Title
Brain Trauma Disrupts Hepatic Lipid Metabolism: Blame It on Fructose?

Journal
Molecular nutrition & food research, 63(15)

ISSN
1613-4125

Authors
Rege, Shraddha D
Royes, Luiz
Tsai, Brandon
et al.

Publication Date
2019-08-01

DOI
10.1002/mnfr.201801054

Peer reviewed

Molecular Nutrition & Food Research
www.mnf-journal.com

Brain Trauma Disrupts Hepatic Lipid Metabolism: Blame It on Fructose?

*Shraddha D. Rege, Luiz Royes, Brandon Tsai, Guanglin Zhang, Xia Yang, and Fernando Gomez-Pinilla**

Scope: The action of brain disorders on peripheral metabolism is poorly understood. The impact of traumatic brain injury (TBI) on peripheral organ function and how TBI effects can be influenced by the metabolic perturbation elicited by fructose ingestion are studied.

Methods and Results: It is found that TBI affects glucose metabolism and signaling proteins for insulin and growth hormone in the liver; these effects are exacerbated by fructose ingestion. Fructose, principally metabolized in the liver, potentiates the action of TBI on hepatic lipid droplet accumulation. Studies in isolated cultured hepatocytes identify GH and fructose as factors for the synthesis of lipids. The liver has a major role in the synthesis of lipids used for brain function and repair. TBI results in differentially expressed genes in the hypothalamus, primarily associated with lipid metabolism, providing cues to understand central control of peripheral alterations. Fructose-fed TBI animals have elevated levels of markers of inflammation, lipid peroxidation, and cell energy metabolism, suggesting the pro-inflammatory impact of TBI and fructose in the liver.

Conclusion: Results reveal the impact of TBI on systemic metabolism and the aggravating action of fructose. The hypothalamic-pituitary-growth axis seems to play a major role in the regulation of the peripheral TBI pathology.

1. Introduction

Traumatic brain injury (TBI) is one of the most complex and common types of brain injuries.[1] TBI research has been centered on the CNS,[2] and little is known about the peripheral alterations that may compromise brain pathology. In particular, the lack of knowledge about the systemic sequel of TBI is a big concern for the understanding and management of metabolic dysfunction that intuitively involves body metabolism. Indeed, metabolic dysfunction is an important aspect of the TBI pathology, and it is at risk of exacerbation by part of metabolic alterations provided by caloric foods.[2] Epidemiological data have demonstrated that the number of diabetic and prediabetic persons in the United States is estimated over 40% of the population[3] and that high fructose consumption contributes to several features of metabolic syndrome including obesity and type 2 diabetes.[4] In fact, fructose, which is highly consumed in soft drinks and processed foods, is widely recognized as a major contributor to the pandemic of metabolic disorders.[5] It has been recently shown that fructose-related alterations in metabolic homeostasis aggravate the pathobiology of TBI.[2] However, the role of systemic physiology on the brain pathophysiology of TBI remains largely unknown.

Recent evidence indicates that focal injury to the brain triggers a quick hepatic response.[6] The liver is also the main organ for the metabolism of fructose,[7] such that the liver is a common target for the combined influences of TBI and fructose on the TBI pathophysiology. The liver plays a major role in control of homeostasis and pathogenesis of the whole organism based on its actions on detoxification, synthesis of lipids, and proteins used across the body and brain.[8] The liver is the principal source of circulating IGF-1, which is under regulatory control of pituitary-made growth hormone (GH).[9] GH can also stimulate hepatic glucose production,[10–12] such that change of GH production can affect insulin sensitivity and glucose homeostasis. Reduced sensitivity to the action of insulin is considered a predictor of poor clinical outcome in TBI patients.[13] Posttraumatic neuroendocrine abnormalities particularly in the somatotrophic axis are commonly observed in TBI patients,[14] which are also likely affected by fructose consumption.

The liver plays a crucial action for the development of metabolic syndrome, and is one of the primary organs affected by overconsumption of fructose resulting in nonalcoholic fatty liver disease.[15] High fructose ingestion is strongly associated with decreased HDL cholesterol and increased hepatic inflammation.[16]

Dr. S. D. Rege, Dr. L. Royes, B. Tsai, Dr. G. Zhang, Dr. X. Yang, Dr. F. Gomez-Pinilla
Department of Integrative Biology & Physiology
University of California, Los Angeles
Los Angeles, CA 90095, USA
E-mail: fgomezpi@ucla.edu
Dr. F. Gomez-Pinilla
Department of Neurosurgery
UCLA Brain Injury Research Center
University of California, Los Angeles
Los Angeles, CA 90095, USA
Dr. L. Royes
Centro De Educacao Fisica e Desportos
Universidade Federal de Santa Maria
Santa Maria, Rio Grande do Sul 97105, Brazil

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mnfr.201801054

DOI: 10.1002/mnfr.201801054

Since fructose is metabolized by the liver, fructose can affect de novo lipogenesis (DNL) and hepatic lipogenic enzymes.[17] These conditions sensitize the liver to inflammatory stress involving activation of c-Jun N-terminal kinases, mitochondrial dysfunction, reduced insulin signaling, and subsequent hyperglycemia.[18] Body homeostasis is under master control of hypothalamic circuits regulating food intake, energy expenditure, synthesis of protein and lipids, and hepatic glucose production.[19,20] The hypothalamus is an important target for the actions of TBI and fructose,[21,22] and subsequent liver failure can be critical for systemic homeostasis; however, the roles of hypothalamus and liver on the pathophysiology elicited by fructose and TBI are poorly understood.

2. Experimental Section

Twenty-four male Sprague–Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) ≈2 months old were housed in polyacrylic cages and maintained under standard housing conditions (room temperature 22–24 °C) with 12 h light/dark cycle. All experiments were performed in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animal. Animal studies and experimental procedures were approved by the University of California at Los Angeles Chancellor's Animal Research Committee (ARC # 2015-034-11A).

Essential methods in the main text and detailed experimental procedures for glucose tolerance test (GTT), insulin tolerance test (ITT), oil red O staining, and RNA sequencing (RNA-seq) are described in the Supporting Information.

2.1. Dosage Information/Dosage Regimen

Rat chow diet and water were supplied ad libitum and the body weight was measured. After the acclimatization period, the animals were randomly assigned to either regular or fructose (15% w/v) drinking water. It was previously established that 15% fructose for 3 weeks is a suitable stimulus to elicit metabolic disturbances in rats.[23] At 3 weeks of fructose intervention, all animals were subjected to either sham or fluid percussion injury (FPI). After 1 week of injury, animals were tested for glucose tolerance (GTT) and were sacrificed immediately by decapitation. Body weight, food intake, water intake, and calorie intake were measured throughout the study. There were four experimental groups I) Sham plus water (SW); II) TBI plus water (TW); III) Sham plus Fructose water (SF); and IV) TBI plus fructose water (TF).

2.2. Fluid Percussion Injury

Fluid percussion injury (FPI) was performed as previously described.[24] In brief, animals were maintained in a deep anesthetic state during surgery using a Laboratory Animal Anesthesia System (VetEquip Inc., CA, USA). A 3.0-mm-diameter craniotomy was made over the left parietal cortex, 3.0 mm posterior to bregma, and 6.0 mm lateral (left) to the midline with a high-speed drill (Dremel, WI, USA). At the first sign of hind-limb withdrawal to a paw pinch, a moderate fluid percussion pulse (2.3–2.5 atm) was administered to the epidural space. Sham animals underwent an identical preparation with the exception of the lesion.

2.3. Plasma GH Levels

Blood plasma was used in a GH ELISA kit (Invitrogen, KRC5311) according to the manufacturer's instructions to calculate circulating levels of GH ($n = 6$).

