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● *Original Contribution*

TOPICAL FISH OIL APPLICATION COUPLING WITH THERAPEUTIC ULTRASOUND IMPROVES TENDON HEALING

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Abstract—Fish oil has been shown to promote collagen synthesis, and hence, connective tissue healing. Therapeutic ultrasound is commonly used to treat soft tissue injuries. This study aimed to investigate the therapeutic effect of topical fish oil on the management of Achilles tendon rupture, comparing normal therapeutic ultrasound with a combination of ultrasound and fish oil. Eighty-five Sprague-Dawley rats underwent surgical hemitenotomy of the right medial Achilles tendon. The rats received daily treatment of either topical placebo ointment (control group [CON]), topical fish oil (FO), therapeutic ultrasound (US) or ultrasound with fish oil as the coupling medium (FU). The treatment started on post-surgical day 2 over a 2-wk or 4-wk period. On days 15 and 29, the rats were sacrificed and their Achilles tendons were tested for structural stiffness, ultimate tensile strength (UTS) and energy absorption capacity. At 2 wk, only US showed higher normalized UTS compared with CON ($p < 0.05$). At 4 wk, both US and FU demonstrated better UTS ($p < 0.05$), while both FO and FU had improved in structural stiffness ($p < 0.05$). Four wk of treatment with ultrasound using fish oil as coupling medium showed improvement in both structural stiffness and UTS ($p < 0.05$). (E-mail: cckarly@hkcc-polyu.edu.hk) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Fish oil, Omega-3, Achilles tendon rupture, Therapeutic ultrasound, Repair.**

INTRODUCTION

The Achilles tendon (AT) is the largest and strongest tendon in the body, which bears up to 12.5 times of the body's weight during running and jumping (Doral et al. 2010). However, the incidence of spontaneous rupture of the AT is particularly high (Kannus and Józsa 1991; Rees et al. 2006), and it is believed that degenerative changes and repeated mechanical loading that reduce the tensile strength in this tendon are the causative factors of AT rupture (Tallon et al. 2001). Because of the relatively low metabolic rate and long turnover time for tendon collagen synthesis (Abate et al. 2009), natural healing is often very slow and the repaired tissue may never attain the normal pre-injury level of function (Doroski et al. 2007).

There is no consensus on the treatment of acute AT rupture (Nandra et al. 2012). The treatment options include two main approaches, namely operative and conservative. The operative approach is associated with higher risk of post-surgery infection (Bhandari et al. 2002), while the conservative approach may have higher risks of re-rupture (Khan et al. 2005; Weber et al. 2003). Both require 7–9 wk of immobilization, which may compromise the strength of the calf muscles (Ding et al. 2013). Additionally, it has been reported that Achilles tendons cannot fully recover even at 2 y after rupture, irrespective of the type of treatment (Olsson et al. 2011). Due to the deficiencies of current treatments, there is a need to explore potential treatment alternatives that can shed new light on current practices.

Oil from deep-sea fish contains high proportions of polyunsaturated fatty acids (PUFAs) of the omega-3 series that are particularly rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Cleland et al. 2006). Omega-3 fatty acids are believed to have anti-inflammatory properties essential for the healing of rheumatoid arthritis, osteoarthritis and chronic tendonitis (Berbert et al. 2005; Caturla et al. 2011; Mavrogenis et al. 2004). Furthermore, omega-3 PUFAs promote collagen synthesis in connective tissues such as in the

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porcine ligament fibroblasts (Hankenson et al. 2000), mouse fibroblasts (Jia and Turek 2004, 2005) and mouse and rat cutaneous wounds (Cardoso et al. 2004; Otranto et al. 2010). The omega-3 PUFAs are lipophilic and possess high epithelial penetration abilities, meaning they can even serve as a skin drug permeation enhancer for ketoprofen (Heard et al. 2003) and para-aminobenzoic acid (PABA) (Tanojo et al. 1997).

