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Muscle ultrasound density in human fetuses with spina bifida aperta

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ABSTRACT

Background: In fetal spina bifida aperta (SBA), leg movements caudal to the meningomyelocele (MMC) are transiently present, but they disappear shortly after birth. Insight in the underlying mechanism could help to improve treatment strategies. In fetal SBA, the pathogenesis of neuromuscular damage prior to movement loss is still unknown. We reasoned that prenatal assessment of muscle ultrasound density (fetal-MUD) could help to reveal whether progressive neuromuscular damage is present in fetal SBA, or not.
Aim: To reveal whether prenatal neuromuscular damage is progressively present in SBA.
Patients/methods: In SBA fetuses ($n=6$; 22–37 weeks gestational age), we assessed fetal-MUD in myotomes caudal to the MMC and compared measurements between myotomes cranial to the MMC and controls ($n=11$; 17–36 weeks gestational age). Furthermore, we intra-individually compared MUD and muscle histology between the pre- and postnatal period.
Results: Despite persistently present fetal leg movements caudal to the MMC, fetal-MUD was higher caudal to the MMC than in controls ($p<0.05$). Fetal-MUD caudal to the MMC did not increase with gestational age, whereas fetal-MUD in controls and cranial to the MMC increased with gestational age ($p<0.05$). In 5 of 6 patients assessed, comparison between pre- and postnatal MUD and/or muscle histology indicated consistent findings.
Conclusions: In fetal SBA, persistent leg movements concur with stable, non-progressively increased fetal-MUD. These data may implicate that early postnatal loss of leg movements is associated with the impact of additional neuromuscular damage after the prenatal period.

1. Introduction

In spina bifida aperta (SBA), defective neurulation is associated with a motor deficit caudal to the meningomyelocele (MMC) [1]. Notwithstanding eventual postnatal motor function loss, perinatal leg movements are often still transiently present [1–3]. Insight in the underlying mechanism could help to improve treatment strategies. Several explanations for the disappearance of leg movements have been proposed. During embryogenesis, skeletal muscles develop from paraxial mesoderm at the dorsal part of the somite [4,5]. As a consequence of the neural tube defect, early mesodermal muscle development may become hampered. In a fetal SBA study, we showed that muscle histology is affected from the first trimester onwards [1,6].

In addition to the congenital defect, neural exposure by the open MMC could also progressively hamper neural function (by the neurotoxic content of amniotic fluid and by traumatic mechanical forces [2,7]). In sheep fetuses with surgically removed vertebral arches, Meuli et al. showed that spinal cord exposure to amniotic fluid causes progressive neurological damage [8]. In order to protect vulnerable neural connections at the MMC, these findings induced fetal therapy by coverage of the MMC [9,10]. Until now, actual proof for preservation of motor function by prenatal coverage of the MMC is still unconvincing [10–12]. This could be attributed to the prenatal surgical procedure itself (by sub-optimal timing and/or iatrogenic damage) [11,13,14]. However, we have also shown that neural conduction through the MMC is still present in un-operated human SBA neonates during the first week of life [15]. From this perspective, it still remains unclear whether fetal neuromuscular damage in human congenital SBA is similarly progressive as in operated spinal sheep fetuses.

Postnatal human muscle maturation is characterized by a gradual process with a decrease in water and an increase in peptide content of the muscle [16]. Under physiological circumstances, this corresponds with an increase in postnatal muscle ultrasound density (MUD) [17].

Abbreviations: SBA, spina bifida aperta; MMC, meningomyelocele; MUD, muscle ultrasound density.

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MUD values in children with neuromuscular disorders exceed those of normal, age-matched controls by additional fat and collagen deposition [6,18,19]. In this perspective, MUD may provide a non-invasive diagnostic tool for the assessment of neuromuscular damage [17,19–24]. To the best of our knowledge, MUD has never been applied as a diagnostic tool in fetuses before. In fetuses with SBA, we reasoned that assessment of MUD could provide insight in the onset and progression of neuromuscular damage prior to movement loss. We hypothesized that if MUD caudal to the MMC is increased in a non-progressive way (compared to normal controls), stable (non-progressive) dysfunction by the congenital neurulation defect is likely to be involved. However, if MUD caudal to the MMC would increase with gestational age, superimposed secondary neuromuscular damage could also be involved. In order to obtain insight in the onset and progression of neuromuscular damage in fetal SBA, we assessed fetal-MUD cranial and caudal to the MMC and compared outcomes with age-matched fetal control myotomes.

