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● *Original Contribution*

DETERMINATION OF THE AUGMENTATION EFFECTS OF HYALURONIC ACID ON DIFFERENT HEEL STRUCTURES IN AMPUTATED LOWER LIMBS OF DIABETIC PATIENTS USING ULTRASOUND ELASTOGRAPHY

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Abstract—This study measured tissue properties of different anatomies of heels in amputated lower limbs of diabetic patients before and after hyaluronic acid (HA) or normal saline (NS) injections. Seven amputated lower limbs from six diabetic patients constituted the experimental group and one amputated lower limb from a diabetic patient served as the control. The limbs were placed in a fixation platform. A 5–12 MHz linear-array ultrasound transducer controlled by a stepping motor was used to load and unload tested heels. The loading-unloading velocity was 6 mm/s and the maximum loading stress was 178 kPa. Loading-unloading tests were performed before and after 1 mL HA injections into heels in the experimental group. The control limb underwent the same test before and after 1 mL NS injection. The unloaded thickness and Young's modulus of the macrochambers, microchambers and heel pads were determined before and after the interventions. The unloaded thickness of the macrochambers and the heel pad increased significantly ($p = 0.012$) after HA injection. The Young's modulus of the macrochambers decreased nonsignificantly after HA injections. Similar thickness and tissue stiffness changes were observed in the control limb. The baseline heel-pad energy dissipation ratio (EDR_{hp}) was 81.3 ± 1.3% and decreased significantly ($p = 0.012$) to 73.1 ± 1.7% after HA injections. The EDR_{hp} in the control increased after NS injection. Histologic examinations revealed localized HA accumulation in the macrochambers with an extension into the adjacent fibrous septa. Injection of HA can increase tissue thickness and enhance heel-pad tissue resilience. (E-mail: steele0618@gmail.com)

Key Words: **Diabetic foot, Hyaluronic acid, Biomechanics, Elastography, Ultrasonography.**

INTRODUCTION

Diabetes mellitus is a chronic metabolic syndrome characterized by altered glucose metabolism secondary to diminished insulin secretion and/or ineffective insulin action. An estimated 347 million of persons worldwide suffered from this disease (Danaei et al. 2011). The prevalence of foot ulcers in diabetic patients ranges from 4% to 10% (Singh et al. 2005) and 12% to 25% of diabetic patients are at the risk of developing such lesions during their lifetime (Andersen and Roukis 2007). The exact incidence of diabetic heel ulcers is not known but the limb salvage rate in diabetic patients with heel ulcers is two to three times lower than that in diabetic patients with metatarsal ulcers (Cevera et al. 1997).

Custom footwear and orthotics have been advocated for off-loading plantar pressures in diabetic patients and for accommodating disease-related foot deformities (Tyrrell 1999). However, the incidence of foot ulcer recurrence in diabetic patients who wore therapeutic footwear was similar to those wearing normal footwear in a large-scale randomized control trial (Reiber et al. 2002). The result suggests that factors other than high plantar pressures can contribute to the development of foot ulcers. The diabetic foot ulcers have long been known to be the result of hyperglycemia induced angiopathy, peripheral neuropathy, high plantar pressure and altered plantar soft tissue properties (Caputo et al.

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1994; Hsu et al. 2000; 2002). Therefore, early detection of deteriorating tissue properties in heels of diabetic patients to prevent impending heel ulcers is an important issue in current diabetic foot cares.

The heel pad contains organized fibrous compartments retaining adipose tissue and is anatomically divided into a superficial microchamber layer, an inherent heel cup and a deep macrochamber layer, a native shock absorber (Blechschmidt 1982; Hsu et al. 2007). The unloaded tissue thickness, Young's modulus representing tissue stiffness (E) and the energy dissipation ratio (EDR) reflecting nonlinear soft tissue properties, are important parameters for evaluating heel-pad biomechanical behaviors (Hsu et al. 2000). *In vivo* measurements of unloaded heel-pad thickness (UT_{hp}) in diabetic patients have been reported. Ultrasound assessments have reported both increased (Gooding et al. 1985; Hsu et al. 2000) and decreased UT_{hp} (Zheng et al. 2000) in diabetic patients. Increased heel-pad stiffness (Brink 1995; Klaesner et al. 2002; Zheng et al. 2000) and elevated EDR in diabetic heels (Hsu et al. 2000, 2002) have also been observed in diabetic patients. In another study (Hsu et al. 2009), tissue properties of different heel-pad layers in diabetic patients were evaluated using an ultrasound based loading-unloading device with a loading velocity of 6 mm/s and a maximum loading stress of 78 kPa. The results showed that decreased microchamber stiffness and increased macrochamber stiffness resulted in diminished heel-cushion ability in diabetic patients.

