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Tenotomy of Gluteus Medium and Gluteus Minimus is Associated with Dysplasia of the Hip: An Experimental Study in Rabbits

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Abstract

Background: Atrophy of the gluteus medius and gluteus minimus has been reported in patients with developmental dysplasia of the hip (DDH). We aimed to investigate whether atrophy of the gluteus medius and gluteus minimus is associated with DDH.

Methods: Twenty white New Zealand rabbits were divided into 2 groups in our experiment. In group 1, one limb of each rabbit was randomly selected for tenotomy of the gluteus medius and gluteus minimus, with the other side serving as a sham control. In group 2, one limb of each rabbit was randomly selected for intramuscular injection of botulinum toxin to induce atrophy of the gluteus medius and gluteus minimus, with the other side serving as a sham control by injection of equal volumes of normal saline. Acetabulum and muscles were evaluated by ultrasound, X-ray imaging, shear wave elastography, and histology with hematoxylin-eosin (H&E) staining.

Results: Atrophy of the gluteus medius and gluteus minimus was observed on the experimental side in both groups. Early dysplastic changes in the hips on the tenotomy side was observed on the radiographs with an increased acetabular index (40.0 ± 6.7° vs 24.1 ± 1.9°, $P < 0.001$) compared with the control side in group 1. No dislocation of the hips was observed. No dysplasia of the hips was observed on the botulinum-toxin side in group 2 with similar acetabular index on both sides (25.0 ± 3.7° vs 24.1 ± 1.9°, $P = 0.13$).

Conclusion: Tenotomy, not atrophy, of the gluteus medius and gluteus minimus is associated with early changes of DDH.

Keywords: Developmental dysplasia of the hip, Gluteus, Tenotomy, Atrophy, Ultrasound

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Introduction

Developmental dysplasia of the hip (DDH) is a common disease in children with an incidence of 7.6% in Native Americans[1]. DDH refers to dysplasia that occurs progressively in the neonatal period. It includes a series of lesions ranging from mild acetabular dysplasia to dislocation of the hip.[2] The early detection rate of DDH was 2.3% in infants with universal ultrasonographic screening[3]. If not treated in time, some children with DDH will experience severe long-term complications. DDH is the main cause of disability and hip replacement in young and middle-aged people (21%-29%).[4] The exact cause and pathogenesis of DDH are unclear. There are several risk factors for DDH[4-9]. Ortiz-Neira et al[5] reported the relative risk ratio for each factor in newborns was: breech presentation 3.7[5], females 2.5[4], left hip side 1.5[4], first born 1.4[4], and family history 1.3[9]. The higher risk ratio for breech presentation before birth or at delivery indicates that there are mechanical factors involved in the occurrence of DDH.[8,9]

In patients with DDH, the mechanical characteristics of the hip muscles, especially the abductor muscles (such as the gluteus media and gluteus minimus), are altered.[10-12] CT of the hip muscles of adult patients with DDH revealed significant atrophy and fat infiltration in the gluteus medius on the affected side[13, 14], and the density, length and movement of the arm on the affected side were significantly reduced[15]. Tomaru et al[16] used MRI to measure the volume of the gluteus medius and gluteus minimus in children with unilateral DDH who underwent closed reduction of the dislocated hip at the age of 2 years. They found that the volume of the hip abductor gluteus muscle at 2 years of age was associated with subsequent hip development. Promoting the healthy development of the gluteus muscles is important for healthy hip development.

Injury of the gluteus medius may lead to abnormal gait, artificial joint instability and even dislocation after hip replacement. [17-19] However, whether atrophy of the gluteus medius and gluteus minimus is

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associated with DDH has not been reported. We hypothesized that atrophy of the gluteus medius and gluteus minimus was associated with DDH. We will induce atrophy of the gluteus muscles in young rabbits by tenotomy of gluteus muscles or by intramuscular injection of botulinum toxin to determine whether it is associated with DDH.

