GW501516 Ameliorates A Fructose-Induced Inflammation Independent of AT1r Downregulation in Kidney

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Abstract. AT1r high activation is linked to low-grade inflammation and oxidative stress, which yield impaired renal function. This study aimed to verify if GW501516 could improve damage in the kidney of mice with high activation of AT1r. Mice were fed a high-fructose diet (HFru) for eight weeks to induce an activation of the AT1r, whereas the control group received standard chow. The animals were randomly divided into four groups and the administration of GW501516 lasted three weeks. Morphological outcomes, urine and plasma determinations were assessed. Renin and ACE/AT1r axis protein and gene expression were evaluated as well as inflammatory cytokines and proteins. Also, the protein and gene expression of the antioxidant enzymes were verified. GW501516 improved systolic blood pressure and urinary parameters in HFru group. Although GW501516 had no effects either on ACE/AT1r axis or renin expression, it improved the inflammatory state, with increased IκB-α protein expression and decreased ERK and JNK phosphorylation. No differences were found in oxidative stress. We conclude that GW501516 acts downstream AT1r activation, improving inflammatory pathways in the kidney of HFru fed model. This is the first report demonstrating the anti-inflammatory actions of GW501516 upon kidney independently of AT1r downregulation in an HFru model.

Keywords: ACE/AT1r axis; GW501516; Inflammation; Kidney; High-fructose

1. Introduction

A change in dietary intake worldwide has been accompanied by an increase obesity-related diseases and metabolic syndrome (MS), as cardiovascular disease and chronic kidney disease (CKD). The introduction of fructose in feeding habits over the last 30 years, mainly as high-fructose (HFru) corn syrup, found in soft drinks and processed food, has contributed to elevating the number of such comorbidities [1]. Several studies have shown that the HFru intake can lead to CKD in animals [2, 3] and humans [4] as well as to high activation of angiotensin converting enzyme (ACE)/angiotensin 2 receptor type 1 (AT1r) axis in metabolic organs [5]. Fructose-induced metabolic changes are not mediated by excessive simple carbohydrate intake in general but seem to be exclusively linked to fructose intake, once similar effects do not occur following the intake of starch or glucose [6].
The angiotensin (Ang)-II is a product of ACE enzyme action upon Ang-I. The Ang-II binds to AT1r and promotes physiological effects as vasoconstriction and releasing of aldosterone [7]. But when Ang-II bindings to AT1r also yields the development of renal failure by causing hypertrophy of renal cells, increasing renal microvascular pressure, and inducing apoptosis, reactive oxygen species (ROS) and inflammation [8]. The HFru diet is well characterized to generate uric acid [9], and its production is accompanied by ROS formation [10] and tubular injury in kidney through increased monocyte chemoattractant protein-1 (MCP-1) expression and cell proliferation [11].

The GW501516 is a peroxisome proliferator-activated receptor (PPAR)-beta/delta agonist that has an anti-inflammatory effect in liver, white adipose tissue and muscle [12], [13]. In the kidney, GW501516 could attenuate tubulointerstitial inflammation in proteinuric disease [14], and reduce the growth of inner medullary collecting duct cells [15]. However, it is not known the action of the GW501516 administration on the HFru-induced kidney inflammation and oxidative stress. Thus, the present study was proposed to verify whether GW501516 could improve inflammatory state and oxidative stress in the kidney in an HFru-diet model.

2. Materials and Methods

2.1. Animals, Diet, and GW501516 Administration. The Ethics Committee for Animal Experimentation of the State University of Rio de Janeiro approved the study under the protocol CEUA/071/2012. The study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH Publication number 85-23, revised 1996).

Male C57Bl/6 mice (3-mo-old) were in an environment with a 12 h light/dark cycle, controlled temperature (20 ± 2 °C), humidity (60 ± 10 %) and had free access to food and water. Mice were randomly divided into two groups (n=20), standard-chow (SC) or HFru (47 % of fructose) [13], [16] and were fed these diets for eight weeks. The diets were manufactured by PragSolucoes (São Paulo, Brazil) and were consistent with the recommendations of the AIN (93M). After eight weeks, animals were redivided randomly into four groups (n=10) and treated with the GW501516 (Enzo Life Science, Farmingdale, NY, USA) dissolved in a carboxymethylcellulose (CMC) medium viscosity (w/v 0.5%) at the dose of 3 mg/Kg/day [17].

