Obesity Induced Tendinopathy-like Histopathological Changes in Tendon and Maintained TDSCs in a Healing State with Low Regenerative Capacity

Yuk Wa Lee[1], Cheuk Hin Kot[1], Ssu Chi Chen[1], Chi Ming Wong[2], Pauline Po Yee Lui[1,3,*]

[1] Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China

[2] Department of Health and Information Technology, The Hong Kong Polytechnic University, Kowloon, Hong Kong SAR, China

[3] Center for Neuromusculoskeletal Restorative Medicine, Hong Kong Science Park, Shatin, New Territories, Hong Kong SAR, China (* Corresponding author)

Email of Presenting Author: angellee@link.ort.cuhk.edu.hk

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INTRODUCTION: Degenerative tendinopathy is a tendon overuse disorder with disappointing treatment outcomes and unclear etio-pathogenesis. Obesity increases tendinopathy's risk but its influences on tendon's health is unclear. This study aimed to examine the impacts of obesity on the histopathology of tendons and its influences on the inflammation pro-resolving power, cell fate, and regenerative capacity of tendon-derived stem / progenitor cells (TDSCs) in vitro.

METHODS: The clinical and animal research ethics committees approved the study. Tendon samples were collected from patients after obtaining their written consent. The effects of obesity on tendon histopathology were examined in hamstring tendons of individuals with normal BMI and overweight/obese. Mice were fed with standard chow (SC) or hight-fat diet (HFD) for 12 weeks. The Achilles tendons were harvested for (1) histology/immunohistochemical staining of inflammatory markers and matrix remodeling enzymes; or (2) TDSC isolation and characterization. Self-renewal capacity, proliferation, apoptosis, autophagy, and cellular senescence of TDSCs were assessed by colony-forming unit (CFU) assay, Alamar blue reduction assay, TUNEL assay, immunofluorescent staining of LC3 autophagosome marker, and β-galactosidase activity assay, respectively. IL-1β was used to mimic overuse-induced inflammation in tendons. The mRNA expression of proliferation marker, pluripotency markers, apoptotic markers, autophagy markers, inflammatory markers, matrix remodeling markers with/without IL-1β treatment was assessed by qRT-PCR. The osteogenic and adipogenic differentiation potential of HFD-TDSCs and SC-TDSCs were compared after induction with Alizarin Red S staining and Oil Red O staining, respectively.

RESULTS: HFD induced histopathological changes in mouse tendons resembling tendinopathy (n=4-5/group) **(Figure 1)**. There were an increase in proteoglycan deposition as shown by Alcian blue staining and presence of ectopic bone as confirmed by von Kossa staining in the HFD group. Similar histopathological changes were observed in hamstring tendons of individuals with obesity (30.7±3.2 kg/m^2) but were not observed in individuals with normal BMI (22.1±0.8 kg/m^2) (n=5/group).

The balance of pro- and anti-inflammatory cytokines is perturbed, favouring a pro-inflammatory status in both HFD tendons and HFD-TDSCs. The expression of pro-inflammatory cytokine (IL-6) and anti-inflammatory cytokine (IL-10) was upregulated in HFD tendons compared to SC tendons (n=4-5/group). Similarly, HFD-TDSCs showed higher expression of *Il6* ($p<0.05$), *Tnfa* ($p<0.01$) and *Il33* ($p<0.05$) compared to SC-TDSCs at the basal state (n=4-5/group) **(Figure 2)**. While IL-1β only slightly increased inflammation in SC-TDSCs, it dramatically increased tendon inflammation in HFD-TDSCs, with much higher expression of pro-inflammatory cytokines (*IL6*, *Tnfa*) and reduced expression of anti-inflammatory cytokine (*Il33*) **(Figure 2)**.

The expression matrix matelloproteinase (MMP-3) was upregulated while the expression of inhibitor of matrix metalloproteinase (TIMP-1) remained unchanged in HFD tendons compared to SC tendons (n=4-5/group). Similarly, HFD-TDSCs showed elevated expression of *Mmp3* (basal: $p<0.05$; IL-1β: $p<0.01$) and *Mmp3*/*Timp1* (basal: $p<0.05$; Il-1β: $p<0.01$) but similar expression of *Timp1* compared to SC-TDSCs with/without IL-1β treatment (n=4-5/group). The fold-change of *Mmp3* and *Mmp3*/*Timp1* after IL-1β treatment was significantly higher in HFD-TDSCs compared to SC-TDSCs.

HFD-TDSCs showed higher CFU compared to SC-TDSCs under both basal ($p<0.05$) and inflammatory ($p<0.01$) conditions (n=6/group). HFD did not affect the expression of pluripotency markers in TDSCs at basal state. However, HFD-TDSCs showed higher expression of pluripotency markers *Nanog* ($p<0.01$) and *Sox2* ($p=0.05$) compared to SC-TDSCs after IL-1β treatment (n=4-5/group).

HFD-TDSCs showed higher cell proliferation compared to SC-TDSCs (n=6/group) ($p<0.01$). This was consistent with higher expression of *Pcna* in HFD-TDSCs compared to SC-TDSCs under basal condition (n=4-5/group) ($p<0.05$). However, IL-1β increased the mRNA expression of *Pcna* in HFD-TDSCs ($p<0.05$) but not in SC-TDSCs (n=4-5/group) ($p>0.05$).