2.4. Immunoblotting

The liver tissues ($n = 6$ per group) were homogenized in 10 mL of lysis buffer containing 137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% NP40, 10% glycerol, 1 mM phenylmethylsulfonylfluoride (PMSF), 10 µg mL^{-1} aprotinin, 0.1 mM benzethonium chloride, and 0.5 mM sodium vanadate. The homogenates were then centrifuged at 12 000 × g (4 °C) for 30 min, and total protein concentration was determined according to MicroBCA procedure (Pierce, IL, USA). Briefly, 40 µg of total protein was loaded in each well and resolved on a 10% polyacrylamide gel, transferred onto PVDF membrane (Millipore, MA, USA). Membranes were rinsed in 0.05% Tween-20 buffer and then incubated with appropriate primary antibodies pIRS1 (Millipore 05–1086), anti-IRS1 (Millipore 05–1085), anti-InR (sc- 711), anti-GHR (sc-57161), anti-GH (sc-10365), anti-IGF1 (Abcam- 63926), anti-TLR4 (sc- 10741), anti-4HNE, anti-uMitCK (sc- 15166), anti-LXR α (sc-1202), anti-FAS (sc-48357), anti-ABCA1 (Novus NB 400-105), anti-CD36 (sc-7309), and anti-actin (sc-1616), followed by secondary antibodies (1:10 000; SC Biotechnology), the immunoreactive bands were visualized with an enhanced chemiluminescence reagent (Millipore) and were then scanned (Image Lab Software, Version 3.0, Bio Rad). The relative density of each band of interest was measured with Image J v1.46, and bands were normalized to β-actin. 4-HNE conjugates to many different proteins across a range of molecular weights.[25] The anti-4-HNE antibody recognizes multiple bands as part of the 4-HNE complexes, and the multiple bands were grouped and quantified together using Image J software.

2.5. Cell Culture

A nontumorigenic mouse hepatocyte cell line, i.e., AML12 (alpha mouse liver 12) (CRL- 2254) was maintained in a humidified incubator at 37 °C, 5% CO_2. The cell line was maintained in DMEM medium (GIBCO-11995-065) supplemented with 10% fetal bovine serum (Invitrogen), 4.5 g L^{-1} D-glucose, L-glutamine, 110 mg L^{-1} sodium pyruvate, MEM NEAA (GIBCO 100X- 11140-050), penicillin (100 U mL^{-1}), and streptomycin (100 µg mL^{-1}; GIBCO, NY, USA) (Complete Growth Medium). AML12 cells were seeded at ≈60% confluency in complete growth medium. Twenty-four hours later, the cells were changed to serum-free medium, after washing twice with calcium and magnesium-free PBS, the cells were treated with fructose (5 mM) or bovine GH (MP Biomedicals, CAT-160074) (50 mM) alone or fructose in

combination with GH for 48 h. After the treatment, cells were lysed in RIPA buffer (1 M tris-Cl pH 7.4, 5 M NaCl, 10 % Triton-X, 10% sodium deoxycholate, 20% SDS 1 mM Na_3VO_4, 1 mM phenylmethyl sulfonyl fluoride, 10 µg mL^{-1} leupeptin, and aprotinin.), the supernatants were collected, and total protein concentration was estimated according to MicroBCA procedure (Pierce, IL, USA), using bovine serum albumin (BSA) as standard.

2.6. Statistical Analysis

All data were expressed as mean ± SEM. Protein results are expressed as percentage (%) of sham–water group, and analyzed using analysis of variance (ANOVA) and post hoc analyses were conducted using Bonferroni's multiple comparison tests or unpaired Student's *t*-test (GraphPad Prism, San Diego, CA).

3. Results

3.1. Fructose Aggravates the Effects of TBI on Hepatic Insulin Signaling

TBI or fructose alone reduced levels of the activation stages of insulin receptor substrate 1 (pIRS1/IRS1; **Figure 1**A) and insulin receptor (pInR/InR; Figure 1B) compared to the control group (sham–water; $p < 0.01$). The combination of fructose and TBI reduced these levels even further ($p < 0.01$; Figure 1A,B). The ratio of phosphorylated IR versus total IR provides an indication of the signaling level of the IR.

3.2. Fructose Potentiates the Effects of TBI on Systemic Glucose Regulation

Animals fed fructose showed increased tendency toward glucose intolerance as evidenced by an elevated area under the curve (AUC) in blood glucose GTT. However, animals subjected to TBI or fructose alone had elevated blood glucose levels in the GTT ($p < 0.01$; Figure 1C). The combined actions of TBI and fructose were found to exacerbate the effects of TBI alone ($p < 0.05$; Figure 1D). Moreover, exposure to fructose elevated the levels of plasma insulin ($p < 0.05$; Figure 1E) even in rats exposed to TBI.

3.3. Fructose Potentiates the Effects of TBI on Hepatic and Circulating Levels of GH, and Hepatic IGF-1 Levels

GH is released by the pituitary gland under hypothalamic command, and the hypothalamus is often affected by TBI.

