Manuscript Details

Manuscript number	JNB_2017_744
Title	Differential effects of high consumption of fructose and glucose on mesenteric endothelial function in female rats
Article type	Research Paper

Abstract

We have recently shown that type of supplemented simple sugar, not merely calorie intake, determines adverse effects on metabolism and aortic function in female rats. The aim of the current study was to investigate and compare the effects of high consumption of glucose and fructose on mesenteric arterial reactivity and systolic blood pressure (SBP). Sprague-Dawley female rats were supplemented with 20% w/v glucose or fructose in drinking water for 8 weeks. Here, we show that both sugars alter insulin signaling in mesenteric arteries (MA), assessed by a reduction in phosphorylated Akt, and increase SBP. Furthermore, ingestion of glucose or fructose enhances inducible nitric oxide synthase (iNOS) expression and contractile responses to endothelin (ET-1) and phenylephrine (PE) in MA of rats. The endothelium-dependent vasodilation (EDV) to acetylcholine (ACh) and bradykinin (BK) as well as the relaxation responses to the nitric oxide donor sodium nitroprusside (SNP) are impaired in MA of fructose-, but not glucose-supplemented rats. In contrast, only glucose supplementation increases the expression of phosphorylated endothelial NOS (eNOS) in MA of rats. In conclusion, this study reveals that supplementation with fructose or glucose in liquid form deranges MA insulin siganlling, enhances vasocontractile responses and increases iNOS expression in MA, effects which are accompanied by increased SBP in those groups. On the other hand, the preserved vasodilatory responses in MA from glucose-supplemented rats could be attributed to the enhanced level of phosphorylated eNOS expression in this group.

Keywords	Rat mesenteric arteries; Fructose; Glucose; Endothelial dysfunction; Blood pressure

Corresponding Author	Roshanak Rahimian
Corresponding Author's Institution	Department of Physiology and Pharmacology, Thomas J. Long School of Pharmacy and Health Sciences, University of the Pacific
Order of Authors	Sonali Shaligram, Gemma Sangüesa, Farjana Akther, Marta Alegret, Juan C Laguna, Roshanak Rahimian
Suggested reviewers	Mohammed Moghadasian, María Soledad Fernández Alfonso, María Teresa Tejerina, Haifei Shi, Lynda Brown

Submission Files Included in this PDF

File Name [File Type]

Cover Letter_Oct2017.pdf [Cover Letter]

Highlights.docx [Highlights]

Fructose final version MS oct5-amb biblio.docx [Manuscript File]

Figure 1.pdf [Figure]

Figure 2.pdf [Figure]

Figure 3.pdf [Figure]

Figure 4.pdf [Figure]

Figure 5.pdf [Figure]

Table 1.docx [Table]

Table 2.docx [Table]

Table 3.docx [Table]

Supplemental Table 1.docx [Table]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

UNIVERSITY OF THE
PACIFIC
Thomas J. Long School of
Pharmacy & Health Sciences

October 9, 2017

Journal of Nutritional Biochemistry

Dear Editorial Board Members:

Enclosed please find the online copy of a full manuscript entitled "Differential effects of high consumption of fructose and glucose on mesenteric endothelial function in female rats" for your consideration as an original research article.

All authors have read and approved its submission to the Journal of Nutritional Biochemistry. The authors hereby declare that manuscript, or part of it, neither has been published (except in form of abstract) nor is currently under consideration for publication elsewhere.

The basis of this study is the several epidemiological reports providing evidence that in humans, an excessive intake of added sugars has been linked to the development of metabolic disturbances and therefore to an increase in the risk for CVD mortality. Despite a large number of studies on the association of consumption of excess sugar with the development of cardiometabolic disease, there is little data on differential effects of dietary sugars on blood pressure and vascular reactivity. In a recent report, we showed that high fructose consumption impairs aortic function and alters metabolic parameters, including insulin signaling in liver and aorta, to a greater extent than glucose consumption in female rat (Sangüesa et al., Am J Physiol Hear Circ Physiol 2017;312:H289–304). Nevertheless, it remains to be established whether the above-mentioned differences in the effects of fructose and glucose are specific to large conduit arteries or whether they are generalizable effects extending to smaller arterial beds, such as mesenteric arteries. Thus, we aimed to determine the effects of supplementation of 20% w/v glucose or fructose in drinking water for 8 weeks on systolic blood pressure (SBP) and mesenteric arterial reactivity in female rats. Our data suggest that that the intake of liquid fructose or glucose increases SBP, possibly by altering insulin signaling and vasculature function in mesenteric arteries in female rats. Furthermore, we have shown that the mesenteric arterial vasodilatory function was impaired in fructose-, but not glucose-supplemented groups. The preserved vasodilatory responses in mesenteric arteries from the glucose group could be attributed to the enhanced phosphorylation of eNOS in this group.

Please do not hesitate to contact me if you have any questions or concerns at (209) 946-2373 or by email at rrahimian@pacific.edu. Thank you for your consideration.

Kind regards,

Roshanak Rahimian, *PharmD., MSc., PhD.*
Professor
Department of Physiology & Pharmacology
Thomas J. Long School of Pharmacy and Health Sciences
University of the Pacific

Highlights

- Female rats were supplemented with 20% w/v glucose or fructose for 8 weeks
- Both sugars caused insulin signalling impairment in mesenteric arteries (MA)
- Both sugars enhanced vasocontractile responses and increased iNOS expression in MA
- These effects were accompanied by increased systolic blood pressure in both groups
- Only glucose supplementation enhanced phospho-eNOS and preserved vasodilatory responses in MA

Differential effects of high consumption of fructose and glucose on mesenteric endothelial function in female rats

Sonali Shaligram[1a] Gemma Sangüesa[2a], Farjana Akther[1], Marta Alegret[2,3,4], Juan C Laguna[2,3,4b], Roshanak Rahimian[1b]

[1]Department of Physiology & Pharmacology, Thomas J. Long School of Pharmacy & Health Sciences, University of the Pacific, Stockton, CA 95211,[2]Department of Pharmacology Toxicology and Therapeutic Chemistry, School of Pharmacy and Food Sciences, University of Barcelona, [3]IBUB (Institute of Biomedicine, University of Barcelona), and [4]CIBERobn (Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición).

[a] Equally contributing authors.

[b] Equally contributing senior authors

* To whom correspondence should be addressed: Roshanak Rahimian Ph. D., Department of Physiology & Pharmacology, Thomas J. Long School of Pharmacy & Health Sciences, University of the Pacific, 3601 Pacific Ave, Stockton, CA 95211. Telephone: (209) 946-2373, Fax: (209) 946-2857, Email: rrahimian@pacific.edu

Running Title: Simple sugar effects on mesenteric arteries in female rats

Key words: Rat mesenteric arteries; Fructose; Glucose; Endothelial dysfunction; Blood pressure

Abstract

We have recently shown that type of supplemented simple sugar, not merely calorie intake, determines adverse effects on metabolism and aortic function in female rats. The aim of the current study was to investigate and compare the effects of high consumption of glucose and fructose on mesenteric arterial reactivity and systolic blood pressure (SBP). Sprague-Dawley female rats were supplemented with 20% w/v glucose or fructose in drinking water for 8 weeks. Here, we show that both sugars alter insulin signaling in mesenteric arteries (MA), assessed by a reduction in phosphorylated Akt, and increase SBP. Furthermore, ingestion of glucose or fructose enhances inducible nitric oxide synthase (iNOS) expression and contractile responses to endothelin (ET-1) and phenylephrine (PE) in MA of rats. The endothelium-dependent vasodilation (EDV) to acetylcholine (ACh) and bradykinin (BK) as well as the relaxation responses to the nitric oxide donor sodium nitroprusside (SNP) are impaired in MA of fructose-, but not glucose-supplemented rats. In contrast, only glucose supplementation increases the expression of phosphorylated endothelial NOS (eNOS) in MA of rats. In conclusion, this study reveals that supplementation with fructose or glucose in liquid form deranges MA insulin siganlling, enhances vasocontractile responses and increases iNOS expression in MA, effects which are accompanied by increased SBP in those groups. On the other hand, the preserved vasodilatory responses in MA from glucose-supplemented rats could be attributed to the enhanced level of phosphorylated eNOS expression in this group.

