

GLUCOSE CONCENTRATION INCREASES IGF EXPRESSION FROM SYNOVIAL MEMBRANE

Final Report

Aug 17 2009

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INTRODUCTION

There is some clinical evidence to suggest that 10% dextrose injected into a joint may have a therapeutic effect on osteoarthritis. We hypothesized that the increased sugar concentration may stimulate the synovium cells to secrete anabolic growth factors that would have a therapeutic effect on degenerating cartilage. To test this hypothesis we harvested synovial tissue from patients undergoing total knee arthroplasty and exposed it to different concentrations of glucose. We selected insulin-like growth factor as the most likely to respond to glucose and measured gene expression as well as protein release in the culture media.

This phase was to provide proof of concept for a larger more definitive study that would monitor changes in normal and arthritic cartilage co-cultured with synovial tissue.

METHODS

1. Tissue Source and Harvest

-Synovial explants (n=6) were harvested from human donors undergoing either uni-compartmental or total knee replacement. In all but the first experiment, the specimens were trimmed of extraneous adipose tissue and washed in low-glucose DMEM (1 g/L). The specimens in the first experiment were not trimmed of excess adipose tissue and were washed in high-glucose DMEM (4.5 g/L).

2. Glucose Treatment

In the first experiment (n=2), the specimens were cultured for 48 hours in 0.1% calf serum and either high- or low-glucose DMEM. In the second two experiments (n=4), the specimens were cultured for 48 hours in serum-free ITS (Insulin, Transferrin, Selenium) media diluted with either high- or low-glucose DMEM.

3. IGF-1 gene expression

Briefly, total RNA was extracted from synovial tissue using RNeasy Total RNA Kit (Qiagen Inc., Santa Clarita, CA). Real-time quantitative RT-PCR was done using Taqman RT-PCR reagents (Applied Biosystems, Foster City, CA). Expression of IGF-1 was normalized to that of the housekeeping gene GAPDH.