Therapeutic ultrasound has been used to treat soft tissue injuries for over six decades. Therapeutic ultrasound improves tendon healing by increasing biomechanical strength (Ng et al. 2003; Yeung et al. 2006), collagen synthesis (Jackson et al. 1991) and collagen fibril size (Ng and Fung 2007).

Taking into account the wide use of fish oil on different connective tissues and the popularity of ultrasound in treating tendon injuries, the aims of the present study were to (i) investigate the effects of topical fish oil application, (ii) compare the effect of topical fish oil with normal therapeutic ultrasound and (iii) examine if there is synergistic effect on therapeutic ultrasound coupled with fish oil for treatment of AT rupture.

METHODS

Experimental Design

Eighty-five female Sprague-Dawley rats (mean weight, 243.14 g ± 25.64 g; range, 325.00–208.76 g) aged 12 wk at the time of surgery were used. Ethics approval was obtained from the Animal Ethics Review Committee of the administrating institution before the study.

All rats underwent the same surgical procedures modified from a previous report by Ng and Fung (2008). The rats were anesthetized with isoflurane through a nose cone during the surgery. Skin on the anterior aspect of the right calf was shaved, incised and retracted to expose the AT. The tendon of the medial gastrocnemius was identified and separated from the lateral tendon with a blunt probe, and the medial tendon was cut with a scalpel at its mid-point without suturing. The lateral tendon was left intact to simulate a partial tendon rupture, and to prevent retraction of the cut ends. The skin wound was closed by non-absorbable suture that was removed 3 d after surgery. The limbs were not immobilized and the rats were allowed to have free activity inside their cages. The rats were kept in an animal house with a 12-h light-dark cycle, and temperature was maintained at about 20°C. Water and food were given *ad libitum* during the study. All treatments were given on the second post-surgical day.

Treatment

The animals were allocated randomly into eight groups. All animals received same amount of topical ointment daily, but with different compositions and treatment protocols according to their group assignment for either 2 wk or 4 wk (Table 1). Vaseline plain petroleum jelly acted as a control (CON) and was applied over the skin of the right AT and secured further with adhesive dressing. For the fish oil group (FO), fish oil ointment was applied over the skin of the right AT and secured further with adhesive dressing. A cone collar was put on the neck of each rat to prevent the animals from removing the dressing which would dry off the other day. The fish oil ointment was prepared by mixing 40% fish oil (F8020; Sigma-Aldrich, UK) with 60% Vaseline. The fish oil was standard refined Menhaden oil and comprised 10–15% EPA and 8–15% DHA. This ointment was changed daily for each animal. The animals in the ultrasound group (US) received pulsed ultrasound at a duty cycle of 50% at 1 MHz, at an intensity of 0.5 W/cm^2 for 4 min daily (spatial average temporal average of 0.25 W/cm^2) (Ng and Wong 2008; Ng et al. 2003), with Vaseline acting as the coupling medium. An ultrasound machine (Dynatron 150 Plus, Dynatronics, Salt Lake City, Utah, USA) with a 2.0 cm^2 soundhead was used throughout the study. The machine was calibrated with an ultrasound wattmeter (UW4 Ultrasound Wattmeter, Fluke Biomedical, Carson City, Nevada, USA) before the experiment. The combination group (FU) received similar treatment as the US group, except that fish oil

Table 1. Details of the three treatment groups and the control group

Group	Treatment	Details of the ointment/coupling agent	Ultrasound parameters
Control at 2 wk and Control at 4 wk	Topical Vaseline	Vaseline plain petroleum jelly	NA
Fish oil at 2 wk and Fish oil at 4 wk	Topical fish oil ointment	Fish oil ointment which consisted of 40% fish oil and 60% Vaseline	NA
Ultrasound at 2 wk and Ultrasound at 4 wk	Ultrasound and Vaseline	Vaseline plain petroleum jelly	50% duty cycle, 1 MHz at the intensity of 0.5 W/cm^2 for 4 min
Combination at 2 wk and Combination at 4 wk	Ultrasound and fish oil ointment	Same as fish oil group	Same as ultrasound

NA = not applicable.