving steroids after renal transplantation [11, 12] or for rheumatoid arthritis [13] have shown an increase in FM and a decrease in lean tissue mass (LTM). In DMD patients, weight gain during steroid treatment seems to result from FM and LTM. Indeed, urinary creatinine excretion over 24 hours after 18 months of steroid treatment helped demonstrate a 36% increase in muscle mass in the DMD prednisone group vs. the DMD
placebo group [8]. It was then suggested that prednisone-induced muscle mass in DMD was mediated by inhibition of proteolysis [14].

Dual X-Ray absorptiometry (DXA) has been already used in DMD patients for accurate assessments of total body composition and muscle mass; besides, significant correlations with function and strength could be also demonstrated [15, 16]. Yet, additional data are still awaited.

The present study analyses the changes in body composition as measured by DXA in DMD patients treated with steroids over a two-year follow-up and compares these changes with those seen in steroid-naïve DMD patients. It also investigates the relationship between body composition and motor function as assessed by the Motor Function Measure (MFM).
Materials and Methods

Ethics
This prospective study was approved by an ethics committee (Comité de Protection des Personnes Sud-Est II, Lyon, France) and informed consents were obtained from the children parents.

Patients and setting
All children included in the present study were seen and followed-up for two years in a single centre. All were boys aged 5 to 15 years old with a DMD diagnosis confirmed by muscle biopsy, absence of dystrophin (by immunohistochemistry or Western blotting analysis), and/or molecular biology (mutation or deletion of the dystrophin gene).

After an initial clinical examination, twenty patients started a steroid treatment with 0.75 mg/kg/day prednisone according to recommendations of the DMD Care Considerations Working Group [17] and the French Observatory of Steroid Treatment in DMD. One boy was given 1 mg/kg prednisone every other day because of pre-existing obesity. Steroid-naïve patients were from a prior historical control group.

All the participants (steroid-treated and steroid-naïve) benefited from the current recommendations concerning diet. In our setting, this is a standard management of patients, especially circa the time of loss of ambulation. In particular, all received an oral vitamin D supplementation (100,000 IU every 3 to 6 months) according to the blood levels of 25-hydroxyvitamin D and oral calcium supplementation (500 to 1,000 mg/day) in case of insufficient dietary intake.
Anthropometric measurements

Weight (without braces but with light underwear) was measured using a digital chair scale. Height was measured in the standing position in ambulant patients and in the lying position in non-ambulant patients using the convenient scale and a standardized procedure [18]. In patients with significant joint retractions, height was extrapolated from the segmental measurements of the ulna [19].

The body mass index (BMI) was calculated as body weight (in kg) divided by height squared (in m²). We did not consider a BMI correction for height because only one patient had a slightly delayed growth (-2.1 SD). All the others had rather "normal" growth curves (within the 2 SD boundaries).

The theoretical weight gain (+2% weight per cm height) was calculated for each patient and compared with the observed weight gain.

Motor function assessment

Motor function was assessed with the Motor Function Measure (MFM), a functional measurement tool validated in children with neuromuscular diseases, DMD included. The MFM consists of 32 items (tasks) divided into 3 domains that provide a detailed profile of the physical impairment: D1 for standing and transfers, D2 for axial and proximal motor capacity, and D3 for distal motor capacity [20]. Its sensitivity to change in DMD is known and has been the object of a specific publication [21].

Body composition assessment
Body composition was determined with DXA (Hologic Discovery A, Hologic Inc, Bedford, MA, USA). In 7 patients, all steroid-treated (3 ambulant and 4 non-ambulant), the first assessment was made by another machine (Norland XR-36 DXA system); this imposed the use of a correction factor based on the results obtained from measurements of a full-body anthropomorphic phantom [22].

A standard procedure was followed for positioning the patient and running the software. The DXA scans were analyzed using a three-compartment model of body composition: LTM, FM, and bone mineral content (BMC). The percentage of body fat mass (%BFM\textsubscript{DXA}) as determined by DXA, was calculated with the following formula: \( \% \text{BFM}_{\text{DXA}} = \frac{\text{FM}}{\text{Weight}} \). All scans were performed and analysed by a certified technician.

Medical visit course

For steroid-treated patients, the visits were scheduled at inclusion (M0), and 12 and 24 months later (M12 and M24). For steroid-naïve patients, the visits were scheduled at inclusion (M0) and 24 months later (M24) only.

