Resveratrol prevents the development of hypertension programmed by maternal plus post-weaning high-fructose consumption through modulation of oxidative stress, nutrient-sensing signals, and gut microbiota

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Abbreviation:

8-OHdG= 8-hydroxydeoxyguanosine

ADMA= asymmetric dimethylarginine

AMPK= adenosine monophosphate-activated protein kinase

DOHaD= the developmental origins of health and disease

HF= high-fructose

LDA= linear discriminant analysis

ND= normal diet

NO= nitric oxide

Nrf2= nuclear factor erythroid-derived 2-related factor 2

PPAR= peroxisome proliferator-activated receptor

ROS= reactive oxygen species

SD= Sprague Dawley

SDMA= symmetric dimethylarginine

SIRT= silent information regulator transcript

Keywords: fructose, gut microbiota, hypertension, nutrient-sensing signal, oxidative stress

Abstract

Scope: High-fructose (HF) intake, oxidative stress, nutrient-sensing signals, and gut microbiota dysbiosis are closely related to the development of hypertension. We investigated whether resveratrol can prevent hypertension induced by maternal plus post-weaning HF diets in adult offspring via the above-mentioned mechanisms.

Methods and Results: Female Sprague-Dawley rats received either a normal (ND) or 60% high-fructose (HF) diet during gestation and lactation. Male offspring were assigned to five groups (maternal diet/post-weaning diet; n = 8/group): ND/ND, ND/HF, HF/ND, HF/HF, and HF/HF+ Resveratrol. Resveratrol (50 mg/L) was administered in drinking water from weaning to three

months of age. We found that HF/HF induced hypertension in adult offspring. Maternal HF diet altered gut microbiota composition in adult offspring, including decreasing the abundance of genera *Bacteroides, Dysgonomonas,* and *Turicibacter*, while increasing phylum *Verrucomicrobia* and *Akkermansia muciniphila.* Additionally, HF/HF diets increased oxidative stress and decreased renal mRNA expression of *Prkaa2*, *Prkag2*, *Ppara*, *Pparb*, *Ppargc1a*, and *Sirt4*. Resveratrol reduced renal oxidative stress, activated nutrient-sensing signals, modulated gut microbiota, and prevented associated HF/HF-induced programmed hypertension.

Conclusion: Targeting oxidative stress, nutrient-sensing signals, and gut microbiota by resveratrol might be a useful therapeutic strategy for treatment of hypertension induced by excessive consumption of fructose in the adult rat offspring.

1. Introduction

Fructose is a monosaccharide naturally present in fruits and vegetables. However, current excessive consumption of refined fructose mainly comes from high fructose corn syrup and produced foods. Global fructose consumption has risen steeply over the past century [1], paralleling the epidemics of hypertension and its related diseases [2]. Adulthood hypertension can be programmed in response to nutritional insults in early life, which can be considered under the concept of the developmental origins of health and disease (DOHaD) [3]. We have previously shown

that, in rats, offspring of mothers exposed to 60% high-fructose (HF) diet during pregnancy and lactation developed hypertension [4,5].

Hypertension is a multifactorial condition that includes major contributions from the kidney. The developing kidney is particularly vulnerable to early-life insults resulting in morphological and functional changes. This process is formally known as renal programming [6]. This and other phenomena falling under the concept of DOHaD suggest the potential of reprogramming strategies aimed at shifting therapeutic interventions from adulthood to early life, even before clinical phenotypes are evident [7].

Resveratrol, a natural phytoalexin, has found applications as a nutraceutical with a wide range of therapeutic effects [8]. It has been used to prevent hypertension associated with excess fructose consumption in adult rats [9]. We previously demonstrated that resveratrol prevents hypertension of developmental origin induced by combined pre- and post-natal high-fat consumption [10]. Whether resveratrol can prevent hypertension of developmental origin induced by early-life high-fructose consumption remains unclear.

Recently published studies demonstrate that oxidative stress, nutrient-sensing signals, and dysbiosis of the gut microbiota are related to the development of hypertension [11-14]. Nitric oxide (NO) controls blood pressure (BP). Imbalance between NO and reactive oxygen species (ROS) signaling resulting in oxidative stress has been linked to programmed hypertension [12].

Additionally, several nutrient-sensing signals exist in the kidney, including SIRT (silent information regulator transcript), adenosine monophosphate (AMP)-activated protein kinase (AMPK) pathway, and the peroxisome proliferator-activated receptor (PPAR) pathway. In addition to its antioxidant property [15], resveratrol is considered as a potential reprogramming agent to prevent hypertension for its ability to mediate nutrient-sensing signals, including SIRT1 and AMPK [16]. Furthermore, it was observed that in rats that are fed a fructose-rich diet, the development of metabolic syndrome is correlated with dysbiosis of the gut microbiota [17,18]. Additionally, HF diet may alter intestinal barrier function and increase bacterial endotoxin levels, thereby leading to nonalcoholic fatty liver disease [19]. Hence, we determined whether resveratrol therapy can prevent maternal plus post-weaning HF diets-induced programmed hypertension, and whether the protective effects of resveratrol are associated with mediation of oxidative stress, nutrient-sensing signals, and gut microbiota.

2. Materials and Methods

2.1 Animal study

This study was approved by the Institutional Animal Care and Use Committee of the Kaohsiung Chang Gung Memorial Hospital (Approval number: 2015120102). All experiments were performed in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Virgin Sprague Dawley (SD) rats (12–16 weeks old) were

obtained from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan), and were housed and maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Female rats were weight-matched and assigned to receive either a normal diet (Fwusow Taiwan Co., Ltd., Taichung, Taiwan) or 60% high-fructose (HF) diet (Harlan Teklad, Madison, WI, USA) during gestation and lactation [3]. Diet composition is shown in Supplementary Table 1. The content of fat, protein, and carbohydrate in the normal diet represented 14.1%, 27.95%, and 58%, respectively of the total energy content of the diet. In the HF diet, fat, protein, and carbohydrate were 12.5%, 23.3%, and 66.7%, respectively of the total energy content of the diet. Male SD rats were housed with individual females until mating was confirmed by observation of a vaginal plug. Because cardiovascular events occur at a higher rate and at an earlier age in males than females [20], only male offspring from litters that were culled to eight pups after birth were used in subsequent experiments. The male offspring were weaned at three weeks of age and placed onto either the ND or HF diet ad libitum from weaning to three months of age. Male offspring (n = 40) were assigned to five experimental groups (maternal diet/post-weaning diet; n=8/group): ND/ND, ND/HF, HF/ND, HF/HF, and HF/HF+R. Male offspring in the HF/HF+R group were treated with resveratrol (50 mg/L; Sigma-Aldrich, St. Louis, MO, USA) or vehicle in drinking water from weaning to three months of age (i.e. a total of nine weeks). Resveratrol (Sigma-Aldrich) was prepared twice weekly by dissolving the drug in ethanol and then diluting with water to a final concentration of 50

mg/L [21]. Water bottles were wrapped with aluminum foil to protect the solution from light. BP was measured in conscious rats at 12 weeks of age using an indirect tail-cuff method (BP-2000, Visitech Systems, Inc., Apex, NC, USA) after being systematically trained as previously described [4]. All rats were sacrificed at three months of age. Fresh feces samples were collected before sacrifice, frozen, and stored at −70 °C until use. Kidneys and heparinized blood samples were collected at the end of the study.

