Emerging Ideas

The Effect of Hypercholesterolemia on Tendons

Joseph A. Abboud MD, David P. Beason MS,
Louis J. Soslowsky PhD

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Abstract

Background High levels of total cholesterol, triglycerides, and low-density lipoprotein are toxic to the vascular endothelium and thus have long been associated with atherosclerosis. Several clinical studies have suggested that elevated cholesterol also has a negative effect on tendon structure and function. Data from our preliminary studies show that the patellar tendons of hypercholesterolemic knockout mice exhibit reduced baseline elastic modulus and strength postinjury compared with controls.

Questions/hypotheses We therefore hypothesized elevated cholesterol would be associated with diminished tendon mechanical properties.

Method of Study Using hypercholesterolemic (B6.129P2-Apoetm1Unc/J) mice, patellar tendons from control (C57BL/6) and knockout mice will be injured surgically at two different times. Subsequently, mechanical testing will be performed and data evaluated for differences in baseline and in healing between cholesterol groups. For healing assessment, data from the injured limb of each animal will be normalized to that of the sham-operated contralateral limb.

Significance We anticipate that such studies eventually will enable us to elucidate the link between elevated cholesterol and tendon disease. If a mechanistic cause of hypercholesterolemia causing tendon disease is clarified, then potentially therapeutic interventions such as the use of pharmacotherapy may be used to help combat this. In the future, it may become worthwhile to consider the presentation of tendinopathy as a trigger to measure serum cholesterol just as one might consider measuring serum glucose in patients presenting with adhesive capsulitis.

Hypothesis

Starting from the observation that high levels of total cholesterol, triglycerides, and low-density lipoprotein (LDL) are toxic to the vascular endothelium, we propose elevated cholesterol may have deleterious effects on tendons. In particular, we propose elevated cholesterol is a risk factor for tendinopathy and in turn hypothesized elevated cholesterol has a deleterious effect on tendon mechanical properties.

Each author certifies that he or she has no commercial associations (eg, consultancies, stock ownership, equity interest, patent/licensing arrangements, etc) that might pose a conflict of interest in connection with the submitted article.

J. A. Abboud (✉)

Department of Orthopaedic Surgery, University of Pennsylvania Health System, 3B Orthopaedics, Pennsylvania Hospital, 800 Spruce Street, 8th Floor Preston, Philadelphia, PA 19107, USA
e-mail: Joseph.Abboud@uphs.upenn.edu; abboudj@gmail.com

D. P. Beason, L. J. Soslowsky

McKay Orthopaedic Laboratory, University of Pennsylvania, Philadelphia, PA, USA

Background

Musculoskeletal disorders were reported by more than 100 million adults in the United States in 2005 alone [4]. Because most tendon injuries occur in middle-aged or older persons, it has been hypothesized that the decreased vascularity of older tendons contributes to increased attenuation rates in the setting of repetitive microtrauma [14]. Decreased vascularity may not be the only factor.

Hypercholesterolemia has been reported to affect approximately 17% of the US population 20 years and

older (National Center for Health Statistics, 2006 [15]), even in the presence of numerous cholesterol-lowering treatments. High levels of total cholesterol, triglycerides, and LDL have long been associated with a myriad of cardiovascular disorders, such as atherosclerosis; the proposed relationship is damage to the vascular endothelium—ie, soft tissue.

Clinical reports have noted a link among Achilles tendon thickness, hypercholesterolemia, and intima-media thickness of the carotid artery, suggesting thickening of the Achilles as a potential indicator of atherosclerosis [7]. Elevated cholesterol also has been implicated clinically in the formation of Achilles tendon xanthomas, pain, and ruptures. Ozgurtas et al. reported patients with Achilles ruptures had substantially increased total cholesterol, triglycerides, and LDL compared with control subjects [12]. High-density lipoprotein (HDL, commonly known as good cholesterol) was abnormally low in patients with tendon ruptures. Mathiak et al. reported elevated cholesterol in 83% of patients with Achilles tendon ruptures, whereas only 19% were aware of their condition [10]. Symptoms have been reported to improve in more than half of patients after lipid-lowering treatment [8].

In a previous study, we measured serum lipid profiles for patients presenting with full-thickness rotator cuff tears [1]. Patients with rotator cuff tears showed increased total cholesterol, triglycerides, and LDL in addition to decreased HDL compared with control subjects. These clinical findings have implicated hypercholesterolemia as a risk factor in rotator cuff tears and served as the impetus for our current study.