Despite higher proliferation of HFD-TDSCs (n=6/group) ($p<0.01$), these cells also displayed higher cellular senescence ($p<0.05$) (n=6/group), autophagy (n=9/group) ($p<0.01$), and apoptosis (n=3/group) ($p<0.05$) compared to SC-TDSCs **(Figure 3)**. The results were confirmed by the mRNA expression of autophagy and apoptotic markers in HFD-TDSCs at basal state and after IL-1β treatment. HFD-TDSCs expressed higher levels of autophagy markers (*Nbr1*, *Atg14*, *Atg7*) compared to SC-TDSCs after IL-1β treatment (n=4-5/group) (all $p<0.01$), and IL-1β increased the expression of autophagy markers (*Nbr1*, *Atg7*) in HFD-TDSCs, but not in SC-TDSCs. HFD-TDSCs showed higher expression of pro-apoptotic markers *Bax* (both conditions $p<0.05$) and *Bak1* (both conditions $p<0.01$) (n=4-5/group) under both basal and inflammatory conditions. They also showed higher expression of anti-apoptotic markers compared to SC-TDSCs under inflammation. Specifically, while HFD-TDSCs showed lower expression of *Bcl2* at basal state ($p<0.05$), the expression of anti-apoptotic markers, *Bcl2* ($p<0.05$), *Bcl2l1* ($p<0.01$) and *Mcl1* ($p<0.01$), was significantly higher in HFD-TDSCs compared to SC-TDSCs after IL-1β treatment.

Compared to SC-TDSCs, HFD-TDSCs showed higher ostengenic and adipogenic differentiation upon induction.

DISCUSSION: HFD induced histopathological changes resembling degenerative tendinopathy. HFD tendon and TDSCs showed higher inflammation and matrix degeneration. The inflammation-resolving power of HFD-TDSCs was significantly lower compared to SC-TDSCs after IL-1β treatment. IL-1β exacerbated the expression of pro-inflammatory cytokines and matrix degrading enzymes in HFD-TDSCs. This finding might explain the higher risk of tendinopathy in obese patients. HFD-TDSCs showed higher CFU, proliferation and expression of pluripotency markers under an inflammatory condition. This is likely due to chronic inflammation in these cells, which maintain them in an active healing state. The activation threshold of HFD-TDSCs after tendon injury is hence lower. However, they showed lower regenerative capacity, as revealed by altered cell fate, higher cellular senescence, apoptosis and autophagy. The impaired healing response of HFD-TDSCs might predispose microinjury accumulation after repeated microtrauma and hence tendinopathy development.

CLINICAL RELEVANCE: This study provided a metabolic perspective for the increased risk of tendinopathy in obese patients and the roles of inflammation and impaired response of TDSCs in its pathogenesis. HFD- tendons and TDSCs are useful models to explore the pathogenesis and treatment of tendinopathy.

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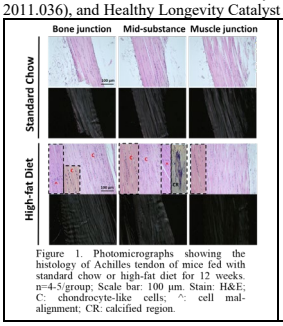

Figure 1. Photomicrographs showing the histology of Achilles tendon of mice fed with standard chow or high-fat diet for 12 weeks. n=4-5/group; Scale bar: 100 μm. Stain: H&E; C: chondrocyte-like cells; ^: cell mal-alignment; CR: calcified region.

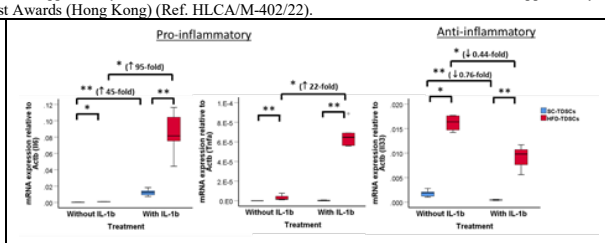

Figure 2. Boxplots showing the expression of *Il6*, *Tnfa* and *Il33* in SC-TDSCs and HFD-TDSCs under basal and inflammatory conditions, n=4-5/group, *p<0.05; ** p<0.01

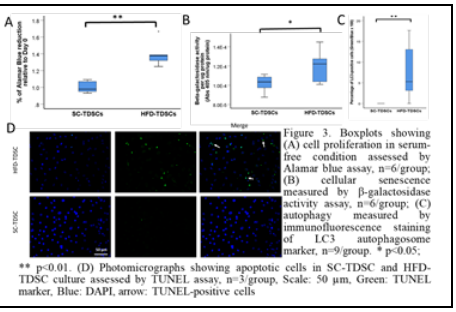

Figure 3. Boxplots showing (A) cell proliferation in serum-free condition assessed by Alamar blue assay, n=6/group; (B) cellular senescence measured by β-galactosidase activity assay, n=6/group; (C) autophagy measured by immunofluorescence staining of LC3 autophagosome marker, n=9/group. * p<0.05; ** p<0.01. (D) Photomicrographs showing apoptotic cells in SC-TDSC and HFD-TDSC culture assessed by TUNEL assay, n=3/group, Scale: 50 μm, Green: TUNEL marker, Blue: DAPI, arrow: TUNEL-positive cells