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# Fructose Alters Cell Survival and Gene Expression in Microglia and Neuronal Cells Lines

Drew P. Seidel
*St. John Fisher College*, dps02008@students.sjfc.edu

Susan DeNapoli
*St. John Fisher College*, smd02656@students.sjfc.edu

Thomas S. Maciulewicz
*St. John Fisher College*, tsm01174@students.sjfc.edu

Melinda E. Lull
*St. John Fisher College*, mlull@sjfc.edu

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**Abstract**
Purpose: Microglia are macrophages that are found primarily in the CNS and play a crucial role in maintaining a healthy brain by engulfing invading microorganisms, releasing inflammatory mediators, and pruning dead cells. Microglia can become activated in response to certain stimuli which causes them to transition into a pro-inflammatory state, and can sometimes become chronically activated which can result in neuronal damage. Studies have shown a causal relationship between this activation and sugars such as fructose and glucose. We sought to understand the role of sugars in microglial activation and the subsequent effects on neuron health.

Methods: Rat microglia (HAPI) and neuronal (B35) cell lines were treated with varying concentrations of fructose (25 mM, 12.5 mM, and 6.25 mM) or glucose (25 mM and 12.5 mM)as a positive control to determine their effects on the cells. Following treatment and incubation for 3 or 24 hours, the cells were analyzed using an MTT assay to measure cell survival or real-time polymerase chain reaction (RT-PCR) to measure gene expression levels. Effects of fructose were measured in HAPI microglia after direct treatment with the sugar. The genes investigated by the RT-PCR in the HAPI cells included: glucose transporter 5 (GLUT5), and the inflammatory markers high mobility group box 1 (HMGB1), and prostaglandin E receptor 2 (Ptger2). To evaluate the effects of microglial activation on neuronal function, the B35 neurons were treated either directly with sugars or with the supernatant collected from fructose-treated HAPI microglia. This allows examination of the effects of soluble neuron-injury factors released by microglia. The genes investigated by RT-PCR in B35 neurons included nuclear factor-κB (NFκB) and enolase 2 (Eno2).

Results: Cell survival assays showed that 24-hour direct fructose treatment increased B35 cell survival by up to 13%, while groups treated with microglia supernatant increased cell survival by up to 33%. In HAPI microglia, 3 hours of treatment with fructose caused GLUT5 expression to be suppressed by up to 32% in all treatment groups except for 6.25 mM fructose, while Ptger2 and HMGB1 expression was increased by as much as 65% and 15%, respectively. After 24-hours of treatment with fructose, the HAPI microglia showed a maximum of 80% increased expression of HMGB1, while Ptger2 expression was mostly unchanged. In B35 neurons, 3 hours of treatment with fructose caused a decrease of up to 26% in NFκB and an increase of up to 46% in Eno2 expression.

Conclusion: Cell survival results indicate that the microglia may provide a short term protective effect on the B35 neurons. However, data from the gene expression assays show evidence of cellular dysfunction in neurons and pro-inflammatory activity in microglia which may lead to neuronal death on a longer timeline. As seen in the gene expression results, microglia had increased expression of pro-inflammatory genes and B35 neuronal cells had increased expression of markers of cellular damage. Future studies will further explore the effects of fructose on expression of other genes and examine the effects on neuron survival at later time points.

**Keywords**
fsc2017

**Disciplines**
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# Fructose alters cell survival and gene expression in microglia and neuronal cell lines

Drew P. Seidel[1], Susan M. DeNapoli[1], Thomas S. Maciulewicz[1], Melinda E. Lull, Ph.D. [2]
[1]Pharm.D. Candidate. [2]Department of Pharmaceutical Sciences
**St. John Fisher College, Wegmans School of Pharmacy, Rochester, NY**

## Introduction

Microglia are macrophages that are found primarily in the CNS and play a crucial role in maintaining a healthy brain by engulfing invading microorganisms, releasing inflammatory mediators, and pruning dead cells[1]. Microglia can become activated in response to certain stimuli which causes them to transition into a pro-inflammatory state, and can sometimes become chronically activated which can result in neuronal damage[1,2]. Studies have shown a causal relationship between this activation and sugars such as fructose and glucose[3].

## Objective

We sought to understand the role of sugars in microglial activation and the subsequent effects on neuron health. We hypothesized that B35 cell survival would be decreased with treatment of microglial supernatant, and gene markers of cellular damage would be increased.

## Methods

Rat microglia (HAPI) and neuronal (B35) cell lines were treated with varying concentrations of fructose (25 mM, 12.5 mM, and 6.25 mM) or glucose (25 mM and 12.5 mM) as a positive control to determine their effects on the cells. Following treatment and incubation for 3 or 24 hours, the cells were analyzed using an MTT assay to measure cell survival or real-time polymerase chain reaction (RT-PCR) to measure gene expression levels. Effects of fructose were measured in HAPI microglia after direct treatment with the sugar. The genes investigated by the RT-PCR in the HAPI cells included: glucose transporter 5 (GLUT5), and the inflammatory markers high mobility group box 1 (HMGB1) and prostaglandin E receptor 2 (Ptger2). To evaluate the effects of microglial activation on neuronal function, the B35 neurons were treated either directly with sugars or with the supernatant collected from fructose-treated HAPI microglia. This allows examination of the effects of soluble neuron-injury factors released by microglia. The genes investigated by RT-PCR in B35 neurons included nuclear factor kappa B (NFkB) and enolase 2 (Eno2).