Materials and Methods

All experiments were performed in accordance with the U.S. National Institutes of Health's Guide for the Care and Use of Laboratory Animals and were approved by the Animal Welfare and Ethical Committee of our hospital (accreditation number: 134-20230509). All rabbits (New Zealand rabbits, 4 weeks old, weighing approximately 550 - 655 g) were procured from the animal breeding center of WQJX Bio-technology, Wuhan, China. The limb selected for surgery was randomly assigned for each rabbit using a computer-generated random number sequence. The operators performing ultrasound and the data analyst were blinded to the group allocation and side of the limbs throughout the data collection and analysis phase.

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Experimental protocols

Twenty white New Zealand rabbits of either sex (detailed sex distribution per group is provided in Table 1) were randomly divided into 2 groups in our study. For the 10 rabbits in group 1, tenotomy of the gluteus tendon was performed on one side. Gluteus tendons on the other side were exposed without tenotomy, and used as sham control, and the wound was sutured. For the 10 rabbits in groups 2, one limb was intramuscularly injected with botulinum toxin, with the other side serving as a sham control by injection of equal volumes of normal saline.

Acetabulum development and gluteus muscles were evaluated by ultrasound and X-ray 4 weeks after surgery. Histopathological changes in the gluteus muscles and acetabulum were analyzed by optical

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microscopy with H&E staining. (Figure 1)

Tenotomy of gluteus muscles

The rabbits in group 1 were anaesthetized by intramuscular injection of 30 mg/kg sodium pentobarbital. The rabbit was placed lying on the side, the skin around the femoral trochanter was depilated by using hair clippers and depilatory cream. After the skin was disinfected with iodine tincture, a longitudinal skin incision approximately 1 cm in length was made over the femoral greater trochanter. After the gluteus maximus fascia was dissected, the gluteus medius tendon attached to the posterolateral upper facet of the femoral greater trochanter was exposed. The gluteus medius tendon was then cut off with scissors. Next, the gluteus minimus tendon attached to the anterior facet of the greater trochanter was exposed and cut off with scissors. The opposite hind limb was treated similarly to expose the tendons of the gluteus medius and gluteus minimus without tenotomy. The incision was sutured. Penicillin (800,000 IU) was injected intramuscularly once a day for 3 days to prevent bacterial infections. Postoperative analgesia was provided with oral Ibuprofen (4 mg/kg) administered once daily for 3 days. The effectiveness of analgesia and the animal's recovery status were monitored by assessing weight recovery, spontaneous activity, food and water intake, and fecal output.

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Injection of botulinum toxin

In group 2, the rabbits were anaesthetized and depilated. Under the guidance of ultrasound, 2 units/0.1ml of botulinum toxin type A (Botox, Allergan; Lot: C7566C3; Clicinal grade) were injected into the gluteus medius and gluteus minimus in the superior, inferior, deep and superficial regions of the muscles on one limb. A total of 8 units of botulinum toxin type A were injected in each rabbit [20,21]. Equal volumes of normal saline were injected into the gluteus medius and gluteus minimus on the control side of limb.

Ultrasound and X-ray imaging

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The gluteus muscles were evaluated with an Aixplorer ultrasound system (Supersonic Imagine, Aixen-Provence, France) equipped with a 20-MHz transducer (SLH20-6) 4 weeks after the operation. Muscle thickness and shear wave elastography were measured in the longitudinal plane of the gluteus medius and gluteus minimus with the rabbit lying on one side and the hip in a neutral position. The depth of detection for shear waves was 0.5 – 1.0 cm. A 3mm-diameter region of interest was placed within the belly of the gluteus muscles, avoiding visible fascia and blood vessels. The same settings were used for all examinations. All the indexes were measured 3 times, and their mean values were used for the final analysis.

Anteroposterior X-rays of the pelvis were obtained 4 weeks after the operation. DDH was identified through the presence of an increased acetabular index or dislocation of the femoral head.

After the rabbits were euthanized by intravenous injection of 150 mg/kg phenobarbital sodium, the acetabulum was removed to determine the gross morphological changes. The three-dimensional diameter and cartilage thickness of the acetabulum were measured by a Vevo2000 ultrasound system (Visualsonics Inc., Toronto, Canada) equipped with a 30-MHz transducer (MS400).

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To evaluate the impact of operator-dependent variability, imaging data were independently acquired and analyzed by a second operator.