For all patients, each visit included a clinical examination with anthropometric measurements, a functional motor assessment with the MFM, and a whole body DXA scan. This follow-up is classically proposed for all patients with DMD in accordance with the international recommendations [17].

In the steroid-treated group, VZV-nonimmune children were vaccinated with Varilrix\textsuperscript{®} prior to steroid treatment. Steroids were started one month after the two vaccine injections. At M0 and M12, a visit was planned with a dietician for customized dietary advices, review of dietary intakes, determination of the adequacy of calcium intake, and prescription of convenient hyponatremic or hypoglucidic diets.
At M12 and M24 visits, the patients were interviewed about compliance and tolerance to the treatment. The following investigations were carried out to detect side-effects of steroid treatment: a detailed medical history and clinical exam, an echocardiography, an ophthalmologic examination, a blood test for phosphocalcic metabolism and inflammatory markers, a urine test, and a column X ray (only in case of suspicion of vertebral fracture).

Statistical analysis
The medians, means, and SDs were calculated for continuous variables. Proportions were calculated for categorical variables.

A non-parametric Mann-Whitney U test for two independent samples was performed to compare the two groups (steroid-treated vs. steroid-naïve).

A non-parametric Wilcoxon test for paired samples was used to compare the anthropometric characteristics and body composition parameters between the two groups at different time points (M0, M12, and M24). Individual changes were represented graphically.

Linear regressions were used to analyse the effect of age on body composition parameters (%BFM\textsubscript{DXA}, BMI, and FM/LTM) at inclusion. Correlation between body composition and motor function was evaluated graphically and by Pearson’s r test of correlation.

Data analysis was performed with SPSS 10.7 software (SPSS, Chicago, Il, US). Differences associated with p < 0.05 were considered significant
Results

Patient characteristics at baseline

From May 2003 to April 2010, the study included 21 boys in the steroid-treated group (5.4 to 14.6 years old, mean ± SD: 8.1 ± 2.6) and 8 boys in the steroid-naïve group (6.5 to 13.3 years old, mean ± SD: 9.6 ± 2.1).

At the initial visit (M0), 5 patients in the steroid-treated group and 4 in the steroid-naïve group were non-ambulant. The mean age at loss of ambulation was similar between the two groups (9.3 ± 1.4 and 9.4 ± 0.8, respectively). The participants’ characteristics are shown in Table 1. At the initial visit (M0), there were no significant differences between the two groups in terms of age, age at loss of ambulation, and motor function performance as measured by D2, D3, and Total MFM scores. However, significant differences were found regarding MFM D1 score (p = 0.04), BMI (p = 0.008), and %BFM_{DXA} (p = 0.009).

At M0 (baseline), alterations in body composition were seen in the two groups but were more prominent in the steroid-naïve group: the latter group had significantly higher BMI and %BFM_{DXA} (Table 1). The ranges of BMI and %BFM_{DXA} were 13.2-27.6 and 16-72% in the steroid-treated group vs. 15.1-26.3 and 28.3-71.7 in the steroid-naïve group. The mean FM/LTM ratio in the steroid-treated group was 0.6 ± 0.5 [0.2 to 2.5] (Table 2). At the initial visit, some anthropometric and body composition parameters were statistically worse in the oldest vs. the youngest patients. As shown in Figure 1, there were strong positive correlations between age and i) %BFM_{DXA} (R = 0.81, n = 29, p < 0.0001); ii) BMI (R = 0.62, n = 29, p = 0.0004); and iii) FM/LTM ratio (R = 0.73, n = 29, p < 0.0001).
Changes in body composition

The changes in body composition parameters are shown in Table 2. One year after steroid-treatment onset, the mean weight gain in the treated group was 18.4 ± 12.8% vs. an expected theoretical gain of 10.9 ± 6.3%. At the final visit (M24), after two years of treatment, the mean weight gain reached 33.3 ± 15.4% in the steroid-treated group (vs. an expected theoretical gain of 20 ± 8.8%) whereas it was only 23 ± 20.5% in the steroid-naïve group.