2. Patients

The medical ethical committee of the University Medical Center Groningen, the Netherlands, approved the present study. After informed consent by the parents, fetal-MUD was assessed in six SBA (22–37 weeks gestational age; median 34 weeks) and 11 control fetuses (17–36 weeks gestational age; median 28 weeks). MMC was at thoracic ($n=1$), lumbar ($n=1$) or lumbar-sacral ($n=4$) level. All six SBA patients were delivered vaginally. Three of six fetuses were spontaneously delivered (patients 4, 5 and 6) and the other three fetuses were delivered after induction (patients 1, 2 and 3; by prostaglandine-E2 medication and/or additional cephalocentesis, respectively). The three fetuses that were delivered after induction, died during delivery. In these patients obduction and histological muscle assessment were performed. Patient 4 died within two weeks after birth due to severe illness (by the consequences of extensive hydrocephalus, microcephaly and Chiari II malformation). Parents gave no permission for obduction. In the other two surviving neonates (patients 5 and 6), pre- and early postnatal MUD could be assessed. Clinical data are summarized in Table 1. In all fetal controls, neurological pathology was absent.

3. Methods

In accordance with the previously described method of MUD assessment in children [17,20], we assessed fetal-MUD. To exclude for alterations of the ultrasound signal by the maternal abdominal wall and fetal position, we expressed fetal-MUD as a ratio between muscle and bone density: [mean muscle pixel value]/[mean bone pixel value]. In order to obtain fetal control data, we determined the cross-sectional relationship between fetal-MUD and gestational age in 11 healthy fetuses (17–36 weeks gestational age), first. Consecutively, we cross-sectionally assessed and compared fetal-MUD between SBA and age-matched control fetuses (six age-matched pairs; 22–38 weeks gestational age; median 33.5 weeks). In SBA, MUD cranial to the MMC can be altered by cerebral pathology, whereas MUD caudal to the MMC can be altered by both cerebral and spinal (i.e. the MMC) pathology. By comparison of MUD caudal to the MMC with MUD cranial to the MMC (in relation with healthy age-matched controls), the impact of the MMC upon MUD caudal to the MMC can be derived.

We assessed MUD in arm (biceps or triceps (C5–C8)) and leg (quadriceps (L2–L4), tibialis anterior (L4–L5), gluteus (L4–S1), hamstrings (L5–S2) and calf (gastrocnemius or soleus (L5–S1)) muscles and categorized outcomes according to segmental muscle innervation (i.e. either cranial or caudal to the MMC). Additionally, we assessed the occurrence and quality of leg movements caudal to the MMC (see reference [2] for description of the methods). The time interval between the last prenatal ultrasound recording and birth varied between 0 and 6 weeks.

In five of six SBA patients, we were able to obtain postnatal muscle parameters to serve as intra-individual controls. These postnatal muscle parameters consisted of neonatal-MUD assessment (in 2/2 surviving patients) and muscle histology (in 3/4 obducted patients). In the two surviving patients, postnatal MUD data were assessed within four days. The time interval between pre- and postnatal MUD assessment was 5 and 6 weeks. The ratio of MUD before and after birth (perinatal-MUD ratio) is expressed as: [neonatal-MUD]/[fetal-MUD]. Since it takes more than 1–2 weeks before MUD increases after acute neuromuscular injury, a perinatal-MUD ratio of approximately 1.0 would indicate that fetal-MUD outcomes are reproducible (by the postnatal technique) and non-progressive.

In the succumbed fetuses, myotomes cranial and caudal to the MMC were histologically assessed. Post mortem time before autopsy was 0–3 days. Muscles were stained by haematoxylin–eosin (H&E) and qualitatively classified as “discretely abnormal” (incidental muscle fiber hypertrophy), “moderately abnormal” (pronounced muscle fiber a- and hypertrophy), or “severely abnormal” (muscle atrophy and interspersed fat and collagen deposition (fibrosis)). ATP-ase staining was applied for assessment of muscle fiber type differentiation and type grouping. We mathematically compared fetal-MUD caudal to the MMC with fetal-MUD in age-matched control myotomes, according to the formula: ([fetal-MUD caudal to MMC]/[fetal-MUD control]×100%). Furthermore, we associated outcomes with histological assessments.