Pathological analysis

Histologic findings were evaluated qualitatively. A standardized sampling strategy was employed: the acetabulum was sectioned along its maximum diameter through the superior lateral margin, and tissue samples were harvested from the midportion of the gluteus medius and gluteus minimus muscles along the direction of the muscle fibers. The tissues were fixed with 4% paraformaldehyde, embedded in paraffin, and cut into 8 μm thick sections for H&E and Masson staining. The acetabulum was cut along

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the maximum diameter through the superior lateral margin and stored in a 4% formaldehyde solution for 4 hours. The samples were then decalcified in 10% EDTA solution for 2 to 4 weeks. The specimens were then embedded in paraffin and cut into 4 μm thick sections for H&E staining. The sections were observed under an optical microscope.

Statistical analysis

All statistical analyses were performed using SPSS 22.0 (IBM) and Prism 8.0 (GraphPad) software. The primary endpoint of this study was the acetabular index, as it is the key radiographic metric for assessing DDH. In addition, we assessed a set of primary outcomes that included morphologic and biomechanical parameters: muscle thickness, shear wave speed, acetabular cartilage thickness, and acetabular length, width, and depth. A paired *t* test was used to analyze differences between groups for the primary endpoint, primary outcomes, and secondary outcomes (e.g., histological findings). The sample size of 10 per group was initially selected based on common practice in similar previous experimental studies and resource constraints. We performed a post-hoc power analysis based on the observed effect size in the shear wave speed of muscles. The mean difference between the groups was 0.3m/s with a standard deviation of 0.21m/s. With a sample size of 10 and $\alpha = 0.05$ (two-tailed paired *t*-test), the achieved power ($1-\beta$) is >85%. This confirms that our sample size was sufficient to detect the observed large effect. To control for the familywise error rate due to multiple comparisons across the seven primary outcome parameters, we applied the Bonferroni correction. The significance level (α) was adjusted to $0.05 / 7 \approx 0.007$. Therefore, for all primary outcomes, a *P* value < 0.007 was considered statistically significant. For results that approached but did not meet this adjusted threshold, precision was conveyed by reporting mean paired differences with 95% confidence intervals (CI).

Results

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Four weeks after the procedure, atrophy of gluteus medius and gluteus minimus was observed on the experimental sides in both groups. The gluteus medius and gluteus minimus were thinner on the tenotomy side than on the control side (9.1 ± 0.2 mm vs 10.7 ± 0.2 mm, $P < 0.001$) with a shear wave speed of 1.9 ± 0.2 m/s vs 1.7 ± 0.1 m/s, $P = 0.03$ (Figure 2, Table 2). Similar results were observed in group 2, with thinner gluteus muscles on the botulinum-toxin side than on the control side (8.9 ± 0.3 mm vs 10.3 ± 0.3 mm, $P < 0.001$) and a shear wave speed of 2.0 ± 0.3 m/s vs 1.6 ± 0.2 m/s, $P = 0.03$ (Table 3).

Four weeks after the operation, dysplasia of the hips on the tenotomy side was observed on the radiographs, with an increased acetabular index compared to that on the control side ($P < 0.001$, Table 2, Figure 2A) in group 1. No dysplasia of the hips was observed in group 2 (Table 3). No dislocation of the hips, defined as lateralization of the femoral head beyond the lateral edge of the acetabulum, was observed in our study.

The thickness of the cartilage at the superior lateral part of the acetabulum in the hips on the tenotomy side was greater than that on the control side, as measured by ultrasound ($P < 0.001$, Figure 2B, 2C, 3A-3D, Table 2).

The morphology of the acetabulum changed from spherical to ellipsoid after tenotomy, with decreased depth ($P < 0.001$) compared to that of the control side. There was no significant difference in the width of the acetabulum between the tenotomy side and the control side ($P = 0.12$). (Figure 3, Table 2)

Histological examination of the acetabulum revealed cartilage thickening at the superior lateral margin and a dull bony border. Concurrently, pathological analysis confirmed gluteal muscle atrophy on the experimental side in both groups. This muscular atrophy was marked by extensive fat infiltration (adipocyte deposition between muscle fibers) and fibrosis, which was positive for Masson staining (blue)

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(Figure 4).

