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Mineralocorticoid Receptor Antagonism Prevents Obesity-Induced Cerebral Artery Remodeling and Reduces White Matter Injury in rats.

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Abstract

Midlife obesity is a risk factor for dementia development. Obesity has also been linked to hyperaldosteronism, and this can be modeled in rats by high fat (HF) feeding from weaning. Aldosterone, or activation of the mineralocorticoid receptor (MR) causes cerebrovascular injury in lean hypertensive rats. We hypothesized that rats fed a HF diet would show inward middle cerebral artery (MCA) remodeling that could be prevented by MR antagonism. We further proposed that the cerebral artery remodeling would be associated with white mater injury. Three-week-old male Sprague-Dawley rats were fed a HF diet ± the MR antagonist canrenoic acid (Canr) for 17 weeks. Control rats received normal chow (Control NC). MCA structure was assessed by pressure myography. The MCAs from HF fed rats had smaller lumens and thicker walls when compared to arteries from Control NC rats; Canr prevented the MCA remodeling associated with HF feeding. HF feeding increased the mRNA expression of markers of cell proliferation and vascular inflammation in cerebral arteries and Canr treatment prevented this. White mater injury was increased in the rats fed the HF diet and this was reduced by Canr treatment. The expression of doublecortin, a marker of new and immature neurons was reduced in HF fed rats, and MR antagonism normalized this. These data suggest that HF feeding leads to MR dependent remodeling of the MCA and this is associated with markers of dementia development.

Keywords

obesity; vascular remodeling; middle cerebral artery; mineralocorticoid receptor

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Introduction

The incidence of obesity is increasing at an alarming rate worldwide, particularly in developed countries. According to the Center for Diseases Control, in the United States of America 71% of the population is overweight and 38% are considered obese, and the prevalence of childhood obesity is approximately 17%. Importantly, childhood obesity increases the risk of developing cardiovascular diseases later in life [57]. Childhood obesity also impairs mental flexibility, attention, disinhibition and academic performance [33,34,39,78]. In adults, obesity causes up to a 5-fold increase in the risk of dementia development [2,4,10,19,24,36,63,68,74,75]. The association between overweight / obesity and dementia is strongest when midlife adiposity (40-55 years) is considered [10,19,24,36,63,74]. This association occurs independently of other vascular risk factors [9] including hypertension, diabetes, and stroke [18]. Cerebral blood flow is reduced in obese patients [1,5,66,76] and this could be the cause of the cognitive decline [29,36] because mild hypoperfusion impairs learning and memory by reducing protein synthesis and synaptic plasticity [27]. The reduction in blood flow in obese patients suggests that dementia development may have a vascular component in this population. Vascular cognitive impairment is the 2nd most common cause of dementia [14] and a key cause of early onset dementia [26]. Five percent of the population over 65 [62] suffers from vascular cognitive impairment that will eventually progress to dementia [43].

To study the links between weight gain and dementia we developed a rodent model that is analogous to an overweight adult who began overeating as a child. We have previously shown that this model of HF feeding results in an approximately three-fold increase in plasma aldosterone and in the aldosterone-to-renin ratio. These changes in hormone levels were associated with hypertrophy of the adrenal zona glomerulosa and an increase in the expression of aldosterone synthase [48]. Importantly, hyperaldosteronism is also observed in overweight / obese patients [7,16,22,23,37,40,47,50,61,71]. In a previous study we showed that HF feeding causes marked remodeling of the middle cerebral artery (MCA), the underlying mechanisms for this remodeling were not elucidated [11]. We hypothesized that MR activation contributes to the MCA inward hypertrophic remodeling in HF fed rats. We further proposed that this remodeling would be associated with white matter injury, a hallmark of dementia development. We tested this hypothesis by blocking the MR with canrenoic acid (Canr), the active metabolite of spironolactone [32].