Statistical analysis was performed by SPSS version 12.0.1 (SPSS, Chicago, IL). For correlations between gestational age and fetal-MUD, Kendall's tau was used. The Mann Whitney Test was applied to compare fetal-MUD in the 2nd and 3rd trimesters in controls and between SBA and age-matched controls.

Table 1
Clinical data of included SBA patients.

Case nr	Fetal US at GA	Partus at GA	Level MMC	Cerebral pathology	FM level	Postnatal data	AS 3′ and 5′	PM level
1	22	22	L_5–S_1	HC, ChII	L_5–S_1	H	†	†
2	37	37	L_5–S_1	HC, ChII, DG, B, EC	L_5–S_1	H	†	†
3	36	41	Th_{12}–L_2	HC, ChII	L_5–S_1	H	†	†
4	35	40	L_4–S_4	HC, ChII, MC	L_5–S_1	–	–	L_1–L_2
5	33	38	L_4–L_5	HC, ChII	L_5–S_1	US	9/10	L_2–L_3
6	32	38	L_5–S_1	ChII	L_5–S_1	US	6/8	L_5–S_1

SBA = spina bifida aperta, nr = number, US = ultrasound assessment, GA = gestational age in weeks, MMC = meningomyelocele, FM level = lowest segmental level of fetal motor behaviour, L = lumbar, S = sacral, Th = thoracic, HC = hydrocephalus, ChII = Chiari II malformation, DG = dysgyration abnormality, B = bleedings, EC = encephalocele, MC = microcephaly, H = histological muscle assessment, – = no data, AS = Apgar score, † = perinatal death, PM level = lowest segmental level of postnatal motor behaviour.

4. Results

4.1. Prenatal muscular assessments

In all six SBA fetuses, leg movements caudal to the MMC were present. Movement quality was abnormal in 4 of 6 fetuses (hardly discernible). In SBA, fetal-MUD caudal to the MMC (calf muscle) was higher than in age-matched controls (medians 0.41 (range 0.36–0.45) and 0.30 (range 0.14–0.44) respectively; $p<0.05$), Fig. 1a. In 5 of 6 SBA patients, segmental innervation of quadriceps muscle was located cranial to the MMC. Comparing fetal-MUD in SBA cranial to the MMC (quadriceps muscle) with age-matched controls, indicated no significant differences (medians 0.34 (range 0.24–0.71) and 0.38 (range 0.21–0.52) respectively), Fig. 1b. In control fetuses, cross-sectional fetal-MUD of quadriceps muscle increased from the 2nd to 3rd trimester of pregnancy (medians 0.21 (range 0.15–0.29) and 0.46 (range 0.30–0.52) respectively; $p<0.01$), Fig. 1c. In SBA myotomes

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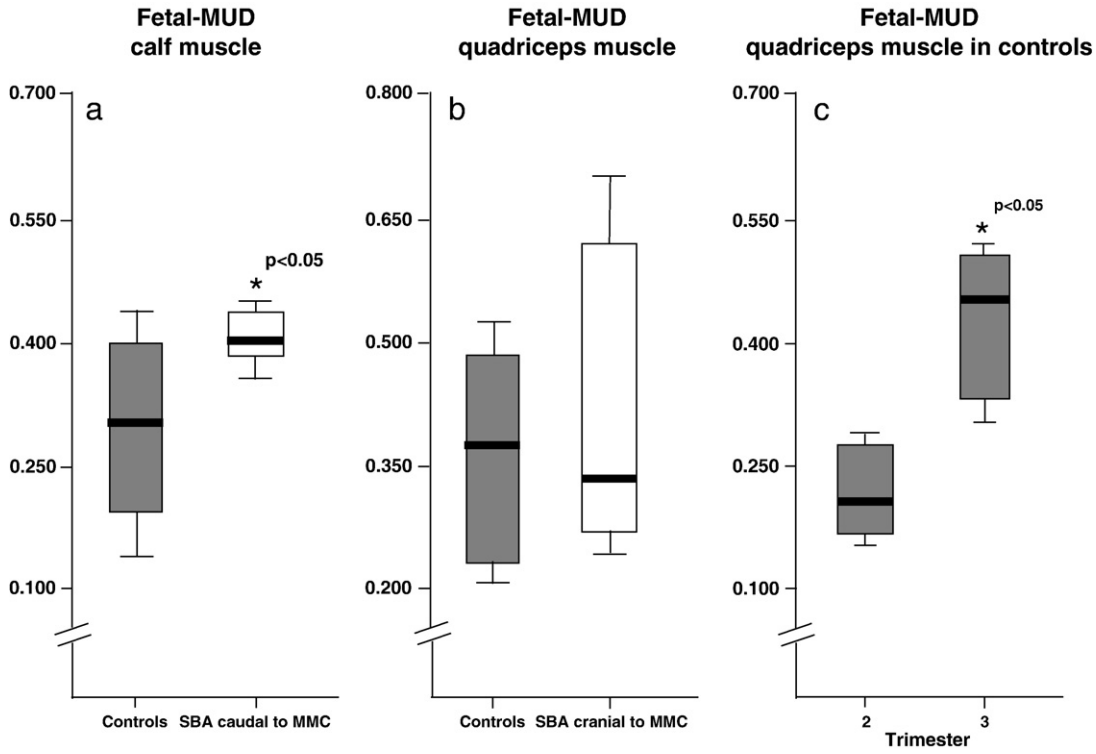

