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ECCENTRIC TRAINING FOR TENDON HEALING AFTER ACUTE LESION A RAT MODEL

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Background: The tendon is a dynamic entity that remodels permanently. Platelet-rich plasma (PRP) injection has been shown to have a beneficial effect on tendon healing after lesion in rats. Furthermore, eccentric exercise seems to improve the mechanical quality of the tendon.

Hypothesis: A combination of PRP injection and eccentric training might be more effective than either treatment alone.

Study Design: Controlled laboratory study.

Methods: Adult male rats were anesthetized, an incision was performed in the middle of their left patellar tendon and an injection of physiological fluid (PF) or homologous PRP was randomly made at the lesion level. The rats were then divided into 2 groups: the eccentric group, undergoing eccentric training 3 times a week, and the untrained group, without any training. Thus, 4 groups were compared. After 5 weeks, the tendons were removed and their ultimate tensile strength and energy were measured. Tendons were frozen for proteomic analyses when all biomechanical tests were completed. Statistical analysis was performed with linear mixed effect models.

Results: No significant difference was found between the treatments using PF injection or PRP injection alone. However, the value of the ultimate tensile force at rupture was increased by 4.5 N (108% of control, P = .006) when eccentric training was performed. An intragroup analysis revealed

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that eccentric training significantly improved the ultimate force values for the PRP group. Proteomic analysis revealed that eccentric training led to an increase in abundance of several cytoskeletal proteins in the PF group, while a decrease in abundance of enzymes of the glycolytic pathway occurred in the PRP-treated groups, indicating that this treatment might redirect the exercise-driven metabolic plasticity of the tendon.

Conclusion: Eccentric training altered the metabolic plasticity of tendon and led to an improvement of injured tendon resistance regardless of the treatment injected (PF or PRP).

Clinical Relevance: This study demonstrates the necessity of eccentric rehabilitation and training in cases of tendon lesion regardless of the treatment carried out.

Keywords: tendon; platelet-rich plasma; PRP; eccentric; biomechanics; proteomics

The tendon, a connective fibrous tissue that is mechanically responsible for the transmission of muscle strength to bones, is a dynamic entity that remodels permanently, according to mechanical stimuli or stresses, as a result of various metabolic and mechanical changes.[14,23] However, this tissue has a low metabolism, and its lesions are often difficult to treat.[18] In particular, a study that analyzed the tendon proteome of young and old horses indicated a correlation between an increased risk of injury with aging and a decrease in the levels of proteins involved in the maintenance and the repair of tendons.[25]

Investigators using a rat model observed that load application, especially in eccentric mode, could lead to intratendinous modifications related to the mechanotransduction process.[17] Indeed, the higher resistance of eccentrically trained tendons mostly results from an increase of the cross-sectional area, although small modifications of the tissue architecture may also play a role.[22] In an in vitro study of tendon engineering from human tenocytes grown in a bioreactor system, investigators analyzed the proteomic modification occurring after 12 weeks with and without dynamic loading. After loading, a significant upregulation of collagens I and VI was observed, which suggested that the production of this more mature tendon tissue may involve the mechanotransduction process and lead to intratendinous modifications.[11]

Moreover, investigators have demonstrated, also using a rat model, that the healing process of sectioned tendons can be improved by an injection of platelet-rich plasma (PRP) through the release of platelet growth factors.[15] This cicatrization is much improved if the injured tendon is not immobilized.[26]

We therefore hypothesized that the healing process of sectioned patellar tendons in rats and the mechanical strength of the restored tissue could be improved by an injection of PRP combined with eccentric training, compared with the use of each treatment alone. To our knowledge, no study has investigated these 2 approaches together. We tested this hypothesis by measuring the force at rupture, also called the ultimate tensile strength (UTS), and by proteomic analyses of healing tendons during repair process.

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METHODS

All experimental procedures and protocols used in this investigation, inspired by our previous studies,[15,17,20] were reviewed and approved by our Institutional Animal Care and Use Ethics Committee. The Guide for the Care and Use of Laboratory Animals[24] was followed carefully, as were European and local legislations. Animal welfare was assessed at least once per day, with humane end points applied when necessary as described in the ethical form.