The intra-observer intraclass correlation coefficients (ICC) for acetabular-index measurement was 0.92 and the inter-observer ICC was 0.88. The intra-observer ICC for shear-wave elastography was 0.90 and the inter-observer ICC was 0.85.

Discussion

The generation of a dysplastic hip has been achieved by maintaining the knee in extension in rats[22], rabbits[23] and other species[24, 25]. The laxity of the capsule has been thought to be a major contributing factor to DDH.[26, 27] Muscular imbalance may induce dislocation of the hip.[23] In our study, for the first time, we successfully produced a DDH model by detaching the tendons of the gluteus medius and gluteus minimus in young rabbits. The primary function of the gluteus medius and gluteus minimus is to abduct the hip and stabilize the hip joint. The hip abductor muscles stabilize the hip while standing on one limb. They contract to keep the hip leveled so it won't tilt toward the unsupported limb[17,18]. In our study, tenotomy of the gluteus medius and gluteus minimus decreased the function of the gluteus muscles, and reduced the lateral stability of the hip.

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The acetabular indexes of the hips on the tenotomy side measured in our study (40.0 ± 6.7 degrees) were slightly smaller than those measured in the study (44.4 ± 15.3 degrees) by Moraleda et al[28]. In Moraleda's study[28], the knees of rabbits were immobilized in extension with an intramedullary K-wire for 11 weeks before the hips were assessed, which was much longer than the 4 weeks of observation in our study. The longer the period the hips were maintained under the experimental conditions, the greater the severity of the pathological changes[22]. No dislocation of the hips was observed in our study. In a study by Michelsson et al[23], knees of young rabbits (1 to 8 weeks of age) were immobilized in extension for 1 to 8 weeks. The results showed that the younger the animal was, the more often dislocation

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developed in the hip. In our study, we used 4-week-old rabbits. The relatively old age of the animals used may be the reason why no hip dislocation was found. Therefore, the pathological changes observed in our model, particularly within the 4-week timeframe, should be interpreted primarily as early acetabular changes indicative of the initial stages of dysplastic development, rather than representing full-blown DDH progression which might require a longer duration to manifest completely.

In our study, the length of the acetabulum on the tenotomy side increased compared to that on the control side, while the width did not change significantly; that is, the shape of the acetabulum on the tenotomy side changed from round to oval, which was consistent with the findings of Greenhill et al[29]. The acetabular depth on the tenotomy side was shallow, which was consistent with the findings of Moraleda et al[28]. In our study, ultrasound, instead of a calliper or micrometer, was used to measure the dimensions of the bone structure of the acetabulum. The diameters measured did not include soft tissue or cartilage components. The thickness of the acetabular cartilage was also measured via ultrasound. The increased thickness of the superior lateral acetabular cartilage on the tenotomy side was consistent with the results observed by Ding et al[30] and Liu et al[31] via MRI.

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The shear wave speeds of the gluteus medius and minimus muscles were greater on the experimental side than on the control side in our study. Pathological analysis revealed that muscle atrophy was accompanied by fat infiltration and fibrosis, and the increase in shear wave velocity may be caused by the increase in fibrotic components.

In our study, tenotomy of gluteus medium and gluteus minimus resulted in a biomechanical imbalance of the hip joint, placing it in an adducted position. This may share similar underlying mechanisms with DDH resulting from infant swaddling with adducted legs[22] and dysplastic hips secondary to adductor spasticity in cerebral palsy[32]. These findings not only highlight the importance of

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avoiding prolonged adducted positioning in infants, but also suggest that enhancing gluteus medius and minimus strength through rehabilitation may be beneficial for DDH treatment[33]. The atrophy of gluteus muscles induced by injection of botulinum toxin did not lead to DDH, which may be attributed to the fact that muscle strength was not completely abolished after injection, thereby preserving a relative biomechanical balance between hip adduction and abduction.