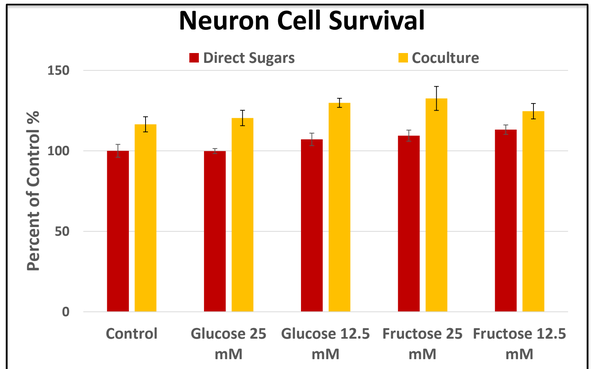


**Figure 1: Effect of glucose and fructose on B35 cell survival.** Direct fructose treatment increased cell survival by up to 13%, while groups treated with microglia supernatant increased cell survival by up to 33%.

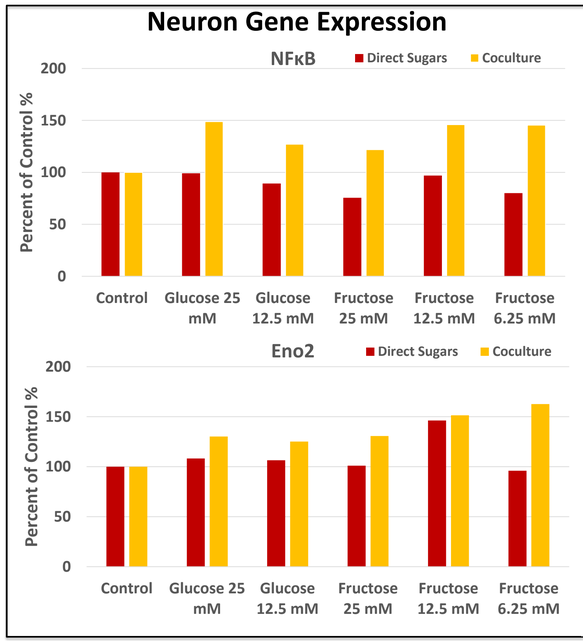


**Figure 2: Gene expression changes in HAPI cells after 3 and 24 hour treatment.** GLUT5 expression was suppressed by up to 32% following treatment with all treatment groups except for 6.25 mM fructose. Ptger2 and HMGB1 expression was increased by as much as 65% and 15%, respectively. After 24 hours, GLUT5 expression was increased by as much as 60% after 24 hour treatment with fructose. HMBG1 expression was increased to a maximum of 81%, while Ptger2 expression was mostly unchanged.

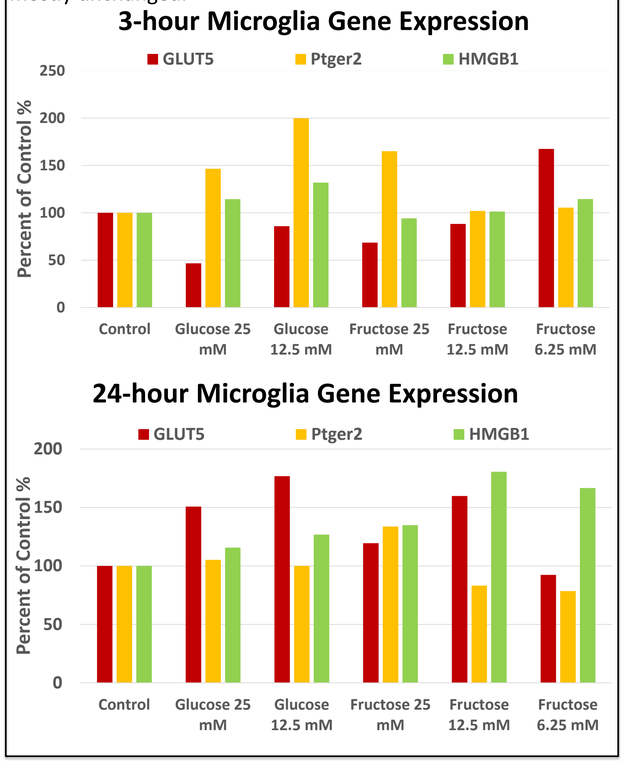


**Figure 3: Gene expression changes in B35 cells after 3 hour treatment.** NFkB expression was decreased by up to 26%, while Eno2 expression was decreased by up to 46% after 3 hours of treatment with glucose or fructose.

## Conclusions

- Microglia may have a short term protective effect on survival of B35 neurons.
- Gene expression assays show evidence of pro-inflammatory activity in microglia and cellular dysfunction in neurons after treatment with glucose or fructose.
- In HAPI microglia cells, GLUT5 expression was suppressed at the 3 hour time point, but was increased at 24 hours.

## References

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## Disclosures

The authors of this presentation have no personal or financial interest in the subject matter of this presentation.
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