2.3 Quantitative Real-time Polymerase Chain Reaction (qPCR)

RNA was extracted from kidney cortex according to previously described methods [4]. Two-step quantitative reverse transcription PCR (qRT-PCR) was conducted using Quantitect SYBR Green PCR Reagents (Qiagen, Valencia, CA) on an iCycler iQ Multi-color Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Several genes related to nutrient-sensing signals were analyzed in this study, including sirtuin-1 (*Sirt1*), sirtuin-4 (*Sirt4*), peroxisome proliferator-activated receptor (PPAR)-α (*Ppara*), -β (*Pparb*), and -γ (*Pparg*), PPARγ coactivator 1-α (PGC-1α encodes for *Pargc1ap*), and protein kinase, AMP-activated, subunit-α2 (*Prkaa2*), -β2 (*Prkab2*), and -γ2 (*Prkag2*). Additionally, NF-E2-related factor-2 (*Nrf2*), a key regulator of antioxidants, was analyzed. The 18S rRNA gene (*Rn18s*) was used as a reference. Primer sequences are provided in Supplementary Table 2. The cycling conditions were one cycle of three-minute denaturation at 95 °C, followed by 45

three-segment cycles of amplification (95 °C/10 sec, 55 °C–60 °C (gene dependent)/20 sec, 72°C/one sec), and one three-segment cycle of product melting (95 °C/5 sec, 65 °C/30 sec, 97 °C/five min). All samples were run in duplicate. For the relative quantification of gene expression, the comparative threshold cycle (C_T) method was employed. The averaged C_T was subtracted from the corresponding averaged *Rn18s* value for each sample, resulting in ΔC_T. $\Delta\Delta C_T$ was achieved by subtracting the average control ΔC_T value from the average experimental ΔC_T. The fold-change was established by calculating $2^{-\Delta\Delta_{CT}}$ for experimental vs. reference samples.

2.4 High-performance liquid chromatography (HPLC)

The levels of several components of the nitric oxide (NO) pathway, including L-arginine, L-citrulline, asymmetric dimethylarginine (ADMA, an endogenous inhibitor of nitric oxide synthase), and symmetric dimethylarginine (SDMA, an isomer of ADMA), were measured using HPLC (HP series 1100; Agilent Technologies Inc., Santa Clara, CA, USA) with the o-phtalaldehyde-3-mercaptoprionic acid derivatization reagent described previously [4]. Standards contained 1–100 mM L-arginine, 1–100 mM L-citrulline, 0.5–5 mM ADMA, and 0.5–5 mM SDMA. The recovery rate was approximately 95%.

2.5 Western blotting

Western blot analysis was performed using methods published previously [4]. Briefly, samples (200 µg of kidney cortex) were loaded on 10% polyacrylamide gel and separated by electrophoresis

(200 volts, 90 min). Following transfer to a nitrocellulose membrane (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA), the membranes were incubated with Ponceau S red (PonS) stain solution (Sigma-Aldrich, St. Louis, MO, USA) for 10 minutes on a rocker. After blocking with phosphate-buffered saline-Tween (PBS-T) containing 5% dry milk, the membranes were incubated with primary antibody. We used the following primary antibodies: goat polyclonal anti-rat AMPKα2 antibody (SC-19131, 1:1000, overnight incubation; Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit monoclonal anti-rat PPARγ antibody (ab191407, 1:1000, overnight incubation; Abcam, Cambridge, MA, USA), and rabbit polyclonal anti-rat PGC-1α antibody (SC-13067, 1:1000, overnight incubation; Santa Cruz Biotechnology). Following five washes with 0.1% Tween-Tris-buffered saline (TBS-T), the membranes were incubated for 1 h with horseradish peroxidase-labeled secondary antibody diluted 1:1,000 in TBS-T. Bands were visualized using SuperSignal West Pico reagent (Pierce; Rockford, IL, USA) and quantified by densitometry as integrated optical density (IOD), normalized to PonS staining to correct for variations in total protein loading and for an internal standard. Protein abundance was represented as IOD/PonS.

2.6 Immunohistochemistry staining

Paraffin-embedded tissue was sectioned at 3 μm thickness. Tissue slides were deparaffinized with xylene and rehydrated in a series of ethanol solutions with decreasing concentrations. Following blocking with immunoblock (BIOTnA Biotech., Kaohsiung, Taiwan), the sections were

incubated with an anti-8-hydroxydeoxyguanosine (8-OHdG) antibody (clone #N45.1, 1:100, JaICA, Shizuoka, Japan) at room temperature for 2 h. Immunoreactivity was detected using the polymer-horseradish peroxidase (HRP) labelling kit (BIOTnA Biotech) and 3,30-diaminobenzidine (DAB) as the chromogen. The sections were lightly counterstained with hematoxylin and preserved under cover glass. Identical staining protocol omitting incubation with primary antibody was employed to prepare samples that were used as negative controls. Reagent incubation times and antibody dilutions were identical in all experiments. Quantitative analysis of 8-OHdG-positive cells per microscopic field (400×) in the renal sections was performed as we described previously [10].

2.7 Gut Microbiota Profiling

Bacterial DNA was extracted from feces. Stool samples were washed with DNase-free water and centrifuged. DNA was then amplified from the pellets using specific forward and reverse primers as follows: 5'- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3' respectively, which targeted the V3-V4 region of the bacterial 16S rRNA gene. We prepared the amplicons according to the 16S Metagenomics Sequencing Library Preparation protocol (Illumina, California, USA), and sequenced them with the Illumina MiSeq platform (Illumina, California, USA) in paired-end mode with 600-cycle sequencing reagent.

Next generation sequencing data were analyzed with the Microbial Genomics Module of CLC Genomics Workbench 9.5.4 (Qiagen, Stockach, Germany). Differences in the relative abundance of bacterial DNA at the phylum, family, genus, and species levels were compared using the Student's *t*-test for independent samples. We next used linear discriminant analysis (LDA) effect size to agnostically identify microbial biomarkers [22]. The LDA score represents an estimation of the magnitude of the difference between grouping categories (e.g., ND/ND vs. HF/ND). For stringency, microbial biomarkers in our study were retained if they had a (log10) LDA score >1.5.

