Original Article

Combined fructose and sucrose consumption from an early age aggravates cardiac oxidative damage and causes a dilated cardiomyopathy in SHR rats

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Obesity increases the risk of arterial hypertension in young adults and favors an early-onset cardiomyopathy by generating oxidative stress. In this sense, indiscriminate consumption of sucrose and fructose sweetened beverages from early ages causes obesity, however its consequences on the heart when there is a genetic predisposition to develop hypertension are not clear. We compared the effects of sucrose, fructose, and their combination in weanling male spontaneously hypertensive rats to determine the relationship between genetic hypertension, obesity, and consumption of these sugars on the degree of cardiac hypertrophy, oxidative stress and Ca^{2+}/calmodulin dependent protein kinase II delta oxidation. Histological, biochemical, and Western blot studies were performed 12 weeks after treatment initiation. We found that chronic consumption of sucrose or fructose leads to obesity, exacerbates genetic arterial hypertension-induced metabolic alterations, and increases cardiac oxidative stress, Ca^{2+}/calmodulin dependent protein kinase II delta oxidation and cardiac hypertrophy. Nonetheless, when sucrose and fructose are consumed together, metabolic alterations worsen and are accompanied by dilated cardiomyopathy. These data suggest that sucrose and fructose combined consumption starting from maternal weaning in rats with genetic predisposition to arterial hypertension accelerates the progression of cardiomyopathy resulting in an early dilated cardiomyopathy.

***Key Words*: arterial hypertension, cardiac hypertrophy, dilated cardiomyopathy, oxidative stress, $CaMKII_\delta$, high sucrose diet, high fructose diet**

Arterial hypertension (AH) and obesity are independent risk factors for cardiac disease (CD) and mortality.[1,2] AH is a complex disease that arises from both genetic predisposition and exposure to environmental factors.[3] It has been reported that early AH is mostly due to genetic predisposition, but the increase in unhealthy lifestyle has favored its prevalence in young adults (<45 years), and notably, in children and adolescents.[2–4] Furthermore, obesity due to unhealthy lifestyle, combined with a genetic susceptibility to AH has been associated with higher levels of arterial pressure and greater risk of CD in adults, indicating that the detrimental effect of genes on arterial pressure and on the subsequent CD risk are increased by obesity.[3] This issue is particularly alarming given that coexistence of AH and obesity has become increasingly common in children and adolescents,[4] and genetic predisposition to AH might most likely increase the risk for an early onset of CD in childhood and, thus for poor health and mortality at early ages.

Excessive consumption of sugar-sweetened beverages during early life is an important contributory factor of childhood obesity.[5,6] It has been reported that consumption of sugar-sweetened beverages at early ages leads to metabolic disorders such as dyslipidemia, hyperglycemia, insulin resistance (IR) and oxidative stress (OS), which in turn increase the risk of AH and CD in adulthood.[4,7,8] Sugar-sweetened beverages are usually prepared using sucrose (S) and fructose (F),[7,8] and recently, we reported that combined consumption of these sugars during early life aggravates obesity-induced OS and cardiac hypertrophy (CH) in normotensive rats.[8] Nonetheless, investigation regarding the effects of combined consumption of S and F in animal models of genetic hypertension are scarce. Sharma and coworkers reported that in adult hypertensive Dahl salt-sensitive rats, both S and F, but especially S, increases cardiac dysfunction and mortality.[7] In this study, however, AH was initiated once obesity was established with high sugar diets, and thus, the contribution of a genetic predisposition to AH to CD was not established. Accordingly, although it is well established that genetic AH leads to CH by inducing the development of OS and IR independently of obesity,[9,10] it is still unknown whether these conditions may worsen when childhood obesity is favored by consumption of S and F under the setting of a predisposition to develop AH.

The aim of this work was to study the relationship between metabolic and cardiac damage induced by consumption of S, F, and their combination (S + F) when having a genetic predisposition to AH and when consumption of these sugars is started from early age. To this end, we evaluated the effects of S, F, and S + F on IR, OS markers and cardiac structure in freshly weaned male spontaneous hypertensive rats (SHR). Moreover, we assessed the oxidation state of Ca^{2+}/calmodulin-dependent protein kinase II isoform delta ($CaMKII_\delta$) since the oxidation state of this protein is susceptible to OS and is related with an overactivation of this protein,[1,11] which in turn has been associated with the development, progression, and severity of CD in different cardiomyopathies, including hypertensive and obesity cardiomyopathies.[1,11–14]

Materials and Methods

Experimental animals. Forty-eight male SHR rats, freshly-

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weaned (21 days old) from the Cinvestav-IPN Pharmacobiology Department's animal facility were used. The rats were housed under a normal 12 h light–dark cycle and controlled temperature–humidity (22 ± 2°C and 60%, respectively). SHR rats were randomized into four (n = 12) groups as follows: Control group (C) with normal diet, Sucrose group (S) with sucrose diet, Fructose group (F) with fructose diet, and Sucrose + Fructose group (S + F) with sucrose and fructose diet. All animal procedures and the protocols of the present investigation were approved by our Institutional Ethics Committee (CICUAL-Cinvestav-IPN) and followed the regulations established by the Mexican Official Norm for the Use and Welfare of Laboratory Animals (NOM-062-ZOO-1999).