Fig. 1. Fetal-MUD in SBA and control fetuses. (a). Fetal-MUD in SBA caudal to the MMC compared with age-matched controls. The *x*-axis indicates fetal controls (left) and SBA fetuses (right). The *y*-axis indicates fetal-MUD of the calf muscle. Fetal-MUD reflects the mean pixel value of the muscle divided by the mean pixel value of the bone (pixel value range 0–255). Fetal-MUD in SBA (caudal to the MMC) is higher than in age-matched controls ($p<0.05$). (b). Fetal-MUD of quadriceps muscle (L2–L4) in SBA cranial to the MMC compared with age-matched controls. The *x*-axis indicates fetal controls (left) and SBA fetuses (right). The *y*-axis indicates fetal-MUD of the quadriceps muscle. Since comparison of fetal-MUD is performed between SBA fetuses with an innervation of the quadriceps muscle cranial to the MMC, only the 5 (of 6) SBA fetuses with a MMC at, or caudal to L4 are shown. Fetal-MUD in SBA cranial to the MMC does not significantly differ from fetal-MUD in age-matched controls. (c). Relationship between fetal-MUD and gestational age in controls. The *x*-axis indicates the trimester of pregnancy; the *y*-axis indicates fetal-MUD of the quadriceps muscle. Fetal-MUD is higher in the 3rd compared to the 2nd trimester of pregnancy ($p<0.05$). MUD = muscle ultrasound density; SBA = spina bifida aperta; MMC = meningomyelocele; L = lumbar.

cranial to the MMC, fetal-MUD was also associated with gestational age ($n=5$; for quadriceps muscle: $r=0.50$; $p<0.05$). Fetal-MUD in SBA myotomes caudal to the MMC did not increase with gestational age ($n=6$; $r=0.26$; $p=0.13$).

4.2. Postnatal muscular assessments

Postnatal muscle parameters were obtained in 5 of the 6 SBA patients, consisting of neonatal-MUD or muscle histology (in 2/2 and 3/4 patients; respectively).

Intra-individually, perinatal-MUD ratio (i.e. [neonatal-MUD]/[fetal-MUD]) approximated 1.0 (1.0–1.3), Fig. 2a. Histological assessment varied between severely abnormal (patient 1: diffuse muscle fiber atrophy and interspersed fat and collagen deposition (fibrosis)), moderately abnormal (patient 2: pronounced muscle fiber a- and hypertrophy) or discretely abnormal (patient 3: normal muscle fibers with incidental fiber hypertrophy). In these patients, fetal-MUD caudal to the MMC in comparison with age-matched controls ([fetal-MUD caudal to the MMC]/[fetal-MUD control]×100%) was 150%–300% increased. The quantitative increase in fetal-MUD corresponded with the severity of histological alterations (i.e. patient 1, severely abnormal muscle alterations; patient 2, moderately abnormal muscle alterations; and, patient 3, discretely abnormal muscle alterations). ATP-ase staining did not indicate abnormal type grouping. Muscle histology did not indicate abnormalities in SBA muscles cranial to the MMC and fetal controls.