In the steroid-treated group, the mean BMI values increased significantly between M0 and M12 (p = 0.003) and even more so between M0 and M24 (p = 0.0005) whereas it remained relatively constant between M0 and M24 in the steroid-naïve group (p = 0.87). The mean LTM values increased significantly over the first then the two-year treatment period (p < 0.0001 in both comparisons); however, the %BFM_{DXA} remained practically constant between M0 and M24 (p = 0.98) (Table 2). The mean FM/LTM ratio decreased somewhat between M0 and M12 but did not change significantly between M0 and M24. Over the two-year period, 7 out of the 8 steroid-naïve patients showed an increase in the FM/LTM ratio vs. only 4 out of 21 patients in the steroid-treated group.

No significant increases in the LTM were observed over the two-year follow-up in the steroid-naïve group. Instead, a deterioration in body composition was noticed with significant increases in the %BFM_{DXA} and the FM/LTM ratio (p = 0.025 and p = 0.05, respectively) (Table 2).

Changes in motor function and correlations with body composition parameters

The MFM scores at inclusion are shown in Table 1. In the steroid-treated group, there were no significant changes in MFM scores over the two-year treatment period, which indicated a relative
stabilisation of the motor function (Figure 2). This contrasted with a trend toward a decrease in all MFM scores in the steroid-naïve group (no statistically significant differences). At M24, 15 patients (mean age 9.1±1.71) were still ambulant in the steroid-treated group whereas the four ambulant patients of the steroid-naïve group had lost the ability to walk at ages ranging between 8.4 and 10 years old.

As shown in Figure 3, considering all DXA measurements performed in all patients, a negative correlation was found between %BFM_{DXA} and the MFM total score (R = - 0.79, n = 76, p < 0.0001).
Discussion

Steroid treatment is usually started in patients aged 6 years old, before loss of ambulation, and when patients show functional capacity plateau. Encouraging results were obtained even before 5 years old [23]. During the initial phase, prednisone at 0.75 mg/kg/day or deflazacort at 0.9 mg/kg/day appear to be optimal [17]. The ideal treatment duration is currently unknown but steroids can be continued for several years [5].

To our knowledge, the impact of steroid treatments on body composition parameters, as measured by DXA, in patients with DMD has not been studied yet. The present study found that a 2-year steroid treatment at least stabilizes body composition of boys with DMD through a significant increase in LTM. This finding was supported by a significant decrease in LTM found in steroid-naïve patients. More interesting, the increase of LTM in steroid-treated patients was associated with a halting or slowing down of the deterioration of motor function in comparison with the steroid-naïve group. The strong negative correlation between $\%\text{BFM}_{\text{DXA}}$ and MFM Total score in both groups confirms the link between improvement of body composition and stabilisation of motor function. Patients with DMD under steroid treatment do gain weight but our present results demonstrate that this corresponds to a gain in LTM (leading to a stable value for the ratio FM/LTM) and that it should not necessarily imply a steroid discontinuation but rather to a thorough check of body composition.

The abnormal body composition parameters we found in the patients with DMD at inclusion (i.e., increased FM and decreased LTM) are in line with those already found by several authors [15, 24-26]. These disturbances begin usually before the age of loss of ambulation and keep worsening with age [24, 26]. They correspond to a progressive destruction of muscle and its gradual replacement by adipose and fibrous tissue.
Compared to healthy boys, DMD boys had a deeply altered body composition. Indeed, the average FM/LTM ratio before steroid treatment was 0.61 [0.2 - 2.7] in the steroid-treated group and 1.34 [0.4 - 2.5] in the steroid-naive group whereas the standard ratio in a similar-age general population is 0.3 [unpublished data]. Regarding %BFM\textsubscript{DXA}, the present study found 33% [16 - 72%] in the steroid-treated group and 52% [28 - 72%] in the steroid-naive group whereas the standard percentage in a similar-age general population is 17% [unpublished data]. Here, the worse body composition parameters found in the steroid-naive patients could be linked to the higher proportion of non-ambulant patients in this group (4/8, 50%) vs. the steroid-treated group (5/21, 24%), overweight being classically observed in DMD patients just after loss of ambulation.

Our results differ from those found in children with transplanted organs or suffering from a steroid-dependent nephrotic syndrome because, in such patients, long-term steroid treatments have rather negative impacts: i) increasing LTM values with age and growth --though to a lower level than that reached in absence of treatment; ii) more quickly increasing FM values that lead to excessive adiposity [11-13].