1. Introduction

Over the past decade obesity and type 2 diabetes (T2D) have reached epidemic levels in developed countries, becoming one of the most serious and challenging health problems in the 21st century. Excessive intake of sugar-sweetened beverages has been implicated to the development of obesity and metabolic disturbances [1,2]. Recent data provided by the National Health and Nutritional Examination Survey show that almost half of the USA population consumes sugar-sweetened beverages daily, with a 25% obtaining a minimum of 200 kcal/day, and a 5% obtaining more than 576 kcal/day from these beverages [3].

High fructose corn syrup (HFCS), which mainly consists of 55% fructose and 45% glucose, has become a predominant sweetener in soft drinks replacing sucrose (50% glucose and 50% fructose) in the US market. It has been suggested that fructose in 'free' form, which is mainly present in HFCS, might be an important contributing factor for the high incidence of obesity and cardiometabolic diseases such as diabetes than 'bound' disaccharide form in sucrose [4]. Liquid energy intake through these sugar-sweetened beverages (SSB) is considered to evoke weak satiety signals and incomplete compensation for total energy compared to solid energy intake [5,6].

Fructose has unique biochemical, metabolic and endocrine responses compared to glucose, both in experimental animal models and in humans [7–10]. However, controversy remains whether glucose or fructose differentially impacts cardiometabolic risk factors such as weight gain and cardiovascular disease (CVD) [10–12].

Despite a large number of studies on the association of consumption of excess sugar with

the development of cardiometabolic disease, there is little data on differential effects of dietary sugars on blood pressure and vascular reactivity. In a recent report, we showed that high fructose consumption impairs aortic function and alters metabolic parameters, including insulin signaling in liver and aorta, to a greater extent than glucose consumption in female rats [13]. Nevertheless, it remains to be established whether the above-mentioned differences in the effects of fructose and glucose are specific to large conduit arteries or whether they are generalizable effects extending to smaller arterial beds, such as MA. These vessels are important for maintaining vascular tone and regulation of blood pressure in the basal state.

Animal studies have shown an association between sugar ingestion and hypertension development [14,15]. A number of studies have reported the development of hypertension accompanied by insulin resistance and hyperinsulinemia in rats fed fructose-rich diet [16], however, no differences in mean arterial blood pressure have also been reported [17,18]. Epidemiological studies in humans also indicate a positive association between SSB ingestion and hypertension development [19].

There is accumulating evidence showing a possible link between hypertension, insulin resistance, and endothelial dysfunction [16,20,21]. Endothelial dysfunction is defined as reduced endothelial-dependent vasodilation (EDV) to vasodilators such as acetylcholine (ACh) and bradykinin (BK). EDV is used as a reproducible parameter to probe endothelium function under pathological conditions. Impaired EDV may in part result from either a decreased synthesis or release of endothelium-derived relaxing factors (EDRFs), including prostacyclin (PGI2), nitric oxide (NO), and endothelium-derived

hyperpolarizing factor (EDHF), or an increased release of endothelium-derived contracting factors such as endothelin-1 (ET-1).

Here, we studied the effects of supplementation of 20% w/v glucose or fructose in drinking water for 8 weeks on systolic blood pressure (SBP) and mesenteric arterial reactivity in female rats. The majority of studies in animals are performed in males [22–24], which calls for an attention to characterize and compare the effects of dietary sugars in females. A 8-week duration of sugar supplementation was selected to simulate duration equivalent to 6 years of daily consumption of sugars in humans [13,25]. The mesenteric arterial reactivity was examined by performing ACh-, BK-, and sodium nitroprusside (SNP)-mediated relaxation. Vasoconstrictor responses to phenylephrine (PE) and ET-1 were also obtained. Furthermore, the expression level of proteins associated with vascular function and cellular energy homeostasis such as endothelial NO synthase (eNOS) and inducible NO synthase (iNOS), phosphorylated and total AKT and adenosine monophosphate activated protein kinase (AMPK) were determined.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), and dissolved in water, unless otherwise stated.

2.2. Animals and experimental design

Female Sprague-Dawley rats, aged 9–11 weeks (Simonsen Laboratories, Gilroy, CA, USA) were maintained with water and standard rodent chow food *ad libitum* at constant

humidity and temperature, with a light/dark cycle of 12 h. After acclimation for 1 week, the animals were randomly categorized into a control, a glucose-supplemented or a fructose-supplemented group (14 rats per group). Sugars (fructose or glucose) were supplied as a 20 % (w/v) solution in drinking water for 8 weeks. Body weight, food and drink intake were monitored throughout the experiment. After 8 weeks, the rats were fasted for 12 h and euthanized using carbon dioxide as euthanasia agent, according to the recommendations from the 2013 AVMA Guidelines on Euthanasia and the NIH Guidelines for the Care and Use of Laboratory Animals: Eighth Edition (US National Institutes of Health 2011). All animal protocols were approved by the Animal Care Committee of the University of the Pacific and complied with the Guide for the Care and Use of Laboratory Animals: Eighth Edition (US National Institutes of Health 2011) and with ARRIVE guidelines.

2.3. Blood/plasma analysis

Blood samples were obtained by intracardiac puncture and collected in tubes containing anticoagulant. Plasma was obtained by centrifugation at 10,000 *g* for 5 min at 4°C and stored at -80°C until used. Glucose, triglycerides, and cholesterol were measured in 12-h-fasted rats using an Accutrend Plus System glucometer and specific test strips (Roche Farma, Barcelona, Spain) with blood collected from the tail vein. Insulin levels, leptin and adiponectin levels were also assessed by using ELISA kit (Mercodia, Uppsala, Sweden) according to the manufacturer's instructions. Insulin sensitivity index (ISI) was determined from fasting plasma glucose and insulin using the following formula:

ISI = [2/ (blood insulin (nM) x blood glucose (μM) + 1].

2.4. Blood pressure measurement

Blood pressure was measured in unanaesthetized rats by non-invasive tail cuff method as described previously [26]. Animals were placed in restraints and heating chambers in order to be acclimatized in a warm, quiet and dark environment for 30 min prior actual measurement. Blood pressure (BP) was measured between 9 am and 3 pm, before sugar supplementation and every 2 weeks throughout the study. Rats were allowed to habituate to the procedure one week before experiments were conducted. Once the cuffs were attached to the tail, followed by stabilization period of 5-10 minutes, readings were taken in an interval of 5 minutes. The mean of 6 readings within range of ± 5-10 mmHg was considered the final reading.

2.5. Measurement of Mesenteric Arterial Tension

The branches of MA of second and third order were separated from veins, cleared of fatty and adhering tissues and cut into 2 mm rings with internal diameter ranging between 250-350 μm. Each 2-mm segment was mounted between two jaws with use of tungsten wire (40 μm diameter) in organ bath of myograph (model 610M; Danish Myo Technology, Denmark). The organ bath contained Krebs solution of (in mM) 119 NaCl, 4.7 KCl, 1.6 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, 0.023 EDTA, and 6 glucose at 37°C, bubbled with 95% O2-5% CO2. The arterial tension was monitored with a computer-based data acquisition system (Chart5, Powerlab; ADInstruments, Colorado Springs, CO). The rings were normalized to a resting tension of 13.3 kPa and equilibrated for 30 min to obtain a basal tone. Arterial segments were then stimulated with 80 mM KCl

solution for a couple of times. To test the viability of the endothelium, ACh (10 μM)-induced relaxation was recorded in PE (2 μM) pre-contracted vessels.

2.6. Relaxation Responses to ACh

Mesenteric arterial rings were contracted with PE (2 μM), which produced about 80% of the maximal contraction. The concentration response curve (CRC) was obtained by the addition of increasing concentrations of ACh (10^{-8} to 10^{-5} M).

2.7. Relaxation Responses to BK

The CRCs to BK were measured following the addition of increasing concentrations of BK (10^{-9} to 10^{-5} M) in U46619 (9,11-dideoxy-9α,11α-methanoepoxy Prostaglandin $F_{2\alpha,;}$ 100 nM) pre-contracted mesenteric rings.