There are limitations in our study. First, no dislocation of the hips was found during the experiment. We will extend the observation time and use newborn rabbits as animal models to determine whether hip dislocation can occur in subsequent studies. As most clinical cases of children with DDH only have mild acetabular dysplasia without dislocation[2], this experimental model should be at least suitable for the study of this mild acetabular dysplasia. Second, it is well-known that DDH is more common in females. As this study focused on the effect of muscle biomechanics on DDH, we did not test for differences between the sexes. We used the intra-individual contralateral limb as a control. This approach eliminates the potential influence of gender factors on the experimental results, thus the gender effect was not evaluated. Third, although using the contralateral limb as a control is a powerful design for reducing inter-animal variability, we acknowledge that compensatory gait patterns and altered loading mechanics in the rabbit following unilateral tenotomy could theoretically introduce systemic biomechanical effects influencing the contralateral, sham-operated limb. The sham measurements may underestimate true between-group differences.

In summary, tenotomy of the gluteus medius and minimus (abductor continuity loss) induced early dysplastic acetabular remodeling in a juvenile rabbit model, whereas toxin-induced muscle weakness (partial functional reduction) did not produce comparable bony changes within the study window. This indicates that the loss of mechanical continuity of the abductors, rather than muscle atrophy/weakness

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alone, is a key mechanistic driver of early acetabular dysplastic changes.

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Abbreviations

DDH, developmental dysplasia of the hip

H&E, hematoxylin-eosin

EDTA, ethylenediaminetetraacetic acid

Authors' contributions

Author Y.Z. and Q.F. wrote the main manuscript text and prepared the tables. Authors L.Y. and K.H. established the animal models. P.L. imaged and interpreted the hips. T.X. and X.W. performed the histological examination of the hips. C.Z. guaranteed integrity of entire study. The author(s) read and approved the final manuscript.

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Availability of data and material

The datasets generated and analyzed during the current study are available in the Figshare repository (https://doi.org/10.6084/m9.figshare.30069139).

Declarations

Ethics approval and consent to participate

The experiments were approved by the Animal Welfare and Ethical Committee of our hospital (accreditation number: 134-20230509).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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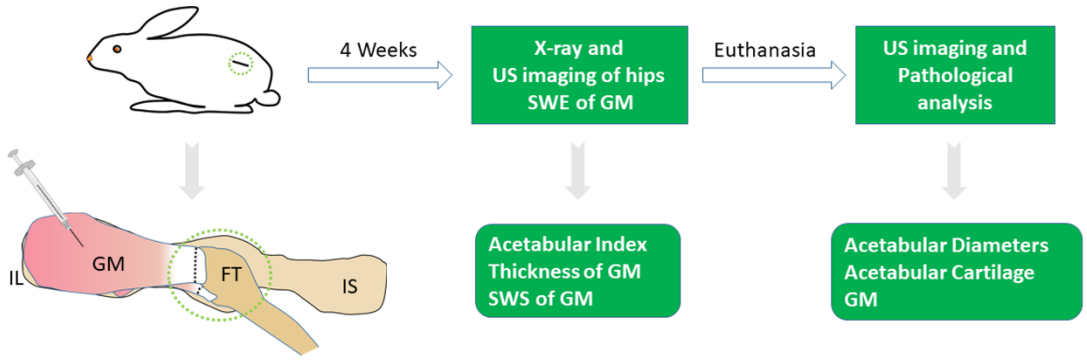

Figure 1. The schedule of atrophy of gluteal muscles in rabbits. The animals underwent tenotomy of tendons or local injection of botulinum-toxin on one side of the gluteus medius and gluteus minimus (GM). The other side of the gluteus muscles underwent a sham operation or injection of equal volumes of normal saline. Hips and muscles were examined 4 weeks after the operation via X-ray imaging, ultrasound (US), and shear wave elastography (SWE). The diameter of the acetabulum and thickness of the acetabular cartilage were measured via ultrasound in vitro. Pathologic changes in the acetabulum and muscles were analysed via microscopy. IL, ilium; FT, greater trochanter of the femur; IS, ischium; SWS, shear wave speed