Because subcutaneous tissue fat is not different from between DMD and healthy boys, the increased fat mass in DMD children is most probably mainly due to an increase in intramuscular fat accumulation [3, 24]. Recently, there has been an increasing interest in muscle magnetic resonance imaging for the diagnosis and assessment of disease progression in various neuromuscular disorders, including DMD [27]. The technique aimed to distinguish muscle contractile from non-contractile tissue and differentiate fat tissue or oedema from muscle. A recent study [28] used this technique to compare 28 boys with DMD treated with steroids with 10 control subjects. It concluded that boys with DMD had a significantly higher proportion of non-contractile tissue than control subjects and that the proportion of non-contractile tissue increased
significantly with age in steroid-treated patients but less than in steroid-naïve patients [29]. These results tend to confirm the present findings.

As measured by DXA, LTM consists primarily of muscle mass but includes also water, fibrous tissue, and internal organs. It has been shown in DMD patients that the percentage of total body water is reduced and that it increases with growth but to a lesser extent than in healthy children [30]. Besides, the water compartment may be increased by added salt (parents’ cooking habits or frequent fast food consumption); thus, measuring the water compartment would have helped a better determination of lean mass composition. However, in the present study patients, it appears unlikely that the observed increase in LTM be simply related to fluid retention because the clinical and biological monitoring has shown no changes in blood pressure or blood electrolytes and no oedema of the soft tissues (data not shown). In addition, the increase in LTM was significantly associated with a stabilisation of motor function compared to what is expected in DMD [21]. In other words, this improvement in LTM was compatible with a steroid-induced increase in muscle mass. However, the definite proof requires other investigation techniques (e.g., MRI). Measuring the water compartment by bioelectrical impedance and bioimpedance spectroscopy may help elucidating the effects of steroids on muscle [31] and a better determination of lean mass composition.

Investigating the relationships between body composition parameters and motor function, we found a significant negative correlation between the MFM total score and the $\%\text{BFM}_{\text{DXA}}$. This result is potentially important because it suggests that these measures are convenient as an outcome measures in future clinical trials of candidate therapeutic agents in DMD.

One major limitation of this work is the relatively small number of patients, especially in the steroid-naïve group. In fact, the parents of DMD patients who refuse a child steroid treatment are rare; the group of steroid-naïve patients is mainly composed of a historic cohort of patients
seen when steroids were not systematically proposed in DMD, at least at our Department (2002-2003). Some differences, at baseline, between the two groups in terms of body composition may represent another limitation. At baseline, the two groups had significant differences in MFM D1, BMI, and %BFM. For example, when only non-ambulant patients at inclusion are considered, BMI and %BFM values remain significantly higher in the corticoid-naïve group than in the corticoid-treated group (23.8 vs. 18.2 and 67% vs. 51%, respectively). A more appropriate "control" (i.e., steroid-naïve group) to the 16 walking patients of the steroid-treated group would be boys closer in age and walking during the 2 years. A better patient matching would be the use of a placebo group but this would have been unethical with regard to the current recommendations. This was the reason for the use of a historical cohort from an era during which corticosteroid were not widely used. Another limitation was that the small sample size hindered an analysis of the impact of loss of ambulation on body composition through comparisons of control vs. treatment groups within ambulant and within non-ambulant patients. Nevertheless, it remains true that a significant improvement in body composition was observed in the steroid-treated group but not in the steroid-naïve group.

**Disclosure of conflict of interest**

The authors declare no financial or other conflict of interests.
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Figure legends

Figure 1 - Regression line between age and $\%\text{BFM}_{\text{DXA}}$ (A), BMI (B) and FM/LTM ratio (C) in 29 DMD patients (black circles for 21 steroid-treated patients and grey circles for 8 steroid-naïve patients) at M0 (baseline).

Figure 2 - Bar graphs (mean + SD) showing the progress of MFM Total score (A) and MFM subscores D1 (B), D2 (C), and D3 (D) over two years in the steroid-treated and the steroid-naïve group.