2.8. Relaxation Responses to SNP

The CRCs to SNP (10^{-9} to 10^{-5} M), a NO donor, were generated in mesenteric arterial rings pre-contracted with PE (2 μM).

2.9. Constrictor Responses to PE and ET-1

The CRCs to PE and ET-1 were obtained by the addition of increasing concentrations of PE (10^{-8} to $3X10^{-5}$ M) or ET-1 (10^{-10} to $3X10^{-7}$ M) in pre-contracted MA.

2.10. Western blot analysis

Mesenteric tissue samples were micronized through freezing with liquid nitrogen and grinded with a mortar. For total protein extraction, lysis buffer with proteases, phosphatases and acetylases inhibitors (50 mM Tris– HCl pH=8, 150 mM NaCl, 1% Igepal, 10mM NaF, 1 mM EDTA, 1mM EGTA, 2 mM Nappi, 1mM PMSF, 2 μg/mL

leupeptin, 2 μg/mL aprotinin, 1 mM Na_3VO_4, 10mM NaM, 1μM TSA) was used. Samples were homogenized for 1.5 h at 4°C, centrifuged at 15,000×g for 15 min at 4°C and supernatants were collected. The homogenates were kept on ice for 10 min and centrifuged at 1000xg for 10 min at 4°C. Lysis buffer was added to the pellet obtained and samples were incubated for 1.5 h at 4°C, centrifuged at 25,000×g for 30 min at 4°C and supernatants were collected. Protein concentrations were determined by the Bradford method [27].

20-30 μg of protein was subjected to SDS-polyacrylamide gel electrophoresis. Proteins were then transferred to Immobilon polyvinylidene difluoride transfer membranes (Millipore, Billerica, MA, USA), blocked for 1 h at room temperature with 5% non-fat milk solution in 0.1% Tween-20-Tris-buffered saline (TBS), and incubated overnight at 4°C with primary antibodies. Detection was performed using the Pierce® ECL Western Blotting Substrate (Thermo Scientific, Waltham, MA, USA) and Immobilion™ Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA, USA). To confirm the uniformity of protein loading, blots were incubated with β-actin antibodies (Sigma-Aldrich, St. Louis, MO, USA) as a control. Primary antibodies for p-eNOS (Ser1177), total eNOS, iNOS, p-Akt (Ser473), total Akt, p-AMPK (Thr172) and total AMPK were supplied by Cell Signaling (Danvers, MA, USA).

2.11. RNA isolation and real-time PCR

The whole MA bed was submerged in RNAlater (Life Technologies, Carlsbad, CA) shortly after dissection. Total RNA was extracted from MA using an RNeasy Mini Kit with on-column DNase treatment (Qiagen, Valencia,CA). cDNA was synthesized by

reverse transcription using the Omniscript reverse transcriptase kit (Qiagen). The gene fragments were specifically amplified with the iQ SYBR Green Supermix (Bio-Rad, Hercules, CA) using StepOnePlus Real-Time PCR System Thermal Cycling Block (Applied Biosystems, Foster City, CA). Internal variations were normalized to rat GAPDH or β actin. The following primers were used for detection of gene expression: *bkr1*-Forward: 5'-CAGCGCTTAACCATAGCGGAAAT-3', Reverse: 5'-CCAGTTGAAACGGTTCCCGATGTT-3', *bkr2*-Forward: 5'-TTTGTCCTCAGCGTGTTCTG-3', Reverse: 5'-TCACAAGCATCAGGAAGCAG-3'.

2.12. Statistical Analysis

The ACh- and SNP-induced relaxations were expressed as the percentage of relaxation from maximum PE contraction at each concentration. Similarly, the recorded increase in the force of contraction was calculated as the percentage of maximum contraction obtained with PE at the highest dose. Statistical analyses were performed by one-way ANOVA test, followed by Bonferroni's or Tukey's *post-hoc* analysis. Comparison of CRCs between two groups was done using two-way ANOVA, with one factor being concentration and the others being groups (control vs. sugar- supplemented). EC_{50}, the concentration of the agonist which produced half of the maximum effect (E_{max}) was calculated by a sigmoidal dose-response model (for variable slope) using GraphPad Prism 6.01 (GraphPad Software Inc., San Diego, CA). The sensitivity of the agonists was expressed as pD_2 values (-log [EC_{50}]), which were normally distributed. The area under the curve (AUC) was determined using GraphPad Prism 6.01 with trapezoidal method. Data were reported as the mean ± standard error of the mean (SEM). Student's

unpaired t-test was used for comparisons of two group means. A probability value of less than 5% ($P<0.05$) was considered significant.

3. Results

3.1. Both glucose and fructose supplementation impair insulin signaling in MA and increase SBP

As previously reported by us [13] and shown in the supplementary data, both fructose and glucose (20% w/v in drinking water for 8 weeks) increased the total amount of ingested calories, but only fructose supplementation caused hypertriglyceridemia and increased body weight (Supplemental Table 1). On the other hand, supplementation with glucose, but not fructose, increased plasma adiponectin level (Supplemental Table 1). Furthermore, the insulin sensitivity index (ISI) was significantly reduced in both glucose- and fructose-supplemented rats (Supplemental Table 1). The reduced ISI and the impairment of insulin signaling in aortic tissue observed in our previous study [13] prompted us to examine whether sugar supplementation caused similar effects on MA. As shown in Fig. 1A, both fructose and glucose supplementation reduced p-Akt protein expression levels while total Akt expression was not affected, suggesting that Akt activity was reduced in MA of both sugar-supplemented groups.

It has been proposed that fructose-induced insulin resistance and hyperinsulinemia can lead to hypertension [16]. We, therefore, measured blood pressure in sugar-supplemented rats throughout the intervention period. The changes in SBP are shown in Figures 1B&C. As assessed by the 2-way ANOVA analysis, only simple sugar supplementation

($p<0.0001$), but not time ($p=0.243$), significantly increased SBP. SBP is elevated in glucose-supplemented rats as early as the 2nd week, and the 4th week in the fructose-supplemented group (Figure 1B). Both sugars similarly increased the AUC for the SBP for the entire supplementation period (Figure 1C).

3.2. Supplementation with fructose but not glucose impairs EDV in MA

The EDV was determined by assessing the relaxation responses to ACh (10^{-8}-10^{-5} M) and BK 10^{-9}-10^{-5} M) (Figures 2A and B, respectively). There was a significant rightward shift in the ACh relaxation responses in MA of fructose-supplemented rats compared with the control rats (Figure 2A). As shown in Table 1, the pD2 to ACh was decreased significantly only in fructose-supplemented rats. Moreover, the E_{max} to BK was significantly reduced in the fructose, but not glucose group when compared to control rats (0.42-fold, Table 1). To investigate a mechanism by which the relaxation to BK might have been affected in the MA of fructose-supplemented rats, the mRNA expression of BK receptors (*bkr1* and *bkr2*) was assessed. As shown in Figure 2C&D, the mRNA expression of *bkr1* receptors was significantly lower in MA from fructose-supplemented rats compared with controls; the expression of *bkr2* showed a similar pattern, but the reduction induced by fructose supplementation did not reach statistical significance.

3.3. Fructose supplementation impairs relaxation response to SNP in MA

Although the CRC to the NO donor SNP (10^{-9}-10^{-5} M) in mesenteric arterial rings tended to shift to the right in both fructose and glucose-supplemented groups (Figure 3), the sensitivity of MA to SNP as assessed by -log[EC50] (pD2) was similar in all groups

(Table 2). The maximal response to SNP (E_{max}), however, was significantly reduced in the fructose-, but not in the glucose-supplemented group compared with controls (0.6-fold, Table 2).

3.4. Both sugars enhance contractile responses in MA

To examine whether simple sugar supplementation affect the responses to contractile agents, CRCs to PE (10^{-7} to $3X10^{-5}$ M) or ET-1 (10^{-10} to 10^{-7} M) were generated in rat mesenteric arterial rings (Figures 4A and B). The maximal responses ($tension_{max}$) to both contractile agents in the arteries of glucose- and fructose-supplemented rats were higher than those in controls (Table 3). However, the sensitivity to PE or ET-1 was not changed in either group.