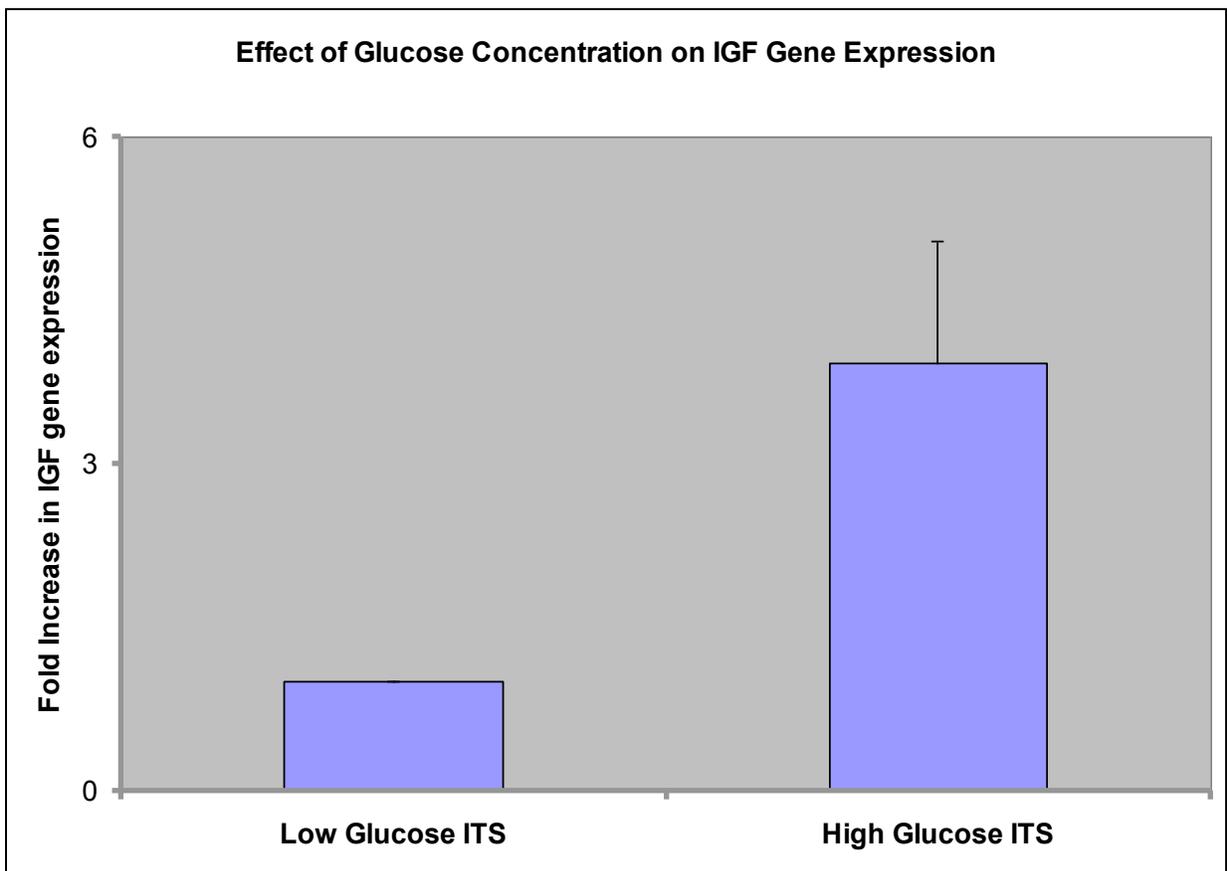
4. IGF-1 ELISA

Human IGF-1 protein secretion into the culture media was measured by ELISA (R & D Systems, Minneapolis, MN). Synovial culture media was centrifuged to remove any particulates. Controls and samples were added to ELISA wells which were pre-coated with a monoclonal antibody specific for IGF-1. Any IGF-1 present would be expected to be bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IGF-1 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells. The media developed a color intensity in proportion to the amount of IGF-1 bound in the initial step. Optical density of each well was determined by using a microplate reader set to 450 nm. IGF-1 concentration in the sample was calculated by a standardized curve of IGF-1 activity obtained by serial dilution of known protein concentrations.

RESULTS

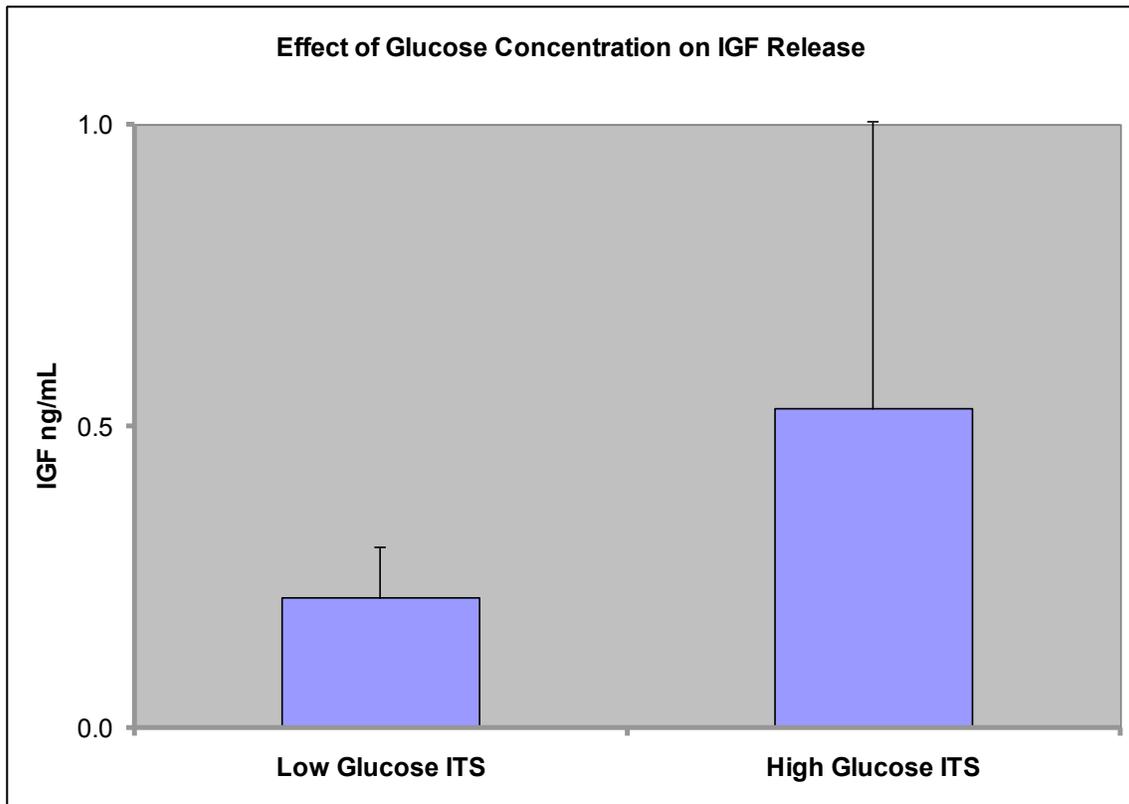
1. Increasing glucose concentration increases IGF-1 gene expression

We tested the effect of glucose concentration on synovial tissue explants in three separate experiments (three donors). Synovial explants were treated with media containing either low (1g/L) or high glucose (4.5g/L) in media for 48 hours. In all three donors, glucose concentration increased IGF-1 gene expression by up to 5 fold.



2. Increasing glucose concentration increases IGF-1 secretion.

We tested the effect of glucose concentration on synovial tissue explants in 6 separate experiments (6 donors). Synovial explants were treated with media containing either low (1g/L) or high glucose (4.5g/L) in media for 48 hours. On average, IGF-1 levels in media more than doubled. However, we noted a donor-dependent response to glucose concentration. In two donors, glucose concentration had no effect on IGF-1 protein release in media, while in four donors there was a significant response. Since the tissue came from patients undergoing knee arthroplasty, disease status may play a role. The tissue that responded the most to high glucose (5x increase) came from a donor undergoing unicompartmental arthroplasty and appeared the healthiest on visual examination.



CONCLUSIONS

1. Increasing glucose concentration consistently increased IGF-1 gene expression in synovial tissue.
2. Increasing glucose concentration increased IGF-1 secretion in synovial tissue, but in a donor dependent fashion.
3. The response of synovial tissue to glucose concentration may be dependent on osteoarthritis disease status.