Compared to SC-TDSCs, HFD-TDSCs showed higher osteogenic and adipogenic differentiation upon induction. While HFD-TDSCs showed lower expression of autophagy markers, HFD-TDSCs showed higher expression of pro-inflammatory cytokines (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 Il13 (p<0.05) compared to SC-TDSCs at the basal state (n=4-5/group) (Figure 2). The fold-change of Mmp3 and Mmp13 changes in HFD-TDSCs compared to SC-TDSCs under basal condition (n=4-5/group) was significantly higher in HFD-TDSCs compared to SC-TDSCs. 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).

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 Il13 (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-TSCDs, with much higher expression of pro-inflammatory cytokines (IL-6, IL-7) and reduced expression of anti-inflammatory cytokine (IL-10) (Figure 2). The expression matrix metalloproteinase (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 Mmp13/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 inflammation-resolving power of HFD-TDSCs was significantly lower compared to SC-TDSCs after IL-1β treatment (n=4-5/group).

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).

The expression of pluripotency markers in HFD-TDSCs was consistent with higher expression of Pcn 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 Pcn 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), 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) (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) 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 senescence and apidogenic 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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**Figures:**

- Figure 1: Phenotypic and immunohistochemical analysis of HFD and SC TDSCs, showing the expression of TNFα, TGFβ1, and TIMP1 in HFD and SC TDSCs. TNFα expression was higher in HFD TDSCs compared to SC TDSCs.
- Figure 2: mRNA expression of osteogenic and adipogenic markers in HFD and SC TDSCs. HFD TDSCs showed higher expression of osteogenic markers compared to SC TDSCs.
- Figure 3: CFU assay showing the colony formation capacity of HFD and SC TDSCs. HFD TDSCs showed higher CFU compared to SC TDSCs.

**Disclosure:** Nil.