3.5. Differential effects of fructose and glucose supplementation on mesenteric arterial expression of eNOS and iNOS

To study potential underlying mechanisms that could explain the differences in responses of MA taken from glucose- and fructose-supplemented rats, the expression of eNOS, p-eNOS and iNOS were measured by Western blot analysis. As shown in Figure 5A, although total eNOS expression was not modified by sugar supplementation, p-eNOS levels were significantly elevated in MA taken from glucose-, but not fructose-supplemented rats compared with those seen in the MA from controls (7.8-fold increase). We also determined the expression of total and phosphorylated adenosine monophosphate activated protein kinase (AMPK). As shown in Figure 5B, there is a trend towards a higher level of phosphorylated, active AMPK protein in the glucose

group, although it did not reach statistical significance. On the other hand, iNOS expression was increased in the MA of both fructose- and glucose-supplemented groups (1.4 and 1.5-fold, respectively, Figure 5C).

4. Discussion

A number of studies have examined the influence of excessive sugar consumption on blood pressure in different animal models, however, the results have been inconsistent [16–19], and the underlying mechanisms involved are not fully elucidated. Here, we show that the ingestion of simple sugars (glucose or fructose) in liquid form for 8 weeks alters insulin signaling in MA, and increases SBP in female rats. The contractile responses and iNOS protein expression are also increased in MA taken from both sugar-supplemented groups. However, only fructose-supplementation impairs mesenteric arterial endothelial vasodilatory function. This differential effect could be attributed to the increased p-eNOS expression in MA of glucose-, but not fructose-supplemented animals.

Several studies have shown that feeding rats a solid diet containing 60-66% of fructose for 4-8 weeks causes hypertension [28–32]. However, when fructose is provided as a sweetened beverage, the major source of fructose consumption in humans, the hypertensive effect is not always observed. In a recent report, Sousa et al showed that supplementation with 10% w/v fructose for 6 weeks did not alter SBP in Wistar rats [33]. Similarly, Gordish et al found that SBP was not altered after 2 weeks of 20% fructose supplementation in Sprague-Dawley rats [34]. Both reports, as the majority of the studies on the effects of high fructose diets in rodents, have been performed on male

rats, while in our study we used females. Female animals are underrepresented in preclinical studies, although the metabolic alterations and the mechanisms involved may differ in males and females. We previously reported that female rats supplemented with liquid fructose for 2 weeks displayed glucose intolerance and insulin resistance, effects that were not observed in males [35]. Here, we show that fructose- or glucose-supplementation in liquid form impair insulin signaling, as assessed by reduced Akt phosphorylation in MA of these female rats. Along similar lines, we recently reported an impairment of insulin signaling in aorta and liver of sugar- supplemented female rats [13]. In contrast to studies performed on male rats [33,34], in the current study the impairment in insulin signaling was accompanied with a significant increase in SBP in female rats. The fact that insulin signaling was affected by fructose or glucose supplementation suggests that the insulin resistance may in part contribute to the elevated blood pressure in both sugar- supplemented groups. Insulin resistance has been proposed as one of the mechanisms by which fructose increases blood pressure [16,36]. Furthermore, our results suggest that the decreased insulin sensitivity and the increase in SBP are not specific to fructose consumption, but extending to excessive simple sugar consumption.

Endothelial dysfunction may be an underlying mechanism which links insulin resistance and hypertension [16,37]. Our data show that EDV was impaired in the rats supplemented with fructose, but not glucose, as assessed by rightward shift in the CRC to ACh or reduction of E_{max} to BK in MA of this group (Figure 2). This excludes endothelial dysfunction as a sole cause of the increased blood pressure, since it did not occur in MA of glucose supplemented rats. These data are, in part, consistent with our

recent report demonstrating impaired aortic responses to BK in fructose, but not glucose-supplemented female rats [13]. To identify possible changes in receptor level, we measured the expression level of bradykinin receptors. The decrease of mesenteric *bkr1* expression levels in fructose-supplemented rats may in part explain the significant reduction of maximum response to BK as well as the ACh shift to the right which was observed only in this group. Previously published data demonstrated that impaired vascular response to ACh is also present in BK-1$^{-/-}$ mice [38]. Both bkr1 and bkr2 receptors are located in the endothelium and in vascular smooth muscle cells [39,40]. Loiola et al. [38] demonstrated that deletion of these receptors impairs EDV by reducing NO bioavailability, which may occur via reduced eNOS enzymatic activity or increased NO inactivation. Miatello et al. showed that eNOS activity decreases in mesenteric vasculature isolated from fructose-fed rats [41]. Here, we did not assess enzymatic eNOS activity, but we measured the expression of the active, phosphorylated form of eNOS at Ser1177 in MA. Our data show that p-eNOS expression was not altered in the fructose-supplemented group, suggesting that in our experimental model, the vascular effects of fructose cannot be attributed to reduced eNOS activation. Although the eNOS protein or activity was not changed in MA of fructose-supplemented rats, our data reveals the elevation of iNOS in MA of both glucose and fructose groups. Along similar lines, Sousa et al. [33] observed no changes in eNOS, but an elevation of iNOS in mesenteric vascular bed of male rats following 6 weeks high fructose intake. Any increase in NO production resulting from iNOS has the potential for free radical-mediated damage, particularly under conditions of oxidative stress where peroxynitrate is formed more easily [42]. Increased vascular iNOS activity and/or protein expression have been also described in

hypertension [43,44]. Thus, the elevated iNOS could in part contribute to increased blood pressure in sugar-supplemented female rats.

On the other hand, we showed that p-eNOS levels were significantly increased in MA from glucose-supplemented rats, an effect that was also observed in our earlier studies in the aortic vasculature and was attributed to the hyperadiponectinemia caused by glucose supplementation [13]. In the same study, we showed that the *in vitro* incubation of endothelial cells with adiponectin increases cellular NO levels, however, this effect was absent when cells where incubated with only fructose or glucose solution [13]. It has been shown that adiponectin stimulates eNOS activity via increasing AMPK phosphorylation [45]. In our study, glucose- but not fructose-supplemented female rats exhibit elevated plasma adiponectin levels (Supplemental Table 1) along with a tendency to increase the phosphorylation of AMPK in MA (Fig 5A), leading to a significant increase in p-eNOS in these tissues (Figure 5B). Taken together, our results suggest a specific role of adiponectin/AMPK/eNOS axis in mediating the vascular effect of glucose. It is important to note that this mechanism did not protect the glucose-supplemented rats from increased blood pressure; however, our data suggest that an increased NO resulting from p-eNOS may in part contribute to preserved responses to ACh and BK in MA from glucose-supplemented rats.

Vascular dysfunction in metabolic syndrome may also be associated with an increased vasoconstrictor sensitivity, through an enhanced release of contracting factors or a decreased sensitivity of smooth muscle to NO [29]. In the current study, we show that the contractile responses to PE and ET-1 were enhanced in MA of either glucose- or

fructose-supplemented groups (Figure 4). These results are in accordance with observations of Sousa et al, who showed that high fructose intake increased norepinephrine-induced vasoconstriction in MA of male rats [33]. However, our data are in contrast with studies by Navarro-Cid et al. [46] and Iyer et al. [30] showing no differences in the vasoconstrictor responses to PE in MA from fructose-fed male rats. The source of these discrepancies may be related to the duration of exposure, concentration and form of sugar-supplementation or the sex of animals used.

Verma et al. [47] have shown that the ET-1 content in rat MA was higher when fed with 66% fructose diet compared to control rats. This increased ET-1 is thought to arise from fructose-induced hyperinsulinemia, since insulin has been shown to stimulate the production and secretion of ET-1 *in vitro* and *in vivo* [48,49]. In the current study, we did not directly measure ET-1 (or any other contracting factors), but observed increased response to ET-1 in both fructose- and glucose-supplemented groups. This enhanced response may further contribute to increased blood pressure that is detected in both sugar-supplemented rats. Accordingly, Verma et al reported that the treatment with bosentan – a dual ET_A and ET_B receptor blocker – reduced the hypertension in fructose-fed rats [47]. Finally, the fact that the SNP-induced relaxation was affected only by fructose supplementation suggests that the decreased smooth muscle response to NO may in part contribute to the increased contractile responsiveness in this group (Fig. 3).