It is believed that soft tissue properties provide not only basic information about the material itself but also indicate the presence of a disease (Jahss et al. 1992; Ophir et al. 2000). Collagen fibrils with varying degrees of deterioration and a heterogeneous distribution of the involved fibrils are typical connective tissue features in diabetic heel pads (Hsu et al. 2002). To improve plantar soft tissue properties in diabetic patients, soft tissue augmentation therapy has been attempted. Liquid silicone has been used for soft tissue augmentation, in an off-label manner, for decades (Narins and Beer 2006). In one clinical study, the recurrence of foot ulcers and plantar keratosis was examined in 30 diabetic patients with healing ulcers and healing keratosis who received injections of 0.2–1.2 mL liquid silicone into the plantar subdermal soft tissues. Among these patients, about 80% showed no post-therapy recurrence (Balkin and Kaplan 1991). Nonsignificant reduction in callus formation has also been reported in 14 diabetic patients received 0.2 mL liquid silicone injections in comparison with the other 14 diabetic patients who had equal amount of saline injections. Liquid silicone injections are associated with a significant increase in plantar soft tissue thickness and a significant decrease in peak plantar pressures (van Schie et al. 2000). The complications of small-volume liquid silicone injections are mild and include fluid migration, skin pigmentation and inflammation (Balkin and Kaplan 1991). Nevertheless, renal failure may occur in patients receiving large volumes of injected silicone (Khan and Sim 2010). Therefore, plantar soft tissue augmentation with silicone injections is still controversial and has not been approved by the United States Food and Drug Administration.

Hyaluronic acid (HA), a major component of the extracellular matrix, is nonimmunogenic and binds a large amount of water to form a viscous gel (Fujii et al. 2002). Intra-articular viscosupplementation therapy with HA is gaining popularity as a treatment option in patients with knee osteoarthritis (Strauss et al. 2009). As a result, it is conceivable to use HA for plantar soft tissues augmentation to prevent the occurrence of foot ulceration in diabetic patients. HA injection into heels is not an approved technique and is not allowed by the Taiwanese Department of Health. *In vivo* trials cannot be proposed until sufficient *in vitro* data has been obtained. High-resolution ultrasonography is a reliable tool to measure human heel-pad thickness (Rome 1998). Ultrasound elastography is an imaging technique used to calculate tissue displacement before and after tissue compression and allows a noninvasive estimation of tissue stiffness. This is a versatile technique in diagnosing soft tissue pathologies in different locations (Ophir et al. 2000). This study attempted to measure tissue properties of different anatomic structures in heels of amputated lower limbs from diabetic patients using a loading-unloading device and ultrasound elastography. Assessments were done before and after HA or normal saline (NS) injections into tested heels.

MATERIALS AND METHODS

Four below-knee and three above-knee amputated lower limbs of one female and five male patients with type 2 diabetes mellitus comprised the experimental group. An additional right below-knee amputated lower limb from a male diabetic patient served as the control. All data were presented as mean ± standard error of mean. The control limb was taken from a 62-year-old diabetic patient with a body mass index (BMI) of 25.8 kg/m^2. The disease duration and glycohemoglobin (Hb_{a1c}) level of this patient were 5 years and 8.7%, respectively. The mean age of the diabetic patients in the experimental group was 65.8 ± 5.5 years and this group of patients had an average BMI of 22.3 ± 6.0 kg/m^2. In this group, the average disease duration and Hb_{a1c} level were 17 ± 2 years and 8.0 ± 0.9%, respectively. The enrolled amputated lower limbs had forefeet gangrene complicated with uncontrolled infection. The heels in these patients