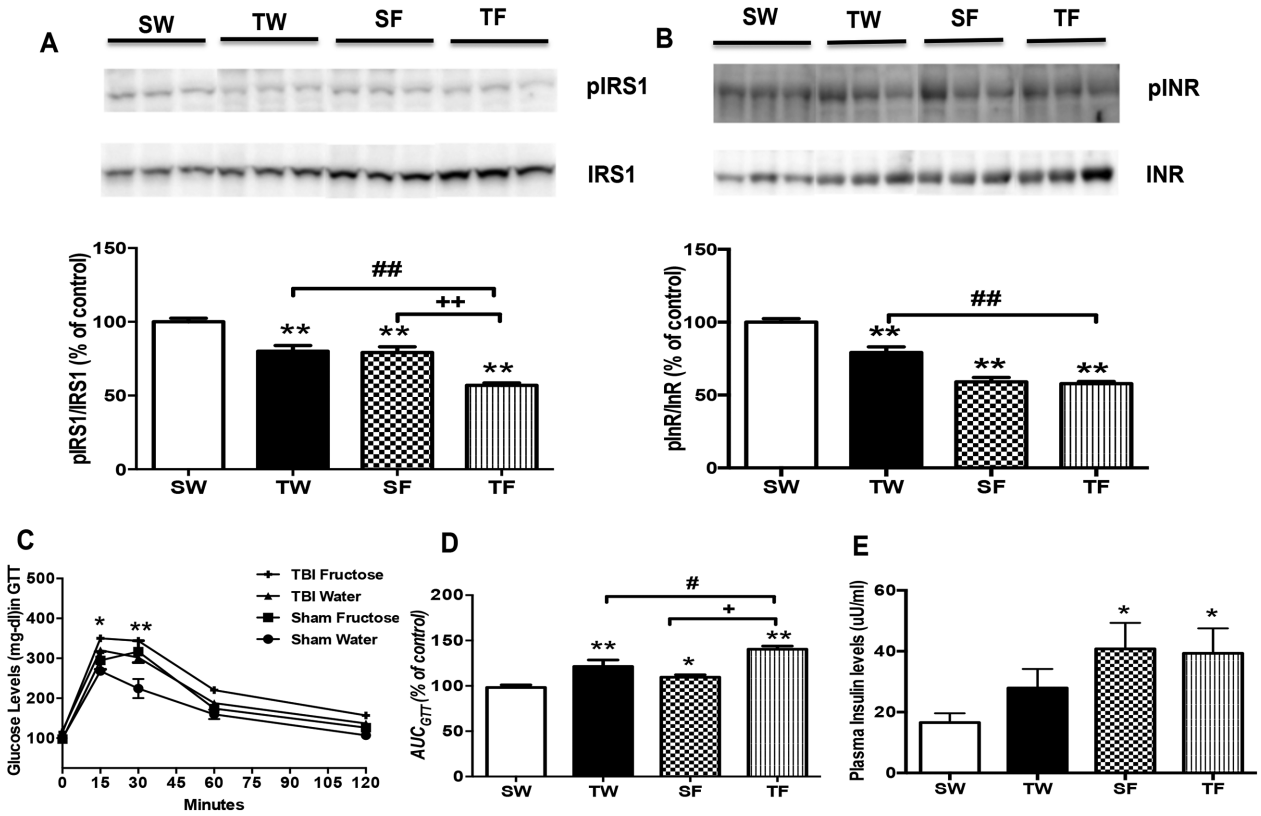

Figure 1. Fructose and TBI impair hepatic insulin signaling and systemic glucose regulation. Representative western blot images showing that fructose together with TBI reduce the levels of A) pIRS1 and B) pINR beyond than TBI alone, and C) Glucose tolerance test (GTT) indicates that fructose-fed TBI animals had significantly higher blood glucose levels than TBI animals. D) Fructose-fed TBI animals had a larger AUC, indicating reduced capacity of glucose clearance compared to Sham or TBI animals. E) Increased plasma insulin levels in fructose and TBI animals as compared to sham animals. Data are expressed as percentage of control (mean ± SEM) $^{*}p < 0.05$; $^{**}p < 0.01$ versus SW group; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ versus TW; $^{+}p < 0.05$; $^{++}p < 0.01$ versus SF group. ANOVA (one-way) followed by post hoc test with Bonferroni's comparisons.

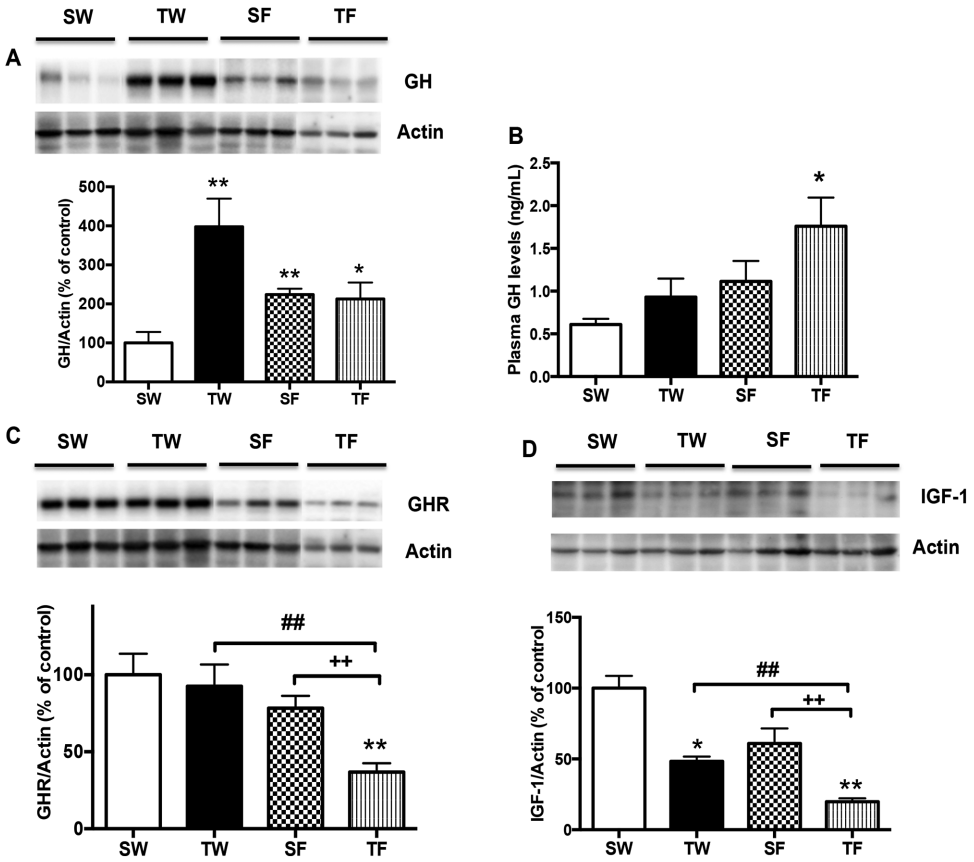

Figure 2. Fructose and TBI affect hepatic GH and IGF-1 levels, and plasma GH. Representative western blot images show increased hepatic levels of A) GH in TBI animals. B) Bar graph depicts elevated plasma GH levels in fructose-fed TBI animals as compared to sham animals. Representative western blot shows that fructose together with TBI reduces the levels of C) GHR and D) IGF-1 beyond than TBI alone. Proteins shown in (A) and (C) were probed on the same membrane. Data are expressed as percentage of control (mean ± SEM). $^{*}p < 0.05$; $^{**}p < 0.01$ versus SW group; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ versus TW; $^{+}p < 0.05$; $^{++}p < 0.01$ versus SF group. ANOVA (one-way) followed by post hoc test with Bonferroni's comparisons.

Interestingly, hepatic GH levels were significantly elevated in rats exposed to TBI or fructose ($p < 0.01$) as well as rats exposed to the combined actions of TBI and fructose ($p < 0.05$), as compared to the control group (sham–water; **Figure 2**A). Rats exposed to TBI and fructose showed an increase in plasma GH levels, as compared to the control group ($p < 0.05$; Figure 2B). However, the levels of GH receptor (GHR) were decreased in fructose-fed animals subjected to TBI, as compared to sham and TBI alone, probably as a result of a state of GH resistance (Figure 2C). The IGF-1 levels were significantly reduced in TBI animals as compared to control group ($p < 0.05$). In addition, levels of IGF-1 were reduced in animals exposed to TBI and even further in animals exposed to fructose and TBI ($p < 0.01$; Figure 2D). These data suggest that fructose worsens the effects of TBI in the liver.

3.4. Fructose Exacerbates the Effects of TBI on Hepatic Inflammation, Oxidative Stress, and Energy Metabolism

We investigated whether fructose influences hepatic inflammatory or oxidative stress states that could provide an indication of liver dysfunction. Our results revealed that fructose exposure increased TLR4 receptor levels, including in rats exposed to TBI as well ($p < 0.05$; **Figure 3**A). Furthermore, rats exposed to fructose showed an increase in 4HNE levels, including those rats exposed to TBI. Significant upregulation of 4HNE levels was noticed in fructose-fed TBI group, as compared to TBI alone ($p < 0.01$; Figure 3B). These data suggest that fructose affects inflammatory status and lipid peroxidation in the liver. Mitochondrial CK enzyme (uMitCk) is crucial for cellular energetics. The levels of uMitCk were significantly increased in both TBI and SF group as compared to the control group ($p < 0.05$; Figure 3C).