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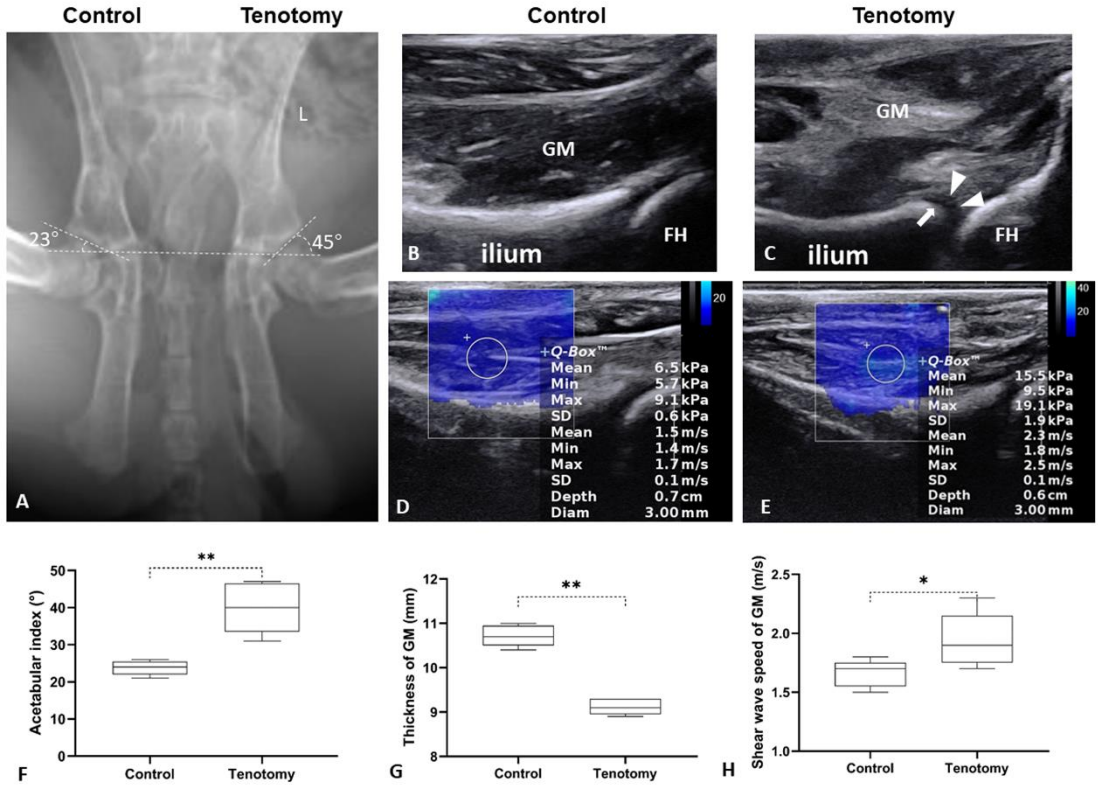

Figure 2. Imaging of the hips 4 weeks postsurgery. X-ray of the hip (A) showing a shallower left acetabular fossa and an enlarged acetabular angle on the tenotomy side (A, F). Ultrasound imaging (B, C) revealed decreased thickness and increased echogenicity of the gluteus muscles, indicating atrophy and fat infiltration of the muscles on the tenotomy side (G). The superior lateral margin of the acetabular cartilage was thickened (arrow head, C), and the bone margin was dull (arrow, C). The margin of the acetabular bone on the sham control side was sharp (B). Shear wave elastography of the gluteus muscles revealed increased shear wave speeds on the tenotomy side (D, E, H). GM, gluteus medius and gluteus minimus muscles; FH, femoral head; *, $P < 0.05$; **, $P < 0.001$.