Obesity induction. All animals received LabDiet 5008® rat chow (Richmond, IN) *ad libitum*, with total calories: 3.19 kcal/g, protein (26.64%), fat (16.51%), and carbohydrate (56.84%). During a 20-week period, C group, were given purified water *ad libitum*, while S group received 30% (w/v) commercially refined sucrose (Ingenio Azucarero Potrero, Veracruz-Mexico), F group received 10% (w/v) fructose (JT-Baker Mexico) and S + F received 30% (w/v) sucrose + 10% (w/v) fructose. Total energy intake received was C: 0 kcal/g, S: 3.87 kcal/g, F: 3.99 kcal/g, and S + F: 7.86 kcal/g. The daily amount of food and liquid consumed was recorded, and food remaining from the previous day was replaced with a fresh supply. Intake (grams), total caloric intake (kcal) and caloric intake (kcal) of each macronutrient (protein, fat, and carbohydrates) as well as intake as percentage (%) of energy were calculated from the daily consumption of food and liquid. Total energy was adjusted according to the "multivariate nutrient density model" (Disease risk = nutrient energy/total energy + total energy)[15] (Table 1). At the end of the experimental period, the rats were fasted for 12 h and then sacrificed by decapitation. Blood samples, visceral adipose tissue, and hearts were obtained. Visceral adipose tissue was weighed to determine the adiposomatic index.

Biochemical blood analysis. Serum concentrations of glucose was determined using enzymatic colorimetric commercial diagnostic kits (Spinreact, Girona, Spain), and triglycerides (TG) and total cholesterol were determined by a colorimetric method (AccuLine plus kit using a Microlab 100; Merck, Dieren, The Netherlands), according to the manufacturer instructions. Serum insulin levels were measured by immuno-enzymatic assay (ELISA) using a commercial kit (Millipore, St. Charles, MO). IR was evaluated by the homeostatic model assessment (HOMA-IR) HOMA was calculated as follows: HOMA = fasting glucose (nM) × fasting insulin (μU/L)/22.5.

Arterial pressure. Arterial pressure was measured after the 12 weeks of treatment with a LE 5002 plethysmograph (Panlab, Harvard Apparatus, Barcelona), which uses the non-invasive "Tail Cuff" method. All animals were habituated for 3 days to reduce stress. Systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP) were recorded. Data recording consisted of 3 to 4 repeated measurements per animal, and the average of those measurements was reported.

Lipid peroxidation assay and total nitrites assay. Assays were performed in the homogenized heart and serum samples as previously reported.[8]

Superoxide dismutase (SOD). SOD activity was measured using a commercial kit (Sigma-Aldrich® kit No. 1916; Sigma-Aldrich, Darmstadt, Germany). The heart and serum homogenate samples were incubated with the chromophore and enzyme, performing spectrophotometric measurements Tecan Infinite 200® microplate reader at 425 nm every 30 s for 5 min to estimate the activity of the enzyme present in the samples.

Western blot. Assays were performed on homogenized hearts to determine the expression of ANP, total $CaMKII_{\delta}$, and ox-$CaMKII_{\delta}$ as previously reported.[8]

Histology. Hearts were fixed in 10% formalin, dehydrated, and embedded in paraffin for histological analysis. The tissues were cut in 7-μm slices, stained with hematoxylin and eosin and Masson's trichrome, and observed with an optical microscope to examine and evaluate any changes in the micro-architecture.

Cardiac hypertrophy determination. Gross determination of CH was obtained by measuring left and right ventricle wall thickness (RVWT and LVWT, respectively), the left ventricular

Table 1. Body weight, means of crude nutrient intakes, and nutrient intakes as a percentage of total energy

Parameter	Exprimental groups			
	C	S	F	S + F
BW (g)	314.3 ± 5.29	370.2 ± 6.32*	348.4 ± 5.48[a]	385.9 ± 6.87*
VATW (g)	3.16 ± 0.16	9.76 ± 0.43*	5.81 ± 0.23*[,]	12.34 ± 0.63*[,a,b]
Adiposomatic Index %	0.99 ± 0.04	2.64 ± 0.13*	1.66 ± 0.94*[,a]	3.20 ± 0.18*[,a,b]
Food consumption (g/day)	18.36 ± 0.33	7.74 ± 0.22*	7.99 ± 0.13*	7.54 ± 0.17*
Liquid consumption (ml/day)	40.25 ± 0.43	41.25 ± 0.54	40.58 ± 0.45	40.83 ± 0.55
Crude intake (day)				
Total Kcal	59.13 ± 1.04	73.44 ± 1.02*	42.42 ± 0.41*[,a]	89.02 ± 1.03*[,a,b]
Total protein (g)	4.89 ± 0.08	2.06 ± 0.05*	2.13 ± 0.04*	2.09 ± 0.05*
Total protein (Kcal)	15.60 ± 0.28	6.58 ± 0.18*	6.80 ± 0.11*	6.41 ± 0.15*
Total fat (g)	3.03 ± 0.05	1.28 ± 0.03*	1.32 ± 0.02*	1.25 ± 0.03*
Total fat (Kcal)	9.67 ± 0.17	4.08 ± 0.11*	4.21 ± 0.25*	3.97 ± 0.10*
Total carbohydrate (g)	10.43 ± 0.18	16.65 ± 0.19*	8.79 ± 0.10*[,a]	20.35 ± 0.35*[,a,b]
Total carbohydrate (Kcal)	33.29 ± 0.59	61.93 ± 0.80*	30.68 ± 0.25*[,a]	77.76 ± 0.84*[,a,b]
Intake as percentage of energy (%/day)				
Total protein	26.64	9.05*	16.28*[,a]	7.26*[,a,b]
Total fat	16.51	5.60*	10.09*[,a]	4.50*[,a,b]
Total carbohydrate	56.84	85.34*	73.62*[,a]	88.23*[,a,b]

Values represent mean ± SEM. ANOVA one way. Post hoc Tukey. *p<0.05 vs Control. [a]p<0.05 vs Sucrose group. [b]p<0.05 vs Fructose group. C: Control, S: Sucrose, F: Fructose, S + F: Sucrose + Fructose. BW, body weight; VATW, visceral adipose tissue weight. Adiposomatic index % = (VATW/BW × 100). n = 12.

