

Reduced Muscle Degeneration and Decreased Fatty Infiltration after Rotator Cuff Tear in a PARP-1 Knock-Out Mouse Model

PARP-1 Regulates Muscular Deterioration After Rotator Cuff Tear

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1 **Abstract**

2 **Introduction:** Disturbed muscular architecture, atrophy and fatty infiltration remain

3 irreversible in chronic rotator cuff tears (RCT) even after repair. Poly (ADP-ribose)

4 polymerase-1 (PARP-1) has shown to be a key regulator of inflammation, apoptosis, muscle

5 atrophy, muscle regeneration and adipocyte development. We hypothesized that the absence

6 of PARP-1 would lead to a reduction in damage to the muscle subsequent to combined

7 tenotomy and neurectomy in a PARP-1 knock-out mouse model.

8 **Methods:** PARP-1 knock-out (PARP-1 KO group) and wild type C57BL/6 (WT group) mice

9 were analyzed at different time points (1, 6 and 12 weeks, total n=84). In all mice the

10 supraspinatus and infraspinatus muscles of the left shoulder were detached and denervated.

11 Macroscopic analysis, magnetic resonance imaging, gene expression analysis,

12 immunohistochemistry and histology were used to assess the differences in PARP-1 KO and

13 WT mice.

14 **Results:** The muscles in the PARP-1 KO group had significantly less retraction, atrophy and

15 fatty infiltration after 12 weeks than in the WT group. Gene expression of inflammatory,

16 apoptotic, adipogenic and muscular atrophy genes was significantly decreased in PARP-1 KO

17 mice in the first 6 weeks.

18 **Discussion:** Absence of PARP-1 leads to a reduction in muscular architectural damage, early

19 inflammation, apoptosis, atrophy and fatty infiltration after combined tenotomy and

20 neurectomy of the rotator cuff muscle. Although the macroscopic reaction to injury is similar

21 in the first 6 weeks, the muscles ability to regenerate is much greater in the PARP-1 KO

22 group leading to a near normalization of the muscle after 12 weeks.

23 **Keywords:** rotator cuff tear; PARP-1; ARTD1; supraspinatus muscle; mouse model;

24 inflammation; muscle atrophy; fatty infiltration

25

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26 Introduction

27 Rotator cuff tears (RCT) cause profound and potentially irreversible structural alterations in
28 the affected muscle. There is significant migration of inflammatory cells within the first few
29 days of a tear and the muscle fibers undergo apoptosis^{27; 32}. The infiltrate of inflammatory
30 cells releases Interleukin1- β (IL1- β) and Tumor Necrosis Factor α (TNF α), which incites the
31 inflammatory cascade³². These factors activate intracellular Nuclear Factor kappa B (NF- κ B)
32 which not only induces apoptosis and muscular atrophy, but also inhibits muscle regeneration
33^{23; 32; 35; 42}. Pro-fibrotic factors from the surrounding extracellular matrix (ECM)²⁴ are released
34 and activated. These factors are members of the Transforming Growth Factor beta (TGF β)
35 superfamily and are key regulators of gene expression in muscle homeostasis²⁰. They lead to
36 the degradation of the injured muscle fibers and the clearance of cellular debris by M1
37 macrophages. Once the cellular debris have been evacuated, the monocytes transform into
38 anti-inflammatory M2_{reg} macrophages to support myogenesis¹ with the expression of
39 myogenic regulatory factors (MRFs)⁴⁵, which in combination with other endocrine growth
40 factors instigate the development mature myocytes from precursor cells⁴⁵. If the tendon
41 remains torn, unloaded and retracted, the macrophages switch to become pro-fibrotic M2_a
42 macrophages and reprogram myogenic precursor cells into the adipogenic pathway, with
43 mature adipocytes infiltrating the free inter- and intramyofibrillar spaces⁹. This phenomenon
44 is termed fatty infiltration^{2; 27}. Although reloading the dynamic musculotendinous units leads
45 to partial recovery of atrophy and retraction, fatty infiltration remains irreversible^{5; 18}. The
46 degree of fatty infiltration in a chronically torn rotator cuff is a negative predictor for a
47 successful surgical outcome⁴⁶.

48

49

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50 The complex interplay of molecular and cellular mechanisms, which leads to potentially
51 irreversible structural alterations in skeletal muscle, is well described ²². However, a single
52 upstream regulator may orchestrate this molecular cascade. The discovery of such a regulator
53 could potentially provide a future target for therapeutic interventions at the molecular level
54 that may enhance the recovery of rotator cuff muscles post surgical repair.

55

56 Poly (ADP-ribose) polymerase-1 (PARP-1), also known as ADP-ribosyltransferase (ARTD1),
57 is a key transcription factor involved in the maintenance of cellular homeostasis ³⁷. It activates
58 NF- κ B transcription during the inflammatory response which not only induces apoptosis and
59 muscular atrophy, but also inhibits muscle regeneration ^{14; 42}; it promotes a caspase
60 independent pathway of apoptosis via the apoptosis inducing factor (AIF) ¹⁵; it regulates the
61 expression of peroxisome proliferator-activated receptor gamma (PPAR γ), which has a role in
62 adipogenesis and may induce fatty infiltration of the muscle ⁷; it also induces muscular
63 atrophy and fibrosis whilst depressing regenerative pathways ^{17; 40}. Hence, PARP-1 may be
64 the upstream regulator that orchestrates the molecular and cellular mechanisms that leads to
65 potentially irreversible structural alterations after RCT.

66

67 We therefore hypothesized that the absence of PARP-1 would lead to a reduction in muscular
68 architectural damage, early inflammation, atrophy and fatty infiltration subsequent to
69 combined tenotomy and neurectomy in an established PARP-1 knock-out mouse model ^{19; 25}.

70 The aim of this study was to investigate the role of PARP-1 in regulating the potentially
71 irreversible structural alterations after RCT utilizing macroscopic, histological, molecular, and
72 radiological techniques.

73

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74 **Methods**

75 **Animals**

76 This investigation gained approval from the federal ethics committee (No. 98/2013). PARP-1
77 knockout mice were originally obtained from Zhao-Qi Wang, PhD (Jena, Germany) and have
78 been crossed back into the C57BL/6 background. These C57BL/6 mice have a PARP-1 gene
79 fragment replaced by the neomycin resistance gene in between the second exon and intron
80 (PARP-1 KO). The wild type (WT) C57BL/6JOLA^{Hsd} mice were obtained from Harlan
81 Laboratories (Netherlands). The animals were housed in a specific pathogen free facility
82 under standard enriched housing conditions. Only female mice between the ages of 6-8 weeks
83 at the time of surgery were included in the study.