POPULATION

An a priori statistical power test was conducted to determine the number of animals to test, and 56 2-month-old male rats (Sprague-Dawley, mean + SD weight 267.5 g ± 72 g) were used in this protocol.

SURGERY

Fifty-two rats were weighed, anesthetized intraperitoneally with pentobarbital (60 mg/kg of body weight), and placed on a warm pad. The skin of the left hindlimb was shaved. Buprenorphine (0.05 mg/kg of body weight) and tetracycline (15 mg/kg of body weight) were administered a priori by subcutaneous route. The complete surgical procedure was performed with aseptic conditions under a dissecting microscope. The skin of the left patellar tendon was incised laterally. The patellar tendon was exposed after dissection of the surrounding fascia. Subsequently, 5 mm proximal to its insertion on the patella, the patellar tendon was partly cut (50%). The tendon was left unsutured. The fascia and the skin were sutured with resorbable Vicryl No. 6/0 (Ethicon, Johnson & Johnson). The animals were placed in clean cages under a heating lamp until awakening and were not subject to postoperative immobilization. The rats were divided into 2 groups that received a local injection (inside the defect) at the 2-hour postoperative time point: One group received 50 µL of a physiological fluid (PF group), and the other group received fresh homologous PRP (made 15 minutes before; see PRP preparation section) after activation with 50 µL $CaCl_2$ for each milliliter of PRP. The animals were checked daily and assessed for well-being, and a global observation of walking and activity was made.

PRP PREPARATION

Whole blood was collected from 4 Sprague-Dawley males. The donor rats were intraperitoneally anesthetized with pentobarbital (60 mg/kg), and blood was collected by cardiac puncture. One milliliter of anticoagulant acid citrate dextrose solution per 4.5 mL of blood was immediately added. Blood samples were centrifuged at low speed (150g) for 10 minutes at room temperature. The upper phase from each sample, consisting of PRP, was gently collected and pooled in a secondary plastic tube. Initial platelet count was measured on a Sysmex XS 800i hematology analyzer. Platelet

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concentration was adjusted to ~2.5 X 10^6/mL by performing a second high-speed centrifugation of the pooled PRP at 1000g for 10 minutes: One-half to two-thirds volume of supernatant was removed to reach the desired concentration. The platelet pellet was then resuspended, and final platelet concentration was assessed.[21] Final platelet counts ranged from 2.2 to 2.9 X 10^6/mL, and no significant amounts of contaminating white blood cells or red blood cells were present in the PRP: White blood cell and red blood cell counts were respectively below 1 X 10^2/mm^3 and 1 X 10^4/mm^3. Pooled rat PRP was used within 3 hours of preparation.

ECCENTRIC PROTOCOL

Twenty-six rats (13 of the PF group and 13 of PRP group) constituted an untrained group and were not subjected to physical exercise. The 26 remaining rats (PF and PRP) ran on a treadmill set at a –15° inclination (similar to running downhill), at a speed of 17 meters/min (1 km/h) for 1 hour, 3 times per week for 5 weeks (after an initial familiarization period of 2 weeks). Thus, we had 4 groups: PF alone (PF), PF + eccentric exercise (PF+E), PRP alone (PRP), and PRP + eccentric exercise (PRP+E).

BIOMECHANICAL TESTING

At the end of this 5-week training, all rats were weighed; while the animals were under general anesthesia (pentobarbital, 60 mg/kg, intraperitoneally), the patellar tendons from each rat (n = 52) were carefully removed along with the distal third of the muscle on which the tendons inserted and the portion of the tibia to which the tendon attached. Tendon samples were also taken from the opposite side (right) of 13 untrained and 13 trained rats to build 2 groups without surgery: without and with eccentric training (C and C+E, respectively). The rats were sacrificed by means of a pentobarbital injection (200 mg/kg, intraperitoneally). The mechanical testing was done with a universal testing machine (106.2 kN, TesT GmbH) and a ''cryo-jaw''–type clamping device.[27] The entire muscular segment (triceps surae) was placed between the cryo-jaws while the bony segment was fixed between the lower clamps. The muscle was subsequently frozen with liquid nitrogen to dramatically increase its stiffness compared with that of the tendon and to ensure that only the tendon underwent a deformation. The tensile test was started as soon as the expansion of the freezing zone reached the border of the metal clamp but did not extend into the tendon tissue. The displacement rate was set to a constant speed of 1 mm/s until rupture. A computer for subsequent data analysis recorded force versus displacement curves. The UTS was expressed in newtons. The mechanical energy at failure, defined as the work of the applied load along the relative displacement of the tendon's ends, was also computed and was expressed in joules. Just after the biomechanical testing, all the tendon samples were isolated from the muscle and bone before being frozen in liquid nitrogen.