5. Discussion

In human fetuses with SBA, we non-invasively assessed fetal-MUD to reveal the onset and progression of muscle damage caudal to the MMC. Our data indicate that fetal-MUD caudal to the MMC is stable and non-progressively increased compared with controls. These observations may implicate that the open defect at the MMC is more strongly associated with stable congenital neuromuscular alterations than with progressive fetal neuromuscular damage. To the best of our knowledge, fetal-MUD has never been assessed before. This technique may find a wider application for prenatal, non-invasive surveillance of other neuromuscular diseases.

In healthy children and adults, MUD has been shown to increase with gestational age [17,20]. Analogous to postnatal assessments, we also observed that fetal-MUD increases with gestational age (in myotomes cranial to the MMC and controls). Before the 20th week gestational age, healthy muscle fibers are still undifferentiated (type IIC). During the 20th–30th week gestational age, muscle fibers mature from undifferentiated type IIC fibers into type II fibers, and, at term age into type I or type II fibers [25]. In healthy fetuses, muscle development involves a gradual process of a decreased water and increased peptide content [16], corresponding with increased fetal-MUD. Thus, in fetal controls and SBA myotomes cranial to the MMC, the positive relationship between fetal-MUD and gestational age apparently reflects physiologic muscle maturation, whereas myotomes caudal to the MMC lack this relationship. It is well known that muscle damage is associated with a decline in water and increase in fat and collagen deposition in the muscle [6,18], causing increased MUD compared with age-matched controls. Throughout gestation, fetal-MUD caudal to the MMC was non-progressively increased (compared with fetal-MUD cranial to the MMC and control myotomes). Present ultrasound findings seem confirmative of our previously published histological data indicating that fetal muscle alterations caudal to the MMC are non-progressively present [1,6]. Thus, these fetal data indicate that the open MMC is more likely to be associated with

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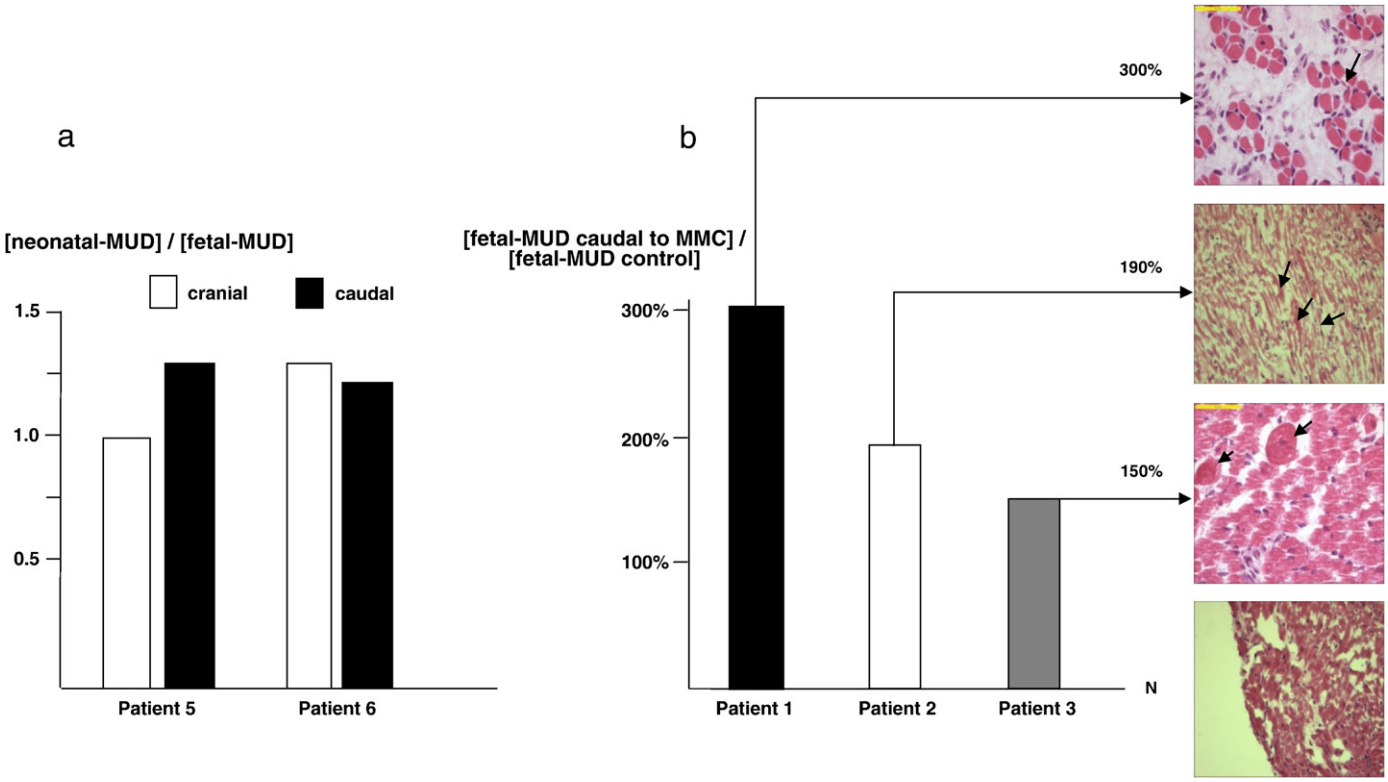