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cavity radius (LVCR), as well the left and right ventricular lumen area (LVLA and RVLA) and the ratio between LVWT and LVCR (LVWT/LVCR). All the data images were analyzed using ImageJ 1.53j.

Statistical analysis. All the data were analyzed using GraphPad Prism 9.0 software (GraphPad Software, Boston, MA). Data are presented as mean ± SEM. To statistically compare experimental groups, analysis of variance (ANOVA) and a post hoc Tukey test were applied. $P<0.05$ was considered significant.

Results

Effect of high-sugar diets on body weight. Table 1 shows the body weight (BW) gain after 20 weeks of high carbohydrate diet treatment. S and F showed an increase of 17.8 and 10.8% respectively, however S + F had the highest BW gain with 22.9% with respect to C. On the other hand, food consumption was significantly lower in all experimental groups compared to C, while fluid intake was not significantly different between them and with C. Nevertheless, visceral adipose tissue weight (VATW) and adiposomal index (VATW/BW ratio) increased significantly in all three hypercaloric groups compared to C, although the increase in these parameters was statistically higher in S + F.

Biochemical profile. Glucose increased significantly with the 3 diets compared to C (by 82, 45, and 87%, for S, F, and S + F, respectively) (Table 2). The same trend was observed with insulin levels, which doubled and tripled in the 3 hypercaloric diets compared to C. According to normal reference values for glucose and insulin, the groups treated with carbohydrate for 5 months could present DM2. The SRH strain *per se* presented a clear IR according to the HOMA index, with values higher than the normal reference value of 3, however, the three experimental groups showed a higher increase in IR due to the hypercaloric diets according to C. As for TG levels, these increased ~2-fold in S and F, while the increase was ~3-fold in S + F, compared to C. Total cholesterol also increased significantly with all 3 diets ~1.5-fold in S and F and ~2-fold in S + F compared to C. Finally, the S + F group had the highest caloric intake from consumption of the combined diet compared to S and F. In all cases, the S + F group was statistically superior to the S and F groups.

Arterial pressure. After 12 weeks of high carbohydrate diet intake, no increase in MAP, SAP, and DAP values was observed in all experimental groups compared to C (Table 3) suggesting that hypercaloric diets did not exacerbate the AH that the animals developed per se due to the genetic predisposition of the SHR strain. On the other hand, the heart rate (HR), measured in beats per minute (BPM), increased significantly in all three experimental groups (16, 25, and 36% for S, F, and S + F, respectively). These results indirectly confirm that the hearts underwent cardiac remodeling by increasing HR to try to compensate for these changes.

Oxidative stress. MDA levels in cardiac tissue and serum were significantly increased in animals fed the hypercaloric diets compared with C, however, the increase was greater in S + F

Table 2. Biochemical parameters

Biochemical parameters	Experimental groups			
	C	S	F	S + F
Glucose (mg/dl)	93.91 ± 2.03	171.4 ± 2.80*	136.5 ± 3.29*,a	176.3 ± 5.24*,b
Insulin (µg/dl)	25.27 ± 1.30	83.46 ± 1.62*	50.51 ± 0.94*,a	86.85 ± 1.75*,a,b
HOMA-RI	5.89 ± 0.30	34.88 ± 2.17*	17.15 ± 0.52*,a	36.39 ± 1.11*,a,b
TG (mg/dl)	71.82 ± 2.37	169.8 ± 3.73*	142.7 ± 2.66*,a	208.6 ± 5.86*,a,b
Total cholesterol (mg/dl)	43.17 ± 2.14	74.53 ± 1.34*	70.95 ± 1.25*	87.54 ± 0.83*,a,b

Values represent mean ± SEM. ANOVA one way. Post hoc Tukey. *$p<0.05$ vs Control. [a]$p<0.05$ vs Sucrose group. [b]$p<0.05$ vs Fructose group. C: Control, S: Sucrose, F: Fructose, S + F: Sucrose + Fructose. BW, body weight; VATW, visceral adipose tissue weight; TG, triglycerides. $n = 6$.

Table 3. Arterial pressure and heart morphometric data

Parameters	Experimental groups			
	C	S	F	S + F
MAP (mmHg)	127.8 ± 3.86	129.5 ± 5.11	128.9 ± 5.17	128.2 ± 2.56
SAP (mmHg)	147.5 ± 4.20	147.8 ± 4.89	148.6 ± 5.14	146.6 ± 2.39
DAP (mmHg)	119.8 ± 4.01	120.9 ± 5.27	121.5 ± 4.86	120.8 ± 2.29
HR (bpm)	351.2 ± 2.48	408.1 ± 1.75*	441.5 ± 7.79*	478.8 ± 5.59*,a,b
HW (g)	1.93 ± 0.03	2.05 ± 0.04	2.08 ± 0.03*	2.18 ± 0.04*
HW/BW (%)	0.61 ± 0.01	0.56 ± 0.02	0.59 ± 0.02	0.56 ± 0.01
LVWT (mm)	4.55 ± 0.13	5.37 ± 0.06*	5.42 ± 0.10*	5.62 ± 0.75*,a,b
RVWT (mm)	2.22 ± 0.11	2.72 ± 0.12*	3.20 ± 0.15*	3.27 ± 0.08*,a,b
LVLA (mm^2)	2.00 ± 0.09	1.89 ± 0.12*	2.50 ± 0.09*	4.28 ± 0.10*,a,b
RVLA (mm^2)	0.97 ± 0.04	1.05 ± 0.02*	2.12 ± 0.08*,a,b	3.02 ± 0.11*,a,b
LVCR/LVWT	0.44 ± 0.01	0.70 ± 0.05*	0.62 ± 0.03*,a,b	0.28 ± 0.07*,a,b