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SAMPLE PREPARATION FOR COMPARATIVE PROTEOMIC STUDIES

Dissected tendons from which all visible traces of blood and muscle tissue had been removed were cut into small pieces and were powdered in liquid nitrogen by use of a ceramic mortar and pestle. The powdered samples were suspended in 3 times the initial weight of lysis buffer (7 M urea, 2 M thiourea, 30 mM Tris, pH 8.5 [all from GE Healthcare] and 2% (wt/vol) ASB-14 [Sigma]) and sonicated for 15 minutes. After removal of the insoluble material by centrifugation (20,000g), 100 µL of supernatant was precipitated by use of the 2D Clean-up kit (GE Healthcare) to remove salts and low-molecular-weight contaminants that could have interfered with CyDye labeling (Lumiprobe) and protein electrophoresis. Protein pellets were resuspended in adequate volume of lysis buffer, and the pH was adjusted to 8.5 with 100 mM NaOH for efficient CyDye labeling. Protein concentration was evaluated with the RC/DC Protein Assay (Bio-Rad Laboratories).

TWO-DIMENSIONAL DIFFERENTIAL IN-GEL ELECTROPHORESIS

Two-dimensional differential in-gel electrophoresis (2DDIGE) comparative proteomic analysis was performed as described by Bentaib et al,[4] with 6 replicates of each experimental condition. For analytical gels, 25 µg of each replicate was labeled separately with 200 pmol of Cy NHS Ester (Cy3, Cy5; Lumiprobe) with dye-swapping to minimize dye-specific labeling effect, and was then vortexed before being incubated 30 minutes in the dark. At the same time, a pool of equal amounts of proteins from all biological replicates was labeled with Cy2 for use as an internal standard. After 30 minutes in the dark, the reaction was stopped with 10 mM lysine. Multiplexing was achieved by pooling 25 µg of Cy3- and Cy5-labeled samples together with 25 µg of Cy2-labeled internal standard, used for matching and normalization between gels, and the volume of the combined labeled samples was adjusted to 450 µL with standard rehydration buffer (7 M urea, 2 M thiourea, 2% wt/vol ASB-14, 25 mM dithiothreitol (DTT), and 0.6% vol/vol pH 3-10 NL IPG buffer [GE Healthcare]). Each mixture of CyDye-labeled samples was used for the rehydration of 24-cm IPG strips (pH 3-10 NL, GE Healthcare) for 10 hours at 20C. Isoelectric focusing (IEF) was carried out at 500 V for 1 hour, followed by 2 successive 3-hour gradients (first gradient, 500-1000 V; second gradient, 10008000 V) and constant setting at 8000 V for 70 kVh at 20C. The maximum current setting was 50 mA per strip in an IPGphor isoelectric focusing unit (GE Healthcare).

Before second dimension separation, the IPG strips were incubated for 15 minutes at room temperature (RT) in equilibration buffer (30% glycerol [vol/vol], 1.6% sodium dodecyl sulfate [SDS] [wt/vol], 6 M urea, 50 mM Tris-HCl pH 8.8) containing 1% DTT and then for 15 minutes in the same solution containing 5% iodoacetamide. They were then sealed with 0.5% agarose in SDS running buffer on top of 12.5% wt/vol polyacrylamide gels. The second-dimension electrophoresis was performed overnight at 30C in an Ettan Dalt-6 system (GE Healthcare) at a 50-V constant voltage. Each gel was finally scanned with the Typhoon 9400 scanner (GE Healthcare) at the emission wavelengths corresponding to each CyDye, namely 520 nm (Cy2), 580 nm (Cy3), and 670 nm (Cy5).