2.8 Statistical Analysis

Statistical analysis was conducted using one-way analysis of variance (ANOVA) with a Tukey post hoc test for multiple comparisons. BP was analyzed with two-way repeated-measures ANOVA, with a Tukey post hoc test. All values are reported as mean ± standard error, with statistical significance inferred where $P < 0.05$. Analyses were performed using the Statistical Package for the Social Sciences software (SPSS, Chicago, IL, USA).

3. Results

3.1. Effect of high-fructose diet and resveratrol on morphological values and blood pressure

Blood pressure and morphological values in the five experimental groups are given in Table 1. Litter sizes were not significantly affected by maternal HF diet (pups per litter: control = 14 ± 0.8; HF

= 14.8 ± 0.7). The mortality rate was 0% in each group. The body weight (BW) was lower in HF/HF animals (355 ± 18 g) than that in the ND/ND group (412 ± 10 g), while resveratrol treatment significantly increased BW (457 ± 16 g). Among the five groups, kidney weight was highest in HF/HF+R. Kidney weight-to-BW ratio (ND/ND: 0.48 ± 0.03) was decreased by maternal HF intake (HF/ND: 0.42 ± 0.01), but increased by post-weaning HF diet (HF/HF: 0.58 ± 0.05). Systolic blood pressure (SBP) significantly increased in ND/HF (155 ± 2 mmHg), HF/ND (154 ± 4 mmHg), and HF/HF animals (155 ± 2 mmHg), but near normal in the group treated with resveratrol (HF/HF/+R: 142 ± 3 mmHg; ND/ND: 140 ± 2 mmHg). Similarly, HF/HF increased diastolic BP and mean arterial pressure. These measures of BP were lower in resveratrol-treated animals than in the HF/HF group, indicating that resveratrol may protect against the development of hypertension induced by combined maternal and post-weaning HF diets. As shown in Figure 1, the elevation of SBP in the ND/HF, HF/ND, and HF/HF groups was similar at eight to 12 weeks of age, indicating absence of a synergistic effect of maternal and post-weaning HF on the elevation of SBP, The reduction in SBP caused by resveratrol was significant for measurements taken at 8 to 12 weeks of age, but not earlier. Our data indicated a lack of synergistic interaction between the maternal and post-weaning HF on the elevation of the SBP, which was prevented by resveratrol treatment.

3.2. Effect of high-fructose diet and resveratrol on NO pathway and oxidative stress

In order to explore the role of oxidative stress in HF-induced hypertension, we first investigated the ADMA-NO pathway (Table 2). In line with our previous observation [4], maternal HF intake had no effect on the ADMA-NO pathway. However, plasma levels of L-citrulline, L-arginine, and ADMA were lower in offspring exposed to post-weaning HF intake. Additionally, the plasma L-arginine-to-ADMA ratio was decreased in the offspring of the ND/HF (145.4 ± 5) and HF/HF+R groups (158.1 ± 6.1) compared to ND/ND (226.2 ± 3). Nevertheless, resveratrol therapy had a negligible effect on the NO pathway in the HF/HF group. We next evaluated oxidative stress in the kidney by using immunohistochemistry to assay 8-OHdG, a marker of oxidative DNA damage. Immunostaining of 8-OHdG in the glomeruli and renal tubules indicated intense staining in the ND/HF (55 ± 15 positive cells), HF/ND (49 ± 19 positive cells) and HF/HF (60 ± 10 positive cells) groups, and little staining in the ND/ND (5 ± 2 positive cells) and HF/HF+R (10 ± 5 positive cells) groups (Figure 2). Nuclear factor erythroid-derived 2-related factor 2 (*Nrf2*) is a key transcription factor in the regulation of several genes involved in the oxidative stress response. We found reduced renal *Nrf2* mRNA expression (fold change [FC] = 0.32) in the HF/HF group, which was partially prevented by resveratrol therapy (FC = 0.61) (Figure 2C).

3.3. Effect of high-fructose diet and resveratrol on the nutrient-sensing pathway

We next analyzed genes involved in the nutrient-sensing pathway. As shown in Figure 3, renal mRNA expression of *Prkaa2* (coding for AMPKα2; FC = 0.29), *Prkag2* (coding for AMPKγ2; FC = 0.08), *Ppara* (coding for PPARα; FC = 0.22), *Pparb* (coding for PPARβ; FC = 0.19), *Ppargc1a* (coding for PGC-1α; FC = 0.31), and *Sirt4* (FC = 0.1), were lower in the HF/HF group compared to the ND/ND group; these effects were prevented by resveratrol treatment. Consistent with the alterations in mRNA levels, renal protein levels of AMPKα2 (Figure 4B), PPARγ (Figure 4C), and PGC-1α (Figure 4D) were reduced by HF/HF exposure, and these reductions were prevented by resveratrol therapy.

3.4. Effect of high-fructose diet and resveratrol on gut microbiota

To determine the role of the gut microbiota in hypertension of developmental origin related to HF and resveratrol exposure, we explored bacterial populations in the gut at the phylum (Figure 5) and genus levels (Figure 6). The results of the current study showed that the main phyla in the different groups studied were *Firmicutes, Bacteroidetes, Verrucomicrobia*, and *Proteobacteria* (Figure 5A). Maternal HF diet showed a decreased abundance of *Bacteroidetes* in the HF/ND (26%), HF/HF (34%), and HF/HF+R groups (26%) with respect to the ND/ND group (47%). In contrast, there was a remarkable increase in *Verrucomicrobia* in the HF/ND (10%), HF/HF (11%), and HF/HF+R

groups (12%) compared to the ND/ND group (1%). Additionally, we observed an increase in the *Firmicutes* to *Bacteroidetes* ratio (HF/ND vs. ND/ND = 2.5 ± 0.6 vs. 1.1 ± 0.2; $P < 0.05$) and a decrease in the *Firmicutes* to *Verrucomicrobia* ratio (HF/ND vs. ND/ND = 22.7 ± 11.3 vs. 376.8 ± 177.6; $P < 0.05$) in offspring exposed to maternal HF consumption (Figure 5B and 5C). Notably, HF/HF diets reduced the *Firmicutes* to *Proteobacteria* ratio (HF/HF vs. ND/ND = 10.7 ± 1.5 vs. 18.5 ± 1.9; $P < 0.05$), which was restored by resveratrol treatment (HF/HF+R= 18.8 ± 3.8; $P < 0.05$) (Figure 5D).