Methods

Animals and treatment

Three-week-old male Sprague-Dawley rats were purchased from Harlan Laboratories (Indianapolis, IN, USA). Rats were housed two per cage and had free access to food and water throughout the duration of the experiment. Rats were randomized into three groups: one group received normal rat chow (control NC, Teklad, 4.4% fat; 2.5% saturated, 1.9% unsaturated; 0.3% sodium; and 0.9% potassium); a second group received a “Western style” high-fat chow (HF, 36% fat; 15.2% saturated, 20.8% unsaturated; 0.056% sodium; and 0.87% potassium; BioServe Biotechnologies, Frenchtown, NJ); and a third group were fed the HF chow and received the MR antagonist canrenoic acid (HF+Canr, 20mg/kg/day) in the

drinking water. Water consumption was monitored weekly, and the Canr dosage was adjusted based on the rat's body weight and water consumption. Rats were treated for 17 weeks as described previously [48]. At 20 weeks of age rats were euthanized after anesthesia with 3% isoflurane followed by exsanguination and decapitation. The experimental protocol was approved by the Michigan State University Institutional Animal Care & Use Committee and was in accordance with the "Guide for the Care and Use of Laboratory Animals" from the National Academy of Sciences, Institute for Laboratory Animal Research.

Blood pressure and plasma insulin measurement

Blood pressure was measured during the last week of treatment by tail-cuff using a RTBP1001 tail-cuff blood pressure system (Kent Scientific, Torrington, CT, USA) as described previously [53]. Fasting plasma insulin levels were measured using a commercially available kit (Cayman Chemicals, Ann Arbor, MI, USA) following the manufacturer's instructions.

Tissue harvesting

At euthanasia fasting blood samples were obtained by cardiac puncture. Kidneys, heart and the visceral adipose tissue were excised and weighed. The brain was removed and placed into ice-cold physiological salt solution (PSS, in mmol: 141.9 NaCl, 4.7 KCl, 1.12 KH_2PO_4, 1.7 $MgSO_4 \cdot 7H_2O$, 2.8 $CaCl_2$, 10 Hepes, 5 Dextrose, 0.5 EDTA, pH 7.4) for isolation of the MCA and other large cerebral arteries.

Pressure myography

Spontaneous myogenic tone generation, contractility to 5-hydroxytriptamine (5-HT), and the structural and mechanical properties of the MCA were assessed by pressure myography (Living Systems Instruments, Burlington, VT) as described previously [55]. MCAs were equilibrated at an intraluminal pressure of 80mmHg in 37°C PSS until development of spontaneous myogenic tone, which was calculated using the formula: %tone = [1−(active lumen diameter/passive lumen diameter)] × 100. MCA contractility to 5-HT was assessed by a cumulative concentration-response curve (1nmol/L to 100μmol/L). The MCA was incubated for 10 minutes at each concentration before measuring the outer and lumen diameter.

The MCA passive structure was assessed by placing the arteries in calcium-free PSS supplemented with 2mmol/L EGTA and 10μmol/L sodium nitroprusside. Under these conditions the intraluminal pressure was increased from 3 to 180mmHg in 20mmHg increments and the lumen and outer diameter and wall thickness was measured at each pressure after a 5-minute equilibration period. The wall-to-lumen ratio and circumferential wall stress were calculated [3]. The elastic modulus (β-coefficient) was calculated from the stress/strain curves using an exponential model ($y=ae^{\beta x}$) where β is the slope of the curve and is directly correlated to vascular stiffness.

Quantitative real-time polymerase chain reaction (qRT-PCR)

mRNA expression in brain tissue and cerebral arteries was measured by qRT-PCR. The MCAs, posterior and anterior communicating arteries, posterior and anterior cerebral

arteries and the basilar artery were collected and pooled, these will be referred to as cerebral arteries. RNA was extracted from cerebral arteries using a standard TRIZOL extraction procedure described previously [52]. A 2mm section from the midbrain of both cerebral hemispheres was used for RNA extraction using a Paris RNA extraction kit (Invitrogen, Carlsbad, CA, USA). In both cases mRNA was reverse-transcribed (Superscript VILO, Invitrogen, Carlsbad, CA, USA) and quantitative PCR was performed using Taqman ABI assays on demand probes in a 7500 Real Time PCR System (Applied Biosystem, Foster City, CA, USA). Fold changes from control were calculated using the $2^{-\Delta\Delta CT}$ method [41] with 2-β microglobulin used for normalization [53].