The groups of the study were: a) the SC group, with standard chow during all experiments and CMC for three weeks; b) the SC+GW group, with standard chow during all experiments and GW501516 treatment for three weeks; c) the HFru group with HFru diet during all experiments and CMC for three weeks; d) the HFru+GW group, with HFru diet during all experiments and GW501516 treatment for three weeks. Both vehicle and GW501516 were administrated by oral gavage once a day at 10 a.m., and body mass (BM) and water intake were controlled weekly.

2.2. Blood pressure. Conscious animals were trained for two weeks, twice a week, in constraint conditions before the measurement of their systolic blood pressure (SBP) using a tail-cuff plethysmography (Letica LE 5100, Harvard/Panlab, Barcelona, Spain). Then, the measurements were made weekly during treatment.

2.3. Urine. Urine samples were taken in the week before euthanasia. One mouse per metabolic cage was kept for 48 h to acclimatize and then left for 24 h to measure urine volume. Urine was collected and centrifuged at 120 g for 15 min to remove any solid waste. Afterward, the 24 h urine output was adjusted for BM (mL/g BM). Protein was evaluated using commercial kits and an automatic spectrophotometer (Bioclin System II, Quibasa, Belo Horizonte, MG, Brazil).

2.4. Kidney. After the treatment period, animals were food-deprived from 1 a.m.-7 a.m. and then deeply anesthetized (sodium pentobarbital, 150 mg/Kg intraperitoneal). Blood samples were rapidly obtained by cardiac puncture, and plasma was separated by centrifugation (120 g for 15 min) at room temperature and stored individually at -20 °C until assay. The kidneys were carefully dissected, weighed, and several fragments were frozen for molecular analysis by immunoblotting and RT-qPCR. We used the tibia length (left tibia) measurement from the condyles to the tip of the medial malleolus to normalize the kidney mass [18]. Mallory’s trichrome stain was performed to address collagen deposition around glomeruli.

2.5. Plasma uric acid analysis. The concentrations of plasma uric acid were measured by an automatic spectrophotometer using a commercial kit (Bioclin System II, Quibasa, Belo Horizonte, MG, Brazil).

2.6. Immunohistochemistry. Five-micrometer-thick kidney sections were obtained from the kidney’s blocks. Antigen retrieval was performed using citrate buffer, pH 6.0, and the endogenous peroxidase was quenched using 3% hydrogen peroxide. Next, the sections were incubated with a monoclonal mouse anti-F4/80 antibody, diluted 1:100, for 2 h. The samples were treated with a biotinylated secondary antibody (K0679, LSAB+Kit peroxidase; Dako Cytomation), which was detected using a horseradish peroxidase–streptavidin–biotin complex. The positive immunoreactions were identified after incubation in DAB (K3466; Dako Cytomation) and counterstaining with Mayer’s hematoxylin.

2.7. RT-qPCR. Levels of mRNA of the kidney were assessed by the RT-qPCR as previously described [19]. The sequences
of sense and antisense primers used for amplification were: PPAR-beta/delta, 5’-GCCACAAACGCACCTTGG-3’ and 5’-CACACCCAGGCCCCCTTTCT-3’; Pyruvate dehydrogenase lipase amide kinase isozyme 4 (PDK4), 5’-CACCACA TGCTTCTGCAACTCT-3’ and 5’-AAGGAAGCAGGTT TTCTTGTAG-3’; Carnitine palmitoyltransferase 1 (CPT1), 5’-GCAGAGCAGGCAAATGA-3’ and 5’-GGCTTCT GCACCCAGAAGAC-3’; Ace, 5’-GTGGCTGGAAGAG CAGAATC-3’ and 5’-GCCCTGGCTTCACTAGTCTC-3’; TNF (SC-7345) – 54kDa and c-Jun N-terminal kinase (JNK) (SC-6254) – 54kDa, total 81492)-42-44kDa and Renin (SC-27318)-38kDa, phospho NF-KB (SC-109)-65kDa, IκB-alpha (SC-847) - 50-65kDa, GPx (SC-133) - 160kDa, GR (SC-133245) - 5’-ACGGATTCCATGGTGAAGTC-3’ and 5’-ACAT AGGTGATTGCGGAAAGG-3’; interleukin (IL)-6, 5’-AGT TGCTTCTGAGGAGTGA-3’ and 5’-ACAGGGCTTGGTGG GAGTTGT-3’; Mpc-1, 5’-GCTGGGAGAGCTACAAGA GAATCA-3’ and 5’-CTCCTCTGAGCTGTTGTGACAA A-3’; Cluster of Differentiation 68 (Cd68), 5’-GCTGGAATC-3’ and 5’-GCCTTGGCTTCATAGTCTC-3’; Monocyte chemotactic protein-1 (Mpc-1), 5’-GCTGGGAGAGCTACA AGGAGATCA-3’ and 5’-CTCCTCTGAGCTGTTGTG CAAA-3’. Efficiencies of RT-qPCR for the target gene and the endogenous control were approximately equal and were calculated through dilution series of cDNA. The results for the expression of specific mRNAs are presented about the expression of the control gene (β-actin).

