Comparison of Basal and Cytokine Stimulated Metabolism of Tendon Autografts used for Anterior Cruciate Ligament Reconstruction

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Introduction and Purpose

- ~ 200,000 anterior cruciate ligament reconstructions (ACLR) are performed annually in the U.S.
- Hamstring tendon (HT), patellar tendon (PT), and quadriceps tendon (QT) autografts are common sources for ACLR.
- The environment of the joint after ACL injury and surgery is pro-inflammatory, which may have negative effects on the early ligamentization process of the tendon autografts.
- This study was designed to assess the metabolic responses of HT, PT, and QT autograft explants cultured with or without IL-1β stimulation to simulate an inflammatory stimulus in a post-surgical knee.

Hypotheses

- There will be no significant differences in basal metabolic profiles among tendon graft types.
- Pro-inflammatory cytokine stimulation will incite production of significantly higher levels of inflammatory and degradative biomarkers from PT grafts when compared to QT and HT grafts.

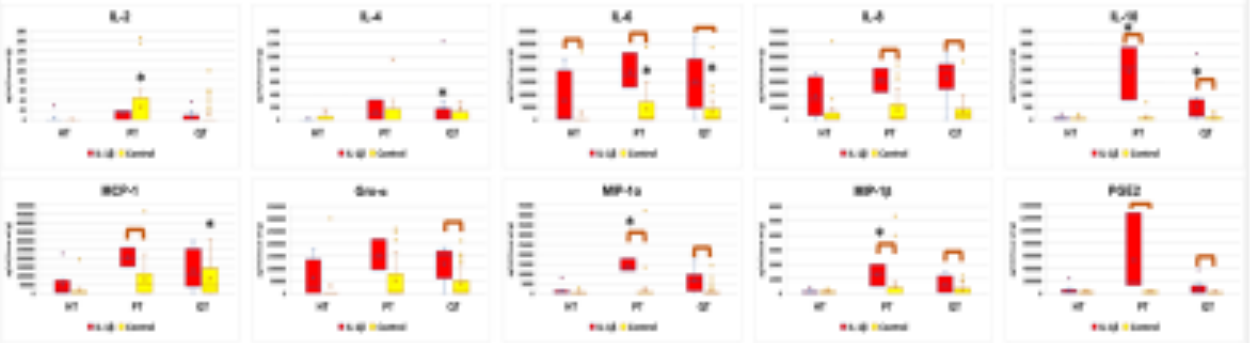
Methods

1) HT (n=8), PT (n=16), and QT (n=16) tissues were collected from patients (n=18; mean age 16.66 range 12-62; 13 females and 16 males, 3 unknown) undergoing anterior or posterior cruciate ligament reconstruction surgeries (IRB 2008878).
2) 4mm explants (n=3/tissue/patient) were created and cultured for 3 days in supplemented DMEM with or without IL-1β. After 3 days, media was collected for biomarker analyses.
3) Media were tested for IL-2, IL-4, IL-6, IL-8, IL-10, MCP-1, MCP-3, GRO-α, MIP-1α, MIP-1β, RANTES, TNF-α, VEGF, PDGF-AA, MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-13, total MMP activity (MMPALT), TIMP-1, TIMP-2, TIMP-3, TIMP-4, ADAMTS4, and PGE2 using commercially available assays according to the manufacturers' protocol.
4) For statistical analysis, the concentrations of media biomarkers were standardized to the wet weights of the explants. Significant differences between IL-1β treated and untreated controls for each tissue type were determined using Mann-Whitney Rank sum tests. Significant differences among tissue types with or without IL-1β stimulation were determined using Kruskal-Wallace tests with post-hoc analyses. Significance was set at p≤0.05.

Conclusions

- Hamstring tendon, patellar tendon, and quadriceps tendon autografts have unique metabolic biomarker profiles with and without pro-inflammatory cytokine stimulation.
- Patellar tendon and quadriceps tendon autografts are associated with higher basal production of inflammatory and degradative biomarkers and are more responsive to cytokine stimulation compared to hamstring tendon autografts.
- Ongoing studies in our lab are aimed at relating these observations to graft healing in patients and comparing clinical responses associated with these common types of tendon autografts in order to improve outcomes for patients undergoing ACL reconstruction.

Results



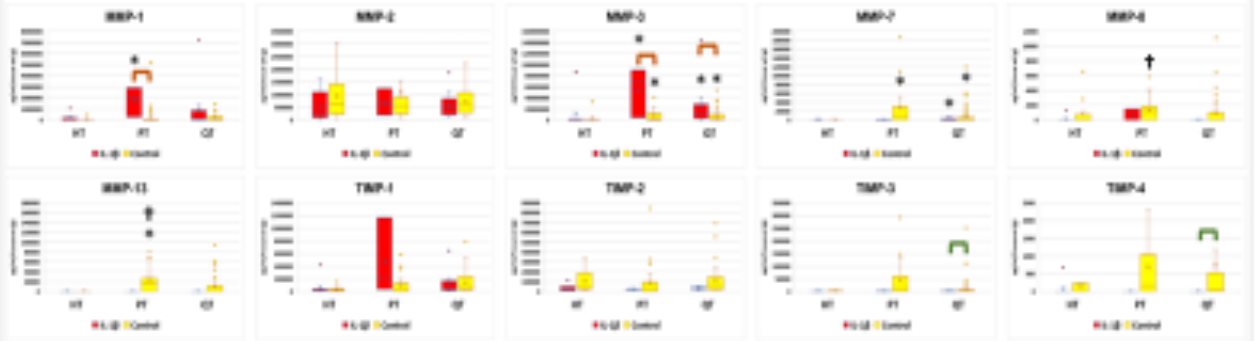
- Within HT group
  - IL-1β > Control: IL-6
  - Control > IL-1β: No significant differences
- Within PT group:
  - IL-1β > Control: IL-6, IL-8, IL-10, MCP-1, MIP-1α, MIP-1β, PGE2
  - Control > IL-1β: TNF-α
- Within QT group
  - IL-1β > Control: IL-6, IL-8, IL-10, GRO-α, MIP-1α, MIP-1β, TNF-α, PGE2
  - Control > IL-1β: No significant differences

Brackets indicate significant differences between treated and untreated ACL or SYN

- Differences between ACL graft types - Basal
  - PT > HT: IL-2, IL-8
  - QT > HT: IL-6, MCP-1
  - PT vs. QT: No significant differences
- Differences between ACL graft types - IL-1β stimulated
  - PT > HT: IL-10, MIP-1α, MIP-1β, VEGF
  - QT > HT: IL-10, IL-6
  - PT vs. QT: No significant differences

(*) Indicates significance compared to the respective HT group

Results



- Within HT group
  - IL-1β > Control: No significant differences
  - Control > IL-1β: No significant differences
- Within PT group:
  - IL-1β > Control: MMP-1, MMP-3
  - Control > IL-1β: No significant differences
- Within QT group
  - IL-1β > Control: MMP-8
  - Control > IL-1β: TIMP-3, TIMP-4

Brackets indicate significant differences between treated and untreated ACL or SYN

- Differences between ACL graft types - Basal
  - PT > HT: MMP-3, MMP-7, and MMP-13
  - QT > HT: MMP-3, MMP-7
  - PT > QT: MMP-8, MMP-13
- Differences between ACL graft types - IL-1β stimulated
  - PT > HT: MMP-1, MMP-3
  - QT > HT: MMP-3, MMP-7
  - PT vs. QT: No significant differences

(*) Indicates significance compared to the respective HT group

(†) Indicates significance compared to the respective QT subgroup