Hypercholesterolemia is believed to contribute to increased tendon injury in several ways. First, deposition of cholesterol byproducts, as mentioned previously, has been implicated in the formation of tendon xanthomas, which may change tendon mechanical properties and result in an increased rate of tendon ruptures [16, 17]. Second, hypercholesterolemia may alter the tendon's extracellular matrix (and in turn, biomechanical properties) in such a way that lends to increased injury or impaired healing. For example, Ronnema et al. [13] found embryonic fibroblasts reacted differently to hypercholesterolemic rat serum than to rat serum with normal cholesterol levels. They found hypercholesterolemic serum was less likely to stimulate the synthesis of noncollagenous proteins or incorporate glucosamine and cytidine (components of the extracellular matrix) compared with serum with normal cholesterol levels. Their study suggests elevated cholesterol levels may alter the biomechanical properties of tendons in a way that could promote injury or retard healing [13]. Third, our preliminary experiments using the patellar tendon of 10-month-old hypercholesterolemic mice have shown reduced baseline elastic modulus and strength compared

with controls [2]. Finally, we know that hypercholesterolemia impairs macrocirculation and microcirculation; however, it is still unclear how this vascular effect translates to tendons and their rate of injury [3, 14].

Proposed Program

Using a hypercholesterolemic mouse model, the biomechanical, histologic, and organizational properties of native and healing tendons will be evaluated. We will be using C57BL/6 control mice (CTL) and C57BL/6 mice deficient for apolipoprotein E (APOE) (B6.129P2-Apoetm1Unc/J) [5, 6]. These APOE mice have markedly elevated total plasma cholesterol levels and reduced HDL-to-LDL ratios and have been used previously as a model for humans with severe hypercholesterolemia [11]. The mice will be divided evenly into two age groups—14 weeks old, representing a mature age, and 10 months old, representing an aging group. Patellar tendons from CTL and APOE mice will be injured, as described previously [9], at either 14 weeks or 10 months of age (Fig. 1). We will use a full-thickness, partial-width (approximately 60%) patellar tendon injury model in mice to assess the effect of the hypercholesterolemic phenotype. The use of a central defect prevents the need for suture repair of the tendon. The right limb undergoes sham surgery, which includes all procedures except for the creation of the central defect. Skin incisions will be closed and mice will be allowed normal cage activity.

Animals will be euthanized 3 weeks postinjury to represent the earliest time that will show enough scar tissue filling the wound space to allow for completion of our assays. The remaining animals will be euthanized at the same ages, without injury, to provide age-matched baseline, uninjured data. For biomechanical evaluation, patellar tendons will be dissected, leaving the patella–tendon–tibia complex intact. Tendon cross-sectional area will be measured and specimens then will be tensile tested. Primary measurements will include maximum (failure) stress, elastic modulus, and percent relaxation.

Limitations

Our proposed studies have limitations that come with any animal model, most importantly the ability to translate this information into data that have clinical relevance for humans. The knockout mouse strain we use has marked hypercholesterolemia; whereas this has a clear benefit in showing potential systemic effects, it is not as clinically applicable because most patients have mixed hypercholesterolemia. To evaluate this limitation, we have been working on using a diet-induced hypercholesterolemic model

Fig. 1 The study design to be used in the testing of our hypothesis is shown.

in pigs and in rats using the rat rotator cuff injury model. Second, we propose two times (14 weeks and 10 months); we may need to use longer times (greater than 10 months) for exposure to elevated cholesterol in the experimental group because there may be a cumulative effect on tendons, which becomes more pronounced as the animal ages. Obviously, the cost of housing and caring for animals for such long periods of time limits our ability to do this.

Implications and Future Directions

The information gained from this study will affect the field of tendon biology and biomechanics through further identification of intrinsic risk factors for tendon disease. Although cholesterol is theorized to have a negative impact on the cardiovascular system, its effects on the musculoskeletal system have been largely ignored. The studies we propose to complete will provide us with better understanding of the implications of mixed hyperlipidemia on the long-term structure and function of tendons. Although many authors have theorized that tendon disease is multifactorial, we should account for these factors one by one in the hopes of reducing the impact of such costly musculoskeletal problems.

If our hypotheses prove correct and we find that some tendon tears are an indication of high cholesterol, we would be able to provide orthopaedic patients with valuable information critical to their musculoskeletal health and provide clinicians with an additional preventive treatment opportunity for patients with previously undiagnosed hyperlipidemia who are at risk for further musculoskeletal injury and potential cardiovascular events. Finally, the results gained from this work hopefully will lead other researchers to investigate the role of various systemic disorders and their effects on musculoskeletal tissues.

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