2.8. Immunoblotting. Total protein was extracted in homogenizing buffer with protease and phosphatase inhibitors. Next, the homogenates were centrifuged at 4°C, and the supernatants were collected. Equal quantities of total protein were separated by SDS/PAGE. After electrophoresis, the proteins were electroblotted onto a polyvinyl difluoride transfer membrane (Amersham Biosciences, Piscataway, N.J., USA). Immunoblotting analysis was performed using antibodies against ACE1 (AB 11734) -170kDa (somatic), AT1R (SC-576) -43 kDa, Catalase (SC-50508) - 64 kDa, SOD2 - (SC-380) - 25kDa, Gpx (SC-133) - 160kDa, GR (SC-133245) - 50-65kDa, NF-KB - (SC-109) - 65kDa, IkB-alpha - (SC-847) - 35-41kDa, ERK1/2-total (SC-135900) e p-ERK1/2 (SC-81492) - 42-44kDa and Renin (SC-27318) - 38kDa, phospho-e-Jun N-terminal kinase (JNK) (SC-6254) - 54kDa, total JNK (SC-7345) - 54kDa and β-actin (SC-47778) – 43kDa. The membrane was developed using ECL detection reagents, and images of the blot were obtained with Bio-Rad’s Molecular Imaging ChemiDoc XRS Systems (Bio-Rad, Hercules, CA, USA). The intensity of the chemiluminescent bands was quantified using ImageJ software, version 1.49 (NIH, imagej.nih.gov/ij, USA).

2.9. Data Analysis. We presented the values as the mean and the standard deviation. During the first eight weeks of the experiment, differences between SC and HFr groups were tested with Student t test. During the treatment phase, the Bartlett test was used to test the homoscedasticity of the variances and once a normal distribution was confirmed, comparisons among groups were made using one-way ANOVA followed by the posthoc test of Holm-Sidak. A P-value< 0.05 was considered statistically significant (Prism version 6.05 for Windows, GraphPad Software, La Jolla California USA).

3. Results

3.1. Effects of GW501516 on SBP, water intake, urine and plasma determinations. During treatment with GW501516, it was not found differences among the experimental groups regarding their BM, but GW501516 treatment rescued HFr diet-induced increase kidney mass (Table 1). As expected, the HFr group presented an elevated SBP from the second week of the diet, and the GW501516 reduced it progressively in the HFr+GW group (Figure 1A).

The HFr diet caused an increase in water intake and urine volume, and GW501516 administration could rescue these parameters. Likewise, the HFr diet increased plasma uric acid in the HFr group as well as proteinuria. The GW501516 administration could reverse these changes. Also, the GW501516 administration was capable of reducing the proteinuria and plasma uric acid between the SC groups (Table 1).

3.2. Effects of GW501516 on PPAR-beta/delta, PDK4, and CPT1 gene expression. To verify if GW501516 was able to activate PPAR-beta/delta in treated groups, we assessed mRNA levels of PPAR-beta/delta. Even though the HFr diet did not cause a significant reduction in PPAR-beta/delta gene expression, the treatment was able to enhance its mRNA expression in the HFr+GW group. To confirm whether the changes observed in this study is due to PPAR-beta/delta activation, we analyzed the mRNA levels of two known PPAR-beta/delta target genes, PDK4, and CPT1. In agreement with PPAR-beta/delta mRNA levels, the GW501516 could increase the expression of both PPAR-beta/delta target genes. It is worth mentioning that GW501516 did not augment the PPAR-beta/delta mRNA in SC+GW group, but it yielded higher expression of its target gene CPT-1 in these animals. These results confirm the activation of PPAR-beta/delta through transcription of its target genes in the HFr+GW group (Figure 1B).

3.3. Anti-inflammatory effects of GW501516 in the kidney are independent of AT1r downregulation. As expected, the HFr diet caused activation of ACE/AT1r axis, verified through increased renin, ACE and AT1r protein levels, which are in agreement with SBP findings (Figure 2).