In conclusion, we have demonstrated that the intake of liquid fructose or glucose increases SBP, possibly by altering insulin signaling and vasculature function in MA in female rats. Particularly, the enhanced contractile responsiveness and iNOS expression in

MA might be involved in the rise in blood pressure in both glucose- and fructose-supplemented groups. Furthermore, we have shown that the mesenteric arterial vasodilatory function was impaired in fructose-, but not glucose-supplemented groups. The preserved vasodilatory responses in MA from the glucose group, in part, could be attributed to the higher level of adiponectin and subsequent enhanced phosphorylation of eNOS in this group. Clearly, the relevance of the present results to understanding how dietary sugar affects vascular system needs further investigation, and it should be discussed with regard to the experimental model (animal studies), type of vascular bed, and sex among other factors.

Acknowledgement

This work was supported by the National Heart, Lung, and Blood Institute Grant R15HL128988 to R. Rahimian and the University of the Pacific. It was also supported in part by grants from the Fundació Privada Catalana de Nutrició i Lípids, SAF2013–42982-R, and European Commission FEDER funds to M. Alegret and J.C. Laguna.

5. References

[1] Malik VS, Schulze MB, Hu FB. Intake of sugar-sweetened beverages and weight gain: a systematic review. Am J Clin Nutr 2006;84:274–88.

[2] Vartanian LR, Schwartz MB, Brownell KD. Effects of soft drink consumption on nutrition and health: a systematic review and meta-analysis. Am J Public Heal 2007;97:667–75. doi:10.2105/AJPH.2005.083782.

[3] Ogden, C L; Kit, B K; Carroll, M D; Park S. Consumption of sugar drinks in the United States, 2005–2008. NCHS Data Brief, No 71 Hyattsville, MD Natl Cent Heal Stat 2011.

[4] Sheludiakova A, Rooney K, Boakes RA. Metabolic and behavioural effects of sucrose and fructose/glucose drinks in the rat. Eur J Nutr 2012;51:445–54. doi:10.1007/s00394-011-0228-x.

[5] DiMeglio DP, Mattes RD. Liquid versus solid carbohydrate: effects on food intake and body weight. Int J Obes Relat Metab Disord 2000;24:794–800.

[6] Almiron-Roig E, Drewnowski A. Hunger, thirst, and energy intakes following consumption of caloric beverages. Physiol Behav 2003;79:767–73.

[7] White JS. Challenging the fructose hypothesis: new perspectives on fructose consumption and metabolism. Adv Nutr 2013;4:246–56. doi:10.3945/an.112.003137.

[8] Sievenpiper JL, de Souza RJ, Cozma AI, Chiavaroli L, Ha V, Mirrahimi A. Fructose vs. glucose and metabolism: do the metabolic differences matter? Curr Opin Lipidol 2014;25:8–19. doi:10.1097/MOL.0000000000000042.

[9] Khan TA, Sievenpiper JL. Controversies about sugars: results from systematic reviews and meta-analyses on obesity, cardiometabolic disease and diabetes. Eur J Nutr 2016;55:25–43. doi:10.1007/s00394-016-1345-3.

[10] Baena M, Sanguesa G, Davalos A, Latasa MJ, Sala-Vila A, Sanchez RM, et al.

Fructose, but not glucose, impairs insulin signaling in the three major insulin-sensitive tissues. Sci Rep 2016;6:26149. doi:10.1038/srep26149.

[11] Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest 2009;119:1322–34. doi:10.1172/JCI37385.

[12] Schaefer EJ, Gleason J a, Dansinger ML. Dietary Fructose and Glucose Differentially Affect Lipid and Glucose Homeostasis1–3. J Nutr 2009;139:1257S–1262S. doi:10.3945/jn.108.098186.WHO.

[13] Sanguesa G, Shaligram S, Akther F, Roglans N, Laguna JC, Rahimian R, et al. Type of supplemented simple sugar, not merely calorie intake, determines adverse effects on metabolism and aortic function in female rats. Am J Physiol Hear Circ Physiol 2017;312:H289–304. doi:10.1152/ajpheart.00339.2016.

[14] Bunag RD, Tomita T, Sasaki S. Chronic sucrose ingestion induces mild hypertension and tachycardia in rats. Hypertension 1983;5:218–25.

[15] Martinez FJ, Rizza RA, Romero JC. High-fructose feeding elicits insulin resistance, hyperinsulinism, and hypertension in normal mongrel dogs. Hypertension 1994;23:456–63.

[16] Tran LT, Yuen VG, McNeill JH. The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension. Mol Cell Biochem

2009;332:145–59. doi:10.1007/s11010-009-0184-4.

[17] Brands MW, Garrity CA, Holman MG, Keen HL, Alonso-Galicia M, Hall JE. High-fructose diet does not raise 24-hour mean arterial pressure in rats. Am J Hypertens 1994;7:104–9.

[18] D'Angelo G, Elmarakby AA, Pollock DM, Stepp DW. Fructose feeding increases insulin resistance but not blood pressure in Sprague-Dawley rats. Hypertension 2005;46:806–11. doi:10.1161/01.HYP.0000182697.39687.34.

[19] Kim Y, Je Y. Prospective association of sugar-sweetened and artificially sweetened beverage intake with risk of hypertension. Arch Cardiovasc Dis 2016;109:242–53. doi:10.1016/j.acvd.2015.10.005.

[20] Takagawa Y, Berger ME, Hori MT, Tuck ML, Golub MS. Long-term fructose feeding impairs vascular relaxation in rat mesenteric arteries. Am J Hypertens 2001;14:811–7.

[21] Pektas MB, Sadi G, Akar F. Long-Term Dietary Fructose Causes Gender-Different Metabolic and Vascular Dysfunction in Rats: Modulatory Effects of Resveratrol. Cell Physiol Biochem 2015;37:1407–20. doi:10.1159/000430405.

[22] Verma S, Skarsgard P, Bhanot S, Yao L, Laher I, McNeill JH. Reactivity of mesenteric arteries from fructose hypertensive rats to endothelin-1. Am J Hypertens 1997;10:1010–9.

[23] Akar F, Uludağ O, Aydın A, Aytekin YA, Elbeg S, Tuzcu M, et al. High-fructose

corn syrup causes vascular dysfunction associated with metabolic disturbance in rats: protective effect of resveratrol. Food Chem Toxicol 2012;50:2135–41. doi:10.1016/j.fct.2012.03.061.

[24] Babacanoglu C, Yildirim N, Sadi G, Pektas MB, Akar F. Resveratrol prevents high-fructose corn syrup-induced vascular insulin resistance and dysfunction in rats. Food Chem Toxicol 2013;60:160–7. doi:10.1016/j.fct.2013.07.026.

[25] Sengupta P. The laboratory rat: relating age with human's. Int J Prev Med 2013;4:624–30.

[26] El-Bassossy HM, Fahmy A, Badawy D. Cinnamaldehyde protects from the hypertension associated with diabetes. Food Chem Toxicol 2011;49:3007–12. doi:10.1016/j.fct.2011.07.060.

[27] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.

[28] Verma S, Bhanot S, McNeill JH. Antihypertensive effects of metformin in fructose-fed hyperinsulinemic, hypertensive rats. J Pharmacol Exp Ther 1994;271:1334–7.

[29] Iyer SN, Katovich MJ. Effect of chronic losartan potassium treatment on fructose-induced hypertension. Life Sci 1994;55:PL139-44.

[30] Iyer, S.N., Katovich MJ. Vascular reactivity to phenylephrine and angiotensin II in

hypertensive rats associated with insulin resistance. Clin Exp Hypertens 1996;18:227–42.

[31] Juan CC, Fang VS, Hsu YP, Huang YJ, Hsia DB, Yu PC, et al. Overexpression of vascular endothelin-1 and endothelin-A receptors in a fructose-induced hypertensive rat model. J Hypertens 1998;16:1775–82.

[32] Galipeau D, Arikawa E, Sekirov I, McNeill JH. Chronic thromboxane synthase inhibition prevents fructose-induced hypertension. Hypertension 2001;38:872–6.