3.5. Fructose Augments the Effects of TBI on Hepatic Lipid Metabolism

To determine the potential action of fructose and TBI on hepatic DNL, we performed Oil Red O staining in liver sections, which provide an indication of lipid accumulation. Control liver displayed very few tiny lipid droplets as compared to TBI alone group ($p < 0.01$; **Figure 4**A). Fructose liver demonstrated multiple lipid droplets as compared to control liver ($p < 0.01$; Figure 4A). However, liver sections from the fructose-fed TBI animals showed more lipid accumulation, especially containing very large lipid droplets, compared to the TBI alone group ($p < 0.01$),

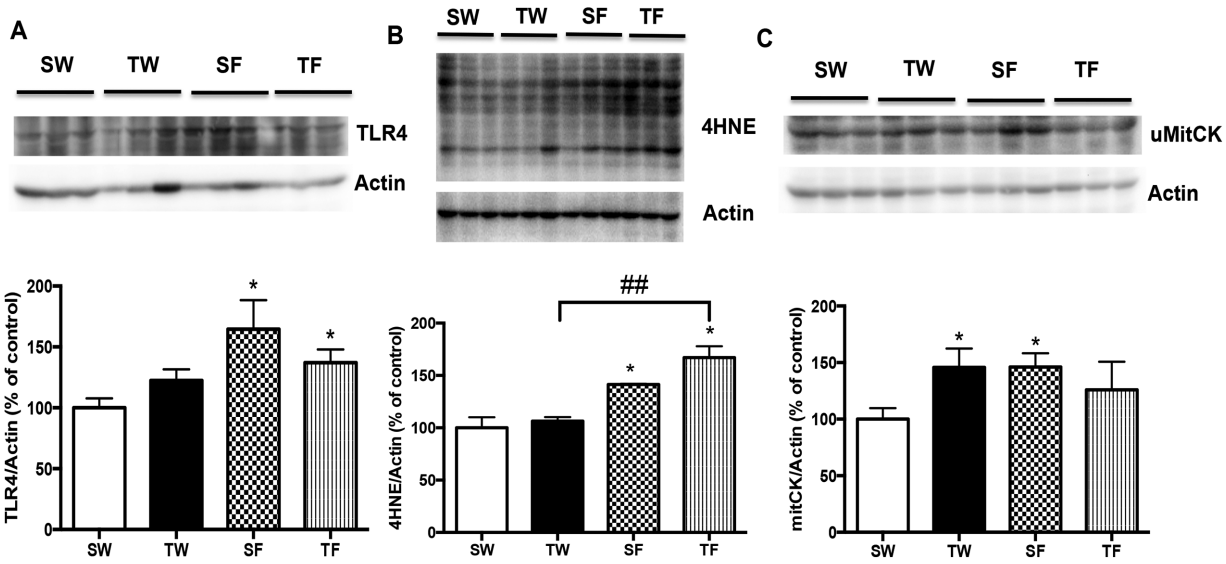

Figure 3. Fructose and TBI promote hepatic inflammation, oxidative stress, and mitochondrial dysfunction. Representative western blot images show fructose and injury increased levels of A) TLR4, as compared to sham animals. Fructose and TBI also increased B) 4HNE levels as compared to TBI animals. Fructose and TBI reduced levels of C) uMitCK as compared to TBI animals. Data are expressed as percentage of control (mean ± SEM). $^{*}p < 0.05$; $^{**}p < 0.01$ versus SW group; $^{\#\#}p < 0.01$ versus TW; $^{++}p < 0.01$ versus SF group. ANOVA (one-way) followed by post hoc test with Bonferroni's comparisons.

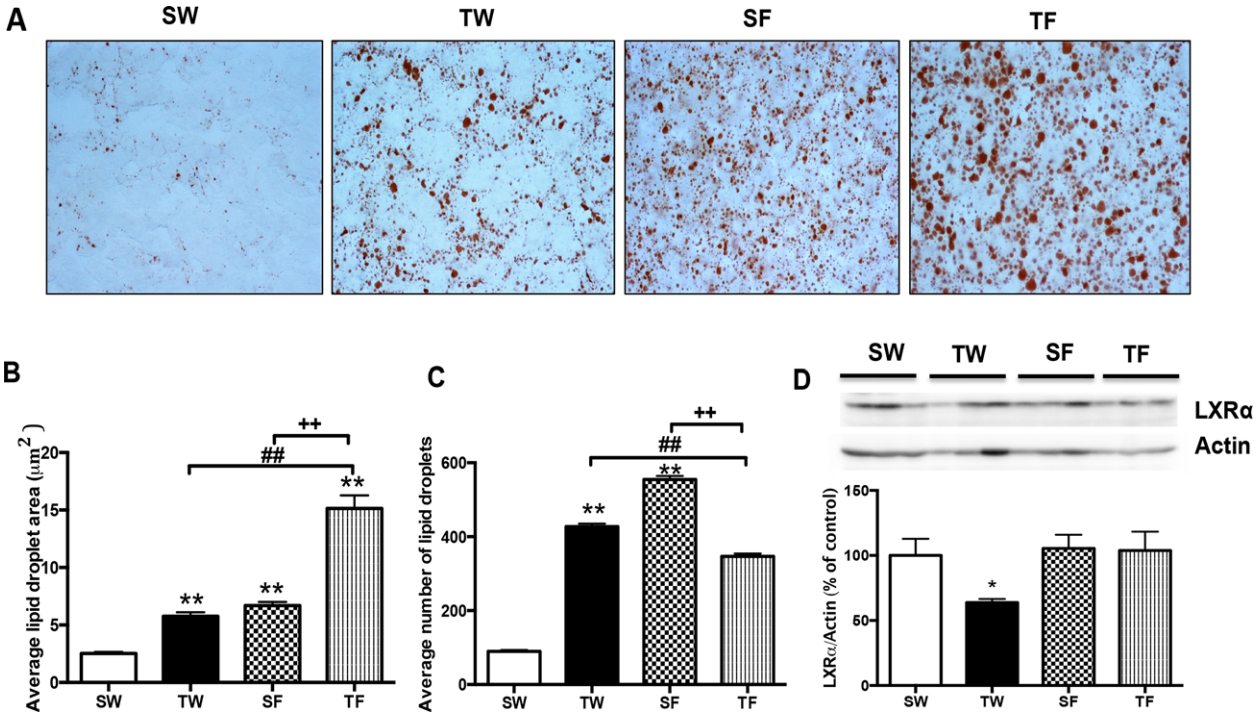

Figure 4. Fructose and TBI contribute to hepatic lipid accumulation. A) Representative photomicrographs of liver sections stained using Oil Red O showed limited tiny lipid droplets in control livers, whereas many large lipid droplets were prominent in fructose-fed TBI livers. Oil Red O staining intensities quantified using imaging software showed B) increased average lipid droplet area in fructose-fed TBI livers as compared to TBI livers, and C) increased number of lipid droplets in both fructose and TBI livers. Representative western blot images show increased levels of D) LXRα in TBI animals. Proteins shown in Figures 4D and 3A were probed on the same membrane. Data are expressed as percentage of control (mean ± SEM). $^{*}p < 0.05$; $^{**}p < 0.01$ versus SW group; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ versus TW; $^{+}p < 0.05$, $^{++}p < 0.01$ versus SF group. ANOVA (one-way) followed by post hoc test with Bonferroni's comparisons.