GW501516 administration had no effects on HFr-induced ACE/AT1r high activation, which suggest that the improvements found in the kidney in the HFr+GW group are independent of AT1r downregulation.
Table 1: Morphological variables, hydric balance and urinary and plasmatic determinations

<table>
<thead>
<tr>
<th>Data</th>
<th>SC</th>
<th>SC+GW</th>
<th>HFru</th>
<th>HFru+GW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Body Mass (g)</td>
<td>29.9±0.73</td>
<td>28.8±0.63</td>
<td>30.1±1.06</td>
<td>28.9±0.56</td>
</tr>
<tr>
<td>Kidney mass/Left Tibia (g/cm, 10-3)</td>
<td>0.61±0.03</td>
<td>0.55±0.02</td>
<td>0.67±0.03 *</td>
<td>0.60±0.02 $</td>
</tr>
<tr>
<td>Water intake (last week, ml/mouse)</td>
<td>5.1±0.31</td>
<td>4.3±0.28</td>
<td>10.5±0.42 ***</td>
<td>7.4±0.33 **††$</td>
</tr>
<tr>
<td>Urine Volume (ml/24h/mouse)</td>
<td>2.1±0.76</td>
<td>1.2±0.12</td>
<td>3.9±0.53 ***</td>
<td>2.8±0.53 †††$</td>
</tr>
<tr>
<td>Proteinuria (mmol/L/24h)</td>
<td>0.27±0.06</td>
<td>0.13±0.06 **</td>
<td>0.39±0.11 **</td>
<td>0.26±0.06 †††$</td>
</tr>
<tr>
<td>Plasma Uric acid (mmol/L)</td>
<td>0.20±0.008</td>
<td>0.20±0.019</td>
<td>0.22±0.005 ***</td>
<td>0.19±0.025 $</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. *, **, *** and **** indicate \( P < 0.05, P < 0.01, P < 0.001 \) and \( P < 0.0001 \), respectively, compared with the SC group; †† and ††† indicate \( P < 0.01 \) and \( P < 0.001 \), respectively, compared with the SC+GW group; $ and $$ indicate \( P < 0.05 \) and \( P < 0.01 \), respectively, compared with the HFru group.

Figure 1: SBP, PPAR-beta/delta and PPAR-beta/delta target genes in the kidney. HFru diet leads to an increase in SBP from the second week in the diet scheme. GW501516 was able to activate PPAR-beta/delta and its target genes PDK4 and CPT1 in the kidney. A: SBP evolution (n=10/group). B: PPAR-beta/delta and its target genes in the kidney (n=5/group). Data are presented as mean ± SD. *, **, *** and **** indicate \( P < 0.05, P < 0.01, P < 0.001 \) and \( P < 0.0001 \), respectively, compared with the SC group; † indicates \( P < 0.05 \) compared with the SC+GW group; $, $$ and $$$ indicate \( P < 0.05, P < 0.01 \) and \( P < 0.001 \), respectively, compared with the HFru group.

Figure 3: mRNA levels (%). A: PPAR-β/δ (n=5/group). B: PDK4 (n=5/group). C: CPT1 (n=5/group). Data are presented as mean ± SD. *, **, *** and **** indicate \( P < 0.05, P < 0.01, P < 0.001 \) and \( P < 0.0001 \), respectively, compared with the SC group; †† and ††† indicate \( P < 0.01 \) and \( P < 0.001 \), respectively, compared with the SC+GW group; $ and $$$ indicate \( P < 0.05 \) and \( P < 0.01 \), respectively, compared with the HFru group.

Considering that AT1r high activation is closely linked to the inflammatory state, IL-1β, IL-6, Mcp-1 and Cd68 mRNA levels were assessed to confirm the HFru inflammatory effects. The HFru diet yielded a significant elevation of two out of four inflammation markers evaluated (IL-1β and Mcp-1), and GW501516 blunted these changes (Figure 3A). HFru diet also diminished the InB-α protein expression, suggesting that nuclear factor kappa B (NF-κB) would be translocated to the nucleus and activating its target genes such as Mcp-1 (Figure 3B). We also assessed others proinflammatory pathways related to AT1r activation. The HFru diet led to an increase in ERK1/2 and JNK phosphorylation, and GW501516 alleviates all these parameters (Figure 3C and D).