The main bacterial genera in the different groups were *Parabacteroides, Lactobacillus, Alkaliphilus, Ruminococcus*, and *Bacteroides* (Figure 6A). Maternal HF, post-weaning HF, and combined HF/HF diets all decreased abundance of genus *Lactobacillus*. Maternal HF intake decreased abundance of genera *Bacteroides, Dysgonomonas,* and *Turicibacter*, whereas the abundance of genus *Akkermansia* was increased. Also, of note is that, at the species level, maternal HF intake significantly increased *Akkermansia muciniphila* in the HF/ND (11%), HF/HF (14%), and HF/HF+R groups (24%) compared to the ND/ND group (1%) (Figure 6B).

The main bacterial genera modified by the maternal HF diet were *Tepidibacter* (LDA score = 3.1), *Mucispirillum* (LDA score = 1.7), and *Lactococcus* (LDA score = 1.6). Post-weaning HF diet showed marked decreases in *Bifidobacterium* (LDA score = -1.8), *Mucispirillum* (LDA score = -1.6), and *Acholeplasma* (LDA score = -1.6) compared to that in the ND/ND group. The HF/HF group showed marked increases in *Tepidibacter, Lactococcus, Serratia, Enterobacter, Erwinia,* and

Mucispirillum, compared to the ND/ND group (Figure 6C). There were remarkable decreases in the genera *Turicibacter, Lactobacillus*, and *Leuconostoc* in the HF/HF group vs. ND/ND (Figure 6C). The HF/HF+R group showed increased abundance of the genera *Flavobacterium, Tepidibacter, Lactococcus*, and *Erysipelothrixas*, and decreased abundance of the genera *Acholeplasma* and *Turicibacter* (Figure 6D).

4. Discussion

This study provides insight into the mechanisms by which maternal and post-weaning high-fructose diets induce hypertension of developmental origin. We provide the first evidence, to our knowledge, that resveratrol protects against HF/HF-induced programmed hypertension via regulating oxidative stress, nutrient-sensing signals, and gut microbiota. The primary novel findings of this study were: 1) combined maternal plus post-weaning HF diets induced hypertension in male adult offspring, which was prevented by resveratrol therapy; 2) resveratrol therapy protected offspring kidney against HF/HF-induced oxidative stress; 3) HF/HF diets decreased renal mRNA expression of *Prkaa2*, *Prkag2*, *Ppara*, *Pparb*, *Ppargc1a*, and *Sirt4*, while these were restored by resveratrol treatment; 4) maternal HF diet had long-term effects on the offspring gut microbiota, including decreasing the abundance of genera *Bacteroides, Dysgonomonas,* and *Turicibacter*, while increasing the abundance of phylum *Verrucomicrobia* and *Akkermansia muciniphila*; 5) resveratrol prevented HF/HF-induced programmed hypertension, which was associated with reduction of renal

oxidative stress, activation of several nutrient-sensing signals, and modulation of gut microbiota including restoration of the *Firmicutes* to *Proteobacteria* ratio,.

The present study is consistent with our previous report showing that maternal HF diet did not amplify post-weaning HF-induced hypertension in adult offspring [23]. Despite maternal and post-weaning HF diets producing similarly elevated BP in adult offspring, they appear to work by independent mechanisms, based on our observations of differential effects on oxidative stress, the NO pathway, nutrient-sensing signals, and gut microbiota. Results of the present study are consistent with those of our previous study showing that maternal HF diet has no effect on body weight of adult offspring [4]. However, the combination of maternal and post-weaning HF diets reduced body weight, which was restored by resveratrol therapy. Resveratrol has been shown to reduce weight in obese rodents [24]. Thus, more detailed studies pertaining to the effects of resveratrol on body weight and fetal growth are warranted.

In the present study, prevention of HF/HF-induced programmed hypertension by resveratrol therapy was related to reduction of oxidative stress (represented as 8-OHdG staining) and activation of several nutrient-sensing signals (e.g. *Prkaa2*, *Prkag2*, and *Sirt4*) and *Nrf2*. A previous report demonstrated that maternal HF diet induced fetal oxidative stress, accompanied by decreased Nrf2 expression [25]. Our results are in agreement with those of previous studies showing that resveratrol reduced oxidative stress by activating the Nrf2/antioxidant defense and the SIRT/AMPK

pathways [26]. We observed that resveratrol therapy had negligible effects on the NO pathway, suggesting restoration of NO might not be a major protective mechanism of resveratrol in this model. It is noteworthy that post-weaning HF diet significantly reduced plasma levels of L-citrulline. Since citrulline is a known marker of altered intestinal permeability that is related to dysbiosis of gut microbiota [27,28], low L-citrulline level might reflect an effect of HF diet on gut permeability leading to dysbiosis, although this remains speculative.

The beneficial effects of resveratrol may also relate to mediation of the composition of the gut microbiota [29,30]. According to our results, several beneficial microbes, such as *Turicibacter,* *Lactobacillus*, and *Bifidobacterium* (LDA score = -1), were depleted in the HF/HF group. Resveratrol therapy increased the proportions of *Lactobacillus* and *Bifidobacterium*, which was in agreement with the findings of previous studies [29,30]. Additionally, we observed that maternal HF diet caused remodeling of the gut microbiota in adult offspring, including decreasing the proportion of phylum *Bacteroidetes*, thus increasing the *Firmicutes* to *Bacteroidetes* ratio. However, resveratrol therapy reduced BP level without affecting this ratio. Our results contradict those previously published using this ratio as a microbial marker for hypertension [13,29], possibly because we were experimenting in the model of developmental programming of hypertension, which is more complex than the hypertensive models. Nevertheless, our results showed that HF/HF diets reduced the *Firmicutes* to *Proteobacteria* ratio, which was restored by resveratrol treatment. Additional study is required to

clarify whether the *Firmicutes* to *Proteobacteria* ratio may serve as a microbial marker for hypertension in other programming models.

In the current study, maternal HF diet increased the abundance of *Akkermansia muciniphila*. This result was unsurprising in view of a previous study showing an increased abundance of *Akkermansia muciniphila* upon the ingestion of a high-fat high-sucrose diet [31]. Although a large body of evidence has demonstrated the beneficial impact of *Akkermansia muciniphila* [32], to the best of our knowledge, our study is the first to show that resveratrol protects against programmed hypertension related to increased abundance of *Akkermansia muciniphila.* Unlike a previous report showing that hypertension-associated dysbiosis is characterized by increases in lactate-producing bacteria and decreases in acetate- and butyrate-producing bacteria [13], we observed reductions in the lactate-producing bacteria *Lactobacillus, Leuconostoc*, and *Turicibacter* in the HF/HF-induced hypertension group. Thus, further studies are needed to elucidate whether the imbalance of gut acetate-, butyrate-, and lactate-producing bacterial populations directly contribute to BP control in a variety of hypertension models.