[33] Sousa GJ, Oliveira PWC, Nogueira B V, Melo AFJ, Faria TO, Meira EF, et al. Fructose intake exacerbates the contractile response elicited by norepinephrine in mesenteric vascular bed of rats via increased endothelial prostanoids. J Nutr Biochem 2017;48:21–8. doi:10.1016/j.jnutbio.2017.06.005.

[34] Gordish KL, Kassem KM, Ortiz PA, Beierwaltes WH. Moderate (20%) fructose-enriched diet stimulates salt-sensitive hypertension with increased salt retention and decreased renal nitric oxide. Physiol Rep 2017;5. doi:10.14814/phy2.13162.

[35] Vila L, Roglans N, Perna V, Sanchez RM, Vazquez-Carrera M, Alegret M, et al. Liver AMP/ATP ratio and fructokinase expression are related to gender differences in AMPK activity and glucose intolerance in rats ingesting liquid fructose. J Nutr Biochem 2011;22:741–51. doi:10.1016/j.jnutbio.2010.06.005.

[36] Klein A V, Kiat H. The mechanisms underlying fructose-induced hypertension: a review. J Hypertens 2015;33:912–20. doi:10.1097/HJH.0000000000000551.

[37] Miller AW, Hoenig ME, Ujhelyi MR. Mechanisms of Impaired Endothelial Function Associated With Insulin Resistance. J Cardiovasc Pharmacol Ther 1998;3:125–34. doi:10.1177/107424849800300205.

[38] Loiola RA, Reis FC, Kawamoto EM, Scavone C, Abdalla DS, Fernandes L, et al. Role of vascular Kinin B1 and B2 receptors in endothelial nitric oxide metabolism. Peptides 2011;32:1700–5. doi:10.1016/j.peptides.2011.06.010.

[39] Bhoola KD, Figueroa CD, Worthy K. Bioregulation of kinins: kallikreins, kininogens, and kininases. Pharmacol Rev 1992;44:1–80.

[40] Leeb-Lundberg LM, Marceau F, Muller-Esterl W, Pettibone DJ, Zuraw BL. International union of pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences. Pharmacol Rev 2005;57:27–77. doi:10.1124/pr.57.1.2.

[41] Miatello R, Risler N, Gonzalez S, Castro C, Ruttler M, Cruzado M. Effects of enalapril on the vascular wall in an experimental model of syndrome X. Am J Hypertens 2002;15:872–8.

[42] Snyder SH, Bredt DS. Biological roles of nitric oxide. Sci Am 1992;266:68-71-77.

[43] Briones AM, Alonso MJ, Marin J, Balfagon G, Salaices M. Influence of hypertension on nitric oxide synthase expression and vascular effects of lipopolysaccharide in rat mesenteric arteries. Br J Pharmacol 2000;131:185–94. doi:10.1038/sj.bjp.0703552.

[44] Briones AM, Salaices M, Vila E. Ageing alters the production of nitric oxide and prostanoids after IL-1beta exposure in mesenteric resistance arteries. Mech Ageing Dev 2005;126:710–21. doi:10.1016/j.mad.2005.01.006.

[45] Deng G, Long Y, Yu Y-R, Li M-R. Adiponectin directly improves endothelial dysfunction in obese rats through the AMPK-eNOS Pathway. Int J Obes (Lond) 2010;34:165–71. doi:10.1038/ijo.2009.205.

[46] Navarro-Cid, J., Maeso, R., Pérez-Vizcaíno, F., Cachofeiro, V., Riulope, L.M., Tamargo, J., Lahera V. Effects of Losartan on Blood Pressure, Metabolic Alterations, and Vascular Reactivity in the Fructose-Induced Hypertensive Rat. Hypertension 1995;26:1074–8.

[47] Verma S, Bhanot S, McNeill JH. Effect of chronic endothelin blockade in hyperinsulinemic hypertensive rats. Am J Physiol 1995;269:H2017-21.

[48] Hu RM, Levin ER, Pedram A, Frank HJ. Insulin stimulates production and secretion of endothelin from bovine endothelial cells. Diabetes 1993;42:351–8.

[49] Juan CC, Shen YW, Chien Y, Lin YJ, Chang SF, Ho LT. Insulin infusion induces endothelin-1-dependent hypertension in rats. Am J Physiol Endocrinol Metab 2004;287:E948-54. doi:10.1152/ajpendo.00536.2003.

Figure captions

Fig. 1. (A) Western blot of phospho- and total Akt in mesenteric arterial samples obtained from control, glucose and fructose-supplemented rats. Representative bands

corresponding to three different rats in each group are shown; bar plots show the level of the phosphorylated protein expressed as the mean (a.u.) ± SEM of the values obtained from 4-5 animals. *P<0.05 vs control, analyzed by one-way ANOVA followed by Sidak's post hoc multiple comparison test. (B) Changes in systolic blood pressure (SBP) expressed as the mean ± SEM of the values obtained from 8 rats/group. $ P<0.05 and $$ P<0.01 glucose vs control, # P<0.05 and ## P<0.01 fructose vs control, analyzed by 2-way ANOVA followed by Tukey's post hoc multiple comparison test. (C) Area under the curve (AUC) for SBP values reported in Fig. 1B. ** P<0.01 vs control, analyzed by one-way ANOVA followed by Sidak's post hoc multiple comparison test.

Fig. 2. Relaxation response to cumulative concentrations of ACh (A) and BK (B) in intact mesenteric arterial rings precontracted with PE (2 uM) and U46619 (100 nM) from control, glucose- or fructose-supplemented rats. Data are expressed as the mean ± SEM from 5-8 animals/group. # P<0.05 fructose vs control, analyzed by 2-way ANOVA followed by Tukey's post hoc multiple comparison test. mRNA levels of *bkr1* (C) and *bkr2* (D) receptors in mesenteric arteries from control, glucose- and fructose-supplemented rats. Each bar represents the mean ± SEM of values obtained from n=8 animals. *P <0.05 versus control. One-way ANOVA followed by Sidak's post hoc multiple comparison test.

Fig. 3. Relaxation response to cumulative concentrations of SNP in intact mesenteric arterial rings precontracted with PE (2 uM) from control, glucose- or fructose-supplemented rats. Data are expressed as the mean ± SEM from 7 animals/group. $

P<0.05 glucose vs control, # P<0.05 fructose vs control, analyzed by 2-way ANOVA followed by Tukey's post hoc multiple comparison test.

Fig. 4. Concentration-response curves to PE (A) and ET-1 (B) in in intact mesenteric arterial rings MA from control, glucose- or fructose-supplemented rats. Data are expressed as the mean ± SEM from 5-7 animals/group. $ P<0.05 glucose vs control, # P<0.05 fructose vs control, analyzed by 2-way ANOVA followed by Tukey's post hoc multiple comparison test.

Fig. 5. Western blot of phospho- and total e-NOS (A), AMPK (B) and iNOS (C) in mesenteric arterial samples obtained from control, glucose and fructose-supplemented rats. Representative bands corresponding to three different rats in each group are shown; bar plots show the level of the proteins expressed as the mean (a.u.) ± SEM of the values obtained from 4-5 animals. *P<0.05 vs control, analyzed by one-way ANOVA followed by Sidak's post hoc multiple comparison test.

