Persistence or reversibility of fructose induced brain alterations after switching to healthy diet

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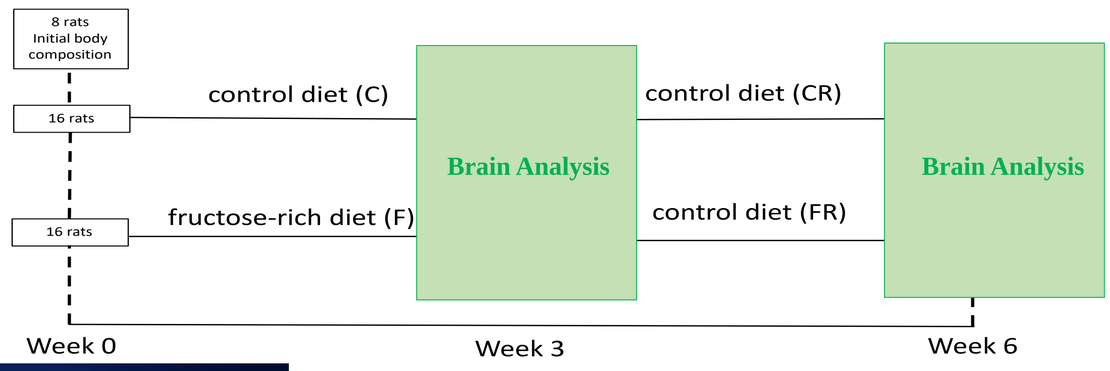
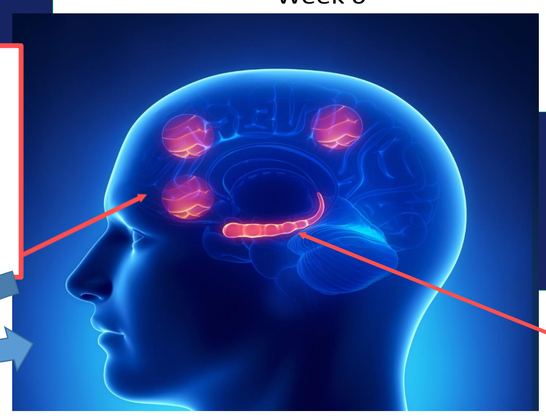
Abstract: In previous decades, a significant increase in the fructose content of the human diet has occurred, far above what is introduced daily with fruits and vegetables, because of the increased consumption of industrial foods and the extensive commercial use of high-fructose corn syrup (HFCS) as a sweetener for beverages, coffee, snacks, and bakery foods. High fructose consumption has long been known to expose the consumer to metabolic health risks, such as obesity, lipid alterations, insulin resistance, and inflammation. Metabolic risk factors related to poor nutrition can arise at a very early age; hence, investigations are required to clarify the brain consequences resulting from a diet rich in fructose at a critical stage of development. Indeed, despite the importance of this issue, few studies have been performed in rodents, providing evidence that, in childhood and adolescence, critical periods of neurocognitive development, the impact of high dietary fructose consumption on hippocampal and cortex function is particularly damaging.

Introduction: The young age is often characterized by a high consumption of processed foods and fruit juices rich in fructose. These habits are critically involved in obesity induction, and also promote alterations in brain function that could persist even with the return to a healthy diet.

Methods: Young rats (30 days old) were fed a high fructose or control diet for 3 weeks. At the end of treatment half of fructose-fed rats were fed a control diet for further 3 weeks to investigate the possible persistence of the brain changes. Glucose transporter-5 (Glut-5), fructose and uric acid levels, oxidative status, inflammation, as well as survival markers of synaptic function were investigated by Western blotting and spectrophotometric or enzyme-linked immunosorbent assays, in the hippocampus and prefrontal cortex, areas of the brain critically involved in learning and memory.