Assessment of white matter injury

20μm thick frozen brain sections were incubated in Sudan Black B solution for 10 minutes in the dark. Slides were washed in ethanol and distilled water to remove excess dye [35]. Sections were then mounted with ProLong Gold mounting media (Life Technologies, Carlsbad CA) and digital images were captured on an upright Axioscope 40 microsocope using an Axiocam MRc 5 color camera and the AxioVision 4.6.3 software (all from Zeiss, Thornwood, NY). White matter injury was assessed in the corpus callosum (CC) and external capsule (EC) using the following scale: grade 0 = no lesions; grade 1 = disarrangement of the nerve fibers; grade 2 = formation of marked vacuoles [38].

Chemical reagents

Unless otherwise stated, chemicals were purchased from Sigma Aldrich (Saint Louis, MO, USA).

Statistical analyses

Body and organ weights, blood pressure, and spontaneous myogenic tone generation data were analyzed by one-way ANOVA or a non-parametric alternative when the data did not fit a normal distribution model. Data for MCA constriction to 5-HT and passive structure were analyzed by two-way ANOVA or a non-parametric test. Tukey's correction for multiple comparisons was performed as a post-test. All statistical analyses were carried out using the GraphPad Prism 6.0 software (GraphPad, San Diego, CA, USA). Difference between means was considered statistically significant when $p < 0.05$.

RESULTS

Physiological variables

The data for the physiological variables measured are presented in Table 1. HF fed rats had higher body weights than Control NC. The body weights of the HF+Canr rats were not different from either Control NC or HF. Abdominal adiposity, assessed by comparing the abdominal fat: body weight ratio, was increased in both HF and HF+Canr compared to the Control NC rats. Interestingly, HF+Canr rats had less abdominal adipose tissue than rats fed the HF diet alone. Heart: body weight ratio was not different between groups. Systolic blood pressure was increased in rats fed a HF diet and Canr did not lower blood pressure. Fasting plasma insulin levels were not different among all experimental groups.

HF induces MCA remodeling that is prevented by MR antagonism

To test the hypothesis that HF feeding induces structural remodeling of cerebral arteries, isolated MCAs were cannulated in a pressure myograph and their structural and mechanical properties were assessed. The MCA outer diameter (Fig 1A) and outer cross-sectional area (Fig 1B) were unchanged by HF feeding, but the lumen diameter (Fig 1C) and lumen cross-sectional area (Fig 1D) were both reduced in the HF rats. Canr prevented the inward remodeling of the MCA. Wall hypertrophy was evident in MCAs from rats fed the HF diet, as evidenced by a significant increase in wall thickness (Fig 2A) and wall cross-sectional area (Fig 2B), and Canr prevented this hypertrophic response to HF feeding. As a consequence of the inward remodeling and wall hypertrophy, the wall-to-lumen ratio was higher in MCAs from rats fed HF alone when compared to rats treated with Control NC or HF+Canr (Fig 2C).

HF did not change mechanical properties of the MCA

The increases in the wall thickness in MCAs from HF fed rats resulted in these arteries having lower wall stress than arteries from Control NC or HF+Canr treated rats (Fig 3A). No differences in the MCA stiffness, as measured by the β-coefficient, were observed among the different treatment groups (Fig 3B and 3C, respectively).

HF increased contractility to 5-HT without altering spontaneous myogenic tone

To evaluate if HF feeding changed the contractile properties of the MCA, isolated arteries were cannulated in a pressure myograph and maintained at 80mmHg intraluminal pressure. There was no difference in spontaneous myogenic tone generation between the groups (Fig 4A). There was a significant increase in the MCA constriction to 5-HT in rats fed a HF diet when compared to control NC-fed rats (Fig 4B). The increased constriction was not reversed by Canr treatment. There was a trend towards a reduction in the logEC50 for 5-HT (Fig 4C). This suggests an increased sensitivity to 5-HT in MCAs from HF+Canr rats.

