Direct Effects of Glucose and Fructose on Proliferation and Activation of Jurkat T-Lymphocytes

C.J. Urso

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Direct Effects of Glucose and Fructose on Proliferation and Activation of Jurkat T-Lymphocytes

by

C.J. Urso

A Master’s Thesis Submitted to the Faculty of

Montclair State University
College of Science and Mathematics
Department of Biology and Molecular Biology

In Partial Fulfillment of the Requirements

For the Degree of

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Physiology Concentration

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Abstract

High-fructose corn syrup has been used with increasing commonality in recent decades. During this interval there is strong evidence of increased occurrence of autoimmune disease. We hypothesized that increased glucose and fructose concentration activates T-cells. Using Jurkat T-cells as a model, we found that increased glucose and fructose concentration decreased Jurkat T-cell proliferation and death rates at pH 7.4 while at pH 7.2 the above effects did not reach significance. Cells in high glucose and fructose concentration formed clusters that were unbreakable by standard trituration techniques suggesting the expression of high-affinity adhesion molecules characteristic of T-cell activation; this effect was more prominent in fructose than glucose formulations. We conclude that exposure of activated T-cells to sustained higher sugar concentration may contribute to the development of autoimmune disease. This thesis continues to discuss suggested future directions of this research, its applications, and implications in the pathophysiology of autoimmune disease.
DIRECT EFFECTS OF GLUCOSE AND FRUCTOSE ON
PROLIFERATION AND ACTIVATION OF JURKAT T-LYMPHOCYTES

A THESIS

Submitted in partial fulfillment of the requirements
For the degree of Master of Science

by

C.J. URSO
Montclair State University
Montclair, NJ
2012
The author wishes to express sincere appreciation to the committee members of this thesis for their criticisms and guidance and especially to Professor Vladislav Snitsarev for his vast reserve of knowledge, experience and patience.

This thesis would never have been completed without the support and encouragement of my parents Charles and Margaret Urso.
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B. List of Abbreviations

(GLUT) Glucose-Family Transporter
(ICAM) Intracellular adhesion molecule
(JTL) Jurkat T-Lymphocyte
C. Thesis Text
   a. Introduction

Hyperglycemia

A higher than normal glucose concentration in plasma is common in certain physiological and pathophysiological states. Glucose concentration is elevated during such conditions as uncontrolled diabetes[2], sympathoexitation[1], systemic inflammation[2] and transiently elevated after consumption of food with high sugar contents[3]. All above-mentioned conditions are shown to be associated with either T_H-cell activation or inflammation where T_H-cells are firmly known to be activated.

Fructose and inflammation

Consumption of food with high fructose contents results in a higher than normal total glucose and fructose, hereafter referred to as simple sugars, concentration in plasma[3]. Fructose has been shown to induce the expression of the inflammatory and adhesion molecule ICAM-1 in endothelial cells[4]. High glucose has also been shown to induce endothelial inflammation[5]. As such, inflamed endothelium is the most likely place where activated T-cells are arrested with homotypic high-affinity adhesion molecules and start extravasation.

Direct effects of glucose and fructose on T-cell activity

It is known that glucose transporter molecules GLUT1 and GLUT3 are expressed in resting lymphocytes, that lymphocytes do not express GLUT2 or GLUT4, and that GLUT5 mRNA but not protein has been detected in resting lymphocytes[6]. There is not, however, clear data available on GLUT family transporter expression in various stages of lymphocyte activation. Though there are
numerous studies and reviews which demonstrate contribution of elevated concentration of simple sugars, especially glucose and fructose, to $T_H$-cell activation and inflammation on the systemic and organ levels, there are no studies on how higher concentrations of glucose and fructose affect $T_H$-cell activity at a cellular level.