Values represent mean ± SEM. ANOVA one way. Post hoc Tukey. *$p<0.05$ vs Control. [a]$p<0.05$ vs Sucrose group. [b]$p<0.05$ vs Fructose group. C: Control, S: Sucrose, F: Fructose, S + F: Sucrose + Fructose. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; HR, heart rate; BW, body weight; HW, heart weight; LVWT, left ventricular wall thickness; RVWT, right ventricular wall thickness; LVLA, left ventricular lumen area; RVLA, right ventricular lumen area; LVCR, left ventricular cavity radius. $n = 12$.

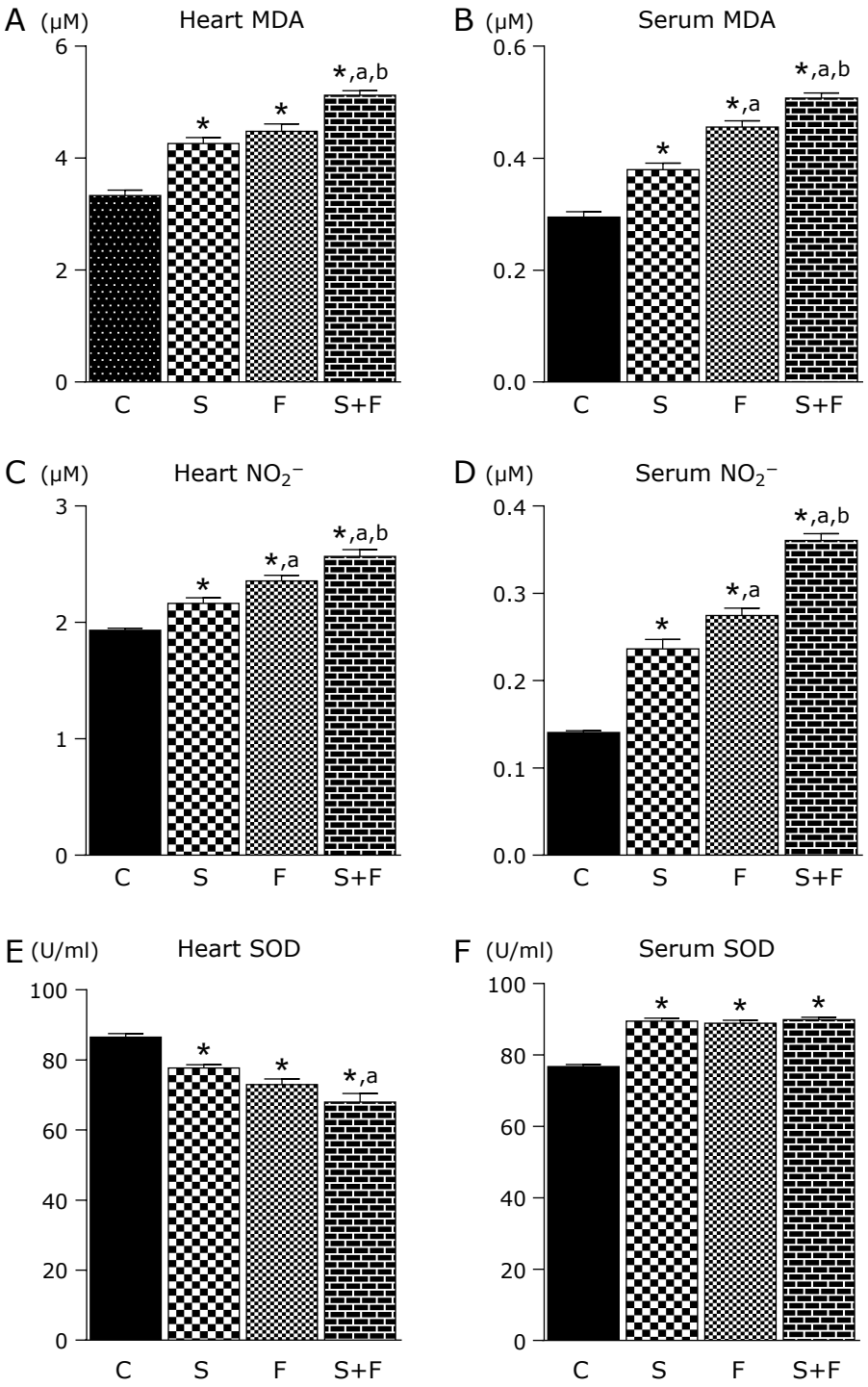

Fig. 1. Oxidative stress. (A) MDA in heart. (B) MDA in serum. (C) NO_2^- in heart. (D) NO_2^- in serum. (E) SOD in heart. (F) SOD in serum. Values represented as mean ± SEM. ANOVA one way. Post hoc Tukey. *p<0.05 vs Control. [a]p<0.05 vs Sucrose group. [b]p<0.05 vs Fructose group. MDA, malondialdehyde; NO_2^-, total nitrites; SOD, superoxide dismutase. n = 8.