84 **Study design (Fig. 1)**

85 A total of 42 PARP-1 KO and 42 WT mice were included in the study. These mice underwent
86 combined tenotomy and neurectomy of the supraspinatus (SSP) and infraspinatus (ISP)
87 muscles. In both groups the animals were randomly assigned to three time points. The 1 week
88 and 6 weeks time points included 12 animals each. These mice were then subdivided for
89 either histological (Histology group) or gene expression (PCR group) analysis (n=6 each).
90 The 12 weeks time point contained 18 animals in each of the PARP-1 KO and WT groups.
91 These were then further subdivided for histological (Histology group), gene expression (PCR
92 group) or MRI (MRI group) analysis (n=6 each).

93 **Surgery**

94 Tenotomy and denervation of the SSP and ISP was performed according to published
95 protocols by Liu et al.²⁵ and Kim et al.¹⁹. Surgery was carried out on the left shoulder and the
96 contralateral shoulder served as an uninjured control. Anesthesia was induced with

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97 intraperitoneal administration of Ketamine 30mg/kg BW and maintained with inhaled
98 Isoflurane. Intraoperative pain was controlled with subcutaneous injections of Buprenorphine
99 01.mg/kg BW when indicated. The surgical site underwent sterile preparation and draping
100 with chlorhexidine. All procedures were performed under a surgical microscope using
101 microsurgical instruments. A 2 cm long skin incision was made over the shoulder joint and
102 the deltoid muscle split parallel to its fibers to expose the underlying rotator cuff insertion.
103 The deltoid was retracted with a forceps and the tendons of the SSP and ISP sharply detached
104 from the humeral head. The trapezius was then split along its fibers over the lateral scapular
105 spine. The SSP muscle was bluntly elevated to reach the suprascapular notch. The
106 suprascapular nerve was identified and a 2 mm segment was resected from a point where it
107 enters the notch to a point beyond its division into supraspinatus and infraspinatus branches.
108 The muscular split in the trapezius and deltoid muscles were then repaired with 10-0 Etibond
109 sutures (Ethicon, USA). The skin incision was closed using staples. The animals were allowed
110 free cage activity with food and water ad libidum post surgery. Postoperative pain was
111 controlled with subcutaneous injections of Buprenorphine 01.mg/kg BW in the first day after
112 surgery followed by Buprenorphine 1ml/50ml H₂O in the drinking water for 3 days.

113 Sacrifice and Sampling

114 At the specified time points post intervention, the mice in the histology group were
115 euthanized with cervical dislocation under anesthesia followed by harvest of the entire upper
116 extremity of both shoulders with the rotator cuff muscles intact. These samples were
117 immediately fixed in 4% Formalin. The animals in the PCR group underwent further
118 Ketamine 30mg/kg BW induction and anesthesia with Isoflurane. The SSP and ISP muscles
119 from both shoulders were carefully dissected and elevated from the scapula and immediately
120 stored in RNAlater (Quiagen) at -20°C for further analysis. After the muscles were harvested
121 these animals were euthanized with cervical dislocation whilst anaesthetized.

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122 A pilot study with 6 WT mice showed severe retraction of the tendon stump in all 6 animals
123 marked with non-absorbable sutures, macroscopic atrophy and fatty infiltration of the muscles
124 12 weeks after surgery (data not shown).

125 Histology

126 For both immunohistochemistry (IHC) and conventional histological analysis the harvested
127 SSP and ISP muscles were fixed in 4% Formalin overnight, washed with deionised water and
128 stored in 70% Ethanol until paraffin embedding. Once embedded in paraffin, they were
129 sectioned, deparaffinized, rehydrated in xylene and ethanol and then incubated with specific
130 antibodies. For routine histology, H&E and Picrosirius Red staining was performed as per
131 institutional standard operating procedure. The slides were digitalized with a NanoZoomer
132 2.0-HT Digital slide scanner C9600 (Hamatsu, Japan) in various magnifications to allow
133 further digital processing and analysis.

134
135 To visualize intramuscular fat deposition, the midportions of SSP cross-sections were stained
136 with a rabbit anti-mouse antibody against Fabp4 (HPA002188, Sigma-Aldrich, USA). Fatty
137 infiltration, measured by the deposition of adipocytes between the muscle fiber bundles
138 (perimysial) or within the muscle bundles due to replacement of muscle fibers (endomysial),
139 was graded from 0 to 5 (0= no intramuscular fat except around the main vessel; 1= Single
140 intramuscular fat cells or fat cells that penetrate from the vessel into the muscle; 2= Streaks of
141 fat cells into the muscle; 3=Fatty streaks in 2 of 4 quadrants of the muscle; 4=fat cells in all
142 quadrants; 5=severe fatty infiltration). This cell surface marker does not differentiate between
143 the two localities.

144
145 The pennation angle was measured three times at different locations and the mean of these
146 measurements used for comparison in the longitudinal sections of the ISP muscle in the

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147 Picrosirius Red stained sections at 20x magnification. The H&E sections underwent semi
148 quantitative analysis. The cross section of the SSP muscle was divided into four quadrants
149 and four images at 20x magnification were taken from each quadrant and analyzed for the
150 frequency of inflammatory cell infiltrate, degenerative cells (hypereosinophilic staining, cell
151 swelling, fragmentation, presence of retraction caps), regenerative cells (rows of myoblast
152 nuclei, cytoplasmic basophilia, internal nuclei), muscular atrophy (rounded to angular cells,
153 hypereosinophilic sarcoplasm, crowded nuclei), fibrosis and fat deposition by a veterinary
154 pathologist who was blinded to the sample group.

155 Gene expression

156 The entire SSP samples for Real Time qPCR (RTqPCR) were stored in RNAlater at -20°C
157 until RNA extraction. The TrizolPlus Kit (Life Technologies) was utilized for RNA
158 extraction. The samples were homogenized in 1ml Trizol per 100mg tissue using a MixerMill
159 (Qiagen). After homogenization RNA was isolated by phase separation with 0.2ml
160 chloroform and incubation. The upper phase, containing the RNA, was then transferred to a
161 new tube and one volume 70% Ethanol was added. The solution was then transferred to the
162 Spin Cartridges for binding and washing as per standard manufacturers protocol, which
163 included DNase digestion. The purified RNA was then eluted in 30µl RNase free water. The
164 relative amount of RNA was measured with a NanoDrop spectrophotometer (Thermo
165 Scientific) and equal amounts of RNA were then reverse transcribed to cDNA with a RNA-to-
166 cDNA Kit (Life Technologies) as per standard manufacturer's protocol. RTqPCR was
167 performed on a 7500 Fast Real-Time PCR system (Applied Biosystems, USA) using TaqMan
168 probes with Fast Advanced Mastermix for the expression of inflammatory (NF-κB, IL1-β,
169 TNFα, IL-6), apoptotic (Caspase3, AIF), atrophic (FOXO1, MuRF, Atrogin1, Ube2b,
170 Ube3a), regenerative (AKT, MyoD₁, Myf-5), fibrotic (TGFβ₁ and MSTN) and fatty
171 infiltration (PPARγ, Fabp4) genes. GADPH serves as the housekeeping gene and relative

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172 levels of gene expression are measured with the $\Delta\Delta\text{Ct}$ method relative to the contralateral
173 uninjured side.