Figure 1

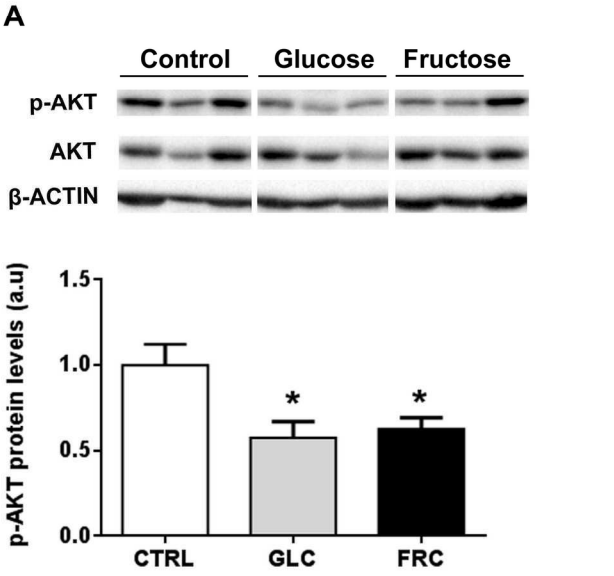
A
Control
Glucose
Fructose
p-AKT
AKT
β-ACTIN
p-AKT protein levels (a.u)
1.5
1.0
0.5
0.0
*
*
CTRL
GLC
FRC
B
Control
Glucose
Fructose
Systolic Blood Pressure (SBP, mmHg)
150
140
130
120
110
$
#
$
##
$$
##
0
2
4
6
8
Weeks
C
mm Hg x rat x 8 weeks
700
650
600
550
500
**
**
CTRL
GLC
FRC

Figure 2

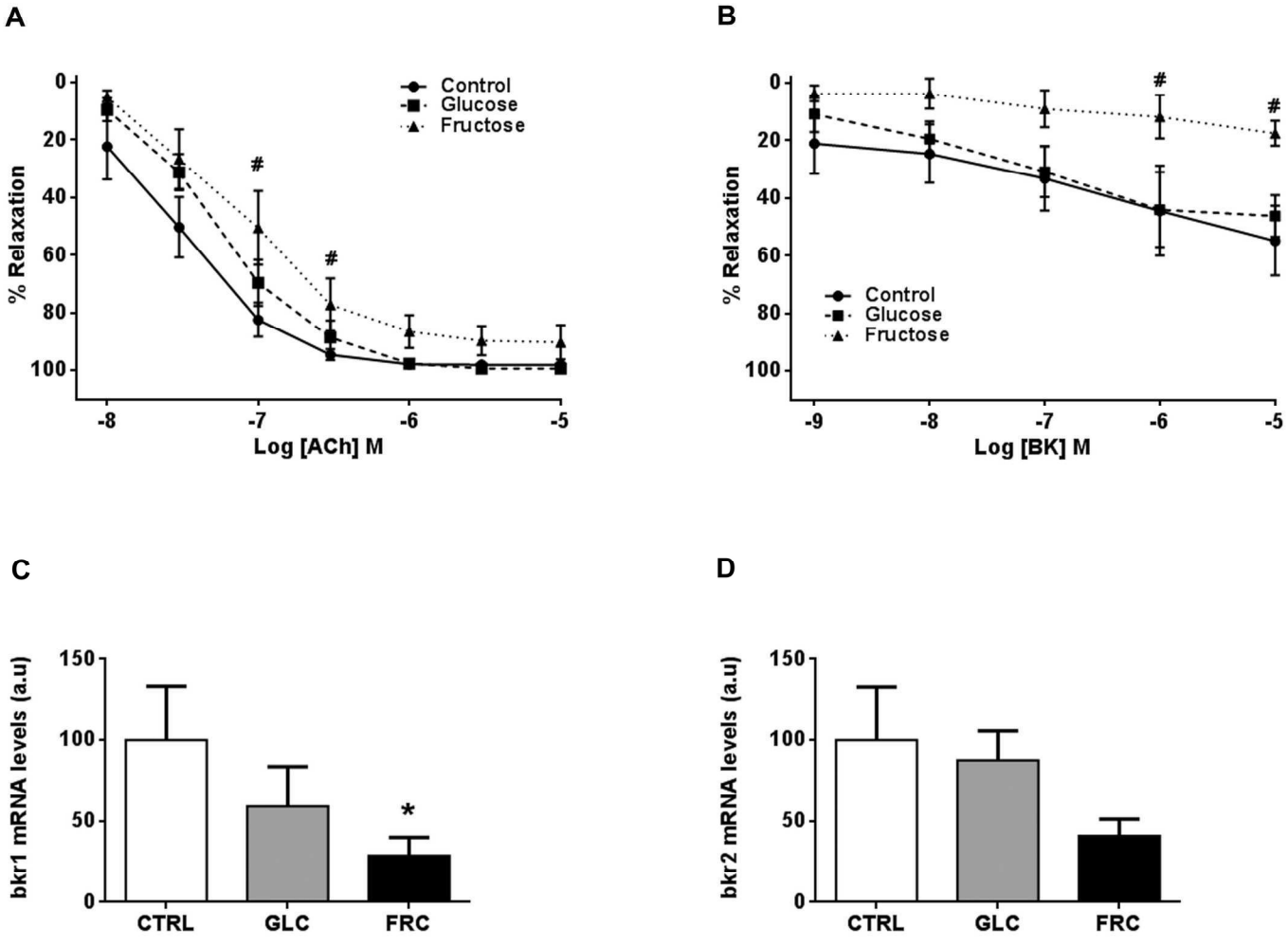
A
B
C
D
Control
Glucose
Fructose
% Relaxation
Log [ACh] M
Log [BK] M
bkr1 mRNA levels (a.u)
bkr2 mRNA levels (a.u)
CTRL
GLC
FRC
#
*

Figure 3

A

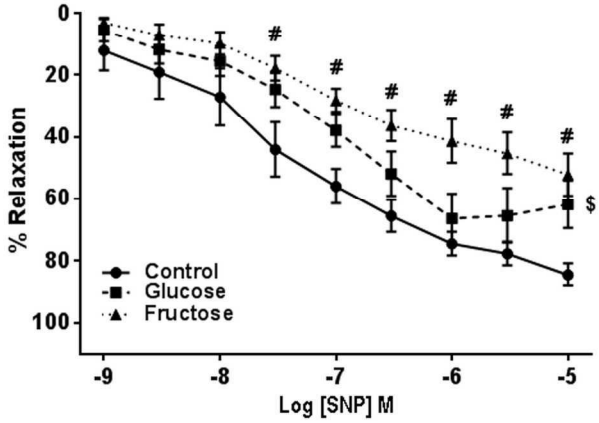
0
20
40
60
80
100
% Relaxation
#
#
#
#
#
#
$
Control
Glucose
Fructose
-9
-8
-7
-6
-5
Log [SNP] M

Figure 4

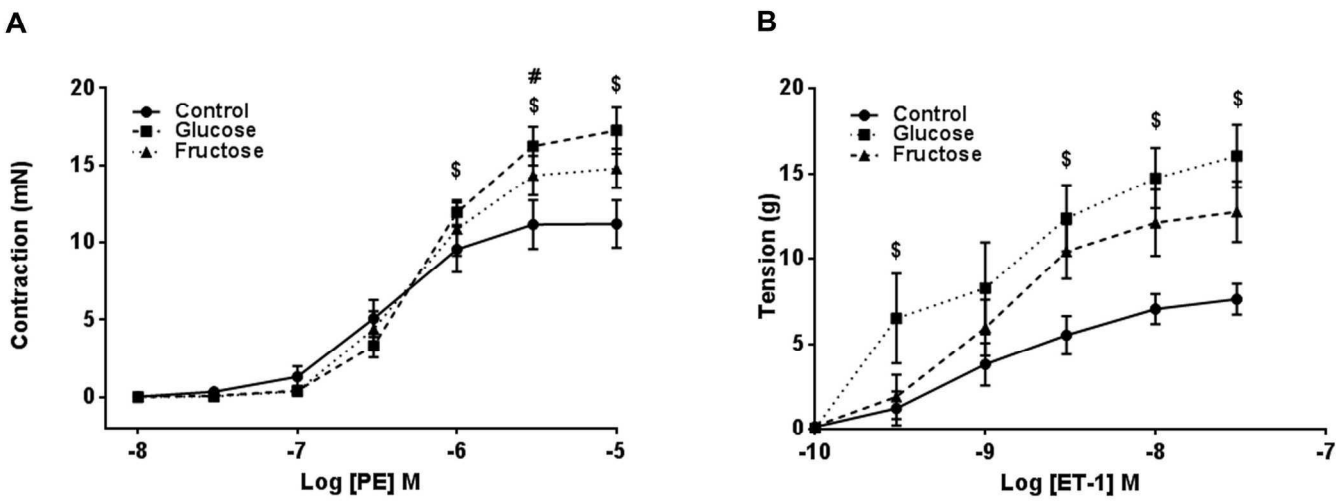
A
Control
Glucose
Fructose
Contraction (mN)
20
15
10
5
0
-8
-7
-6
-5
Log [PE] M
$
#
$
$
B
Control
Glucose
Fructose
Tension (g)
20
15
10
5
0
-10
-9
-8
-7
Log [ET-1] M
$
$
$
$