A potential limitation of this study is the inability to examine serial microbiota changes during multiple developmental windows. The alterations in gut microbiota we found in adult offspring may reflect postnatal plasticity rather than an original programmed process in response to maternal HF consumption. Second, we restricted resveratrol therapy to the HF/HF group. This was because the

effect of resveratrol therapy on HF-induced hypertension in adult rats has been studied [33] and resveratrol has no effect on BP in normotensive controls [34]. However, additional studies are still needed to investigate whether resveratrol affects intestinal microbiota in normal controls. Although 16S rRNA gene amplicon analysis indicates proportional changes among bacterial taxonomies between healthy and diseased groups [35], there is a need to further explore subsequent changes in functional genes to better understand the contribution of gut microbiota to hypertension of developmental origin. Another limitation is that resveratrol dosing was not adjusted overtime to body weight changes due to *ad libitum* drinking water administration of resveratrol. Although body weight and water consumption did not differ among the five groups, the interindividual variability in response to resveratrol may influence efficacy. It is also notable that, despite our results from animal studies in support of resveratrol as a reprogramming strategy to provide long-term protection against hypertension of developmental origin, these results have yet to be confirmed clinically in human subjects. It is important that future programming research should aim to fill the translational gap between animal models and clinical trials.

In conclusion, several important mechanisms are involved in the protective effects of resveratrol on adult offspring exposed to combined maternal and post-weaning fructose-rich diet, including reduction of oxidative stress, restoration of nutrient-sensing signals, and alterations of gut microbiota composition. Targeting these mechanisms by resveratrol might be a therapeutic strategy

for hypertension induced by widespread consumption of food containing high fructose in pregnant mothers and their children. Research into the prevention of hypertension that begins early in life will have a profound impact on the economic burden of hypertension-related disorders.

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Author contributions

Y. T. contributed to concept generation, data interpretation, manuscript drafting, critical manuscript revision, and article approval. W. L. contributed to data interpretation, manuscript drafting, critical manuscript revision, and article approval. K. W. contributed to data interpretation, manuscript drafting, critical manuscript revision, and article approval. S. L. contributed to data interpretation, manuscript drafting, critical manuscript revision, and article approval. J. C. contributed to concept generation, data interpretation, critical manuscript revision, and article approval.

Conflict of interest statement

The authors have declared no conflict of interest.

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Figure Legends

Figure 1 Effects of maternal and post-weaning high-fructose (HF) diets on systolic blood pressure in three-month-old male offspring. *P < 0.05 vs. ND/ND; #P < 0.05 vs. ND/HF; †P < 0.05 vs. HF/ND; ‡P < 0.05 vs. HF/HF. N = 8/group.

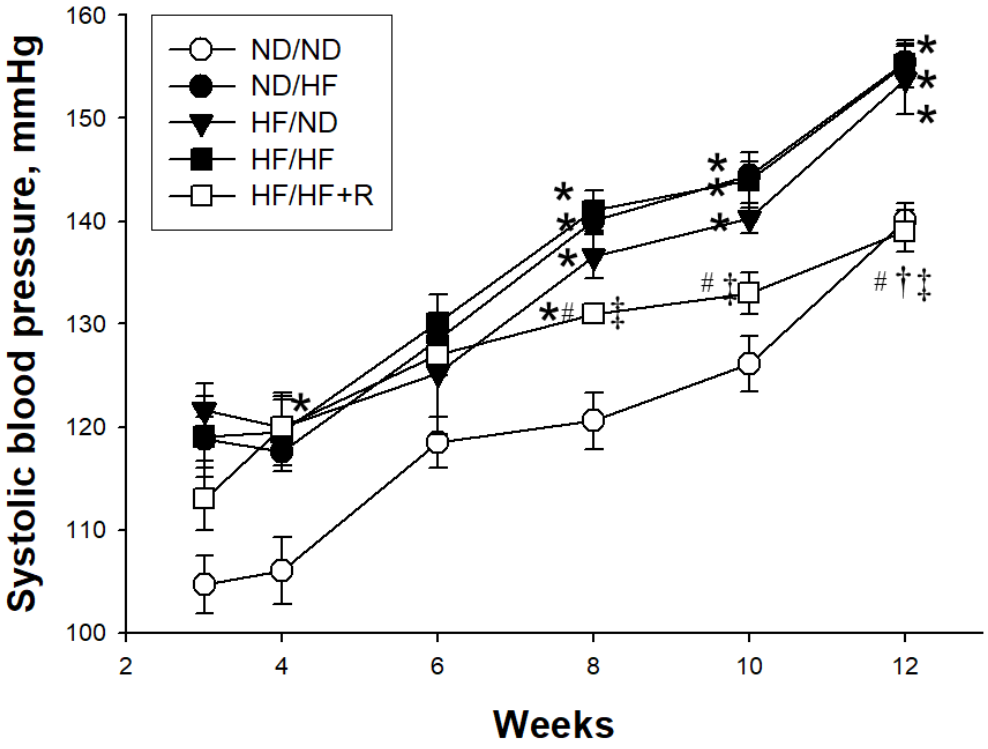


Figure 2 (A) Light micrographs illustrating immunostaining for 8-hydroxydeoxyguanosine (8-OHdG) in the kidney of three-month-old male offspring exposed to maternal and post-weaning high-fructose (HF) diet and resveratrol. Bar = 50 µm. (B) Quantitative analysis of 8-OHdG-positive cells per microscopic field (X400). (C) Effects of maternal and post-weaning HF diet and resveratrol on renal mRNA expression of nuclear factor erythroid-derived 2-related factor 2 (Nrf2). *P < 0.05 vs. ND/ND; #P < 0.05 vs. ND/HF; †P < 0.05 vs. HF/ND; ‡P < 0.05 vs. HF/HF. N = 8/group.