Figure 3 - Scatter plot showing the association between the MFM total score and $\%\text{BFM}_{\text{DXA}}$. (Black circles for steroid-treated patients and grey circles for steroid-naïve patients)
Table 1 - Characteristics of the study groups at inclusion (M0).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Steroid-treated group</th>
<th>Steroid-naïve group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (n)</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>8.1 ± 2.6</td>
<td>9.6 ± 2.1</td>
</tr>
<tr>
<td>Ambulant / Non ambulant</td>
<td>16 / 5</td>
<td>4 / 4</td>
</tr>
<tr>
<td>Age at lost of walking ability (yrs)</td>
<td>9.3 ± 1.4</td>
<td>9.4 ± 0.8</td>
</tr>
<tr>
<td>MFM D1 score (%)</td>
<td>49 ± 28</td>
<td>18 ± 19*</td>
</tr>
<tr>
<td>MFM D2 score (%)</td>
<td>88 ± 16</td>
<td>77 ± 16</td>
</tr>
<tr>
<td>MFM D3 score (%)</td>
<td>83 ± 13</td>
<td>78 ± 10</td>
</tr>
<tr>
<td>MFM Total score (%)</td>
<td>71 ± 18</td>
<td>53 ± 15</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.5 ± 3.5</td>
<td>20.3 ± 4.3*</td>
</tr>
<tr>
<td>%BFM$_{\text{DXA}}$</td>
<td>33 ± 15</td>
<td>52 ± 17*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD - * Significant difference according to a non-parametric t-test for two independent samples - BMI: Body Mass Index - MFM: Motor Function Measure.
Table 2 – Change in body mass composition parameters as measured by DXA over two years of follow-up in steroid-treated and steroid-naïve DMD patients.

<table>
<thead>
<tr>
<th>Patient and follow-up</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>LTM (kg)</th>
<th>FM (kg)</th>
<th>FM/LTM ratio</th>
<th>%BF&lt;sub&gt;DXA&lt;/sub&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid-treated patients (21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>25.3 ±11.5</td>
<td>16.5 ±3.5</td>
<td>14.7 ±3.6</td>
<td>9.6 ±9.3</td>
<td>0.61 ±0.5</td>
<td>33 ±15</td>
</tr>
<tr>
<td>At 12 months</td>
<td>28.9 ±13.9</td>
<td>17.9 ±4.3*</td>
<td>18.6 ±5.0</td>
<td>10.4 ±10.0</td>
<td>0.50 ±0.3</td>
<td>31 ±13</td>
</tr>
<tr>
<td>At 24 months</td>
<td>34.2 ±15.7</td>
<td>18.7 ±4.3*</td>
<td>20.0 ±5.2*</td>
<td>12.9 ±11.2</td>
<td>0.56 ±0.4</td>
<td>33 ±13</td>
</tr>
<tr>
<td>Steroid-naïve patients (8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>37.8±15.1</td>
<td>20.3 ±4.3</td>
<td>14.9 ±2.5</td>
<td>21.7 ±14.2</td>
<td>1.3±0.8</td>
<td>52 ±17</td>
</tr>
<tr>
<td>At 24 months</td>
<td>45.1±15.3</td>
<td>20.6 ±5.2</td>
<td>14.6 ±3.5</td>
<td>29.6 ±13.3</td>
<td>1.8±0.7*</td>
<td>62 ±10*</td>
</tr>
</tbody>
</table>

BMI: Body mass index (kg/m²) – LTM: Lean tissue mass (kg) - FM: Fat mass (kg) - %BF<sub>DXA</sub>: Percentage body fat measured by DXA.

Values are expressed as mean ± SD. * Statistically significant difference vs. the value at baseline (p < 0.05, unpaired t-test, two tailed)
Influence of a two-year steroid treatment on body composition as measured by Dual X-Ray Absorptiometry in boys with Duchenne Muscular Dystrophy

Abstract
Steroids are nowadays routinely used as a long-term treatment in Duchenne muscular dystrophy (DMD). Their effects on body composition were assessed using Dual X-Ray absorptiometry. The study followed over two years 29 genetically confirmed DMD patients: 21 in the steroid-treated group and 8 in the steroid-naïve group. After two years of steroid treatment, the lean tissue mass values increased significantly (p < 0.0001), the percentage of body fat mass remained practically constant (p = 0.94) in comparison with the initial visit. In the steroid-naïve patients, there were no significant increases in the lean tissue mass but deterioration in body composition confirmed by a significant increases in the percentage of body fat mass. Besides, significant negative correlations were found between the percentage of body fat mass and the MFM total score (R = - 0.79, n = 76, p < 0.0001). A 2-year steroid treatment improves significantly body composition of boys with DMD through a significant increase in lean tissue mass. We suggest that a thorough check of body composition should be carried out before steroid treatment discontinuation in case of overweight gain.

Key words
Duchenne muscular dystrophy, steroids, body composition.