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IMAGE ANALYSIS

Images (n = 6 for each experimental condition) were analyzed with the DeCyder 7.0 software (GE Healthcare) according to the manufacturer's instructions. In brief, co-detection and quantification of the 3 CyDye-labeled forms of each spot were performed by use of the Differential In-gel Analysis (DIA) module, allowing the calculation of ratios between samples and internal standard abundances for each spot. The biological variance analysis (BVA) module was used to match spots across all gels and to correct intergel variability through normalization of the Cy2 internal standard spot maps present in each gel. Protein spots that showed a statistically significant Student t test (P \ .05) for an average spot abundance change of at least 1.3 were subjected to identification by liquid chromatography tandem mass spectrometry (LC-MS/MS).

IN-GEL DIGESTION AND MASS SPECTROMETRY ANALYSIS

Preparative gels that contained 200 to 300 µg of either C, C+E, PF, PF+E, PRP, or PRP+E protein samples and 25 µg of the internal standard were run in parallel and matched to the analytical gels master image to generate picking lists of differentially expressed spots used to drive automated gel plug collection by the Ettan Spotpicker (GE Healthcare). In-gel digestion was performed by addition of modified trypsin (Promega) in 50 mM ammonium bicarbonate at 37°C overnight. The tryptic digests were air dried and then dissolved in formic acid (0.1%) for further tandem mass spectrometry (MS-MS) analysis. Each in-gel digest of an individual spot was analyzed by nano-high-performance liquid chromatography (HPLC) electrospray MS-MS through use of an XCT ion trap mass spectrometer (Agilent). The HPLC separations were performed on an RP C18 Zorbax column from Agilent. The mobile phase was a 90-minute gradient mixture formed as follows: mixture A, water–acetonitrile–formic acid (97/3/0.1); mixture B, acetonitrile–water–formic acid (90/10/0.1). The flow rate was fixed at 300 nL/min. The collision energy was set automatically depending on the mass of the parent ion. Each MS full scan was followed by MS-MS scans of the first 4 most intense peaks detected in the prior MS scan. A list of peptide masses was subsequently introduced into the database for protein identification searches using MASCOT (Matrix Sciences).

STATISTICAL ANALYSES

Results are expressed as the mean and standard deviation of the mean. For the biomechanical testing statistical analysis, a Shapiro-Wilks test was performed to verify that the data were normally distributed. A multivariate, linear, mixed effect model was designed, including the treatment (C, PF, and PRP), the participation in eccentric exercise (C+E, PF+E, and PRP+E), and the animal weight as independent variables. The ultimate energy and force were studied together as they are not independent. The model to be tested then reads [Force, Energy] = a Vex + b Vtr + c*Weight + (1|Animal) (1), where Vex indicates whether eccentric training was performed, Vtr expresses the type of treatment (PF or PRP), and a, b, and c are coefficients. The 1|Animal term is included to

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account for the fact that we have 2 values per animal and that the baseline value for each animal is different. The ultimate force (namely energy) is then assumed to be a linear function of those parameters. This analysis was done with the statistical software RStudio and a level of significance set at P \ .05.

RESULTS

Table 1 shows the mean values of the ultimate force and energy that would be sustained by a tendon of a rat from the control, untrained group with a weight equal to the average of all the animals.

This value was incremented by 4.5 N (resp. 36.5 J) for rats that performed eccentric exercise. The weight at time of sacrifice and the application of an eccentric exercise were found to be significant parameters influencing both the ultimate energy and force (Table 1). The value of the ultimate force (resp. energy) was increased by 8%, P = .006 (resp. 15%, P = .03) when eccentric training was performed. For each 100-g increment in weight, the ultimate force increased by 18% (9.7 N, P \ .00001) and the energy by 12% (29.9 J, P = .0008). No significant difference was found between the treatments using PF injection and PRP injection. This might seem unexpected given the results presented in a previous study[15] and shall be discussed in the next section.

An intragroup analysis revealed that eccentric training significantly improved the ultimate force values for the PRP groups (Figures 1 and 2). The complete results are presented in Table 2.