174 MRI

175 We acquired T1 weighted images using a RARE sequence (Rapid Acquisition with
176 Relaxation Enhancement) for the anatomic depiction. For the fat quantification in-phase and
177 out-of-phase sequences were performed³³. The sequences included the following scanning
178 parameters: In-phase (flip angle: 50°; echo time: 2.9 ms; repetition time: 200 ms), Out-of-
179 phase (flip angle: 50°; echo time: 2.2 ms; repetition time 200 ms) and RARE T1 (flip angle:
180 180°; echo_time: 10 ms; repetition time: 1000 ms). All data was acquired on a 4.7-T
181 PharmaScan (Bruker Corporation, Billerica, MA, USA). A linear polarized hydrogen whole-
182 body mouse radiofrequency coil was used. The mice were laid head first and in prone position
183 on an animal bed. We fitted the bed with a pad with continuous flow of warm water in order
184 to avoid cooling of the animals. The animals were anesthetized during the acquisition with
185 isoflurane (Attane, Minrad I, Buffalo, NY) and ophthalmic ointment (Vitamin A Crème,
186 Bausch & Lomb, Steinhausen, Switzerland) was applied to protect the mice from dry eyes.
187 With the acquired data a region of interest (ROI) analysis was done using in house Matlab
188 routines (The MathWorks, Natick, MA) for the fat quantification.

189 Statistics

190 Statistical analysis included analysis of variance (ANOVA) and post Hoc tests to reveal
191 differences between the subgroups with the Fisher's LSD test or Mann-Whitney test for non-
192 parametric measurements. Linear correlation was measured with the Pearson product-moment
193 correlation coefficient. The level of significance was set to $p < 0.05$. Data is reported as the
194 mean \pm standard error of the mean (SEM).

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195 **Results**

196 All animals survived the surgical procedure with no postoperative complications. All mice
197 used their operated left forelimb less than the contralateral side, and the expected gait
198 abnormality secondary to diminished use of the affected limb continued until euthanasia.
199 There was no evidence of any adverse effects (e.g. developmental or reproductive
200 abnormalities) on examination of the PARP-1 KO mice⁴³.

201 **Macroscopic Analysis**

202 All mice in both groups showed retraction of the tendon and muscle of the SSP and ISP at 1
203 week, with further retraction evident at 6 weeks post combined tenotomy and neurectomy.
204 The retraction and atrophy remained unchanged in the WT group at 12 weeks post surgery.
205 In contrast, the PARP-1 KO mice had less retraction and almost normal muscle volume at the
206 12 weeks time point. Sample images are shown in **Fig. 2:A**.

207
208 Retraction was quantified on MRI scans (**Fig. 2B** and **Fig. 2D**) at the 12 week time point.
209 Both tendon and muscle retraction was significantly lower in the PARP-1 KO mice compared
210 to the WT mice ($p = 0.012$ and $p = 0.081$ respectively, **Fig. 2D** and Table 1). The correlation
211 between muscle and tendon retraction reached statistical significance. (PARP-1 KO: $r = 0.91$,
212 $p = 0.001$; WT: $r = 0.98$, $p = 0.0001$).

213
214 The wet weight of the SSP muscle decreased significantly in both the PARP-1 KO and in the
215 WT mice (relative decrease compared to uninjured contralateral side in **Fig. 2C** and effective
216 weight in Table 1) in the first 6 weeks post combined tenotomy and neurectomy compared to
217 the uninjured contralateral side. At 12 weeks post surgery the wet weight of the SSP in PARP-
218 1 KO mice was almost normal in comparison to the contralateral side whilst it remained
219 significantly lower in the WT mice (difference $p < 0.0001$, **Fig. 2C** and Table 1).

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220 **Histology**

221 In comparison to the uninjured contralateral side of all animals (Control: $23.9 \pm 0.9^\circ$; **Fig. 3E**
222 and Table 2) there was a statistically significant increase in pennation angle in the WT mice
223 (1 week: $31.1 \pm 2.4^\circ$; $p = 0.016$, 6 weeks: $36.1 \pm 4.9^\circ$; $p = 0.0002$, 12 weeks: $34.4 \pm 5.9^\circ$; $p =$
224 0.0014 respectively). Conversely, after an initial increase in the pennation angle in the PARP-
225 1 KO mice it remained unchanged at the 6 and 12 weeks time points and did not reach
226 statistical significance when compared to the controls (1 week: $30.0 \pm 3.5^\circ$; $p = 0.088$, 6
227 weeks: $28.1 \pm 4.9^\circ$; $p = 0.155$, 12 weeks: $28.5 \pm 3.9^\circ$; $p = 0.103$ respectively). There was a
228 statistically significant correlation between the pennation angle, and the tendon and muscle
229 retraction measurements in the PARP-1 KO mice ($r = 0.93$, $p = 0.008$ and $r = 0.9$, $p = 0.014$
230 respectively) but not in the WT mice ($r = -0.38$, $p = 0.517$ and $r = -0.36$, $p = 0.546$
231 respectively).

232

233 H&E staining of the SSP cross sections showed a higher inflammatory cell infiltrate at 1 week
234 post injury in the WT mice (**Fig. 3A**). This was followed by an increase in degenerative
235 changes in both groups, with muscle fibers undergoing degradation and atrophy at 6 weeks.
236 PARP-1 KO mice had a higher number of regenerating fibers at this time point. After 12
237 weeks almost no degenerative changes were observed in either group. Muscles of the PARP-1
238 KO group had less fibrosis and better muscle architecture compared to the WT group (**Fig.**
239 **3D**).

240 **Fatty infiltration**

241 Both groups had no fatty infiltration at 1 week (data not shown). Fatty infiltration was present
242 in both groups at 6 weeks with an average grade of 2.7 ± 0.49 in the PARP-1 KO mice and
243 2.3 ± 0.49 in the WT mice (difference: $p = 0.818$ **Fig. 3B** and C). This almost significantly
244 decreased in the PARP-1 KO mice to 1.4 ± 0.25 at 12 weeks post surgery ($p = 0.082$), which

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245 was significantly lower than in the WT mice (2.8 ± 0.37 ; difference $p = 0.032$). Intramuscular
246 fat was also quantified in the In-Phase and Opposed-Phase of the MR scans. The relative
247 amount of intramuscular fat was significantly lower in the PARP-1 KO group ($12.5 \pm 1.82\%$)
248 compared to the WT group ($19.6 \pm 1.96\%$; difference $p = 0.027$).