(Fig. 1A and B). On the other hand, NO_2^- levels in all three hypercaloric diets were significantly increased in both myocardium and serum compared with C, but the increase was greater in animals treated with S + F (Fig. 1C and D). As for antioxidant activity, SOD in cardiac tissue was significantly decreased in S, F, and S + F compared with C (by 10, 15, and 21%, respectively; Fig. 1E) with greater decrease in S + F. In contrast, serum-level of SOD activity increased significantly in all three hypercaloric diets compared with C (by ~15% in all three experimental groups, Fig. 1F).

Western blot. Expression of ANP and oxidation state of $CaMKII_\delta$ were used as biomolecular markers of CH and CD severity.[8,12,14] ANP significantly increased in S, F, and S + F compared to group C, however, the increase was greater in S + F (~2.5-fold increase), than in S and F (1.3- and 1.6-fold increase, respectively; Fig. 2A). There was no change in total $CaMKII_\delta$ in

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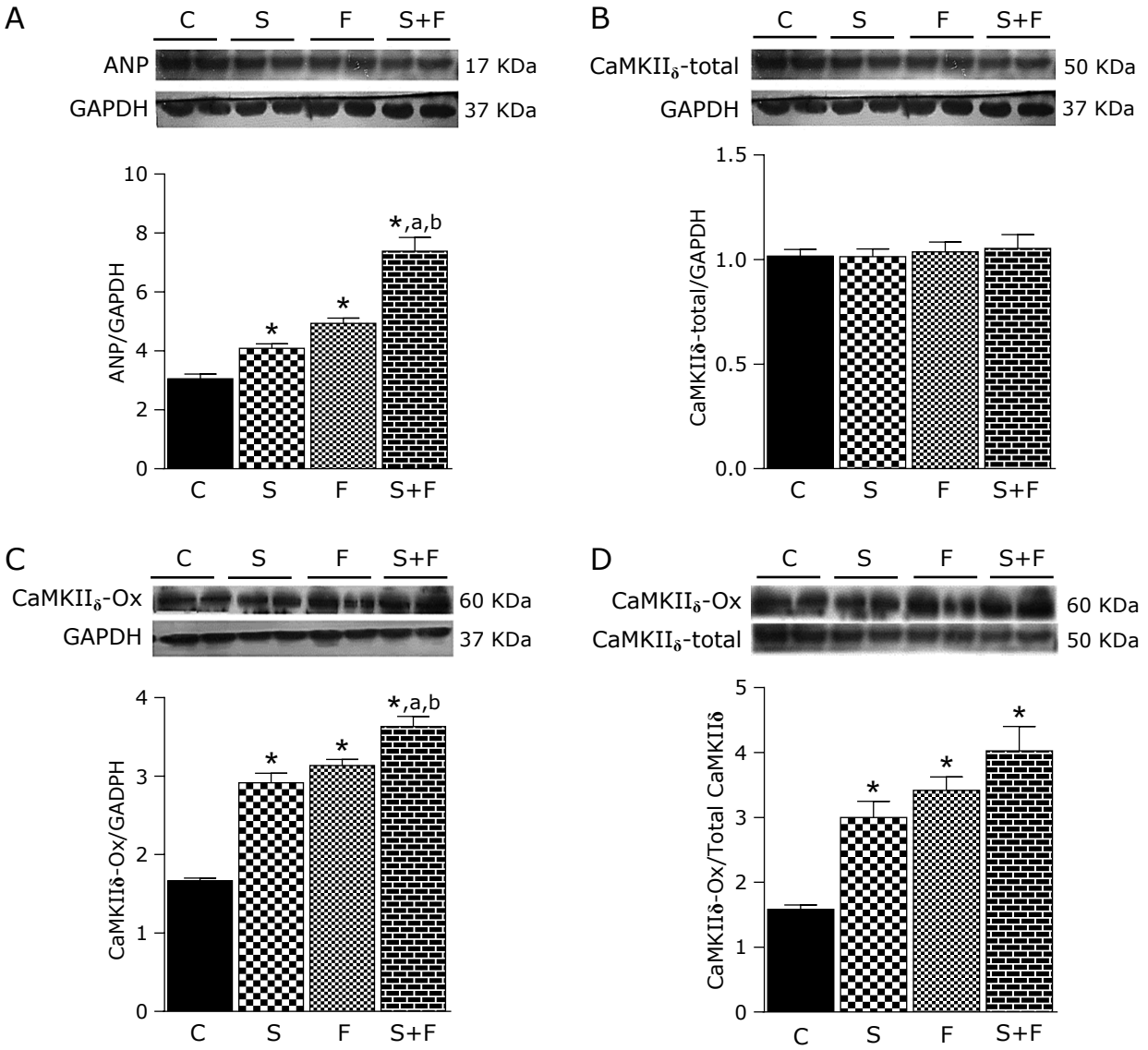

Fig. 2. Immunoblots and Western blot analysis of cardiac hypertrophy. (A) ANP expression. (B) CaMKII$_\delta$-total expression. (C) ox-CaMKII$_\delta$ density. (D) Relation ox-CaMKII$_\delta$/CaMKII$_\delta$-total. Values represented as mean ± SEM. ANOVA one way. Post hoc Tukey. *p<0.05 vs Control. [a]p<0.05 vs Sucrose group. [b]p<0.05 vs Fructose group. n = 4. ANP, atrial natriuretic peptide.

any of the experimental groups (Fig. 2B). However, all hypercaloric diets exhibited significantly increased ox-CaMKII$_\delta$ levels (normalized with respect to GAPDH and total CaMKII$_\delta$, Fig. 2C and D, respectively), with a greater increase in S + F, but no differences between S and F. This suggests that the consumption of high carbohydrate diets does not increase CaMKII$_\delta$ expression but does increase its activation through its oxidation. Moreover, the levels of ox-CaMKII$_\delta$ correlate with the values obtained for OS markers in the different hypercaloric diets and show that the greatest levels of OS and ox-CaMKII$_\delta$ were reached in S + F group.