The proteomic analysis results are presented in Figure 3 and in Table 3. In control tendons, protein amounts of 1 glycolytic enzyme (beta-enolase), of 1 TCA cycle enzyme (isocitrate dehydrogenase), and of 1 of the stress response proteins (heat shock protein) increased after eccentric training (C+E condition), while a relative decrease in the abundance of several cytoskeletal proteins was also observed in the same group. No difference in protein abundance was observed between PF groups with or without eccentric training. When comparing tendons injected with PRP without and with eccentric training, we observed a significant decrease of 4 glucose metabolism enzymes, namely, pyruvate kinase M1/M2 (P = .003), phosphoglucomutase-1 (P = .007), glyceraldehyde-3-phosphate dehydrogenase (P = .017), and triosephosphate isomerase (P = .044) in the eccentric group.

DISCUSSION

Previous research has demonstrated that PRP injection improves the healing process of acute tendon lesions in a rat model,[2,16,28] especially in the early phase of healing,[15] even though the efficacy of this treatment applied to human subjects is still debated.[3,5,6,13] Moreover, a 5-week eccentric training protocol performed on healthy rat tendons significantly increased the force and energy

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required to tear the tendon in a simple tensile test.[17] In human subjects undergoing physical therapy after a tendon injury, eccentric training is a key factor for a successful recovery,[1,7,8] if we assume that the latter is related to the biomechanical properties of the tendon. Eccentric training is also recommended after PRP injection in case of chronic tendinopathies.[12,19]

In this work, it was not possible to prepare an autologous PRP as in a human model. We could, however, prepare a homologous PRP by centrifugation of plasma from 4 inbred donor rats, thus avoiding any histocompatibility problem as in a human model.

We also observed that eccentric training improves tendon resistance whatever the complementary treatment carried out after acute patellar tendon section in rats. However, no significant difference was found for ultimate tensile strength and energy between the PF and PRP groups with respect to the treatment performed. This could be due to the duration of the revalidation period (5 weeks of training), which was sufficiently long to allow for a full recovery. The lack of significant difference between the PF and PRP treatments could also be explained, at least partially, as resulting from a patellar tendon–specific recovery process since in a previous report on rat tendon lesions,[15] PRP injections were found to increase the ultimate force of the Achilles tendon of rats.

The proteomic analysis of the tendons revealed that a combination of PRP treatment and eccentric training would decrease the amounts of 4 enzymes implicated in glucose metabolism and glycolysis and thus would decrease anaerobic adenosine triphosphate (ATP)–producing metabolism. As proposed in a similar study of human muscle subjected to eccentric training, it is likely that the reduction of glycolytic metabolic pathway energy output in the tendon might be compensated by an increased oxidative metabolism output.[9,10] This change in tendon energy metabolism was found to occur only in the PRP-treated group, which indicates the specific effect that this treatment has on both the mechanical properties and physiological plasticity of this tissue. An enhanced oxidative metabolism could be supported by the increase of blood vessel density around the tendon, as observed in histological cross sections in previous studies in healthy tendons after eccentric training.[17]

Surprisingly, even if the PRP 1 eccentric exercise group showed the highest ultimate force at rupture, no significant difference was observed with the other groups. This is contradictory to previous observations showing that PRP and platelet growth factors initiated and stimulated the early phases of collagen synthesis compared with saline injection, leading to an improvement of ultimate force at rupture.[15] In the present study, the delay before sampling was probably too long, allowing a complete reconstruction process to occur. Although we observed no significant differences in biomechanical testing when comparing the combination of PRP and eccentric exercise versus PF and eccentric exercise, we noted a significant difference in some protein abundance, which corresponds to a decrease of the anaerobic metabolic pathway but does not seem to have any consequence on the biomechanics of the tendon at the sampling time.

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Astonishingly, in our proteomic analysis of control tendons we also observed that eccentric training decreases the abundance of proteins of the cytoskeleton and of the glycolytic pathway, the TCA cycle, and the stress response. Such a long period of eccentric training will have a beneficial effect[10] and will make the tendon more resistant, such that it will express a lower abundance of stress proteins, cytoskeletal components, and glycolytic enzymes.