HF feeding increases the mRNA expression of vascular inflammatory and cell proliferation markers

We investigated the effects of HF ± Canr on markers of vascular inflammation, cell proliferation and apoptosis in cerebral arteries by qRT-PCR. HF alone caused a small but insignificant increase in tumor necrosis factor-α mRNA, which was reduced in the HF+Canr rats (Fold change: 1.04±0.09 vs 1.20±0.06 vs 0.97±0.06, Control NC vs HF vs HF+Canr, $p=0.09$). Intercellular adhesion molecule (ICAM)-1 mRNA expression was increased in arteries from HF fed rats compared to control NC and HF+Canr rats (Fig 5A). The mRNA expression of the macrophage marker CD68 followed a similar pattern (Fig 5B). The proliferation marker mKi-67 was elevated in arteries from HF fed rats, and Canr reduced mKi-67 mRNA expression to levels that were not difference from control (Fig 5C). No changes were observed in mRNA expression of BCl-2, an anti-apoptosis protein (Fig 5D). Matrix metalloproteinase (MMP) enzymes have been associated with artery remodeling [55]. We found that HF feeding had no effect on the mRNA expression of MMP-2 and MMP-9, but the mRNA expression of both enzymes was increased in the HF-CANR group (Fig 5E and F). HF feeding did not alter the mRNA expression of the MR in cerebral

arteries, but HF-CANR treatment increased the expression compared to control (in fold-change from control; 1.03±0.08, 1.09±0.09, 1.32±0.04, control-NC, HF, HF+CANR, ANOVA p=0.046, post-hoc analysis shows a significant difference between the control-NC and HF+CANR groups)

HF feeding increases white matter injury and reduces doublecortin mRNA expression

The cerebral cortex was thinner in the rats fed HF±Canr (in μm; Control NC: 1665±51, HF: 1430±38, HF+Canr: 1498±20, ANOVA $p<0.01$). White matter injury evidenced by demyelination was significantly greater in HF fed rats compared to Control NC in both CC and EC, and Canr treatment reduced but did not completely normalize the white matter damage (Fig 6A and B).

Doublecortin is expressed by new and immature neurons. The expression of this marker was reduced in the brains of rats fed the HF diet and this was corrected by Canr treatment (Fig 7A). Synaptophysin is expressed in neuronal synapses [8]. The mRNA expression of synaptophysin was not significantly reduced in the rats fed the HF diet, but Canr treatment significantly increased its expression above the level of the Control NC and HF rats (Fig 7B).

DISCUSSION

The main finding of the present study is that MR activation is required for inward hypertrophic remodeling of the MCA in an overweight model where the HF feeding begins in childhood and persists into adulthood. This MR-dependent remodeling was associated with increased vascular inflammation and cell proliferation in the artery wall. The inward hypertrophic artery remodeling was also associated with increased white matter injury in the HF fed rats and reduced doublecortin expression. These markers of injury were corrected by MR antagonism in the HF fed rats.

Clinical studies show a clear association between overweight / obesity and dementia development. These studies have focused primarily on the population that is currently aging / aged. The individuals in this group were children long before the onset of the obesity epidemic that began in the 1970s. The population that is currently middle-aged or younger contains a higher percentage of individuals who have been overweight since childhood. Our goal was to model this population and develop interventions to mitigate the effects of being overweight on brain function. We developed an overweight rat model by feeding a HF diet to newly weaned rats through adolescence until they are young adults. Using this model, we described the development of hypertension and hyperaldosteronism throughout the rat's lifespan, and showed the impact of life-long obesity in the mesenteric vasculature [48]. Hyperaldosteronism has been reported in overweight / obese humans and individuals with metabolic syndrome [7,16,22,23,37,40,47,50,61,69,71], thus MR antagonism may be a viable therapeutic option to reduce the risk of cerebral vascular diseases during obesity.

Hypertension is commonly associated with overweight / obesity [25], particularly with abdominal fat deposition [67]. In humans, childhood obesity increases the relative risk of developing hypertension during adulthood by approximately 2-fold [30]. Many rodent