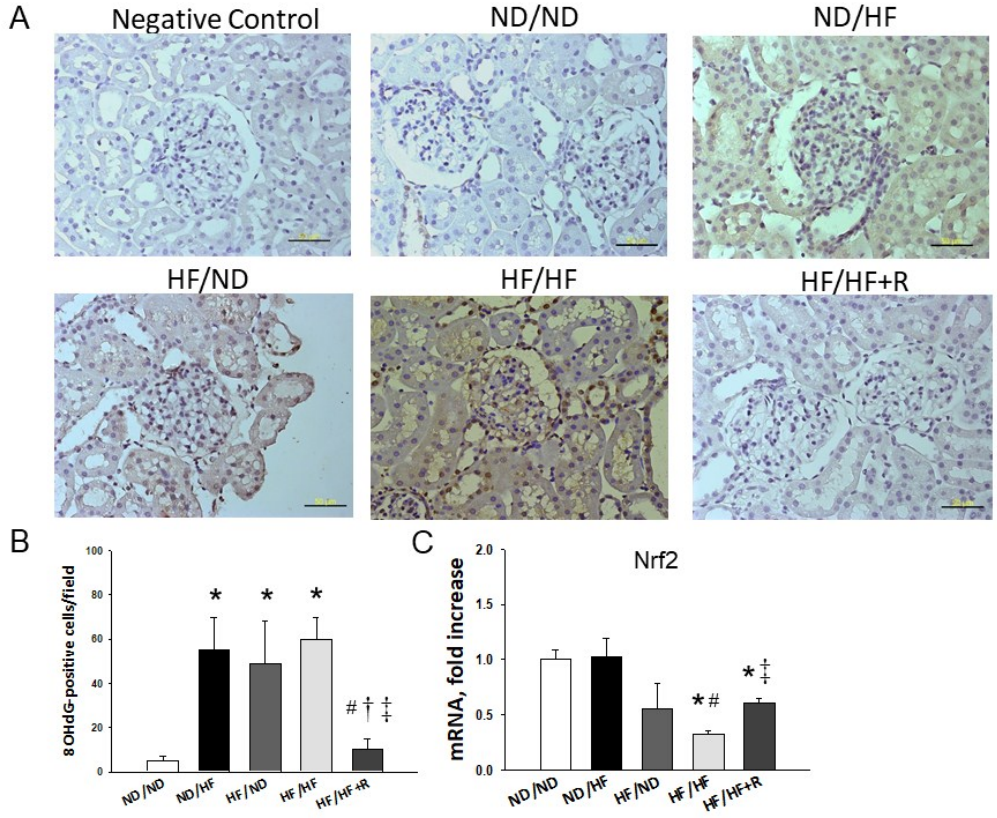


Figure 3 Effects of maternal and post-weaning high-fructose (HF) diet and resveratrol on mRNA expression of (A) AMP-activated protein kinase (AMPK) α-, β- and γ-subunits; (B) Peroxisome proliferator-activated receptor (PPAR) α-, β- and γ-isoforms; (C) PPARγ coactivator-1α (PGC-1α); and silent information regulator transcript 4 (Sirt4) in male offspring kidneys at three months of age. *P < 0.05 vs. ND/ND; #P < 0.05 vs. ND/HF; †P < 0.05 vs. HF/ND; ‡P < 0.05 vs. HF/HF. N = 8/group.

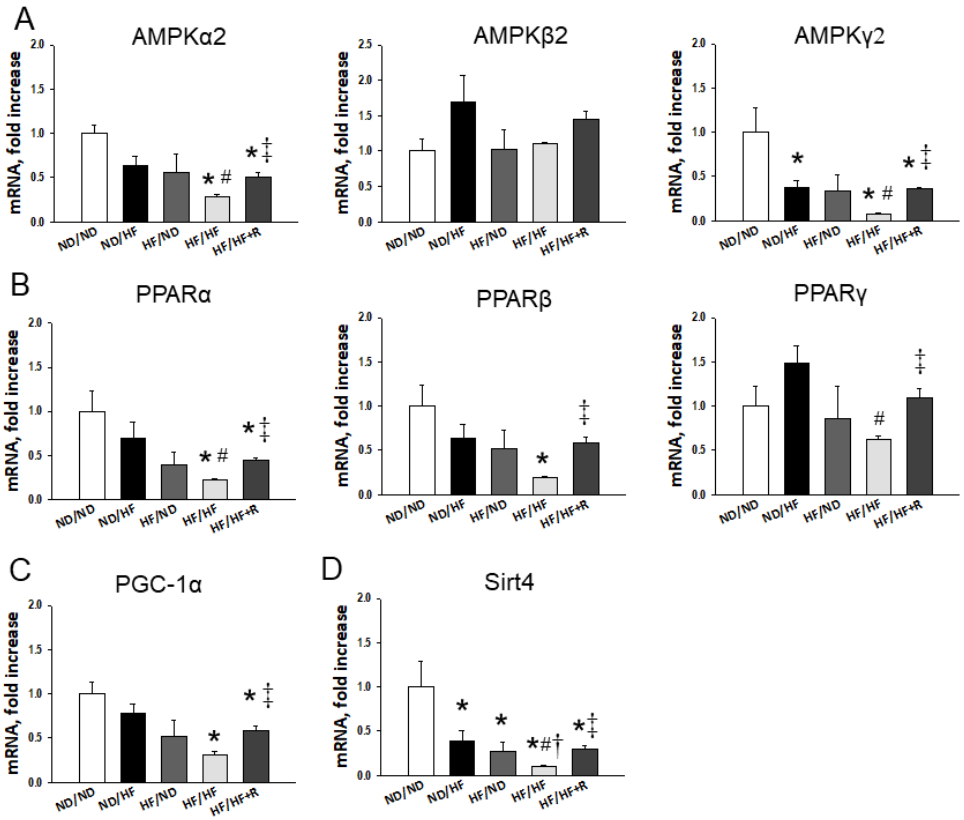


Figure 4 (A) Representative western blots and relative abundance of (B) AMPKα2 (63 kDa); (C) PPARγ (57 kDa); and (D) PGC-1α (90 kDa) in male offspring kidneys at 12 weeks of age. *P < 0.05 vs. ND/ND; #P < 0.05 vs. ND/HF; †P < 0.05 vs. HF/ND; ‡P < 0.05 vs. HF/HF. N = 8/group.