This work is the first animal study analyzing different treatments in combination with eccentric training and analyzing the biomechanical and proteomic modifications related to these managements. However, the study presents some limitations. The a priori statistical power test that was conducted to determine the number of animals to test was based on previous results, as we had no other data to estimate the statistical variables for the present study. Moreover, the final time point for animal sacrifice that was fixed according to our previous experimental schemes was apparently rather late in the reparative process to determine whether any of these treatments had an early effect on tendon healing. For future studies, testing and analysis should be conducted at different time points. Finally, these laboratory results on animal acute tendon lesions may not correspond to the human clinical situation and can explain only partly an acute human tendon lesion, which presents various intrinsic differences compared with an overuse-induced tendinopathy.

CONCLUSION

After an acute tendon lesion in a rat model, eccentric training leads to an improvement of the tendon biomechanical properties during its healing process, whatever the other additional therapeutics used. Moreover, eccentric training could decrease the glycolytic metabolism in the tendon after cicatrization, possibly linked to an improvement of peripheral vascularization of the tendon.

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Tables

Tables 1. *Mean values observed for ultimate force and energy and their respective increments as a function of eccentric training, the weight of the rats, and the type of injection*

Effect	Ultimate Force, N (*P* Value)	Ultimate Energy, J (*P* Value)
Mean value	55.1	246.9
Eccentric training	4.5 (.006)	36.5 (.03)
Weight (100-g increment)	9.7 (1.9e-14)	29.9 (.009)
Physiological fluid injection	1.3 (.49)	–12.9 (.51)
Platelet-rich plasma injection	0.88 (.64)	–3.97 (.84)

Table 2. *Complete results for ultimate force and energy as a function of eccentric training and the weight of the rats for the control group, PF Group, and PRP Group*[a]

Group	Measured Parameters	Ultimate Force, N (*P* Value)	Ultimate Energy, J (*P* Value)
Control	Mean value	55.96	254.26
	Eccentric training	58.88 (.18)	275.77 (.36)
	Weight	+8/100 g (<1e-15)	+35/100 g (.03)
PF	Mean value	57.35	224.33
	Eccentric training	60.19 (.46)	279.50 (.12)
	Weight	+11/100 g (<1e-15)	+26/100 g (.16)
PRP	Mean value	53.60	238.76
	Eccentric training	62.58 (.006)	285.80 (.19)
	Weight	+12/100 g (.0005)	+20/100 g (.58)

[a]PF, physiological fluid; PRP, platelet-rich plasma.

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Table 3. Identified proteins in each treatment condition whose abundance differed by at least 1.3-fold (p\.05, student t test) after eccentric exercise[a]

Master No.	Protein ID	Name	*t* Test	Average Ratio
Control vs Control + Eccentric Training				
Carbohydrate metabolism (glycolysis and TCA cycle)				
1492	ENOB_RAT	Beta-enolase	0.0083	–1.78
1788	IDH3A_RAT	Isocitrate dehydrogenase [NAD] subunit alpha; mitochondrial	0.012	–1.31
Stress response				
1038	HSP71_RAT	Heat shock 70 kDa protein 1A/1B	0.0071	–1.73
Cytoskeleton				
2872	MLRV_RAT	Myosin regulatory light chain 2; ventricular/cardiac muscle isoform	0.043	–1.46
3828	MYL1_RAT	Myosin light chain 1/3; skeletal muscle isoform	0.034	–1.38
2593	TNNI2_RAT	Troponin I; fast skeletal muscle	0.036	–1.39
Miscellaneous				
1257	TRY1_RAT	Anionic trypsin-1	0.03	–1.49
PF vs PF + Eccentric Training				
—	—	—	—	—
PRP vs PRP + Eccentric Training				
Carbohydrate metabolism (glycolysis and TCA cycle)				
1187	KPYM_RAT	Pyruvate kinase isozymes M1/M2	0.0031	1.83
1184	PGM1_RAT	Phosphoglucomutase-1	0.007	1.48
1996	G3P_RAT	Glyceraldehyde-3-phosphate dehydrogenase	0.017	1.57
2618	TPIS_RAT	Triosephosphate isomerase	0.044	2.12

[a]Proteins have been classified into biological processes as defined in the UniProt Database Keywords list. Master No. is the unique identifier of each protein spot provided in the DeCyder analysis. Protein ID is the item entry name in the UniProtKB/SwissProt database. Average ratio is the fold-increased relative abundance of the identified protein in untrained tendon samples (positive values) or in eccentric exercise–trained tendon samples (negative values). PF, physiological fluid; PRP, platelet-rich plasma; TCA, tricarboxylic acid; —, no differentially expressed proteins.

