The speculation of mechanical stress and perceiving pain on the adipose tissue along the posteromedial tibial border - A histological study

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INTRODUCTION: We confirmed the adipose tissue along the posteromedial tibial border via gross anatomical examination [1]. Since the adipose tissue cause pain at the knee and heel, the adipose tissue inflammation along the posteromedial tibial border would cause medial shin pain, such as medial tibial stress syndrome. It has been reported that the adipose tissue absorbs mechanical stress and has nerve endings [2, 3]. However, it is unclear whether the adipose tissue is exposed to mechanical stress and perceives pain along the posteromedial tibial border or not. In order to speculate the functional role of the adipose tissue, a previous study investigated the distribution of fibrotic components around the adipocytes using scanning electronic microscope [2]. The infrapatellar fat pad exposed to mechanical stress had a fibrotic tissue network around the adipocyte; in contrary the adipose tissue on the anterior wall of the abdomen had scarce fibrotic components [2]. These fibrotic components contained collagen fibers of type I and III, which would contribute to mechanical properties [4]. Therefore, we could speculate whether the adipose tissue is exposed to mechanical stress or not by evaluating fibrotic components around the adipocyte. In addition, the adipose tissue of infrapatellar fat pads in patients with anterior knee pain had Substance P positive nerve fiber and would cause pain in the anterior knee [3]. By investigating the presence of Substance P positive reaction on the adipose tissue along the posteromedial tibial border, we could confirm whether the adipose tissue can perceive pain along the posteromedial tibial border or not. Thus, this study aimed to histologically confirm the presence of fibrotic components and Substance P positive reaction around the adipocyte along the posteromedial tibial border histologically.
METHODS: Six specimens of the adipose tissue along the posteromedial tibial border were sampled from cadavers (age at death, 83±10 years; male, 4; female, 2) donated to the University of the Ryukyus. Those were pre-fixed by 10 % formalin solution and were refixed by a half concentration of Karnovsky solution for 3~4 hours. **[Scanning electronic microscope]** After immersing in 2 % Osmium tetroxide post-fixes lipids for 3~4 hours, the specimens were dehydrated with ethanol and replaced t-Butyl Alcohol. Those were treated by a vacuum freeze dryer and carbon and platinum were vacuum-evaporated. We scanned the adipocyte surface. **[Immunohistochemical staining]** Firstly, the specimens were dehydrated with ethanol, and immersed in methyl benzoate and benzene. The specimens were embedded in paraffin wax and were sectioned (thickness 10 µm). The paraffin wax and an endogenous peroxidase were removed from those sections. Next, blocking using 3 % bovine serum albumin, each primary antibody was dropped on the sections (30 µl, 4 ℃, overnight). The sections were reacted by each second antibody (23 ℃, 30 minutes) and were stained using a 3,3'-Diaminobenzidine and methyl green for counterstaining. The collagen fibers of type of I and III, and Substance P were observed histologically. This study was approved by the Institutional Review Board of Waseda University, and University of Ryukyus. Consent was obtained using an opt-out option on Ryukyus institution's website; those who chose to opt-out were excluded.
RESULTS: **[Scanning electronic microscope]** All adipocytes had a basket-like fiber around the cells, and those fiber components formed a three-dimensional network (Figure 1). **[Immunohistochemistry]** We confirmed the positive reactions of collagen fibers of type I, III, and Substance P (Figure 2). Collagen fibers of type I was shown around adipocytes and formed bundles. Collagen fibers of type III was presented not only around adipocytes but also intercellular matrix. Those collagens connected some adipocytes. The immunolocalization of Substance P also was observed around adipocytes, similar to collagen fibers of type III.
DISCUSSION: This study showed that the adipose tissue along the posteromedial tibial border had adipocytes which formed a three-dimensional network and a basket of fibrotic components. These histological findings were similar to the findings of the infrapatellar fat pad in the previous study [2]. These histological characteristics may indicate that the adipocytes along the posteromedial tibial border are exposed to mechanical stress. Furthermore, these fiber components included collagen fibers of type I and III; particularly there was a trend of stronger immunolocalization of collagen fibers of type I. When a material composed of stiff particles embedded within a fibrous network was compressed, fibrous tissue stretched and maintained its structure [5]. Since collagen fibers of type I is abundant in tendon and ligament and resist elongation stress, our results of collagen immunostaining indicate that the adipose tissue along the posteromedial tibial border would be exposed to compression stress. This findings correspond to anatomical feature that the adipose tissue is sandwiched between the tibia and muscle or tendon of the flexor digitorum longus and posterior tibialis muscle [1]. It has been suggested that the infrapatellar fat pad in anterior cruciate ligament injury patients had more collagen fibers of type III than healthy individuals to confer elasticity and flexibility [4]. In addition, the adipose tissues would perceive pain by being exposed to excessive mechanical stress because they had immunolocalization of Substance P. Considering the results of this study, the adipose tissue would be exposed to mechanical stress and perceive pain. The adipose tissue along the posteromedial tibial border would be compressed excessively by the flexor digitorum longus and tibialis posterior muscle in medial tibial stress syndrome patients. This is greater hindfoot eversion and forefoot abduction during running in medial tibial stress syndrome patients would demand activities excessively of these muscles compared with healthy athletes [6] Hence, the adipose tissue would be one of the aetiologies of medial tibial stress syndrome.
SIGNIFICANCE/CLINICAL RELEVANCE: The study indicated that the adipose tissues along the posteromedial tibial border would be exposed to mechanical stress, and would perceive pain. This adipose tissue would be one of the aetiologies of medial tibial stress syndrome. We should investigate the presence of the adipose tissue inflammation in medial tibial stress syndrome patients.
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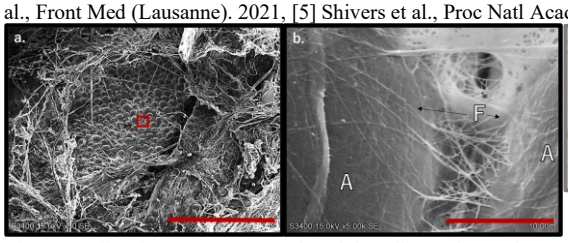

Figure 1. Fibrotic components enclosed the adipose tissue along the posteromedial tibial border. a) bar = 1mm, b) bar = 10µm. Imaging expanding red rectangle. A; adipocyte, F; fibrous tissue

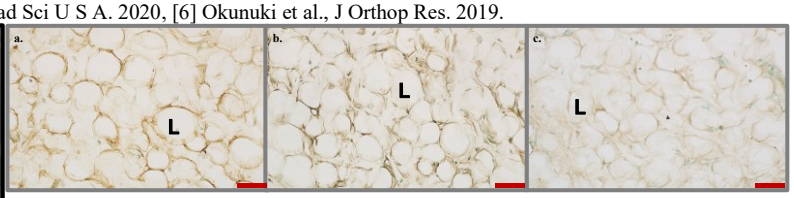

Figure 2. Imunnohistochemical staining. a) Collagen fibers of type I. b) Collagen fibers of type III. c) Substance P. Bar = 50µm.L; Lipid in adipocyte, brown around adipocyte indicated immunolocalization of collagen or Substance P.