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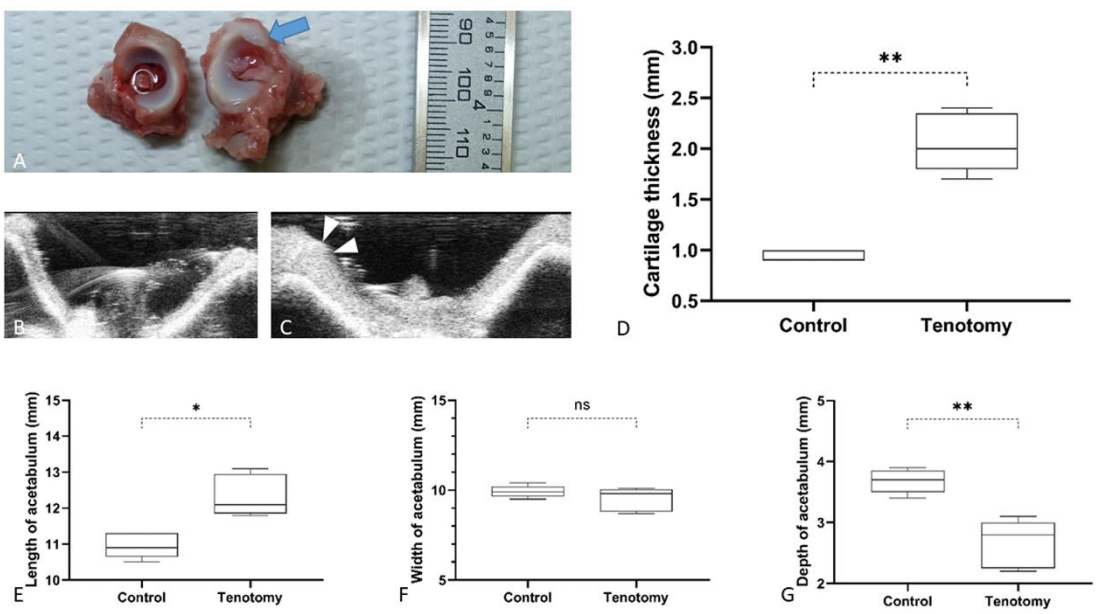

Figure 3. Gross pathology and measurement of the acetabulum with ultrasound. Gross pathology of the hips (A) revealed that the acetabulum on the tenotomy side was ellipsoid instead of spherical in shape with thickened cartilage (arrow head, C) at the superior lateral margin (arrow, A). The increased length and decreased depth of the acetabulum, together with thickened acetabular cartilage, were then measured and confirmed by ultrasound imaging (B-G). *, $P < 0.05$, **, $P < 0.001$.

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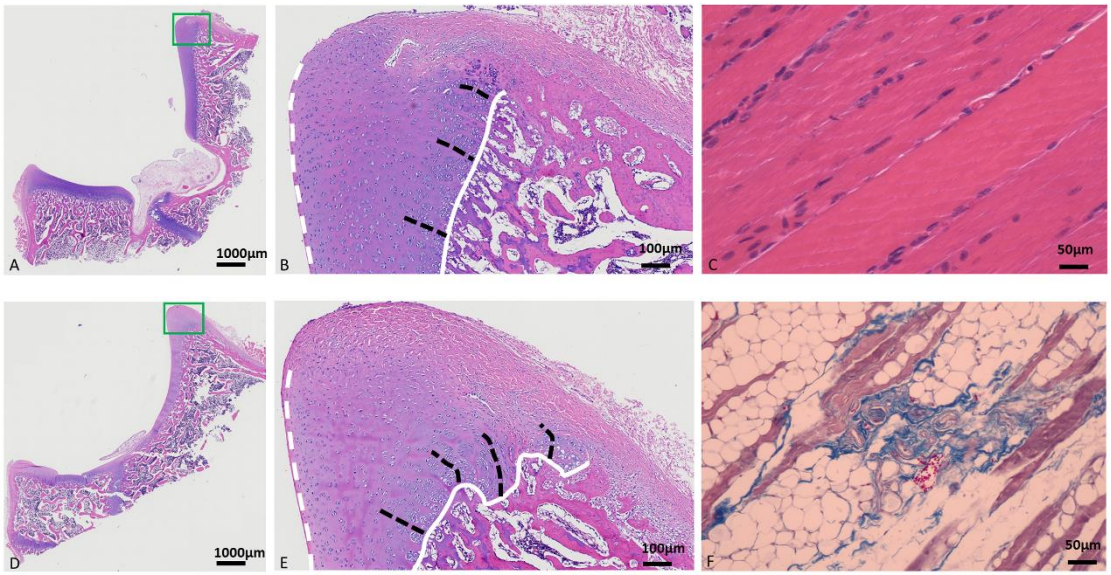