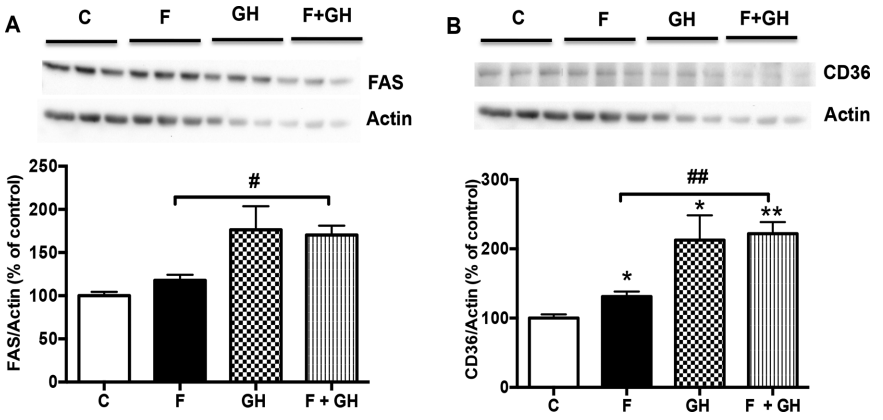

Figure 5. Fructose in combination with GH increases lipid accumulation in liver cells. Hepatocytes were incubated with fructose (5 mM), GH (50 ng mL^{-1}), and fructose (5 mM) in combination with GH (50 ng mL^{-1}) for 48 h. Western blot analyses showed that fructose treatment along with GH increased the levels of A) FAS, and B) CD36, as compared to only fructose treatment. All independent experiments were performed at least three times. Proteins were probed on the same membrane. Data are expressed as percentage of control (mean ± SEM). *$p < 0.05$; **$p < 0.01$ versus control group; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ versus SF group. ANOVA (one-way) followed by post hoc test with Bonferroni's comparisons.

suggesting that fructose exacerbates the effects of TBI on hepatic lipid metabolism (Figure 4A). This was further confirmed by results showing that the average area of lipid droplets was significantly increased in fructose-fed TBI group as compared to the TBI alone ($p < 0.01$) or control group ($p < 0.01$; Figure 4B). However, the number of lipid droplets in fructose-fed TBI group is less than the fructose group, due to the enlargement of lipid droplet size in fructose-fed TBI group (Figure 4C). The liver X receptor α (LXR α), a key regulator of lipid metabolism was significantly decreased in TBI group as compared to the control group ($p < 0.05$) suggesting that TBI impairs hepatic lipid regulation (Figure 4D).

3.6. Fructose in Combination with GH Stimulates Lipid Accumulation in Cultured Hepatocytes

To isolate the direct effects of GH and fructose on de novo fatty acid synthesis, we tested the effects of GH and fructose under controlled conditions in cultured hepatocytes. We performed preliminary dose response experiments by incubating liver cells with different doses of GH (5–500 ng mL^{-1}) or fructose (5–50 mM) for 24, 48, and 72 h. The range 50–100 ng mL^{-1} of GH is equivalent to the mean plasma GH levels in rodents.[26,27] The range 5–20 mM of fructose represents the levels of fructose in the systemic circulation after fructose consumption.[28] We observed maximum increase in the levels of FAS ($p < 0.05$; **Figure 5**A) and fatty acid transporter CD36 ($p < 0.01$) (Figure 5B) with 50 ng mL^{-1} of GH dosage and 5 mM of fructose treatment at 48 h. To further assess whether fructose potentiates the effects of GH on increased fat content, we treated the cells with GH (50 ng mL^{-1}) in combination with fructose (F) (5 mM). Our results showed that although GH in the range of physiological concentrations (50ng mL^{-1}) promoted lipid accumulation in the liver, the same effect was obtained by the combination of fructose and GH (F+GH group) on the levels of FAS and CD36 as compared to F group alone (Figures 5A,B).

3.7. Food Intake, Water Intake, and Body Weight

Fructose-fed animals showed an increase in water intake as compared to the control group ($p < 0.01$; **Figure 6**A), which reflects a compensatory tendency to reduce caloric intake ($p < 0.01$; Figure 6B). Body weights were similar between groups

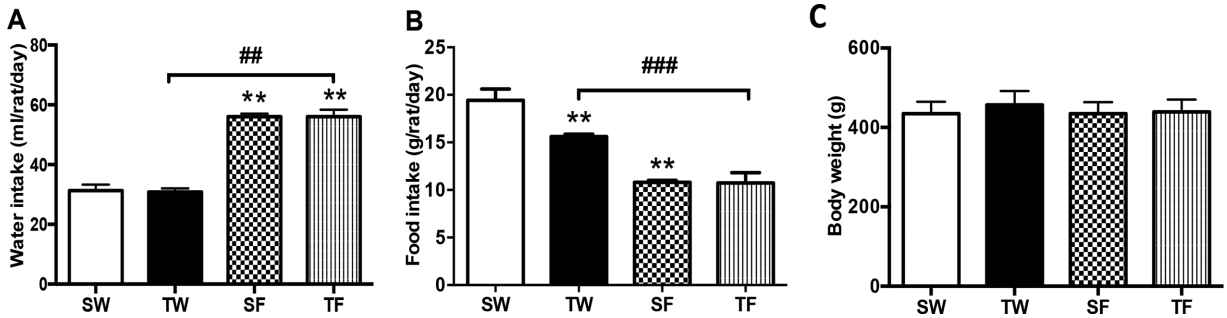

Figure 6. Effects of fructose and TBI on food intake, water intake, and body weight. Fructose-fed TBI animals showed A) increased average water intake as compared to TBI animals and B) decreased average food intake as compared to TBI animals. C) No difference in average body weight was observed between the groups. Data are expressed as percentage of control (mean ± SEM). *$p < 0.05$; **$p < 0.01$ versus SW group; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$ versus TW group. ANOVA (one-way) followed by post hoc test with Bonferroni's comparisons.

(Figure 6C). Our findings also revealed that a single episode of TBI had no effect on water intake, but concomitant fructose consumption was able to decrease food intake as compared to controls ($p < 0.01$), suggesting that there was an additive effect of fructose in aggravating the TBI effects.

3.8. Functional Enrichment Analysis of TBI Transcriptomic Signatures

The hypothalamus is the master regulator of peripheral metabolism. To elucidate how TBI could affect gene control of metabolic processes, we profiled the hypothalamic transcriptome and identified 326 differentially expressed genes (DEGs) ($p < 0.01$) altered by TBI. We annotated the hypothalamic DEG signature genes based on the known pathways or functional categories compiled in KEGG, Biocarta, Reactome, and GO term. At Bonferonni corrected $p < 0.05$, we found 137 overrepresented pathways for hypothalamic DEG signatures. Interestingly, the enrichment of biological pathways affected by TBI in the hypothalamus was mostly associated with lipid metabolism, nonalcoholic fatty liver disease, energy metabolism (oxidative phosphorylation), protein metabolism, oxidative stress, and disease-related gene sets for Alzheimer's disease and Parkinson's disease (Figure S1, Supporting Information).

3.9. Relevance of Transcriptomic Hypothalamic Alterations to Peripheral Metabolism

Given the unique capacity of the hypothalamus to sense metabolic signals from the periphery, we analyzed on detail DEGs related to lipid metabolism affected by TBI in the hypothalamus. We found six upregulated DEGs involved in lipid metabolism that could relate to liver function. Of these, *Fdft1* is involved in cholesterol biosynthetic process. *Sc5d*, *Scd2*, and *Fads2* are involved in fatty acid biosynthetic and metabolic process, and *Acox1* and *Hsd17b10* are involved in fatty acid oxidation. Taken together, these data indicate that the key genes related to lipid metabolism were influenced by TBI indicative of aberration of the lipid metabolic pathways bringing about detrimental changes in peripheral metabolism (Figure S2A and Table S1, Supporting Information). In addition, eight DEGs (*Cat*, *Hmox2*, *Ndufa12*, *Park7*, *Rps3*, *Gatm*, *Sod2*, and *Atm*) involved in anti-oxidation were upregulated likely in response to oxidative stress in TBI suggesting a deleterious impact of TBI on the brain antioxidative defense system (Figure S2B, Supporting Information).

It is clear that redox potential is disrupted following TBI leading to oxidative stress and mitochondrial dysfunction. The KEGG pathway representation of the individual sets of genes showed upregulation of important components of energy metabolism pathways in response to TBI, and included 17 genes related to oxidative phosphorylation (*Cox7a2*, *Ndufs3*, *Cox7b*, *Ndufb9*, *Ugcrh*, *Atp5e*, *Ndufs4*, *Cox6a1*, *Sdhc*, *Ndufa11*, *Ndufa12*, and *Atp6v1g2*). The increase in oxidative phosphorylation genes is in harmony with the counteractive mechanisms in response to the oxidative stress following TBI (Figure S2C, Supporting Information).