249 Gene Expression Analysis

250 Gene expression analysis of various *inflammatory* genes revealed that TNF α mRNA was
251 upregulated at 1 and 12 weeks after injury in both PARP-1 KO and WT mice without
252 reaching statistical significance ($p = 0.775$ and $p = 0.390$ respectively, **Fig 4A**). IL1- β
253 expression was upregulated at 1 and 6 weeks post surgery in the WT group without reaching
254 statistical significance when compared to the PARP-1 KO mice (1 week: $p = 0.197$, 6 weeks:
255 $p = 0.110$). There was a significant upregulation of NF- κ B and the *proapoptotic* factor AIF at
256 the 1-week time point in the WT group ($p < 0.0001$ and $p = 0.005$ respectively). The mRNA
257 of the *proliferative factors* TGF β_1 and MSTN were also significantly upregulated in the WT
258 group at 1 week ($p < 0.0001$ and $p = 0.0038$ respectively, **Fig. 4B**). The *muscle atrophy*
259 related Ubiquitin ligases MuRF1 and Atrogin-1 were present at significantly ($p = 0.048$ and p
260 $= 0.0018$ respectively) higher levels in the WT group consistent with the higher levels of
261 Ubiquitin ligase Ube3a mRNA at the 1-week time point ($p < 0.0001$, **Fig. 4C**). The mRNA
262 level of regulatory protein FOXO1 was also significantly upregulated in the WT mice at 6
263 weeks ($p = 0.013$, **Fig. 4C**). The main regulator of *muscle regeneration* AKT was equally
264 upregulated in the PARP-1 KO and WT group at 1 and 12 weeks ($p = 0.447$ and $p = 0.990$
265 respectively, **Fig. 4D**). Both MyoD and Myf-5 mRNA was upregulated at week 1 and week 6
266 post surgery in both groups. The upregulation of both factors was significantly higher at week
267 1 in the WT group compared to the PARP-1 KO group ($p = 0.0053$ and $p = 0.012$
268 respectively, **Fig. 4D**). The mRNA levels of genes regulating *fatty infiltration* were
269 significantly upregulated at 6 weeks in the WT group (PPAR γ : $p = 0.012$ and Fabp4: $p =$

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270 0.0124

Fig.

4E).

271 Discussion

272 Disturbed muscular architecture, complete atrophy and fatty infiltration remain irreversible in
273 chronic rotator cuff tears even after repair. The complex interplay of molecular and cellular
274 mechanisms, which leads to potentially irreversible structural alterations in skeletal muscle
275 have been described ²². Poly (ADP-ribose) polymerase-1 (PARP-1), also known as ADP-
276 ribosyl-transferase (ARTD1), is a key transcription factor involved in the maintenance of
277 cellular homeostasis ²¹. PARP-1 has shown to be a key regulator of inflammation, apoptosis,
278 muscle atrophy, muscle regeneration and adipocyte development ^{7; 14; 40}. Our study is the first
279 to show that the absence of PARP-1 leads to a reduction in muscular architectural damage in
280 the mice' supraspinatus and infraspinatus muscle. PARP-1 may be the upstream regulator that
281 orchestrates the molecular and cellular mechanisms that leads to these potentially irreversible
282 structural alterations after RCT.

283

284 Macroscopic analysis showed different degrees of tendon and muscle retraction in both WT
285 and PARP-1 KO mice at 1 and 6 weeks post combined tenotomy and neurectomy. After 12
286 weeks retraction of the tendon and muscle was significantly lower in the PARP-1 KO mice
287 compared to the WT mice measured in MRI scans. In a 2006 sheep study, Meyer et al. also
288 showed that the tendon retracts more than muscle in experimental chronic tears of the rotator
289 cuff. This results in an apparently shortened tendon ³¹. In our study, despite the degree of fatty
290 infiltration being less than 50% of the muscle volume (< Goutallier stage 3) in all animals the
291 degree of tendon retraction was consistently much greater than muscle retraction.

292

293 Liu et al. observed significant and consistent muscle atrophy after rotator cuff tendon
294 transection in a mouse model ²⁵. Furthermore they found that denervation significantly
295 increased the amount of muscle atrophy after a rotator cuff tear in a mouse model ²⁵. Muscle
296 atrophy persisted in the WT group in our study whilst the PARP-1 KO mice had almost

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297 normal muscle volume at the 12 weeks time point. This occurrence was further supported by
298 near normalization of the wet weight of the SSP in PARP-1 KO mice, whilst it remained low
299 in the WT mice after the initial decrease in both groups. Only after continuous elongation and
300 subsequent refixation do retracted, fatty infiltrated and atrophied rotator cuff muscles in
301 sheep, achieve partial reversal of muscle atrophy but not fatty infiltration ¹⁰.

302

303 Meyer et al. ³⁰ described the pathomechanical concept of the pennation angle to explain
304 muscle loss and fatty infiltration following RCT. Geometric modeling showed that the
305 increase of the pennation angle separates the muscle fiber bundles mechanically like limbs of
306 a parallelogram. Infiltrating fat cells fill the created space between the reoriented muscle
307 fibers, which may be quantitatively calculated without affecting the structural properties of
308 the muscle cells. Our histological data was consistent with the macroscopic findings. Both
309 groups in our study demonstrated an increase in the pennation angle at 1 week following
310 combined tenotomy and neurectomy. There was a further increase in the pennation angle in
311 the WT mice at 6 weeks and it remained high at 12 weeks. Whilst in the PARP-1 KO mice the
312 pennation angle remained unchanged at 6 and 12 weeks. In contrast to the WT group, the
313 increase in pennation angle in the PARP-1 KO mice did not reach statistical significance
314 when compared to the controls at any time point.

315

316 Fatty infiltration was present in both groups at 6 weeks. The infiltration decreased in the
317 PARP-1 KO mice to 1.4 ± 0.25 at 12 weeks post surgery, which was significantly lower than
318 in the WT mice where the grading conversely increased to 2.8 ± 0.37 from the 6 week time
319 point. The MRI measurement of relative intramuscular fat was also significantly lower in the
320 PARP-1 KO group at 12 weeks. Gerber et al. demonstrated an arrest of fatty infiltration after
321 continuous elongation and refixation in a sheep model ¹⁰. In a sheep study, neither an anabolic
322 steroid nor IGF contributes to regeneration of the muscle once degenerative changes are

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323 established. The findings demonstrated that muscle cells lose reactivity to an anabolic
324 steroid and IGF once retraction has led to fatty infiltration and atrophy of the muscle ¹¹
325 Treatment of mice with Tamoxifen , a competitive estrogen receptor inhibitor, has shown to
326 cause less atrophy and inflammation after RCT but fatty infiltration remained unchanged ⁴. To
327 date there is only one other study in the literature that has demonstrated reversal of fatty
328 infiltration; through local administration of adipose-derived stem cells (ADSCs) into repaired
329 rabbit SSC muscle, Oh et al. demonstrated improvement in fatty infiltration and tendon
330 healing ³⁴. As we have significantly less fatty infiltration and atrophy at 12 weeks in the
331 PARP-1 KO group, one may speculate that outcome post fixation of the RCT in this group
332 may have an improved surgical outcome.