**Hypotheses**

We hypothesize that (1) a higher glucose concentration decreases Jurkat T-Lymphocyte (JTL) proliferation rate and that (2) fructose does not affect JTL proliferation. We also hypothesize that (3) a higher glucose concentration decreases JTL death rate and that (4) fructose does not affect JTL death. Using 5.6 mM glucose as a control, here we report the effects of 11.2mM glucose (double normal) and 5.6mM glucose + 5.6mM fructose on the activity of Jurkat T-cells (JTL).
b. Methods and Materials

Cell Culture

Jurkat T-cells (E6-1 line) stored in liquid nitrogen were rapidly thawed and resuspended in Advanced RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum and 2 mM Glutamax in small culture flasks. Flasks for pH 7.4 formulations were incubated at 37°C with 95% air/5% CO₂. Those for pH 7.2 formulations were incubated 90% air/10% CO₂ with all other parameters being identical, thereby creating an acidic environment without the use of buffers or other chemical additives. Cultures were split and fresh media added to maintain the density of viable cells at approximately 10⁵ cells/mL as needed. The absence of media contamination was visually using light microscopy; biochemical assays for mycoplasmal contamination were not performed. Experimental cultures were placed in a 12-well plate with 4 wells for each control (5.6 mM glucose and 5.6 mM glucose + 5.6 mM fructose), and each treatment group (11.2 mM glucose and 5.6 fructose). Formulations containing 5.6 mM glucose, and 5.6 mM glucose + 5.6 mM fructose were used as controls because neither Glut-5 expression nor fructose uptake by JTL has been demonstrated.

Trypan Blue Assay

Cell viability was assessed by inspection of cells using light microscopy and trypan blue assay. For this, cells 50μL of triturated cell suspension was combined with 50μL of microfiltered trypan blue and mounted on a standard hemocytometer. Counts of live and dead cells for all samples were obtained by the same technician as well as counts of clusters of 3, 4 and 5 or more. Clusters of 2 were counted for total
cell count but not for cluster count as they are visually indiscernible from mitotically diving cells. Manual counting was employed in anticipation of automated counter error due to cluster formation. Micrograph images were obtained using a Nikon Eclipse E200 microscope with a Nikon DN100 digital camera. All images were retained for further analyses including cell diameter distribution and possible recounts (Figure 4a-f).

Formulae

Proliferation rate (PR) and death rate (DR) were determined by the formulae below such that live cell counts at 0 hours (A0) and at 48 hours (A48) and such that dead cell counts at 0 hours (D0) and 48 hours (D48).

\[
PR = \frac{1}{2} \ln \left( \frac{A0}{A48} \right) \times \left( \frac{D48 - D0}{A48 - A0} + 1 \right) \\
DR = \frac{1}{2} \ln \left( \frac{A48}{A0} \right) \times \left( \frac{D48 - D0}{A48 - A0} \right)
\]

Statistical Analysis

Data were evaluated for significance using a two-tailed Student’s T-test with significance threshold p<0.05. Treatment interaction effects were determined by the two-way ANOVA (factorial) method using Statistical Analysis System (SAS) version 9.3.
c. Results

Proliferation Rate

We observed a significant decrease in proliferation rate of glucose treated cells at pH 7.4 compared to control (p < 0.05). The decrease in proliferation of fructose treated cells did not reach significance (p = 0.135). There was no significant differences in the respective proliferation of both glucose and fructose treatments at pH 7.2 (Figure 1).

Death Rate

We next investigated the respective effects of glucose and fructose on cell death. A significant decrease in death rate at pH 7.4 of both glucose (p < 0.05) and fructose (p < 0.05) when compared to 7.4 control was observed. A similar decrease in cell death was observed at 7.2 but it did not reach a significant level. Although death decreased at pH 7.2 compared to pH 7.4, it was not significant (p=0.114) (Figure 2).