3.10. Functional Enrichment Analysis of Fructose Transcriptomic Signatures

To determine the metabolic processes that differed between sham and fructose animals, we annotated the hypothalamic signature genes based on the known pathways or functional categories compiled in KEGG, Biocarta, Reactome, and GO term. The enrichment of biological pathways affected by fructose included those highly associated with insulin signaling, insulin like growth factor (IGF-1) activity, and inflammatory functions (Figure S3A, Supporting Information). We found eight differentially expressed downregulated genes such as *Igf2*, *Igfbp6*, *Igfbp2*, *Igf1*, *Igfbp4*, and *Igfbp3* involved in the regulation of IGF-1 activity (Figure S3B, Supporting Information).

4. Discussion

Here, we show that TBI has a strong impact on peripheral metabolism, and that fructose consumption further aggravates these effects. We report that metabolic perturbations carried by consumption of fructose under the threshold for establishment of metabolic syndrome exacerbates the disruptive effects of TBI on glucose metabolism, inflammation, and lipid peroxidation in the liver. TBI and fructose also promoted alterations in genes that control peripheral metabolism in the hypothalamus suggesting that the hypothalamus can have a pivotal role for the actions of TBI and fructose on brain and body.

4.1. Effects of TBI on Peripheral Metabolism

We previously reported that fructose impaired hippocampal InR signaling and worsened the effects of TBI on behavioral function and plasticity.[2] According to our results in the liver, fructose consumption reduced signaling through the insulin receptors in TBI animals beyond the effects of TBI alone. Animals fed fructose showed a tendency toward glucose intolerance and these effects were exacerbated by TBI. It is important to keep in mind that the energy crisis post-TBI[29,30] increases vulnerability to secondary brain injury such that even a mild injury episode can have severe and long-lasting consequences.[31] Among these secondary complications, the persistent hyperglycemia in TBI patients correlates with the severity of damage and increases incidence of long-term neurological disorders.[32]

Our findings that TBI decreases levels of LXR α support further the impact of TBI on peripheral metabolism. LXRα, specifically expressed in the liver, plays an important role in metabolic regulation of glucose, lipids, cholesterol, and bile acid synthesis.[33] Administration of high cholesterol diet to LXRα deficient mice resulted in vast increase of cholesterol accumulation in the liver.[34]

4.2. Effects of TBI on Hepatic Inflammation

The liver is the organ containing maximum number of resident macrophages, and largely contributes to chemokines and

cytokines in serum post-TBI.[35] Toll-like receptors (TLRs) are signaling receptors in the innate immune system, known for their emerging role in brain injury.[36] We therefore assessed the levels of hepatic TLR4 to see if fructose mediates the development of secondary inflammatory process in TBI. Our results showed that TBI significantly increased the levels of TLR4, and that addition of fructose potentiated the increase caused by TBI, suggesting that fructose sets the stage for inflammatory processes in TBI. One of the most popular explanations for dysregulation of glucose control post-TBI is stress/inflammation-induced hyperglycemia.[37] According to our data, TBI and fructose heavily affected plasma insulin levels and reduced insulin signaling in the liver. Chronic inflammation is most likely linked to insulin resistance, due to inhibition of the insulin pathway at IRS-1.[38] The production of inflammatory proteins, such as tumor necrosis factor-α (TNF-α), IL-1β, and IL-6, after CNS injury promotes systemic inflammatory response syndrome (SIRS) and organ damage.[39]

4.3. Implications for Regulation of Bioenergetics and Oxidative Stress

Our results revealed that fructose exacerbates the effects of TBI on 4HNE levels. The aldehyde 4HNE is the final product of reactive oxygen species formation and its overproduction can result in mitochondria dysfunction, lipid peroxidation,[40] and hepatic damage. It is known that TBI causes structural damage to brain mitochondria resulting in production of reactive oxygen and nitrogen species associated with poor cell energetics, Ca^{2+} overload, and mitochondrial respiratory damage.[41,42] We have previously reported that TBI alters mitochondrial function and oxidative status in the brain,[2] and the current results are novel to show that TBI also disrupts the profile of oxidative-inflammatory status in the liver. Mitochondrial CK (uMitCK) is particularly sensitive to oxidative damage,[43] and we observed increased levels of uMitCk in the liver of both TBI and fructose animals, suggesting an increase in mitochondrial enzymes in response to oxidative damage. These results provide an indication for the effects of TBI on cell energy metabolism and inflammation in the liver, as previously suggested in other disease models.[44,45] Our transcriptomic analysis shows that TBI upregulates oxidative stress genes pathways in the hypothalamus along with an increase in oxidative phosphorylation genes, and suggests an activation of counteractive defense mechanisms in response to oxidative stress.

4.4. Effects of TBI on Liver Lipids

Hepatic lipid droplets (LDs) are major cellular organelles involved in the storage of neutral lipids such as triglycerides, steryl esters, and retinyl esters.[46] LDs are crucial for lipid and energy metabolism; however excessive accumulation of lipids in these organelles is linked to obesity, atherosclerosis, and fatty liver disease.[47,48] Oil Red O staining revealed a widespread accumulation of large lipid droplets in rats either receiving TBI or fructose exposure, and an even larger accumulation in fructose rats exposed to TBI. Fatty acid synthase (FAS) is a key lipogenic enzyme commonly involved in fatty acid synthesis.[49] Moreover, fatty acid (FA) uptake in the liver primarily occurs through the scavenger receptor CD36, also known as fatty acid translocase (FAT).[50] Using in vitro model, we provide evidence that GH enhances hepatic CD36 expression and suggest that GH influences FA uptake and TG accumulation. CD36 expression levels are seen to be upregulated with high fat diets, hepatic steatosis, and NAFLD.[51,52] Additionally, mouse models of obesity, diabetes, and NAFLD have shown elevated hepatic CD36 levels, which correlate with liver TG accumulation, insulin resistance, and hepatic steatosis.[53,54] These results are in general agreement with recent evidence that GH can also regulate liver lipid metabolism.[55–57] Interestingly, GH-dependent spike in plasma FFA and increased hepatic uptake of FFA is induced by CD36, further leading to the development of fatty liver.[58] Taken together, our results support the idea that GH could be exerting direct effects on lipid uptake and DNL in liver during the state of GH resistance and that fructose exacerbates TBI pathology and contributes to hepatic lipid accumulation at large.

4.5. Effects of TBI on Hypothalamic/Growth Axis

Pituitary and/or hypothalamic dysfunction after TBI is commonly associated with increased morbidity and poor recovery.[59] Post-TBI pituitary dysfunction disrupts glucose metabolism and may cause hyperglycemia,[60] provoking imbalance in neuroendocrine function. GH is a key player in liver lipid metabolism such that disturbances in GH signaling promote excessive lipid buildup in the liver as well as in other organs.[55–57] Mice with liver specific GH receptor (GHR) knockout or its downstream effectors JAK2 or STAT5 have been shown to have DNL and lipid uptake resulting from lower liver IGF1 levels and higher GH secretion.[55,56] Recent studies have reported that high fructose solution (30%) results in higher triglycerides as well as hepatic lipid levels.[61] Our results also showed that TBI reduced IGF-1 levels, and that fructose-fed TBI animals showed a larger decrease in IGF-1 levels.