To confirm if the inflammatory state in kidney was related to macrophage infiltration as well, F4/80 immunostaining, a marker of macrophages, was performed. As shown in Figure 4, the HFru group presented an increase of macrophages infiltration around glomeruli. Despite showing a positive reaction to F4/80, the HFru+GW group presented an improvement of this parameter. Figure 4 also depicts increased collagen deposition around glomeruli in HFru and HFru+GW groups after Mallory’s trichrome stain.

3.4. GW501516 does not reduce HFru-induced oxidative stress in the kidney. We verified if GW501516 could restore the expression of proteins and genes related to the oxidative stress once HFru diets administration leads to this unbalance as well. The HFru group presented an elevation of SOD2 expression, confirming the unbalanced antioxidant enzymes.
Figure 2: Renin and ACE/AT1r protein and gene expression in the kidney. The high-fructose administration leads to an increase in protein and gene expression of Renin, ACE and AT1r and GW501516 had no effects on these parameters (n=5/group). A: mRNA levels of renin, ACE, and AT1r. B: RENIN protein expression. C: ACE protein expression. D: AT1r protein expression. Data are presented as mean ± SD. **, *** and **** indicate $P<0.01$, $P<0.001$ and $P<0.0001$, respectively, compared with the SC group; †† indicates $P<0.01$ compared with the SC+GW group.

but GW501516 could not rescue this parameter in the HFrU+GW group (Figure 5A and B). Catalase protein expression was diminished in HFrU and HFrU+GW groups in relation to SC group. However, HFrU+GW group presented with a slight but significant increase in this protein expression compared to HFrU group (Figure 5C). Moreover, no differences in GPx and GR protein expression were found among the groups. Nevertheless, GPx gene expression diminished in both groups that received HFrU diet, and GR gene expression also showed a decrease in the HFrU+GW group (5A, D, and E).
Figure 3: **Inflammatory state in the kidney.** GW501516 could rescue the deleterious effects of HFr u diet upon the inflammatory state (n=5/group). A: mRNA levels of proinflammatory cytokines in the kidney. B: IκB-α protein expression. C: p-ERK/ERK protein expression. D: p-JNK/JNK protein expression. Data are presented as mean ± SD. * and ** indicate $P<0.05$ and $P<0.01$, respectively, compared with the SC group; $\$, $$ and $$$ indicate $P<0.05$, $P<0.01$ and $P<0.001$, respectively, compared with the HFr u group.

Figure 4: **Mallory’s trichrome stain and F4/80 immunohistochemistry in the kidney.** GW501516 decreases F4/80, a macrophage marker, immunostaining and immunodensity. A: Mallory’s trichrome stain. Arrows indicate collagen deposition around glomeruli. B: F4/80 immunohistochemistry labelling. Arrows indicate macrophage infiltration around glomeruli.
Figure 5: Oxidative stress in the kidney. The HFru administration caused an enhanced oxidative stress in the kidney, but GW501516 could not improve these changes. A: mRNA levels of antioxidant enzymes. B: SOD2 protein expression. C: CATALASE protein expression. D: GPX protein expression. E: GR protein expression. Data are presented as mean ± SD. *, **, *** and **** indicate $P < 0.05$, $P < 0.01$, $P < 0.001$ and $P < 0.0001$, respectively, compared with the SC group; †† indicates $P < 0.01$ compared with the SC+GW group; $\$ $ indicates $P < 0.05$ compared with the HFru group.

4. Discussion

The present study demonstrated that GW501516 could improve SBP, hydric balance and inflammatory state in the kidney of HFru-fed mice, independently of AT1r downregulation. Although there are many experimental or clinical studies about HFru diet and its pathophysiology in kidney, a few number of studies correlate GW501516 actions in kidney, and there are no studies in the literature that evaluate the effects of GW501516 in the kidney on an HFru diet model. Previously, our group and others have demonstrated the deleterious effects of HFru diet on SBP, exacerbating ACE/AT1r axis activity in HFru-fed animals model [13, 20, 21], and from this established model we investigate the effects of a short-administration of GW501516.