Histomorphometric changes in the heart. Table 3 shows that heart weight (HW) did not exhibit significant differences between groups S and F with respect to C, however, in S + F, HW increased significantly compared to C. The HW/BW ratio did not differ between the experimental groups and C group. Left ventricular wall thickness (LVWT) and right ventricular wall thickness (RVWT) increased significantly in all experimental groups compared to C, and in both cases, the increases were higher in S + F group (by 18, 19, and 23% for S, F, and S + F, in the case of LVWT, and by 22, 44, and 47%, in S, F, and S + F, in the case of RVWT). (Fig. 3 and Table 3). Similarly, left ventricular lumen area (LVLA) and right ventricular lumen area (RVLA) had a significant increase in S, F, and S + F with respect to C, but the increase was 2–3 times greater in S + F. Left ventricular cavity radius (LVCR)/LVWT ratio increased in S and F groups, but decreased in S + F group compared to C. These data confirmed that CH is exacerbated by the three hypercaloric diets. Nonetheless, in the case of S + F group, cardiac remodeling was also associated with the development of a dilated cardiomyopathy (DC) in both ventricles (Fig. 3A), although, no apparent increase in myocyte size is observed with respect to C (Fig. 3B). Finally, a concomitant increase in interstitial structure was present in all three diets compared to C. Indeed, Masson staining showed increased collagen deposition in all three diets compared to group C (Fig. 3C), which is a characteristic of CH and DC.

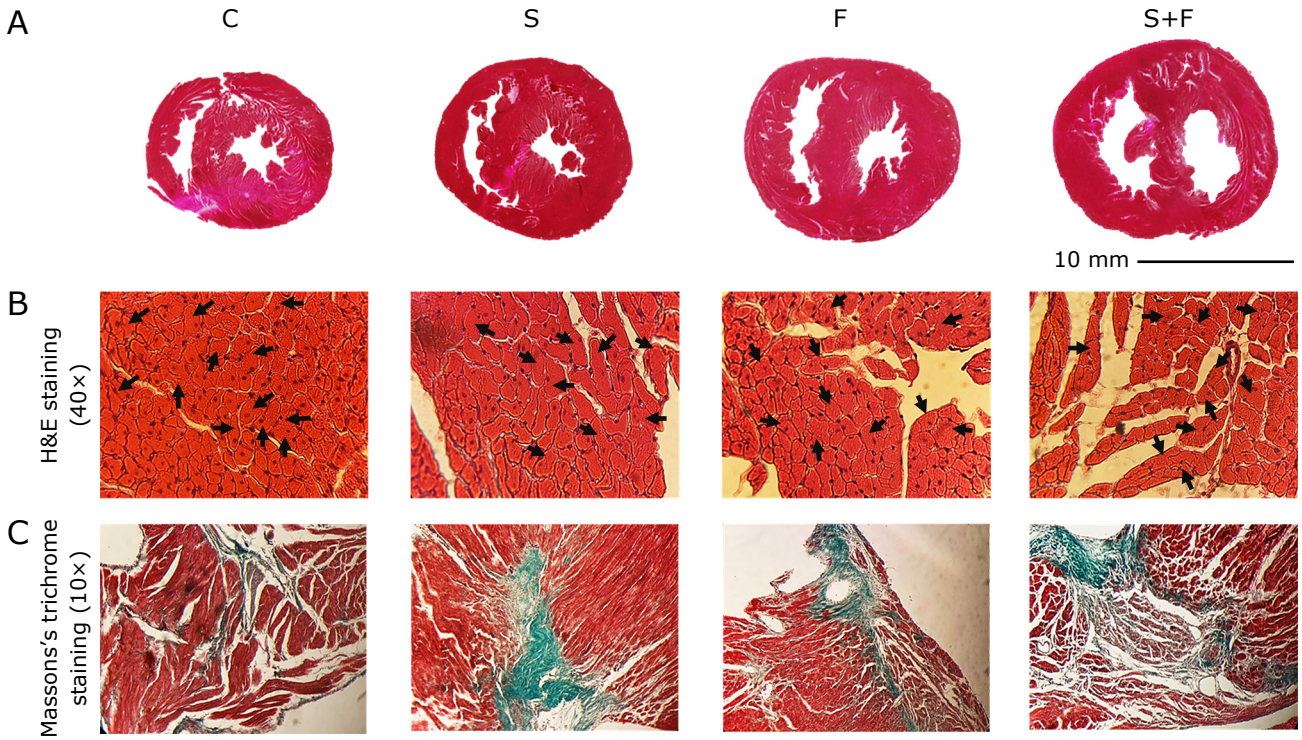

Fig. 3. Histopathological staining of hearts. (A) Cross section of hearts with H & E staining. (B) Cardiac cells of the left ventricular wall with H & E staining. (C) Cardiac cells of the left ventricular wall with Massons's trichrome. C: Control, S: Sucrose, F: Fructose, S + F: Sucrose + Fructose. H & E: hematoxylin and eosin. Viewed at 40× magnification for each slice. ↓ Hypertrophied cardiomyocytes.