GH signaling is often altered following TBI[62] and results in dysregulation of glucose homeostasis.[13] Maintenance of glucose homeostasis principally requires appropriate insulin secretion and normal peripheral tissue insulin response.[63] The liver is the central organ in the GH/IGF-1 axis and deficiency of insulin causes reduction in liver GHR.[64] Furthermore, continuous insulin stimulation as observed in hyperinsulinemia results in GH resistance with increased basal insulin levels.[64] The phenotype of primary GH resistance is similar to that of GH deficiency, and has been linked to defects in GHR signaling and IGF-1 synthesis.[65] Our findings revealed that the levels of GHR were decreased in fructose-fed animals subjected to TBI as compared to sham and TBI alone. The fact that GH levels were significantly elevated in TBI group could infer that a decrease in peripheral GHRs may result in a loss of peripheral sensitivity to GH. This would have eventually led to a feedback-driven spike in the secretion of GH. Moreover, the response of GH axis to trauma is biphasic, with acute and chronic phases.[66,67] The acute phase ranging between 5 and 10 days is characterized by an active secretion of GH by the pituitary,[66,68] which correlates with our tissue

examined 1 week post-TBI model. Excessive amounts of GH oppose the effects of insulin in the liver and peripheral tissues. Reduction of IGF-1 levels could be due to liver inflammation, and the low IGF-1 levels may act as a negative feedback to elevate GH production by the pituitary gland. Similar findings were noted when total deletion of the GHR in liver showed fourfold increase in circulating GH along with insulin resistance, glucose intolerance, and elevated circulating free fatty acids.[55]

In conclusion, our study uncovers the potential bidirectional interactions between the brain and liver after brain injury. These experimental data suggest that an important aspect of the TBI pathology takes place in the periphery with subsequent repercussions for the brain. Furthermore, a metabolic perturbation induced by a short period of high fructose consumption potentiates the effects of TBI on systemic metabolism. These data piece together to reveal the compelling possibility that a metabolic perturbation carried by diet is a predictor of worse outcome in the pathophysiology of TBI.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

F.G.P., S.D.R., and L.R. conceived and designed the experiments. L.R. and S.D.R. conducted the in vivo experiments and S.D.R. conducted the in vitro experiments. X.Y., B.T., and G.Z. conducted bioinformatics analysis. S.D.R., L.R., and F.G.P. wrote the manuscript. All authors reviewed the manuscript. This work was supported by National Institutes of Health NS50465 to F.G.P and DK104363 to X.Y.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

fructose, growth hormone, insulin signaling, lipids, traumatic brain injuries

Received: November 15, 2018
Revised: March 21, 2019
Published online:

[1] H. H. Dash, S. Chavali, *Korean J. Anesthesiol.* **2018**, *71*, 12.
[2] R. Agrawal, E. Noble, L. Vergnes, Z. Ying, F. Reue, F. Gomez-Pinilla, *J. Cereb. Blood Flow Metab.* **2016**, *36*, 941.
[3] C. C. Cowie, K. F. Rust, E. S. Ford, M. S. Eberhardt, D. D. Byrd-Holt, C. Li, D. E. Williams, E. W. Gregg, K. E. Bainbridge, S. H. Saydah, L. S. Geiss, *Diabetes Care.* **2009**, *32*, 287.
[4] K. W. ter Horst, M. J. Serlie, *Nutrients.* **2017**, *9*.
[5] J. D. Botezelli, R. A. Dalia, I. M. Reis, R. A. Barbieri, T. M. Rezende, J. G. Pelarigo, J. Codogno, R. Gonçalves, M. A. Mello, *Diabetol. Metab. Syndr.* **2010**, *2*, 43.
[6] D. C. Anthony, Y. Couch, P. Losey, M. C. Evans, *Brain. Behav. Immun.* **2012**, *26*, 534.
[7] F. Theytaz, S. de Giorgi, L. Hodson, N. Stefanoni, V. Rey, P. Schneiter, V. Giusti, L. Tappy, *Nutrients.* **2014**, *6*, 2632.
[8] L. P. Bechmann, R. A. Hannivoort, G. Gerken, G. S. Hotamisligil, M. Trauner, A. Canbay, *J. Hepatol.* **2012**, *56*, 952.
[9] J. L. Liu, A. Grinberg, H. Westphal, B. Sauer, D. Accili, M. Karas, D. LeRoith, *Mol. Endocrinol. Baltim. Md.* **1998**, *12*, 1452.
[10] N. L. Brooks, C. M. Trent, C. F. Raetzsch, K. Flurkey, G. Boysen, M. T. Perfetti, Y. C. Jeong, S. Klebanov, K. B. Patel, V. R. Khodush, L. L. Kupper, D. Carling, J. A. Swenberg, D. E. Harrison, T. P. Combs *J. Biol. Chem.* **2007**, *282*, 35069.
[11] R. Lindberg-Larsen, N. Møller, O. Schmitz, S. Nielsen, M. Andersen, H. Orskov, J. O. L. Jørgensen, *J. Clin. Endocrinol. Metab.* **2007**, *92*, 1724.
[12] A. A. Sakharova, J. F. Horowitz, S. Surya, N. Goldenberg, M. P. Harber, K. Symons, A. Barkan, *J. Clin. Endocrinol. Metab.* **2008**, *93*, 2755.
[13] J. Shi, B. Dong, Y. Mao, W. Guan, J. Cao, R. Zhu, S. Wang, *Oncotarget.* **2016**, *7*, 71052.
[14] S. Giuliano, S. Talarico, L. Bruno, F. B. Nicoletti, C. Ceccotti, A. Belfiore, *Endocrine.* **2017**, *58*, 115.
[15] M. B. Vos, J. E. Lavine, *Hepatol. Baltim. Md.* **2013**, *57*, 2525.
[16] M. F. Abdelmalek, A. Suzuki, C. Guy, A. Unalp-Arida, R. Colvin, R. J. Johnson, A. M. Diehl, *Hepatol. Baltim. Md.* **2010**, *51*, 1961.
[17] R. Crescenzo, F. Bianco, I. Falcone, P. Coppola, G. Liverini, S. Iossa, *Eur. J. Nutr.* **2013**, *52*, 537.
[18] S. M. Alwahsh, R. Gebhardt, *Arch. Toxicol.* **2017**, *91*, 1545.
[19] H. Münzberg, E. Qualls-Creekmore, H. R. Berthoud, C. D. Morrison, S. Yu, *Handb. Exp. Pharmacol.* **2016**, *233*, 173.
[20] S. D. Jordan, A. C. Könner, J. C. Brüning, *Cell. Mol. Life Sci.* **2010**, *67*, 3255.
[21] Z. Javed, U. Qamar, T. Sathyapalan, *Indian J. Endocrinol. Metab.* **2015**, *19*, 753.
[22] Q. Meng, Z. Ying, E. Noble, Y. Zhao, R. Agrawal, A. Mikhail, Y. Zhuang, E. Tyagi, Q. Zhang, J.-H. Lee, M. Morselli, L. Orozco, W. Guo, T. M. Kilts, J. Zhu, B. Zhang, M. Pellegrini, X. Xiao, M. F. Young, F. Gomez-Pinilla, X. Yang, *EBioMedicine.* **2016**, *7*, 157.
[23] A. Jiménez-Maldonado, Z. Ying, H. R. Byun, F. Gomez-Pinilla, *Biochim. Biophys. Acta Mol. Basis Dis.* **2018**, *1864*, 24.
[24] S. Sharma, Z. Ying, F. Gomez-Pinilla, *Exp. Neurol.* **2010**, *226*, 191.
[25] R. Subramaniam, F. Roediger, B. Jordan, M. P. Mattson, J. N. Keller, G. Waeg, D. A. Butterfield *J. Neurochem.* **1997**, *69*, 1161.
[26] J. O. Jansson, S. Ekberg, O. G. Isaksson, S. Edén, *Endocrinology.* **1984**, *114*, 1287.
[27] B. Contreras, F. Talamantes, *Endocrinology.* **1999**, *140*, 4725.
[28] H. Hui, D. Huang, D. McArthur, N. Nissen, L. G. Boros, A. P. Heaney, *Pancreas* **2009**, *38*, 706.
[29] M. Bergsneider, D. A. Hovda, E. Shalmon, D. F. Kelly, P. M. Vespa, N. A. Martin, M. E. Phelps, D. L. McArthur, M. J. Caron, J. F. Kraus, D. P. Becker, *J. Neurosurg.* **1997**, *86*, 241.
[30] M. Oddo, J. M. Schmidt, E. Carrera, N. Badjatia, E. S. Connolly, M. Presciutti, N. D. Ostapkovich, J. M. Levine, P. Le Roux, S. A. Mayer, *Crit. Care Med.* **2008**, *36*, 3233.
[31] Z. M. Weil, K. R. Gaier, K. Karelina, *Neurobiol. Dis.* **2014**, *70*, 108.
[32] A. Rovlias, S. Kotsou, *Neurosurgery* **2000**, *46*, 335.
[33] M. Baranowski, *J. Physiol. Pharmacol.* **2008**, *59*(Suppl 7), 31.
[34] D. J. Peet, S. D. Turley, W. Ma, B. A. Janowski, J. M. Lobaccaro, R. E. Hammer, D. J. Mangelsdorf, *Cell.* **1998**, *93*, 693.
[35] S. Villapol, *Neural Regener. Res.* **2016**, *11*, 226.
[36] Y. Ye, H. Xu, X. Zhang, Z. Li, Y. Jia, X. He, J. H. Huang, *Int. J. Mol. Sci.* **2014**, *15*, 12651.
[37] P. L. Bosarge, T. H. Shoultz, R. L. Griffin, J. D. Kerby, *J. Trauma Acute Care Surg.* **2015**, *79*, 289.