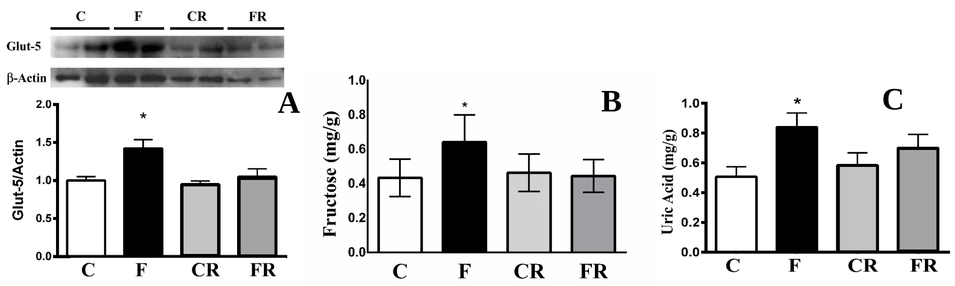


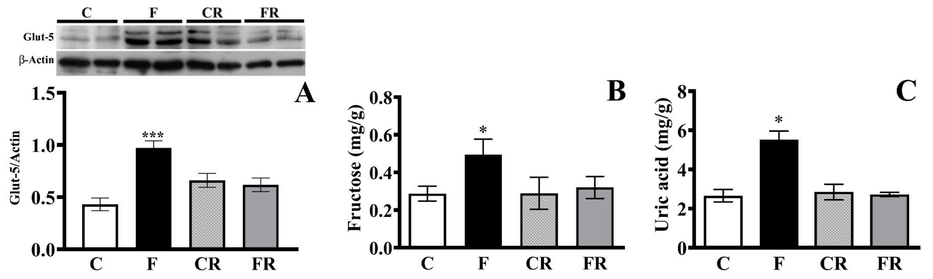


PREFRONTAL CORTEX

HIPPOCAMPUS

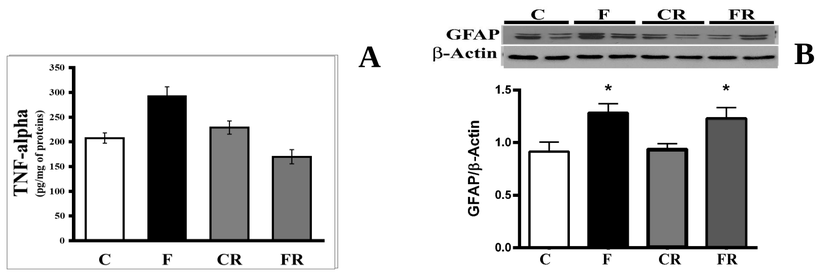
Glut-5 Expression, Fructose and Uric Acid Level

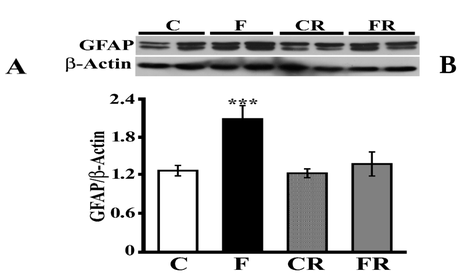




To obtain information on the delivery of fructose to brain cells, we quantified the protein expression of Glut-5, the specific fructose transporter, as well as the levels of fructose and uric acid, one of the main products of fructose metabolism, in the hippocampus and cortex after 3 weeks of consuming a fructose-rich diet. The Glut-5 concentration was significantly higher in the hippocampi ($p < 0.001$; Figure A on the right) and prefrontal cortex ($p < 0.05$ Figure A on the left) of F rats compared to in C rats, while this increase disappeared in FR rats. In line with this result, significant increases in hippocampus and prefrontal cortex levels of fructose ($p < 0.05$; Figure B on the right and left) uric acid ($p < 0.05$; ; Figure C on the right and left) were found in F rats compared to C rats, while no significant differences were found in FR rats (Figure B,C on the left and right). (A) Glut-5 level (with representativeWestern blot), (B) fructose and (C) uric acid concentrations in the hippocampi and prefrontal cortex of control (C), fructose-fed (F), control rescued (CR), and fructose-rescued (FR) rats. Data are the means ± SEM of eight rats/group. * $p < 0.05$ versus control rats; *** $p < 0.001$ versus control rats. Source of variation: one-way ANOVA followed by Tukeys post-test.