333

334 Results of the gene expression analysis further support the hypothesis that PARP-1 may be an
335 instrumental upstream regulator that orchestrates potentially irreversible structural alterations
336 after RCT. Regeneration and degeneration are in harmony during normal muscle homeostasis.
337 RCT incite an inflammatory response that begins with inflammatory cell infiltration and
338 subsequent release of proinflammatory cytokines ³². Intramuscular macrophages release
339 TNF α and IL1- β and thereby stimulate the up-regulation of NF- κ B. NF- κ B has an integral
340 role in influencing muscle degeneration ^{23; 42}; (1) it co-regulates the expression of
341 inflammatory and proapoptotic cytokines that cause muscle damage, (2) promotes muscular
342 atrophy and degradation directly via activation of MuRF1 or indirectly via up-regulation of
343 other cytokines and (3) it inhibits myogenic differentiation and regeneration. PARP-1 has
344 been shown to be an important co-factor for NF- κ B dependent transcription of various genes
345 ¹⁴ and the disturbance of this interaction leads to a lower inflammatory reaction to injury ¹⁴.
346 Studies have shown that inactivation or deletion of PARP-1 protects tissues from damage
347 (review in Kraus and Hottiger, 2013) ²¹.

348

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349 Our results show an inflammatory response at 1 week post combined tenotomy and
350 neurectomy of the SSP and ISP muscles in the PARP-1 KO and the WT mice. TNF α and IL-
351 1 β are extracellular inflammatory cytokines that induce intracellular inflammatory cascades.
352 They were upregulated in both groups but only lead to a significant upregulation of NF- κ B in
353 the muscles of WT mice. This may be explained by a dampened inflammatory response and
354 subsequent reduction in proinflammatory cytokine expression in the muscles of PARP-1 KO
355 mice¹³. Only the WT mice had significantly higher levels of the pro-apoptotic AIF, which is
356 activated by PARP-1 and promotes caspase independent apoptosis. AIF translocates into the
357 nucleus where it triggers apoptosis⁴⁴. The increase in pro-apoptotic gene expression suggests
358 higher apoptosis rates in WT mice leading to a more pronounced cell death in this group.

359

360 Unloading or denervation of the musculotendinous unit initiates complex pathways that
361 eventually result in muscle ubiquitination and degradation⁴¹. Ubiquitination requires ligase's
362 to form complexes with Ube3a (Ubiquitin-protein ligase E3A) that allows recognition and
363 proteasome mediated degradation of muscle fibers⁴¹. The most important of these ligase's are
364 MuRF1 (muscle RING finger 1) and Atrogin-1 (FBX032)³. Their transcription is upregulated
365 by inflammatory, profibrotic, proadipogenic and the forkhead box 0 (FOXO) transcription
366 factors^{23; 29; 36; 38}. The key elements involved in the process of ubiquitination and muscle
367 degradation, Ube3a, MuRF1 and Atrogin-1, were all significantly upregulated in the WT mice
368 at 1 week post combined tenotomy and neurectomy.

369

370 During muscle regeneration satellite cells and mesenchymal stem cells (MSC) are activated
371 and undergo proliferation and differentiation (Review in⁴⁵). This process is orchestrated by
372 the myogenic regulatory factors (MRF), such as MyoD and Myf-5, which are activated
373 through the AKT/mTOR pathway⁴⁵. Additionally it has been shown that NF- κ B has a direct
374 inhibitory effect on muscular regeneration by inhibiting the MRF's, specifically MyoD¹³.

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375 This inhibition of myogenic differentiation and regeneration is also a major effect of NF- κ B
376 in muscle degeneration. We interpret the significant up regulation of MyoD in WT mice after
377 1 week as a failed attempt of the muscle to induce regeneration through stimulation of
378 satellite cells and MSCs. Meanwhile in the absence of PARP-1 in the knock-out group, NF-
379 κ B is not effective in inhibiting MyoD and less muscle fibers were damaged during the initial
380 inflammatory response. Upregulation of MyoD, like in the WT group, is not needed and low
381 levels of MyoD may be sufficient for regeneration of the muscle fibers leading to a
382 normalization of the muscle weight after 12 weeks.

383

384 Both factors, TGF β ₁ and Myostatin, were significantly upregulated in the WT group at 1
385 week. The inflammatory cell infiltrate triggers the release of TGF β ₁ and Myostatin from the
386 fibroblasts in the ECM^{18; 24}. Both factors belong to the Transforming Growth factor
387 superfamily²⁰. Members of this TGF superfamily have been shown to induce fibrosis and
388 regulate muscle mass²⁸. Specifically Myostatin inhibits myogenic differentiation by
389 downregulating the expression of MyoD and Myogenin³⁹. PARP-1 modulates TGF- β ₁
390 activity via negative and positive feedback mechanisms allowing fine-tuning of these
391 pathways^{6; 26}. Our data suggests that the significant early activation of TGF- β ₁ transcription in
392 WT mice directs the balance towards fibrosis and degeneration.

393

394 Our study showed fatty infiltration in both mice groups at 6 weeks but significantly less fatty
395 infiltration in PARP-1 KO mice after 12 weeks. Both proadipogenic factors (Peroxisome
396 proliferator-activated receptor- γ = PPAR γ and Fatty Acid Binding Protein = FABP4) revealed
397 a significantly higher expression in the WT mice compared to PARP-1 KO group at 6 weeks
398 post-injury. These proadipogenic genes are key factors in fat accumulation in between free
399 inter- and intramyofibrillar spaces and also decrease the expression of MRF¹⁶. In addition
400 Myostatin and TGF β reduce the expression of the proadipogenic factors¹². This may be the

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401 reason why PPAR γ is only upregulated at 6 weeks - after the inhibitory effect of Myostatin
402 and TGF β has dissipated. Furthermore, absence of PARP-1 directly inhibits the function of
403 PPAR γ ^{7:17} and is a crucial regulator of adipogenic differentiation⁸.