Clusterization as a measurement of physiological CAM activation

Clusters of three or more cells were counted, excluding clusters of two which are difficult to visually discern from mitotically active cells. Cluster counts of three, four and five or more cells were compiled for graphing and statistical analysis by the original formula below:

\[ \text{Total clustered cells} = (3)(\text{Clusters of 3}) + (4)(\text{Clusters of 4}) + (5)(\text{Clusters of 5}) \]

Significant increases in the number of clusterized cells at pH 7.4 was observed in fructose (p < 0.001) and glucose (p<0.005) when compared to control, and the difference between glucose and fructose was not significant (p=0.260). Similar to pH
7.4, at pH 7.2 the number of clusterized cells in both glucose (p < 0.05) and fructose (p < 0.001) was significantly different from control; however, at this pH the difference between glucose and fructose was significant (p<0.005). Furthermore, when comparing within sugar treatment groups, glucose treatment formed significantly more clusterized cells at pH 7.4 than 7.2 (p < 0.01) while in fructose treatment this difference was not significant (p = 0.106)
d. Discussion

In this study we hypothesized that protracted increases in sugar concentration contribute to activation of T-cells and provide evidence to support this hypothesis in vitro using Jurkat T-cells. To our knowledge this is the first evidence of direct activation of T-cells by commonly used sugars, glucose and fructose.

**The role of simple sugars in T-cell activation**

After activation by an antigen-presenting cell, T-cells undergo a period of clonal expansion in the secondary lymph nodes prior to entering the circulation where they can encounter elevated glucose and fructose concentrations similar to those examined in the present study. T-cells move freely through the circulation until they come in contact with chemokines released from inflamed endothelium which activate already expressed, but inactive, high affinity adhesion molecules on T-cell membranes.

**What does a decrease in Proliferation rate mean for T-cell activation?**

We observed that increased sugar concentration results in decreased cell proliferation rate within 48 hours. This effect supports our hypothesis that increased glucose decreases T-cell proliferation in glucose formulations. A decrease in proliferation was also seen in fructose formulations, possibly suggesting that activated T-cells must up-regulate GLUT5 and are capable of utilizing fructose, contrary to previously published results. A similar suppression of proliferation in association with increased glucose concentration has been reported in fibroblasts[7] and endothelial cells[8] where the effect was reversible by
antioxidant treatment implicating free radicals in the mechanism of this observation. With regard to T-cells, we reason that the observed effect on proliferation may result in lymphopenia in instances of chronic elevation of serum sugar levels. Thereby, our data offer potential evidence that supports a possible developmental mechanism of lymphopenia, which has been recently reviewed and described as elusive[9]. Lymphopenia drives homeostatic proliferation of T-cells which results in an increased population of memory-like T-cells rather than increasing the naïve cell pool[9]. These conditions have been shown to lead to an increase T-cell activation[9].

What does a decrease in death rate mean for T-cell activation?

We observed that increased sugar concentration results in decreased T-cell death rate. This effect was also seen in both glucose and fructose groups, again possibly suggesting that in states of activation lymphocytes change their expression profiles of GLUT family transporters. Similar anti-apoptotic effects have been reported in other cell types in association with increased glucose metabolism[10,11,12]. One study proposes the mechanism that glucose metabolism inhibits apoptosis by redox inactivation of cytochrome c[12]. The above-mentioned role of free radicals and the here referenced effect of redox signaling in chronic inflammatory states have been shown to affect T-cell function[13]. Our data are consistent with the predictions of these reports and may demonstrate that elevated sugar concentrations, resulting in hyperglycemic-induced production of free radicals and redox inactivation of cytochrome c, can lead to T-cell activation.
What does clusterization rate mean for T-cell activation?

We observed that increased sugar concentration promotes cellular adhesion in T-cells which caused the formation of clusters. While intracellular adhesion molecule 1 (ICAM-1) is continuously present in T-cell membranes in low levels, the cluster forming effect seen here is possibly a product of the up-regulating effect of glucose metabolism on ICAM-1 expression. This phenomenon has been reported in other cell types including endothelial cells[14,15] and has been shown to increase leukocyte-endothelial cell adhesion[16].

Our data show that sugar metabolism affects the functioning of cell adhesion molecules. *In vitro*, this resulted in the formation of T-cell clusters potentially by homotypic adhesion, *in vivo* this could result in the adhesion of T-cells to endothelial cells and T-cell extravasation[16]. There is some evidence suggesting a novel model of intracellular signaling which results from ICAM-1 cross linking in endothelial cells[17]. In conjunction with this report, our data further demonstrate how increased sugar metabolism can contribute to T-cell activation.