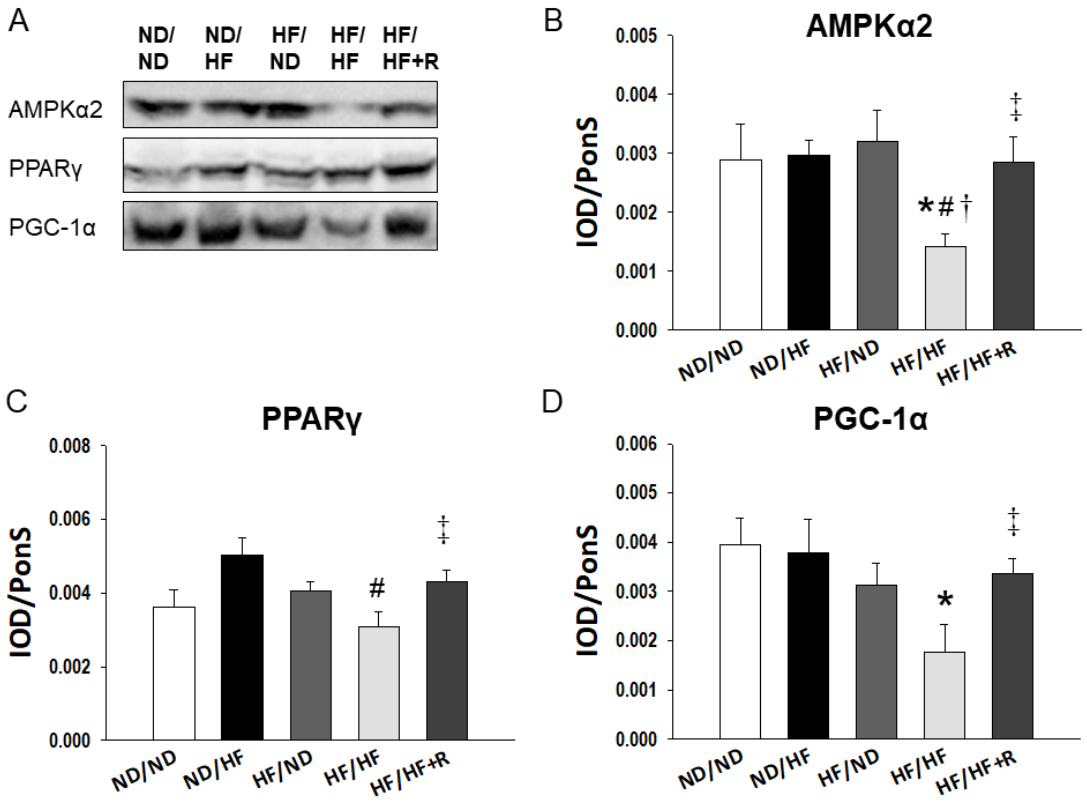


Figure 5 Effects of maternal and post-weaning high-fructose (HF) diet and resveratrol on the gut microbiota: (A) Relative abundances of the gut microbiota at the phylum level; (B) the *Firmicutes* to *Bacteroidetes* ratio; (C) the *Firmicutes* to *Verrucomicrobia* ratio; and (D) the *Firmicutes* to *Proteobacteria* ratio. *P < 0.05 vs. ND/ND; #P < 0.05 vs. ND/HF; †P < 0.05 vs. HF/ND; ‡P < 0.05 vs. HF/HF. N = 8/group.

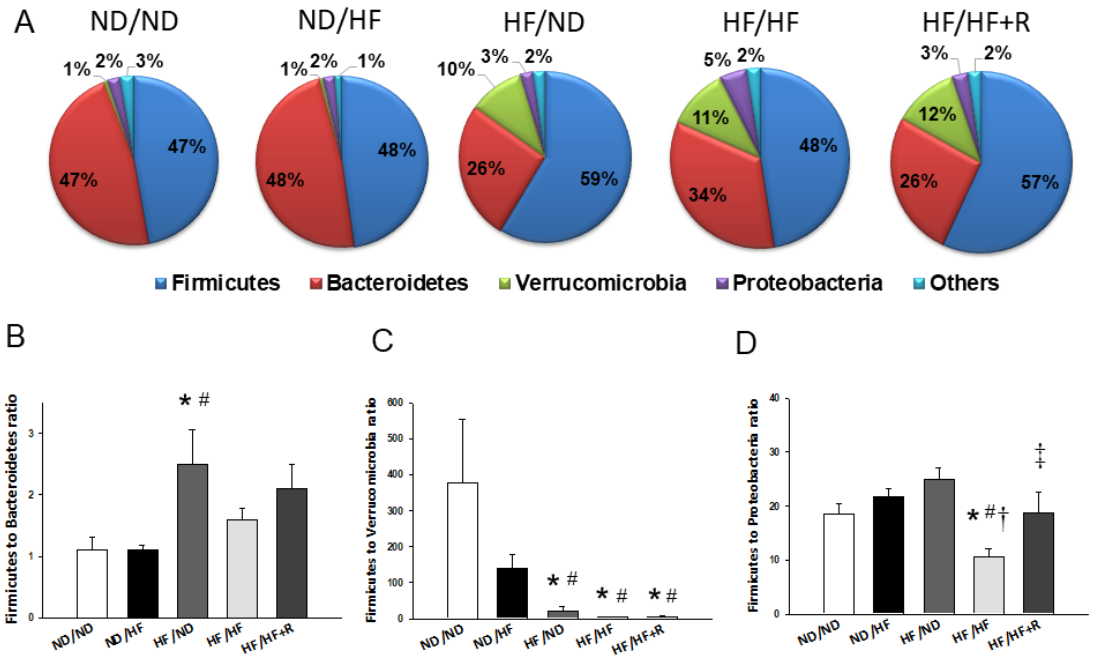
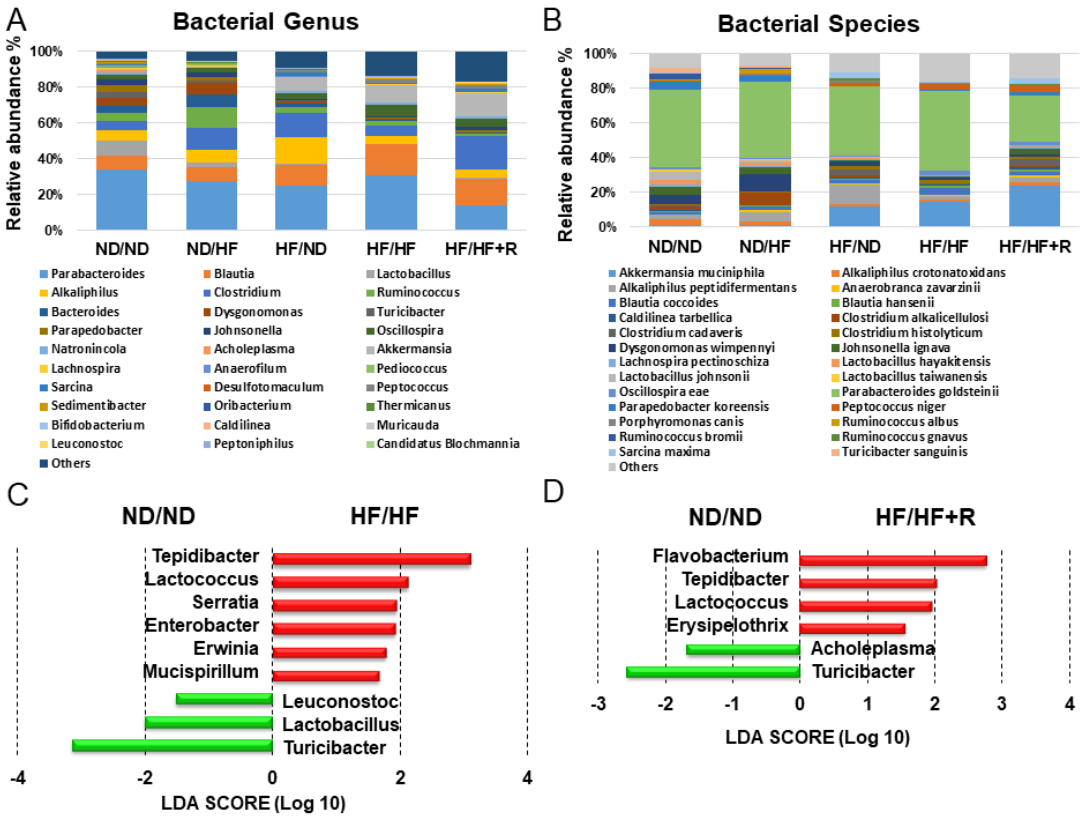