404

405 There are limitations in this study. It could be suggested the differences observed in our study
406 were due to reinnervation. This is not plausible for three reasons. Firstly, a 2 mm length of the
407 nerve was transected from the main branch at its entrance into the scapular notch extending
408 beyond its branches to the SSP and ISP in both the WT and PARP-1 KO mice. Secondly, if the
409 nerves were to reinnervate by chance, then we would expect more outliers in our data - all of
410 our data, including muscle weight measurements, demonstrate no outliers with a narrow
411 standard deviation. Thirdly, why should the reinnervation phenomena be confined to the
412 PARP-1 KO group only and not occur in the WT group? Another possible criticism could be
413 that we analyzed gene expression and not effective protein levels and their activity. This does
414 limit our ability fully interpret the molecular mechanisms at play. We surgically transected the
415 tendons of the SSP and ISP from its origin at the humeral head. This may not accurately
416 mimic degenerative RCT seen in the human population, but to our knowledge there are no
417 degenerative RCT mouse models. There are other animal models of chronic rotator cuff tears,
418 but this would not allow us to use the PARP-1 knockout model. This study relies on gene
419 expression analysis and does not investigate the exact interactions between PARP-1 and the
420 described proteins on a molecular level. Further molecular biological methods would be
421 needed to describe these mechanisms. The first time point of 1 week may be perceived as a bit
422 delayed to assess inflammation, but we were still able to observe significant differences
423 between the PARP-1-KO and WT mice in all the various modes of analyses.

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424 Conclusion

425 Our study is the first to show that the absence of PARP-1 leads to a reduction in muscular
426 architectural damage, early inflammation, apoptosis, atrophy and fatty infiltration after
427 combined tenotomy and neurectomy of the rotator cuff muscle. PARP-1 is one of the
428 upstream regulators that orchestrates the molecular and cellular mechanisms that leads to
429 potentially irreversible structural alterations after RCT. It plays an important role in
430 modulating the muscles reaction to RCT by promoting the immediate inflammatory response.
431 This inflammatory response leads to apoptosis and damage to the muscle fibers and initiates
432 muscular degeneration and atrophy. Architectural changes and loss of myocytes hinders the
433 muscles ability to regenerate and ultimately leads to fatty infiltration. In the absence of
434 PARP-1, the initial inflammatory response is dampened leading to less myocyte degeneration.
435 Although the macroscopic muscles reaction to injury is similar in the first 6 weeks, its ability
436 to regenerate is much greater in the PARP-1 KO group leading to a near normalization of the
437 muscle substance and muscle weight, less retraction, and less fatty infiltration after 12 weeks.
438 We conclude that PARP1 is a molecular regulator of muscular deterioration after RCT.

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439 **References**

- 440 1. Arnold L, Henry A, Poron F, Baba-Amer Y, van Rooijen N, Plonquet A et al.
441 Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory
442 macrophages to support myogenesis. *The Journal of experimental medicine* 2007; 204:1057-
443 69. 10.1084/jem.20070075.
- 444 2. Barry JJ, Lansdown DA, Cheung S, Feeley BT, Ma CB. The relationship between tear
445 severity, fatty infiltration, and muscle atrophy in the supraspinatus. *J Shoulder Elbow Surg*
446 2013; 22:18-25. 10.1016/j.jse.2011.12.014.
- 447 3. Bodine SC, Latres E, Baumhueter S, Lai VK, Nunez L, Clarke BA et al. Identification of
448 ubiquitin ligases required for skeletal muscle atrophy. *Science* 2001; 294:1704-8.
449 10.1126/science.1065874.
- 450 4. Cho E, Zhang Y, Pruznak A, Kim HM. Effect of tamoxifen on fatty degeneration and
451 atrophy of rotator cuff muscles in chronic rotator cuff tear: An animal model study. *J Orthop*
452 *Res* 2015; 33:1846-53. 10.1002/jor.22964.
- 453 5. Choo A, McCarthy M, Pichika R, Sato EJ, Lieber RL, Schenk S et al. Muscle gene
454 expression patterns in human rotator cuff pathology. *The Journal of bone and joint surgery.*
455 *American volume* 2014; 96:1558-65. 10.2106/JBJS.M.01585.
- 456 6. Dahl M, Maturi V, Lonn P, Papoutsoglou P, Zieba A, Vanlandewijck M et al. Fine-tuning
457 of Smad protein function by poly(ADP-ribose) polymerases and poly(ADP-ribose)
458 glycohydrolase during transforming growth factor beta signaling. *PLoS One* 2014; 9:e103651.
459 10.1371/journal.pone.0103651.
- 460 7. Erener S, Hesse M, Kostadinova R, Hottiger MO. Poly(ADP-ribose)polymerase-1
461 (PARP1) controls adipogenic gene expression and adipocyte function. *Mol Endocrinol* 2012;
462 26:79-86. me.2011-1163 [pii] 10.1210/me.2011-1163.

PARP-1 Regulates Muscular Deterioration After RCT

- 463 8. Erener S, Mirsaidi A, Hesse M, Tiaden AN, Ellingsgaard H, Kostadinova R et al. ARTD1
464 deletion causes increased hepatic lipid accumulation in mice fed a high-fat diet and impairs
465 adipocyte function and differentiation. *FASEB J* 2012; 26:2631-38. fj.11-200212 [pii]
466 10.1096/fj.11-200212.
- 467 9. Frey E, Regenfelder F, Sussmann P, Zumstein M, Gerber C, Born W et al. Adipogenic and
468 myogenic gene expression in rotator cuff muscle of the sheep after tendon tear. *J Orthop Res*
469 2009; 27:504-09. 10.1002/jor.20695.
- 470 10. Gerber C, Meyer D, Frey E, von Rechenberg B, Hoppeler H, Frigg R et al. Neer Award
471 2007: Reversion of structural muscle changes caused by chronic rotator cuff tears using
472 continuous musculotendinous traction. An experimental study in sheep. *J. Shoulder Elbow*
473 *Surg.* 2009; 18:163-71. 10.1016/j.jse.2008.09.003.
- 474 11. Gerber C, Meyer DC, Von Rechenberg B, Hoppeler H, Frigg R, Farshad M. Rotator cuff
475 muscles lose responsiveness to anabolic steroids after tendon tear and musculotendinous
476 retraction: an experimental study in sheep. *Am J Sports Med* 2012; 40:2454-61.
477 10.1177/0363546512460646.
- 478 12. Guo W, Flanagan J, Jasuja R, Kirkland J, Jiang L, Bhasin S. The effects of myostatin on
479 adipogenic differentiation of human bone marrow-derived mesenchymal stem cells are
480 mediated through cross-communication between Smad3 and Wnt/beta-catenin signaling
481 pathways. *J Biol Chem* 2008; 283:9136-45. 10.1074/jbc.M708968200.
- 482 13. Guttridge DC, Mayo MW, Madrid LV, Wang CY, Baldwin AS, Jr. NF-kappaB-induced
483 loss of MyoD messenger RNA: possible role in muscle decay and cachexia. *Science* 2000;
484 289:2363-6.
- 485 14. Hassa PO, Hottiger MO. A role of poly (ADP-ribose) polymerase in NF-kappaB
486 transcriptional activation. *Biol Chem* 1999; 380:953-59. 10.1515/bc.1999.118.