Figure 4. Micrographs of sections along major diameters of the hips and gluteus muscles 4 weeks after the operation. On the control side (A, B), the surface of the subchondral bone (solid white line) was regular and was arranged in the same way as the articular cartilage (dashed white line). The cells in the proliferative and hypertrophic zones of the growth plate (dashed black line) were columnar and perpendicular to the surface of the articular cartilage. On the tenotomy side (D, E), the superior lateral acetabular cartilage became thickened. The surface of the subchondral bone was irregular. The cells in the proliferative and hypertrophic zones of the growth plate were arranged abnormally from perpendicular to parallel to the surface of the articular cartilage. Normal muscle fibres could be observed on the control side (C). Atrophy of muscle fibers with fat infiltration and fibrosis (blue, Masson staining) was observed on the tenotomy side (F). Original magnification, X10 (A, D), X100 (B, E), and X200 (C, F)

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Table 1. Sex distribution of experimental animals

Group	Intervention	Number of Rabbits (Male/Female)
1	Tenotomy	10 (5 / 5)
2	Botulinum Toxin Injection	10 (5 / 5)

Table 2. Comparison of indexes of acetabular morphology and muscular biomechanics in group 1

	Control side	Tenotomy side	Mean Paired Difference (95% CI)	*P*
Acetabular Index (°)	24.1 ± 1.9	40.0 ± 6.7	15.9 (10.4 to 21.4)	**<0.001**
Thickness of GM (mm)	10.7 ± 0.2	9.1 ± 0.2	-1.6 (-1.7 to -1.5)	**<0.001**
SWS of GM (m/s)	1.7 ± 0.1	1.9 ± 0.2	0.2 (0.0 to 0.4)	0.03
Acetabular Cartilage (mm)	0.9 ± 0.1	2.1 ± 0.3	1.1 (0.9 to 1.3)	**<0.001**
Acetabular Length (mm)	10.9 ± 0.3	12.3 ± 0.6	1.4 (1.0 to 1.1)	**<0.001**
Acetabular Depth (mm)	3.7 ± 0.2	2.7 ± 0.4	-1.0 (-1.2 to 0.8)	**<0.001**
Acetabular Width (mm)	9.9 ± 0.3	9.5 ± 0.7	-0.3(-0.7 to 1)	0.12

Note, *P* values are based on paired *t*-tests. Statistical significance after Bonferroni correction for multiple comparisons (adjusted $\alpha = 0.007$) is indicated in bold. GM, gluteus medius and gluteus minimus muscles, SWS, shear wave speed

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Table 3. Comparison of indexes of acetabular morphology and muscular biomechanics in group 2

	Control side	Botulinum-toxin side	Mean Paired Difference (95% CI)	*P*
Acetabular Index (°)	25.0 ± 3.7	24.1 ± 1.9	-1.1 (-2.9 to 0.8)	0.21
Thickness of GM (mm)	10.3 ± 0.3	8.9 ± 0.3	-1.4 (-1.7 to -1.0)	**<0.001**
SWS of GM (m/s)	1.6 ± 0.2	2.0 ± 0.3	0.3 (0.1 to 0.6)	0.03
Acetabular Cartilage (mm)	1.0 ± 0.3	1.0 ± 0.3	0.0 (0.0 to 0.1)	0.39
Acetabular Length (mm)	10.1 ± 0.4	9.9 ± 0.3	-0.2 (-0.8 to 0.4)	0.47
Acetabular Depth (mm)	3.6 ± 0.3	3.7 ± 0.4	-0.1 (-0.3 to 0.2)	0.59
Acetabular Width (mm)	10.0 ± 0.4	10.2 ± 0.6	0.3 (-0.3 to 0.7)	0.30

Note, *P* values are based on paired *t*-tests. Statistical significance after Bonferroni correction for multiple comparisons (adjusted $\alpha = 0.007$) is indicated in bold. GM, gluteus medius and gluteus minimus muscles, SWS, shear wave speed

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