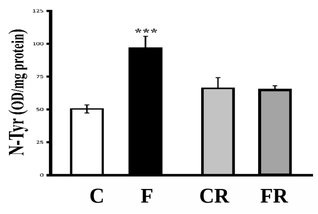
Markers of Inflammation

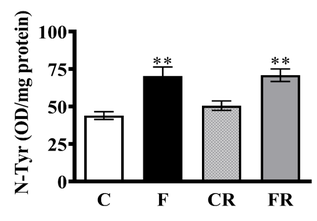




To further clarify the brain inflammatory mechanisms activated by a fructose-rich diet and the putative ability of a subsequent 3-week control diet to rescue the fructose-induced alterations, we evaluated TNF-alpha, and GFAP as marker of astrogliosis. The TNF-alpha level was significantly higher in the hippocampi ($p < 0.01$; Figure A on the right) and prefrontal cortex ($p < 0.01$; Figure A on the left) of F rats, while it returned to control levels in FR rats. In agreement with results from TNF-alpha titration, we found a significant increase of GFAP in the hippocampi ($p < 0.001$; Figure B on the right) and prefrontal cortex ($p < 0.01$; Figure B on the left) of F rats, which returned to levels comparable to those of C rats in FR rats. Overall, these results show that a fructose-rich diet is associated with an increase in key inflammation markers in the hippocampus and prefrontal cortex, and this nutritional insult is fully rescued after switching to a control diet. It is noteworthy that the diet-induced increases in fructose and uric acid levels occurred in parallel with the hippocampal and prefrontal cortex inflammatory status, since the switch to a control diet normalized brain fructose and the uric acid level and brought back almost all the inflammatory parameters to values comparable to those of control rats. (A)TNF-alpha concentration (titrated by sandwich ELISA), (B) GFAP level (with representative Western blot) in protein extracts from the hippocampi and prefrontal cortex of control (C), fructose-fed (F), control rescued (CR), and fructose-rescued (FR) rats. Data are the means ± SEM of eight rats/group. * $p < 0.05$ versus control rats ;** $p < 0.01$ versus control rats; *** $p < 0.001$ versus control rats. Source of variation: one-way ANOVA followed by Tukey's post-test.