PARP-1 Regulates Muscular Deterioration After RCT

- 487 15. Hong SJ, Dawson TM, Dawson VL. Nuclear and mitochondrial conversations in cell
488 death: PARP-1 and AIF signaling. *Trends in pharmacological sciences* 2004; 25:259-64.
489 10.1016/j.tips.2004.03.005.
- 490 16. Hu E, Tontonoz P, Spiegelman BM. Transdifferentiation of myoblasts by the adipogenic
491 transcription factors PPAR gamma and C/EBP alpha. *Proc Natl Acad Sci U S A* 1995;
492 92:9856-60.
- 493 17. Huang D, Wang Y, Wang L, Zhang F, Deng S, Wang R et al. Poly(ADP-ribose)
494 polymerase 1 is indispensable for transforming growth factor-beta Induced Smad3 activation
495 in vascular smooth muscle cell. *PLoS One* 2011; 6:e27123. 10.1371/journal.pone.0027123.
- 496 18. Killian ML, Lim CT, Thomopoulos S, Charlton N, Kim HM, Galatz LM. The effect of
497 unloading on gene expression of healthy and injured rotator cuffs. *Journal of orthopaedic*
498 *research : official publication of the Orthopaedic Research Society* 2013; 31:1240-8.
499 10.1002/jor.22345.
- 500 19. Kim HM, Galatz LM, Lim C, Havlioglu N, Thomopoulos S. The effect of tear size and
501 nerve injury on rotator cuff muscle fatty degeneration in a rodent animal model. *J Shoulder*
502 *Elbow Surg* 2012; 21:847-58. 10.1016/j.jse.2011.05.004.
- 503 20. Kollias HD, McDermott JC. Transforming growth factor-beta and myostatin signaling in
504 skeletal muscle. *Journal of applied physiology* 2008; 104:579-87.
505 10.1152/jappphysiol.01091.2007.
- 506 21. Kraus WL, Hottiger MO. PARP-1 and gene regulation: progress and puzzles. *Molecular*
507 *aspects of medicine* 2013; 34:1109-23. 10.1016/j.mam.2013.01.005.
- 508 22. Laron D, Samagh SP, Liu X, Kim HT, Feeley BT. Muscle degeneration in rotator cuff
509 tears. *J Shoulder Elbow Surg* 2012; 21:164-74. 10.1016/j.jse.2011.09.027.
- 510 23. Li H, Malhotra S, Kumar A. Nuclear factor-kappa B signaling in skeletal muscle atrophy.
511 *Journal of molecular medicine (Berlin, Germany)* 2008; 86:1113-26. 10.1007/s00109-008-
512 0373-8.

PARP-1 Regulates Muscular Deterioration After RCT

- 513 24. Liu X, Joshi SK, Ravishankar B, Laron D, Kim HT, Feeley BT. Upregulation of
514 transforming growth factor-beta signaling in a rat model of rotator cuff tears. *J Shoulder*
515 *Elbow Surg* 2014; 23:1709-16. 10.1016/j.jse.2014.02.029.
- 516 25. Liu X, Laron D, Natsuhara K, Manzano G, Kim HT, Feeley BT. A mouse model of
517 massive rotator cuff tears. *The Journal of bone and joint surgery. American volume* 2012;
518 94:e41. 10.2106/JBJS.K.00620.
- 519 26. Lonn P, van der Heide LP, Dahl M, Hellman U, Heldin CH, Moustakas A. PARP-1
520 attenuates Smad-mediated transcription. *Mol Cell* 2010; 40:521-32.
521 10.1016/j.molcel.2010.10.029.
- 522 27. Lundgreen K, Lian OB, Engebretsen L, Scott A. Tenocyte apoptosis in the torn rotator
523 cuff: a primary or secondary pathological event? *British journal of sports medicine* 2011;
524 45:1035-9. 10.1136/bjism.2010.083188.
- 525 28. McPherron AC, Lawler AM, Lee SJ. Regulation of skeletal muscle mass in mice by a new
526 TGF-beta superfamily member. *Nature* 1997; 387:83-90. 10.1038/387083a0.
- 527 29. Mendias CL, Gumucio JP, Davis ME, Bromley CW, Davis CS, Brooks SV. Transforming
528 growth factor-beta induces skeletal muscle atrophy and fibrosis through the induction of
529 atrogin-1 and scleraxis. *Muscle Nerve* 2012; 45:55-9. 10.1002/mus.22232.
- 530 30. Meyer DC, Hoppeler H, von Rechenberg B, Gerber C. A pathomechanical concept
531 explains muscle loss and fatty muscular changes following surgical tendon release. *J Orthop*
532 *Res* 2004; 22:1004-7. 10.1016/j.orthres.2004.02.009.
- 533 31. Meyer DC, Lajtai G, von Rechenberg B, Pfirrmann CW, Gerber C. Tendon retracts more
534 than muscle in experimental chronic tears of the rotator cuff. *J Bone Joint Surg Br* 2006;
535 88:1533-8. 10.1302/0301-620x.88b11.17791.
- 536 32. Millar NL, Hueber AJ, Reilly JH, Xu Y, Fazzi UG, Murrell GA et al. Inflammation is
537 present in early human tendinopathy. *Am J Sports Med* 2010; 38:2085-91.
538 0363546510372613 [pii] 10.1177/0363546510372613.