In this study, we found an increase in plasma uric acid in HFru-fed animals, which agrees to higher hepatic protein expression of fructokinase after chronic dietary intake of high fructose corn syrup in mice [22]. Fructokinase, an enzyme predominantly expressed in the liver, phosphorylates fructose to produce fructose 1-phosphate. ATP is the phosphate donor in this reaction and is converted into ADP. While the reaction with fructokinase is rapid, it is not associated with negative feedback, which leads to a reduction in intracellular phosphate and ATP when fructose levels are high. A decrease in intracellular phosphate stimulates uric acid production. Emerging data support the notion that fructose metabolism elevates plasma uric acid levels in animals and humans [11]. The uric acid has a significant role in hypertension and CKD progression through stimulation of renin-angiotensin system, induction of oxidative stress and promoting interstitial macrophage and lymphocytes T infiltration [23]. In this way, GW501516 decreased plasma uric acid in the HFru+GW group, which could contribute to the improvement of renal physiology. This finding might be related to diminished hepatic fructokinase expression after PPAR-beta/delta activation in fructose-fed mice [22], which implies reduced plasmatic uric acid in these animals. Also, HFru diets are characterized to induce proteinuria in murine models [24] and GW501516, in this study, could improve this parameter in the HFru+GW group.

In the present study, we demonstrated that HFru diet can induce an increase in renin, ACE and AT1r protein and showed that GW501516 had no effects on gene and
protein expression of renin, ACE and AT1r, but still improved SBP in the HFru+GW group. Recently, we evidenced that GW501516 had no effects upon ACE/AT1r axis in the liver but improved hepatic injury independently of AT1r downregulation [13]. So, the same mechanism would be present in the kidney.

The AT1r activation leads to an intensification in intracellular inflammatory pathways [25]. In the current study, we confirmed that the HFru administration resulted in a proinflammatory state in the kidney through AT1r activation and the HFru+GW group displayed an improvement of the inflammatory state in kidney independently of AT1r downregulation. IκB-α acts as an inhibitor of NF-κB, keeping this transcription factor in the cytosol. When IκB-α is phosphorylated it is ubiquitinated and degraded leading to NF-κB translocation to nucleus which allows transcription of inflammatory target genes [26]. IκB-α protein expression was increased in the HFru+GW group, suggesting that NF-κB transcription factor activity was diminished. It could be confirmed through its target genes IL-6 and Mcp-1 which were downregulated with the administration of GW501516. Previously we showed that in white adipose tissue, GW501516 had the same effects [13] and another study demonstrate that GW501516 had anti-inflammatory effects in the tubulointerstitial proteinuric kidney [14]. IL-1β has a significant role in the development of renal inflammation.

When IL-1β is secreted by macrophages, it recruits others inflammatory cells, amplifying inflammation state [27]. In this study, we demonstrated that IL-1β gene expression was decreased in the kidney of HFru+GW group, confirming the anti-inflammatory effects of GW501516.

We also confirmed that HFru diet can lead to oxidative stress in the kidney. However, the administration of GW501516 had no relevant effects on the disbalance of antioxidant enzymes. SOD2 is the primary antioxidant enzyme that scavenges ROS and acts as the first line of defense against oxidative damage [28]. In the current study, we observed that an HFru diet administered for 11 weeks to animals was capable of increasing the protein but not the gene levels of the enzyme, indicating an initial unbalance among antioxidant enzymes and that GW501516 had no effects in the HFru+GW group. The balance between SOD2 and catalase activities in cells is crucial to determining the steady-state level ROS [29]. Additionally, it was found that catalase protein and gene expression were diminished in the HFru group, and GW501516 could improve this parameter only at the protein level. However, the catalase protein levels in the HFru+GW group was lower than the SC group. Furthermore, no differences were found in the cycle GPx-GR.

Therefore, we suggest that the administration of GW501516 had no effects on the disbalance in antioxidant enzymes in the kidney.

This study presents some limitations. Although we proposed a new insight about GW501516 and its anti-inflammatory effects on kidney in an HFru-fed mice model, it is necessary to verify possible differential actions of GW501516 on kidney’s cortex and medulla. Furthermore, cells that compose filtration barrier could be better investigated in future experiments using cell culture and GW501516 coupled with fructose administration, because data presented here suggest an association between GW501516 administration and improvement of urinary parameters related to filtration barrier.

We propose that GW501516 improves SBP in an HFru fed model though improvement of the inflammatory state in the kidney. An association between hypertension and inflammation has now been clearly demonstrated and, although currently anti-inflammatory drugs are not used to treat hypertension [30], we demonstrated that the anti-inflammatory effects of GW501516 upon the kidney improved the HFru-induced high SBP.

Authors Contributions

1. Conception and design of the study: DCM, IB, VSM
2. Acquisition of data and analysis and interpretation of data: DCM, IB
3. Drafting the article and revising it critically for important intellectual content: DCM, IB, VSM
4. Final approval of the submitted version: DCM, IB, VSM

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Competing interests

The authors declare that they have no competing interests.

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