Discussion

In this study we showed that chronic consumption of S or F from an early age in SHR rats generated several metabolic alterations linked to obesity, such as accumulation of visceral adipose tissue and increased levels of glucose, insulin, total cholesterol, TG, as well as markers of systemic and cardiac OS (MDA and NO_2^-). Furthermore, consumption of S and F aggravated the IR and CH exhibited by SHR rats, and these alterations were accompanied by enhanced levels of ox-CaMKII$_\delta$ in cardiac tissue. Notably, the high carbohydrate diets did not increase the already elevated arterial pressure characteristic of SHR rats, suggesting that severity of CH induced by consumption of S or F is most likely mediated by metabolic abnormalities produced by these sugars. Finally, we demonstrated that combined consumption of S + F aggravated the metabolic alterations caused by these sugars individually and worsen even more the IR and cardiac remodeling exhibited by SHR rats, leading to the development of an early DC.

It has been demonstrated that high carbohydrate diets generate dyslipidemia, obesity, hyperglycemia, hypertriglyceridemia, hyperinsulinemia, IR, glucose intolerance, hepatic steatosis, AH, OS, inflammation, endothelial dysfunction, CD, among others.[8,16–19] However, the possible metabolic alterations and CD caused by S, F and their combination when these sugars are consumed from early ages in animals with a genetic predisposition to develop AH are unknown. For this reason, in this study we evaluated the effects of long-term consumption of S, F, and S + F in recently weaned SHR rats.

Our data show that consumption of S, F, and S + F caused a significant increase in BW, visceral adipose tissue and visceral adiposomal index, confirming the development of obesity in SHR rats. Nonetheless, the greatest degree of obesity was induced by S + F diet. These results coincide with previous reports showing that S and F cause an increase in BW in SHR rats[20] and that fat accumulation depends on the type of sugar ingested.[8] In this sense, although food intake was lower in the three diet groups, the fluid intake was not modified, which is consistent with our previous work showing that high-carbohydrate diets decrease food intake but keep fluid intake constant.[21] This issue seems to be explained by the preference of young animals for the intake of sugar-sweetened beverages.[8,18,19,22] Therefore, the SHR rats that received the sugar diets had a higher caloric intake from the carbohydrates consumed, nonetheless S + F group was the one with the highest caloric intake compared to S and F, which explains why this group was the one that caused the highest increase in BW, visceral adipose tissue and visceral adiposomal index (Table 1).

To evaluate the metabolic alterations caused by chronic consumption of S, F, and S + F in recently weaned SHR rats, we measured different biochemical parameters. Our results show that glucose, insulin, cholesterol, and TG levels, as well as the HOMA index, increased significantly in all three diets. These data are consistent with previous reports where the effects of S and F were evaluated independently.[8,17–19,23,24] However, in this work, we found that combined consumption of S + F aggravates all these metabolic alterations in rats genetically predisposed to HA. Interestingly, although group C did not receive hypercaloric diet, it presented a HOMA value greater than 3, which confirms that control SHR rats exhibits *per se* a decrease in insulin sensitivity,[16] which in turn is worsen by the development of obesity. Finally, hyperglycemia, hyperinsulinemia and IR values obtained in all experimental groups suggest the development of DM2, yet further tests are needed to confirm this idea.

IR and hyperglycemia can alter antioxidant defenses and lead to an overproduction of ROS linked to mitochondrial OS.[16,25–27] Since, the intake of sugar-sweetened beverages has been related to increased lipid peroxidation, we verified the effects of chronic

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consumption of S, F, and S + F on ROS generation by measuring systemic and cardiac MDA levels (Fig. 1). All sugar diets increased systemic and cardiac MDA levels, however, compared with the S and F groups, S + F diet caused a higher increase in MDA levels, suggesting that combined S + F exacerbates OS. Additionally, we measured systemic and cardiac SOD levels. Like our previous results using Wistar rats,[8] the three hypercaloric diets caused a decrease in cardiac SOD and an increase in systemic SOD in SHR rats. These differences in the alterations of SOD in serum and in tissues have been also observed by others[16] and may represent the imbalance between antioxidant enzymes and increased ROS, characteristic of OS.

OS during obesity correlates with the excess of adipose tissue, with the capacity of adipocytes to generate proinflammatory cytokines that induce ROS production[8,24,28] and with the development of different cardiomyopathies such as CH, DC, hypertensive cardiomyopathy, and heart failure (HF).[8,29–32] Moreover, vascular OS due to overexpression and activation of vascular NADPH oxidase as well as uncoupling of endothelial nitric oxide synthase has been reported in SHR rats.[33,34] Therefore, the increase in ROS favored by the three diets and by the strain itself seems to exacerbate even more the OS in SHR rats compared to our previous work in Wistar strain.[8] On the other hand, excessive ROS production induced by obesity and elevated pressure in SHR rats leads to a decrease in NO bioavailability, which results from the rapid reaction between superoxide anion ($O_2^{\bullet-}$) with NO, generating an increase in peroxynitrite ($OONO^{\bullet-}$),[30,35] which together with the decrease in NO, contribute to endothelial dysfunction.[30,31] Our data showed that hypercaloric diets produced an increase in -NO_2 levels, suggesting a decrease in NO as a result of increased vascular OS generation and low activity of the thioredoxin system and other antioxidant agents as observed in our results regarding SOD levels in cardiac tissue.