PARP-1 Regulates Muscular Deterioration After RCT

- 539 33. Nozaki T, Tasaki A, Horiuchi S, Osakabe C, Ohde S, Saida Y et al. Quantification of
540 Fatty Degeneration Within the Supraspinatus Muscle by Using a 2-Point Dixon Method on 3-
541 T MRI. *AJR. American journal of roentgenology* 2015; 205:116-22. 10.2214/AJR.14.13518.
- 542 34. Oh JH, Chung SW, Kim SH, Chung JY, Kim JY. 2013 Neer Award: Effect of the
543 adipose-derived stem cell for the improvement of fatty degeneration and rotator cuff healing
544 in rabbit model. *J Shoulder Elbow Surg* 2014; 23:445-55. 10.1016/j.jse.2013.07.054.
- 545 35. Palumbo C, Rovesta C, Ferretti M. Striated muscle fiber apoptosis after experimental
546 tendon lesion in a rat model. *J Anat* 2012; 221:358-63. 10.1111/j.1469-7580.2012.01554.x.
- 547 36. Penner G, Gang G, Sun X, Wray C, Hasselgren PO. C/EBP DNA-binding activity is
548 upregulated by a glucocorticoid-dependent mechanism in septic muscle. *American journal of*
549 *physiology. Regulatory, integrative and comparative physiology* 2002; 282:R439-44.
550 10.1152/ajpregu.00512.2001.
- 551 37. Pirinen E, Cantó C, Jo YS, Morato L, Zhang H, Menzies KJ et al. Pharmacological
552 Inhibition of poly(ADP-ribose) polymerases improves fitness and mitochondrial function in
553 skeletal muscle. *Cell Metab* 2014; 19:1034-41. 10.1016/j.cmet.2014.04.002.
- 554 38. Reed SA, Sandesara PB, Senf SM, Judge AR. Inhibition of FoxO transcriptional activity
555 prevents muscle fiber atrophy during cachexia and induces hypertrophy. *FASEB J* 2012;
556 26:987-1000. 10.1096/fj.11-189977.
- 557 39. Rios R, Carneiro I, Arce VM, Devesa J. Myostatin is an inhibitor of myogenic
558 differentiation. *American journal of physiology. Cell physiology* 2002; 282:C993-9.
559 10.1152/ajpcell.00372.2001.
- 560 40. Sakamaki J-i, Daitoku H, Yoshimochi K, Miwa M, Fukamizu A. Regulation of FOXO1-
561 mediated transcription and cell proliferation by PARP-1. *Biochemical and biophysical*
562 *research communications* 2009; 382:497-502. 10.1016/j.bbrc.2009.03.022.

PARP-1 Regulates Muscular Deterioration After RCT

- 563 41. Schmutz S, Fuchs T, Regenfelder F, Steinmann P, Zumstein M, Fuchs B. Expression of
564 atrophy mRNA relates to tendon tear size in supraspinatus muscle. *Clin Orthop Relat Res*
565 2009; 467:457-64. 10.1007/s11999-008-0565-0.
- 566 42. Sishi BJ, Engelbrecht AM. Tumor necrosis factor alpha (TNF-alpha) inactivates the PI3-
567 kinase/PKB pathway and induces atrophy and apoptosis in L6 myotubes. *Cytokine* 2011;
568 54:173-84. 10.1016/j.cyto.2011.01.009.
- 569 43. Wang ZQ, Auer B, Stingl L, Berghammer H, Haidacher D, Schweiger M et al. Mice
570 lacking ADPRT and poly(ADP-ribosyl)ation develop normally but are susceptible to skin
571 disease. *Genes & development* 1995; 9:509-20.
- 572 44. Yu SW, Andrabi SA, Wang H, Kim NS, Poirier GG, Dawson TM et al. Apoptosis-
573 inducing factor mediates poly(ADP-ribose) (PAR) polymer-induced cell. *Proc Natl Acad Sci*
574 U S A 2006; 103:18314-19. 10.1073/pnas.0606528103.
- 575 45. Zanou N, Gailly P. Skeletal muscle hypertrophy and regeneration: interplay between the
576 myogenic regulatory factors (MRFs) and insulin-like growth factors (IGFs) pathways. *Cell*
577 *Mol Life Sci* 2013; 70:4117-30. 10.1007/s00018-013-1330-4.
- 578 46. Zumstein MA, Jost B, Hempel J, Hodler J, Gerber C. The clinical and structural long-term
579 results of open repair of massive tears of the rotator cuff. *The Journal of bone and joint*
580 *surgery. American volume* 2008; 90:2423-31. 10.2106/JBJS.G.00677.

581

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582 **Figure legends:**

583 **Fig. 1:** Flow chart of the experimental design including the time points of surgery and
584 sacrifice.

585 **Fig. 2:** Results of the macroscopic and MRI measurements. A: Representative macroscopic
586 images showing less retraction of the tendon in PARP-1 KO mice compared to WT mice. The
587 arrow indicates the distance of the tendon stump to the humeral head. B: Representative
588 images of the radiological retraction measurements in the MR scans. The arrow indicates the
589 distance of the tendon stump to the humeral head. C: Muscle weight measurement. The
590 relative weight to the contralateral uninjured side of the PARP-1 KO and Wild Type mice is
591 shown in the bar graph. D: Bar graphs of the retraction measurements. Statistical significant
592 differences are shown * $p < 0.05$, ** $p < 0.01$ and **** $p < 0.0001$.

593 **Fig. 3:** Representative histological slides and results of the fat quantification and pennation
594 angle measurement. A: Representative histological cross sections of the SSP stained with
595 H&E after 1, 6 and 12 weeks. B: Representative histological cross sections stained with an
596 antibody against Fabp4. C: Fat quantification in the SSP muscles. Relative fat quantification
597 in the MR scans with a 2-Point Dixon Method on a 4.7T small animal MRI scanner and
598 histological grading of the endo- and perimysial fat content in the cross sections of the SSP
599 muscles stained with Fabp4. D: Representative histological cross sections of the SSP stained
600 with Picrosirius Red to visualize the connective tissue. E: Pennation angle measurements in
601 the Picrosirius Red stained longitudinal sections of the SSP muscles of PARP-1 KO and WT
602 mice and bar graphs indicating the degree of the angle. The contralateral side of both groups
603 acted as an uninjured control measurement. Statistical significant differences are shown *
604 $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

605 **Fig. 4:** Results of the gene expression analysis with real time RT-PCR. The increase of
606 mRNA levels is shown as fold expression compared to the uninjured contralateral side with

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607 the Δ Ct method. A: Genes of the inflammatory cascade ($\text{TNF}\alpha$, IL-1 β and NF- κ B) and
608 apoptosis (AIF). B: Proliferative factors of the TGF β superfamily represented by TGF β 1 and
609 Myostatin. C: Genes involved in the degeneration of muscle fibers. Foxo1 is the upstream
610 regulator of the Ubiquitin-Ligases MuRF1 and Atrogin-1, which bind to Ube3a. D: Genes for
611 muscular regeneration. AKT is the upstream regulator of the MRFs here represented by
612 MyoD1 and Myf-5. E: Genes regulating fatty infiltration (PPAR γ) and binding of fatty acids
613 (Fabp4). Statistical significant differences are shown * $p < 0.05$, ** $p < 0.01$ and **** $p < 0.0001$.
614 **Fig. S 1:** Supplemental results of the gene expression analysis with real time RT-PCR. The
615 increase of mRNA levels is shown as fold expression compared to the uninjured contralateral
616 side with the Δ Ct method. A: Genes of the inflammatory cascade (IL6) and apoptosis
617 (Casp3). B: Proliferative factors TGF β 3. C: Genes involved in the degeneration of muscle
618 fibers Foxo3 and Ube2b. D: Gene for muscular regeneration, Myogenins. E: Genes regulating
619 fatty infiltration Leptin. Statistical significant differences are shown with * $p < 0.05$ and ****
620 $p < 0.0001$.
621