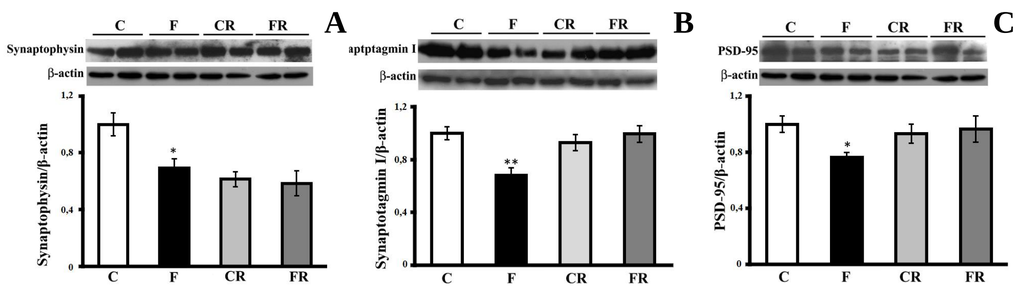
Marker of Oxidative Status

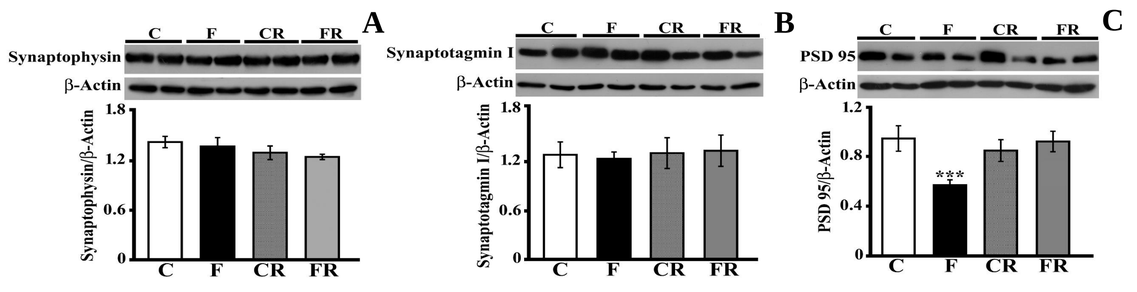




As the strong link between inflammation and reactive oxygen species (ROS) formation is well-known, we investigated the hippocampal and prefrontal cortex oxidative status in the different rat groups and determined whether switching to a control diet can restore redox homeostasis alterations back to physiological levels. N-Tyr, the footprint of protein oxidative damage induced by peroxynitrite, was assessed as a marker of oxidative damage to proteins. Enhanced diet-associated oxidative damage to proteins in hippocampus ($p < 0.01$; Figure on the right), and prefrontal cortex ($p < 0.001$, Figure on the left) were found in F rats. Of note, while the concentration of N-Tyr in hippocampus remained higher in FR with levels comparable to those found of F rats ($p < 0.01$; Figure on the right), in prefrontal cortex the N-Tyr levels in FR rats returned to levels comparable to those in C rats (Figure on the left). This result can be explained by the fact that N-Tyr is a very stable marker of oxidative/nitrative stress and suggests that in hippocampus protein turnover may control the return of the N-Tyr concentration to the initial values. As a matter of fact, brain protein turnover depends on multiple factors, such as the cell type, intracellular environment, specific protein functions, and protein interactions, with the half-lives of neuronal protein ranging from <2 to >14 days. N-Tyr levels in the hippocampi and prefrontal cortex of control (C), fructose-fed (F), control rescued (CR), and fructose-rescued (FR) rats. Data are the means ± SEM of eight rats/group. ** $p < 0.01$, *** $p < 0.001$ versus control rats. Source of variation: one-way ANOVA followed by Tukey's post-test.

Analysis of Synaptic Proteins





The levels of key pre (synaptophysin and synaptotagmin I) and post (PSD-95) synaptic proteins were measured in the hippocampi and prefrontal cortex of all groups of rats. Synaptophysin, the most abundant presynaptic vescicle protein, plays critical dual roles in both exocytosis and exoendocytosis coupling processes. Synaptotagmin I, a major calcium sensor for transmitter release, participates in the clamping of synaptic vesicle fusion in mammalian neurons. The fructose-rich diet did not affect the synaptophysin and synaptotagmin levels in hippocampi (Figure A,B on the right), while the concentration of PSD-95, which is involved in the function of neurotransmitter receptors, was significantly decreased in the hippocampi of F rats ($p < 0.001$, Figure C on the right), but its levels returned to control values in FR rats. In the prefrontal cortex the levels of synaptophysin, synaptotagmin, and PSD-95 were significantly lower ($p < 0.05$, $p < 0.01$, $p < 0.05$; Figure A,B,C on the left) in F rats, while it returned to control levels in FR rats. (A) synapthophysin level (with representative Western blot), (B) synaptotagmin I (with representative Western blot), (C) PSD 95 level (with representative Western blot) in the hippocampi and prefrontal cortex of control (C), fructose-fed (F), control rescued (CR), and fructose-rescued (FR) rats. Data are the means ± SEM of eight rats/group. * $p < 0.05$ versus control rats; ;** $p < 0.01$ versus control rats ; *** $p < 0.001$ versus control rats. Source of variation: one-way ANOVA followed by Tukeys post-test.

Conclusions: The picture that emerges from this study, conducted on a young rodent model, confirms that fructose can have a strong impact on brain function at a young age by promoting inflammation of the hippocampus and prefrontal cortex, oxidative stress and alteration in post-synaptic proteins. These changes could undoubtedly have an important impact on neuronal activity and, in general, on cognitive function, especially at a young age, a very critical phase of brain development. Most of the alterations induced by a high fructose diet can be saved by returning to a control diet. A notable exception is represented by the levels of N-Tyr, a marker of oxidative stress, which remain higher as an imprint of the previous damage in hippocampus. The investigation of the real consequences of persistent alterations in these markers certainly deserves further attention and may represent a problem for further studies. It cannot be ruled out that a longer period of fructose intake may favor brain alterations to a greater extent that are difficult to reverse with the return to a healthy diet.