**Relevance in Disease States**

The findings of our study have potential relevance for a number of disease states including chronic stress, diabetes, chronic inflammation and autoimmune disease as well as various other conditions not discussed at length herein.

**Stress and sympathoexcitation**

Epinephrine acts on α- and β-adrenergic receptors which inhibit insulin release, stimulate glucagon release and stimulate gluconeogenesis and glycolysis. Cortisol has a similar effect on glucose mobilization. These conditions result in increased serum glucose concentration. As described in the previous subsection, our results may present a potential mechanism by which chronic stress can contribute to T-cell activation.

Furthermore, disease states associated with sympathoexcitation include chronic heart failure[18], chronic obstructive pulmonary disorder[19], coronary artery disease[20] and others which we speculate can also contribute to T-cell activation. Accordingly, present study adds to the ongoing discussion of these disorders as a part of the constellation of cardiovascular disorders that often occur with diabetes and are referred to as the Metabolic Syndrome. Our data contribute some causal evidence to the largely undefined understanding of the Metabolic Syndrome by delineating the inter-relationships between sugar concentration and T-cell activation and cardiovascular disease, diabetes, chronic inflammation and autoimmune disease.

**Diabetes and Chronic Inflammation**

Diabetes is a chronic condition that is seen in association with by low-grade systemic Inflammation[21] which is known to promote the release of chemokines by endothelial tissues. Uncontrolled diabetes represents a state of long-term hyperglycemia and is a close *in vivo* manifestation of the experimental conditions we have constructed. The findings of our study are
therefore of particular significance in diabetes. Our data support observations that diabetes suppresses immune status by identifying the anti-proliferatory effect of hyperglycemia and suggest that the physiologic effects of the diabetic condition contribute to activation of T-cells long-term which in turn may lead to conditions such as autoimmune disease. Consistent with this, there is evidence that identifies an increasing population of diabetic patients with comorbid autoimmune diseases[22].

Autoimmune Disease

Our discussion of data has repeatedly enumerated our assertions that chronic elevations in sugar concentration can lead to T-cell activation. This overactivity of the immune system can lead to autoimmune disease. This effect is due in large part to the effects of homeostatic proliferation and decreased T-cell death rate as well as changes in T-cell adhesion molecule activity and increased release of chemokines by inflamed endothelium. High-fructose corn syrup has been used with increasing commonality in recent decades[23]. During this interval there is strong evidence of markedly increased occurrence of a multitude of autoimmune diseases on a global scale[24,25,26,27,28,29,30]. Our data help to explain these trends in public health at the cellular level.

Perspectives

Future directions of this young but compelling research should focus on elucidating the mechanistic enigma of these reported observations at the molecular level. To this end, this research would benefit from further characterizing the activity of T-cells under these experimental conditions. This information can be
obtained through ELISA assays of cell suspension supernatant for interleukins, intracellular calcium studies using Fura2 and immunohistochemical stains for membrane proteins including adhesion molecules. Genetic studies focusing on GLUT family expression should also prove informative. Organismal studies will lend further understanding to the findings we witness at the cellular level.
Tables and Figures

**Figure 2: T-cell proliferation rate**

![Graph showing T-cell proliferation rate](image)

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Table 1: T-cell proliferation rate P-values

**Figure 2: T-cell death rate**

![Graph showing T-cell death rate](image)

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Table 2: T-cell death rate P-values
Figure 3: T-cell cluster formation

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**Figure 4a:** Micrograph of JTL cells, Control 0h

**Figure 4b:** Micrograph of JTL cells, Glucose 0h
Figure 4c: Micrograph of JTL cells, Fructose 0h

Figure 4d: Micrograph of JTL cells, Control 48h
Figure 4e: Micrograph of JTL cells, Glucose 48h

Figure 4f: Micrograph of JTL cells, Fructose 48h
D. Bibliography