Figure 6 Effects of maternal and post-weaning high-fructose (HF) diet and resveratrol (R) on the gut microbiota showing relative abundance at the genus (A) and species (B) levels. Linear discriminant analysis (LDA), along with effect size measurements, was applied to identify enriched bacterial genera. Most enriched and depleted genera (LDA score (log10) > 1.5) in the (C) HF/HF (red) vs. ND/ND (green) and (D) HF/HF+R (red) vs. ND/ND (green). N = 8/group.



Graphic Abstract

Maternal and post-weaning high-fructose diets induces developmental programming of hypertension in adult offspring, which related to oxidative stress, nutrient-sensing signals, and gut microbiota. Resveratrol might be a reprogramming strategy to prevent programmed hypertension induced by widespread consumption of food containing high fructose in pregnant mothers and their children.

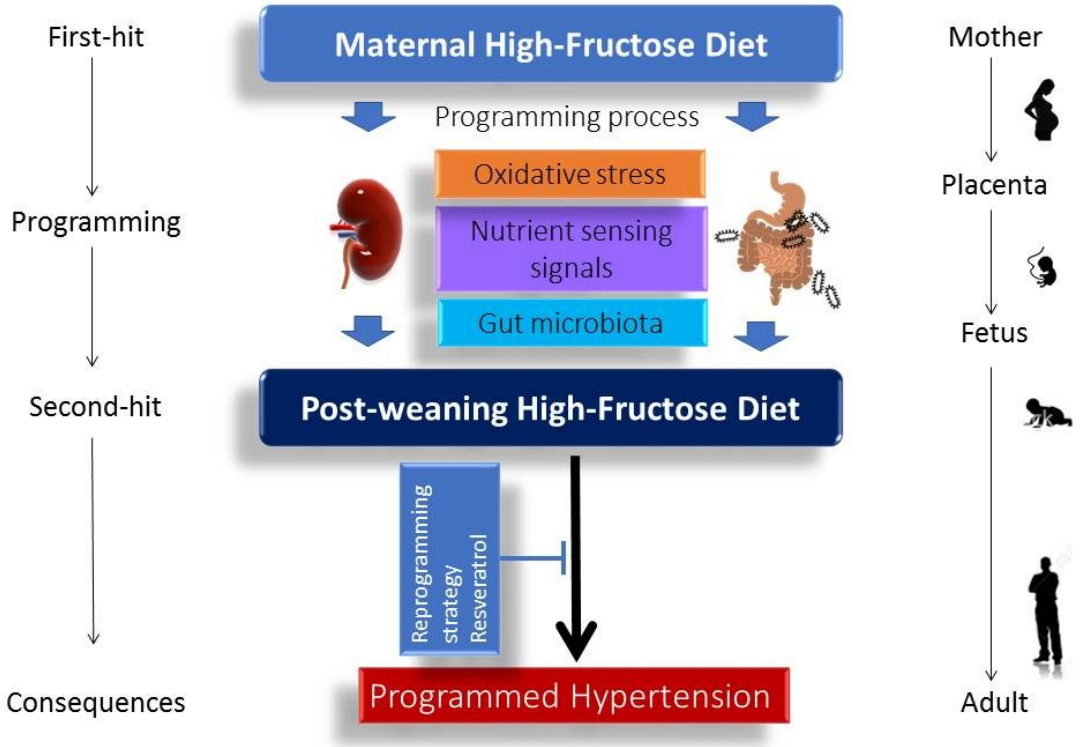


Table 1. Morphological and biochemical measures in each experimental group

	ND/ND	ND/HF	HF/ND	HF/HF	HF/HF+R
Mortality	0%	0%	0%	0%	0%
Body weight (BW) (g)	412 ± 10	370 ± 13	400 ± 10	353 ± 17[a]	457 ± 16[b,c,d]
Left kidney weight (g)	1.96 ± 0.09	2.05 ± 0.1	1.65 ± 0.03	2.03 ± 0.13	2.6 ± 0.16[a,b,c,d]
Left kidney weight/ 100g BW	0.48 ± 0.03	0.55 ± 0.02	0.41 ± 0.01[b]	0.59 ± 0.05[c]	0.58 ± 0.03[c]
Systolic blood pressure (mmHg)	140 ± 2	155 ± 2[a]	153 ± 3[a]	154 ± 3[a]	142 ± 3[b,c,d]
Diastolic blood pressure (mmHg)	82 ± 2	85 ± 5	81 ± 3	91 ± 3[a,c]	77 ± 2[b,d]
Mean arterial pressure (mmHg)	101 ± 1	109 ± 3	105 ± 3	113 ± 2[a]	99 ± 1[b,c,d]

[a]P < 0.05 vs. ND/ND; [b]P < 0.05 vs. ND/HF; [c]P < 0.05 vs. HF/ND; [d]P < 0.05 vs. HF/HF.

Table 2. Plasma L-citrulline, L-arginine, and dimethylarginine levels

	ND/ND	ND/HF	HF/ND	HF/HF	HF/HF+R
L-Citrulline (μM)	57.2 ± 1.1	38 ± 1[a)]	48.5 ± 1.5	43.2 ± 1.6[a)]	39.7 ± 0.6[a]
L-Arginine (μM)	228.4 ± 6.7	101.5 ± 3.4[a)]	226.4 ± 9.1[b)]	135.1 ± 2.3[a),c)]	133.7 ± 5.1[a),c)]
ADMA (μM)	1.01 ± 0.03	0.7 ± 0.01[a]	1.02 ± 0.04[b]	0.81 ± 0.01	0.86 ± 0.03
SDMA (μM)	0.61 ± 0.01	0.49 ± 0.01	0.58 ± 0.01	0.5 ± 0.01	0.52 ± 0.01
L-arginine-to-ADMA ratio (μM/μM)	226.2 ± 3	145.4 ± 5[a)]	228.8 ± 11.3[b)]	166.7 ± 1.3	158.1 ± 6.1[a),c)]

[a)]P < 0.05 vs. ND/ND; [b)]P < 0.05 vs. ND/HF; [c)]P < 0.05 vs. HF/ND; [d)]P < 0.05 vs. HF/HF.