Fig. 2. Longitudinal relationship between fetal and neonatal-MUD and histology in SBA patients. (a). Intra-individual fetal and neonatal-MUD. The *x*-axis indicates neonates five and six that survived after birth. The *y*-axis indicates [neonatal-MUD]/[fetal-MUD]. Both cranial and caudal to the MMC, neonatal-MUD approximated fetal-MUD (indicated by: [neonatal-MUD]/[fetal-MUD] was 1.0–1.3). (b). Relationship between fetal-MUD in SBA caudal to the MMC and histological assessment. The *x*-axis indicates all three neonates that died during delivery (patients 1, 2 and 3). The *y*-axis indicates the extent of fetal-MUD increase caudal to the MMC in relation to an age-matched control ([fetal-MUD caudal to MMC]/[fetal-MUD control] × 100%). On the right side, histology of corresponding muscles is shown (H&E staining). The extent of histological muscle damage appeared related with the proportionally increased fetal-MUD (compared to controls). Patient 1 (fetal-MUD increase of 300%) is associated with severely abnormal histology, patient 2 (fetal-MUD increase of 190%) with moderately abnormal histology and patient 3 (fetal-MUD increase of 150%) with discretely abnormal muscle histology. The associated histological assessments indicate interspersed collagen deposition and fibrosis (calf muscle; 22 weeks gestational age), pronounced fiber a- and hypertrophy (gluteal muscle; 37 weeks gestational age) and normal muscle fibers with an incidental fiber hypertrophy (paravertebral muscle; 41 weeks gestational age), respectively. At the bottom micrograph, a transverse section of a normal (N) paravertebral muscle is indicated. From this figure, it can be derived that the quantitative increase in fetal-MUD is related with the qualitative alteration in histology. MUD = muscle ultrasound density; SBA = spina bifida aperta; MMC = meningomyelocele; HE = haematoxylin–eosin.

congenital, stable muscle alterations than with secondarily progressive neuromuscular damage.

We are aware that the present pilot data are obtained in a small number of patients. Despite this limitation, present results support the concept that fetal-MUD can provide a useful tool for non-invasive prenatal muscle assessment. In accordance with our previous observations, fetal leg movements persisted in all included SBA fetuses and disappeared shortly after birth [1–3,6]. These persistent fetal leg movements caudal to the MMC concurred with non-progressive prenatal muscle alterations. Shortly after birth, fetal leg movements caudal to the MMC disappear [1–3]. Since it takes more than 1–2 weeks before MUD increases after acute neuromuscular injury, it seems likely that the disappearance of postnatal leg movements is associated with acute neuromuscular damage after the prenatal period (for instance during delivery [3,26]).

In conclusion, in SBA, non-progressively increased fetal-MUD caudal to the MMC concurs with persistence of fetal leg movements. These data may implicate that early neonatal movement loss is caused by the impact of additional neuromuscular damage after the prenatal period.

Conflict of interest statement

All authors disclose any financial and personal relationship with other people or organisations that could inappropriately influence (bias) this manuscript.

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