[38] M. Caruso, D. Ma, Z. Msallaty, M. Lewis, B. Seyoum, A.-J. Wissam, M. Diamond, A. B. Abou-Samra, K. Højlund, R. Tagett, S. Draghici, X. Zhang, J. F. Horowitz, Z. Yi, *Diabetes* **2014**, *63*, 1933.
[39] E. Fehrenbach, M. E. Schneider, *Sports Med. Auckl. NZ* **2006**, *36*, 373.
[40] I. N. Singh, L. K. Gilmer, D. M. Miller, J. E. Cebak, J. A. Wang, E. D. Hall, *J. Cereb. Blood Flow Metab.* **2013**, *33*, 593.
[41] P. G. Sullivan, J. N. Keller, M. P. Mattson, S. W. Scheff, *J. Neurotrauma* **1998**, *15*, 789.
[42] I. N. Singh, P. G. Sullivan, Y. Deng, L. H. Mbye, E. D. Hall, *J. Cereb. Blood Flow Metab.* **2006**, *26*, 1407.
[43] U. Schlattner, M. Tokarska-Schlattner, T. Wallimann, *Biochim. Biophys. Acta* **2006**, *1762*, 164.
[44] S. Guo, *J. Endocrinol.* **2014**, *220*, T1.
[45] C. de Luca, J. M. Olefsky, *FEBS Lett.* **2008**, *582*, 97.
[46] D. G. Mashek, S. A. Khan, A. Sathyanarayan, J. M. Ploeger, M. P. Franklin, *Hepatol. Baltim. Md* **2015**, *62*, 964.
[47] J. C. Cohen, J. D. Horton, H. H. Hobbs, *Science* **2011**, *332*, 1519.
[48] T. C. Walther, R. V. Farese, *Annu. Rev. Biochem.* **2012**, *81*, 687.
[49] S. J. Wakil, L. A. Abu-Elheiga, *J. Lipid Res.* **2009**, *50*(Suppl), S138.
[50] P. Steneberg, A. G. Sykaras, F. Backlund, J. Straseviciene, I. Söderström, H. Edlund, *J. Biol. Chem.* **2015**, *290*, 19034.
[51] M. E. Miquilena-Colina, E. Lima-Cabello, S. Sánchez-Campos, M. V. García-Mediavilla, M. Fernández-Bermejo, T. Lozano-Rodríguez, J. Vargas-Castrillón, X. Buqué, B. Ochoa, P. Aspichueta, J. González-Gallego, C. García-Monzón, *Gut* **2011**, *60*, 1394.
[52] D. Greco, A. Kotronen, J. Westerbacka, O. Puig, P. Arkkila, T. Kiviluoto, S. Laitinen, M. Kolak, R. M. Fisher, A. Hamsten, P. Auvinen, H. Yki-Järvinen, *Am. J. Physiol. Gastrointest. Liver Physiol.* **2008**, *294*, G1281.
[53] D. P. Y. Koonen, R. L. Jacobs, M. Febbraio, M. E. Young, C. L. M. Soltys, H. Ong, D. E. Vance, J. R. B. Dyck, *Diabetes.* **2007**, *56*, 2863.
[54] M. Ito, J. Suzuki, S. Tsujioka, M. Sasaki, A. Gomori, T. Shirakura, H. Hirose, M. Ito, A. Ishihara, H. Iwaasa, A. Kanatani, *Hepatol. Res. Off. J. Jpn. Soc. Hepatol.* **2007**, *37*, 50.
[55] Y. Fan, R. K. Menon, P. Cohen, D. Hwang, T. Clemens, D. J. DiGirolamo, J. J. Kopchick, D. Le Roith, M. Trucco, M. A. Sperling, *J. Biol. Chem.* **2009**, *284*, 19937.
[56] Y. Cui, A. Hosui, R. Sun, K. Shen, O. Gavrilova, W. Chen, M. C. Cam, B. Gao, G. W. Robinson, L. Hennighausen, *Hepatology* **2007**, *46*, 504.
[57] A. Vijayakumar, R. Novosyadlyy, Y. Wu, S. Yakar, D. LeRoith, *Growth Horm. IGF Res.* **2010**, *20*, 1.
[58] C. G. Wilson, J. L. Tran, D. M. Erion, N. B. Vera, M. Febbraio, E. J. Weiss, *Endocrinology* **2016**, *157*, 570.
[59] J. R. Dusick, C. Wang, P. Cohan, R. Swerdloff, D. F. Kelly, *Pituitary* **2012**, *15*, 2.
[60] K. Jauch-Chara, K. M. Oltmanns, *Neuroscience* **2014**, *283*, 202.
[61] V. Volynets, A. Spruss, G. Kanuri, S. Wagnerberger, S. C. Bischoff, I. Bergheim, *J. Lipid Res.* **2010**, *51*, 3414.
[62] Z. Karaca, F. Tanrıverdi, K. Ünlühızarcı, F. Kelestimur, *Prog. Mol. Biol. Transl. Sci.* **2016**, *138*, 167.
[63] P. V. Röder, B. Wu, Y. Liu, W. Han, *Exp. Mol. Med.* **2016**, *48*, e219.
[64] S. Ji, R. Guan, S. J. Frank, J. L. Messina, *J. Biol. Chem.* **1999**, *274*, 13434.
[65] R. G. Rosenfeld, V. Hwa, *Eur. J. Endocrinol.* **2004**, *151*(Suppl 1), S11.
[66] R. C. Baxter, *Growth Horm. IGF Res.* **1999**, *9*(Suppl A), 67.
[67] M. P. Rowan, D. J. Beckman, J. A. Rizzo, C. L. Isbell, C. E. White, S. M. Cohn, K. K. Chung, *Scand. J. Trauma Resusc. Emerg. Med.* **2016**, *24*, 119.
[68] R. J. Ross, S. L. Chew, *Eur. J. Endocrinol.* **1995**, *132*, 655.