Figure 5

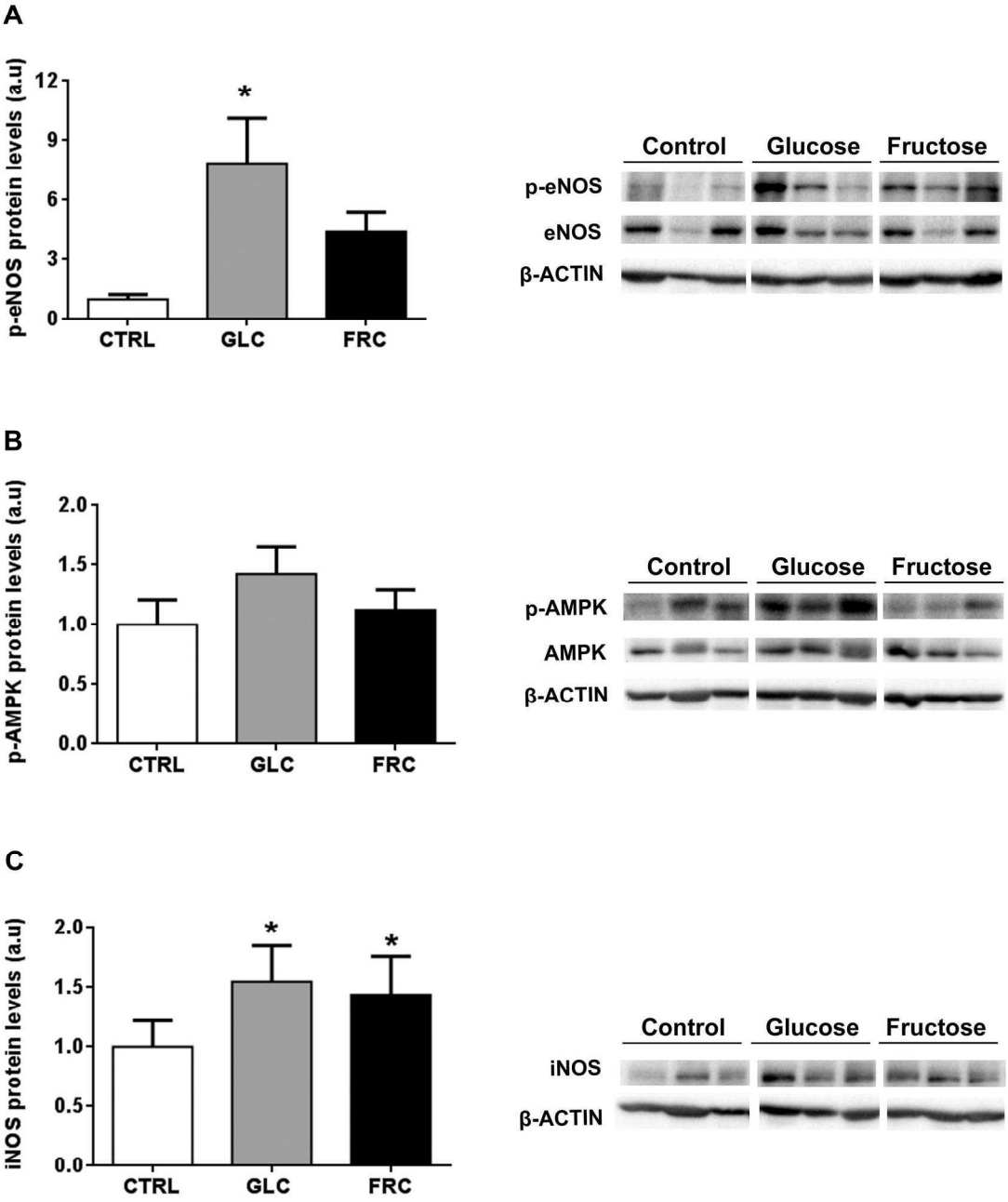
A
p-eNOS protein levels (a.u)
12
9
6
3
0
*
CTRL
GLC
FRC
Control
Glucose
Fructose
p-eNOS
eNOS
β-ACTIN
B
p-AMPK protein levels (a.u)
2.0
1.5
1.0
0.5
0.0
CTRL
GLC
FRC
Control
Glucose
Fructose
p-AMPK
AMPK
β-ACTIN
C
iNOS protein levels (a.u)
2.0
1.5
1.0
0.5
0.0
*
*
CTRL
GLC
FRC
Control
Glucose
Fructose
iNOS
β-ACTIN

Table 1. Sensitivity (pD_2) and maximum response (E_{max}) to acetylcholine (Ach) and bradykinin (BK) in mesenteric arteries from control, glucose and fructose-supplemented rats

		pD2	E max (%)
Ach	Control	7.71 ± 0.25	99.0 ± 0.8
	Glucose	7.25 ± 0.11	99.2 ± 0.3
	Fructose	6.92 ± 0.23*	91.6 ± 4.9
BK	Control	5.77 ± 0.48	67.0 ± 9.1
	Glucose	6.09 ± 0.62	52.0 ± 10.8
	Fructose	4.18 ± 2.31	28.2 ± 11.0*

Data are expressed as mean ± SEM. *P<0.05, one-way ANOVA test followed by Sidak's post hoc múltiple comparison test.

Table 2. Sensitivity (pD_2) and maximum response (E_{max}) to sodium nitroprusside (SNP) in mesenteric arteries from control, glucose and fructose-supplemented rats

		pD2	E max (%)
SNP	Control	7.50 ± 0.30	84.4 ± 3.6
	Glucose	7.15 ± 0.17	68.6 ± 9.1
	Fructose	6.99 ± 0.27	52.6 ± 7.0**

Data are expressed as mean ± SEM. **P<0.01, one-way ANOVA test followed by Sidak's post hoc múltiple comparison test.

Table 3. Sensitivity (pD_2) and maximum tension ($Tension_{max}$) to phenylephrine (PE) and endothelin-1 (ET-1) in mesenteric arteries from control, glucose and fructose-supplemented rats

		pD2	Tension max
PE	Control	6.41 ± 0.16	11.73 ± 0.62
	Glucose	6.89 ± 0.17	16.70 ± 1.43*
	Fructose	6.76 ± 0.09	15.09 ± 1.22*
ET-1	Control	8.87 ± 0.16	7.76 ± 0.90
	Glucose	9.12 ± 0.20	14.70 ± 2.07*
	Fructose	8.96 ± 0.07	12.75 ± 1.79*

Data are expressed as mean ± SEM. *P<0.05 (vs control) analyzed using one-way ANOVA test followed by Sidak's post hoc múltiple comparison test.

Supplemental Table 1. The effect of two months supplementation of 20% glucose or fructose on calorie intake, body weight and metabolic parameters in female rats

	Control	Glucose	Fructose
Total ingested kcal (2 rats x 2 months)[a]	2642 ± 59	4736 ± 158*	4116 ± 129*#
Final body weight	239.1 ± 3.9	245.1 ± 5.5	254.9 ± 5.9*
Blood triglycerides (mg/dl)	116.1 ± 3.9	125.7 ± 2.8	144.4 ± 13.1*
Plasma adiponectin (µg/ml)	22.58 ± 2.4	58.42 ± 5.0*	26.48 ± 2.5
Plasma insulin (mg/dl)	0.67 ± 0.13	1.24 ±0.24	1.64 ± 0.35*
Blood glucose (mg/dl)	101.7 ± 5.1	112.7 ± 5.9	109.7 ± 6.0
ISI	1.23 ± 0.02	0.88 ± 0.03*	0.76 ± 0.02*

[a]AUC: Area Under the Curve. ISI: Insulin Sensitivity Index, calculated as [2/(blood insulin (nM) x blood glucose (µM) + 1]. Values are expressed as mean ± SEM of 14 rats/group *$p<0.05$, Control #$p<0.05$ vs glucose-supplemented rats, analyzed using Student's unpaired t-test. This table is also reported in (Sanguesa et al. 2016).