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Figures

Figure 1. Ultimate energy (joules) in group receiving platelet-rich plasma (PRP) with or without eccentric training. As shown on this figure, eccentric training led to significantly (P = .006) stronger tendons.

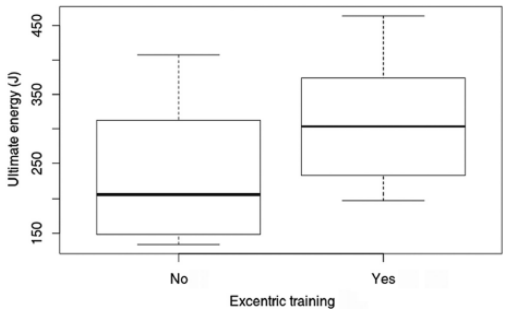

Figure 2. *Ultimate force (newtons) in group receiving platelet-rich plasma (PRP) with or without eccentric training. Eccentric training significantly (P = .006) improved the ultimate force values for the trained groups compared with untrained groups. However, this improvement decreased with the increase of weight of the rats.*

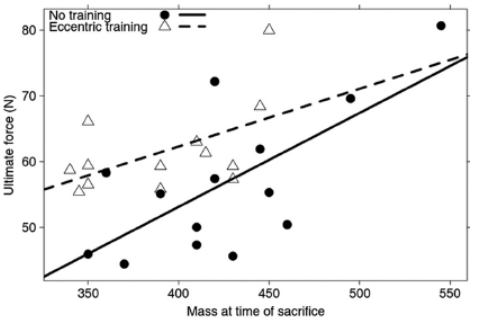

Figure 3. *Gel images illustrating the position of protein spots identified by liquid chromatography tandem mass spectrometry as being more abundant in (A) eccentrically trained vs untrained control tendon and (B) eccentrically trained vs untrained platelet-rich plasma (PRP)–treated tendon.*

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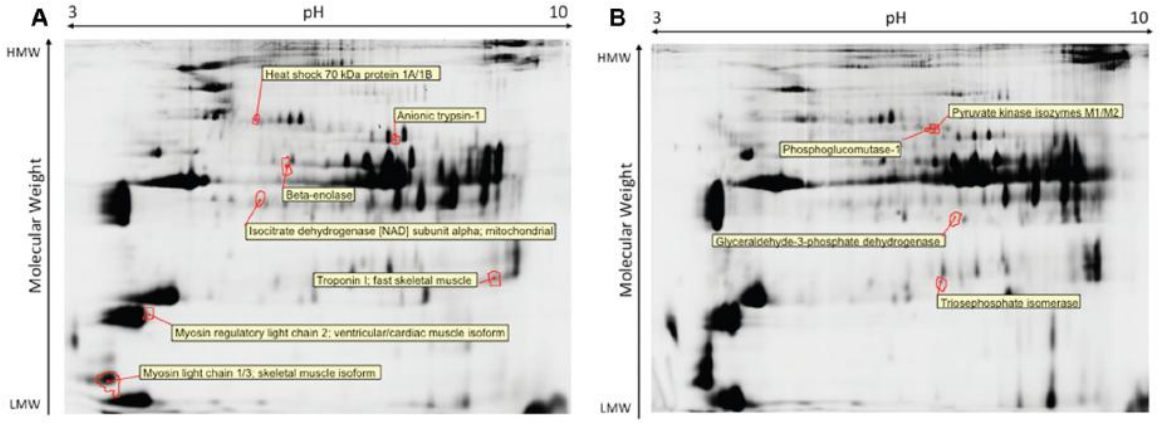

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