SBP, DBP, and MBP were not further increased by sugar diets in SHR rats. These data are consistent with previous reports showing that consumption of hypercaloric carbohydrate diets did not increase AP in this genetic model of AH.[16,36] Although, it is not clear why the sugar diets do not affect the AP, it is probably that the endothelial damage that leads to AH has been already developed by the SHR strain. Nonetheless, further studies are required to determine the possible cause of the absence of an increased in AP. By contrast, HR was significantly increased by all three diets, being higher in S + F. These results indirectly confirm that the hearts underwent cardiac remodeling by increasing HR to try to compensate for the pressure overload caused by AH and hemodynamic alterations produced by obesity.[37–40] In this sense, it has been reported that variability of HR and CH during hypertensive and obesity cardiomyopathy are strongly linked to an overstimulation of sympathetic tone.[37–40]

Pressure overload during AH has been widely reported to induce CH.[1,39,41] In SHR animals, the development of AH has been described as early as 3 months of age with respect to control rats.[38] Genetic predisposition and unhealthy lifestyle support the hypothesis that from early ages, subjects who develop obesity could develop AH and have a high probability of developing CH and later HF.[2–4] According to our histopathological analysis, we confirmed the development of concentric CH *per se* in the SHR strain. Furthermore, we showed that three sugar diets exacerbated CH, nonetheless in the case of S + F, it also caused a DC (Fig. 3A). In this sense, morphometric data (Table 3) indicated that S + F diet generates a higher increase in the walls and lumen of both ventricles and a decrease in the LVCR/LVWT ratio compared to S and F groups. This decrease in the LVCR/LVWT ratio suggest the development of an eccentric cardiac remodeling which is a characteristic of DC. In addition, cardiac remodeling was accompanied by interstitial growth and collagen deposition in control SHR group and in the three sugar diet groups, yet these alterations were greater in S + F (Fig. 3B and C). Interstitial growth and collagen deposition during CH allow the myocyte to have better structural support.[8,42] This collagen deposition originates as a compensatory response to cell loss caused mainly by cardiac cell apoptosis that results from an increase in ROS production.[14,42]

OS in the heart has been related with the development of CH and HF.[1] In a previous study, we reported that consumption of S and F induces CH by a mechanism that involves both the development of OS and activation of $CaMKII_\delta$ due to an increase in oxidation level of this kinase.[8] In this sense, the role of $CaMKII_\delta$ in the development of CH has been well established in several cardiomyopathies.[1,11–14] Accordingly, our data in SHR rats showed that three sugar diets (S, F, or S + F) cause a significant increase in the level of ox-$CaMKII_\delta$ (Fig. 2C and D), that was not accompanied by a change in the expression of this kinase in any of the experimental groups (Fig. 2B), suggesting that the increase in ox-$CaMKII_\delta$ was most likely induced by OS promoted by these sugars. On the other hand, increased ox-$CaMKII_\delta$ levels caused by consumption of S, F, and S + F were associated with a similar increase in ANP expression (Fig. 2A), and importantly, augmented levels of ox-$CaMKII_\delta$ and ANP were higher in S + F compared with S or F. These data confirm the role of ox-$CaMKII_\delta$ in the development of CH,[8,13] and show that there is a direct relationship between the severity of cardiac remodeling and the level of ox-$CaMKII_\delta$, which in turn is also associated with the higher level of OS produced by combined S + F consumption (Fig. 1). These results may explain why S + F leads to DC in SHR rats.

Although the relationship between CH and DC is not clear, it has been reported that $CaMKII_\delta$ is able to activate the response to ventricular hypertrophy, apoptosis, fibrosis, premature death[11–14] and drives DC and HF.[12,14] It has been reported that both nuclear and mitochondrial $CaMKII_\delta$ cause DC,[43] however, overactivation of mitochondrial $CaMKII_\delta$ has been associated with adverse metabolic remodeling and ATP deficiency when it is stimulated by Ca^{2+}/CaM, ROS and/or O-GlcNAcylation.[13,44,45] Thus, it seems that $CaMKII_\delta$ can promote CH and DC depending on its subcellular localization. Although our investigation indicates that ox-$CaMKII_\delta$ is implicated in the development of CH in S and F groups and DC in S + F group, further studies are needed to determine the specific site of $CaMKII_\delta$ activation under our experimental conditions. Nevertheless, accumulated evidence has demonstrated that AH, obesity, and OS play an important role in the development of these cardiomyopathies, and even if the biological connections between CH and DC are uncertain, it has been hypothesized that sustained and excessive hypertrophy may progress to chamber dilation and HF over time.[44] Moreover, it has been shown in several animal models that CH, DC and HF have as a common mechanism the overactivation of $CaMKII_\delta$.[11,12,14,45]

Conclusion

Our study demonstrates that excessive S or F consumption from an early age in rats with a genetic predisposition to AH, leads to the development of obesity and possibly DM2, increases cardiac ox-$CaMKII_\delta$ level and exacerbates OS and CH. However, when these sugars are consumed in combination, all these alterations are exacerbated and give rise to DC. On the other hand, our data suggest that ox-$CaMKII_\delta$ depends on OS level and that the increase of its oxidation plays an important role in the evolution of CH to DC. However, further *in vivo* and *ex vivo* studies as well as clinical trials are required to determine the presence of other possible cardiomyopathies such as arrhythmias or HF in which $CaMKII_\delta$ is key, as well as the signaling pathways involved when these metabolic alterations generated by chronic consumption of S, F and/or their combination.

Author Contributions

GB and NLG-V contributed to the study design and approved the version to be published. DJA-C and PM-S conceived experiments and analyzed data. DJA-C, JL-A and NLG-V contributed to writing and discussion.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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