models of overweight / obesity also show concomitant hypertension, including the obese Zucker rat [49], diet-induced obesity in Sprague-Dawley rats [6], and in the leptin-receptor deficient *db/db* mice [21]. In our model, we previously reported that blood pressure starts to rise 4 weeks after the HF treatment begins, even before a difference in body weight is observed [48]. In the present study, we observed a small but significant increase in systolic arterial pressure in rats fed a HF diet (approximately 15mmHg), and treatment with Canr did not prevent this. Interestingly, the Canr treated rats had a lower level of adiposity than the vehicle treated HF rats. This presents the possibility that in this model the level of adiposity is not directly correlated with blood pressure. This concept is in keeping with our finding that blood pressure increases in the HF fed rats before significant increases in body weight were detectable [48]. In humans, MR antagonists have mild antihypertensive effects, particularly at low doses, such as the one used in this study [72,73]. However, the benefits of MR antagonists go beyond their antihypertensive effects, and their cardiovascular protection is not fully explained by their ability to lower blood pressure [56,65]. The present study is in agreement with this observation, because Canr reduced the cerebral artery remodeling and the white matter injury in the HF fed rats without lowering blood pressure.

Few studies have described the effects of HF feeding or obesity on cerebral artery structure. In the obese Zucker rat, there is inward remodeling of the MCA when compared to the lean Zucker rat, but the authors did not provide data for wall thickness [49]. We showed previously that Sprague-Dawley rats fed a HF diet for 10 weeks also exhibit inward hypertrophic remodeling of the MCA [11]. In the current study the inward remodeling is entirely driven by the increase in wall area / thickness. Arterial remodeling is common in hypertension but it generally includes a reduction in both the lumen and outer diameter of the MCA [51]; however a reduction in lumen diameter without changes in outer diameter is unusual and suggests that the remodeling associated with HF feeding may not be entirely blood pressure dependent. It is important to note that in the aforementioned studies the mechanisms underlying the remodeling were not elucidated. The mechanisms of artery remodeling may also differ between the Zucker rats and our HF fed rats. The Zucker rats are morbidly obese, and their obesity is a consequence of hyperphagia, even if fed only standard rat chow, whereas the model described here reflects exposure to a diet rich in carbohydrates, modeling a different, and perhaps more relevant, population.

Rats fed a HF diet from 3 weeks of age have increased circulating levels of aldosterone [48], a mineralocorticoid hormone linked to remodeling of subcutaneous arteries in hypertensive humans [65] and MCA remodeling in models of essential hypertension [54,58,60]. Thus, it is possible that increased circulating levels of aldosterone lead to excessive MR activation during HF feeding, resulting in MCA remodeling. In agreement with this hypothesis, the present study shows that rats fed a HF diet and treated with the MR antagonist Canr do not show inward hypertrophic remodeling of the MCA. The mRNA expression of the MR was not different between the control-NC and HF rats suggesting that the negative effects of the HF diet were the result of increased circulating aldosterone and not elevated MR expression. The MR is expressed in vascular smooth muscle cells, endothelial cells and in perivascular macrophages. At present, we cannot definitively state which cell types are important for MCA remodeling in the HF rats. Our own recent studies show that the endothelial MR is vital for hypertension associated inward cerebral artery remodeling in both large pial arteries

and parenchymal arterioles [13]. Further studies are required to assess if this is also the case in obesity associated artery remodeling.

Aldosterone is widely recognized as a proinflammatory hormone [45,46]. Markers of vascular inflammation, namely ICAM-1 and CD-68, were elevated in cerebral vessels from HF rats, and normalized to control levels in HF+Canr, suggesting a role for MR activation in mediating vascular inflammation. CD-68 is a marker of macrophages, and we have shown that perivascular macrophages are actively involved in MCA remodeling in rats with essential hypertension [53]. Thus, it seems that MR activation is a key factor in remodeling of cerebral arteries during HF-feeding, likely by direct actions in the vascular wall and *via* inflammatory mediators.

HF feeding caused significant wall hypertrophy in the MCA, a process that was prevented by Canr treatment. MR activation by deoxycorticosterone acetate, without sodium loading, increased wall thickness of the MCA, despite a small increase in systolic arterial pressure [15]. As mentioned previously, it appears that hypertension alone cannot fully explain the wall hypertrophy observed in the HF fed rats. In this study, we observed an approximately 15μm increase in wall thickness, associated to a modest increase in arterial pressure (12mmHg). This same increase in wall thickness is observed in SHRSP when compared to normotensive Wistar-Kyoto rats, even though there is an almost 100mmHg difference in their blood pressures [55]. Thus, the increase in wall mass may be determined by circulating factors, such as aldosterone. Wall hypertrophy can be caused by proliferation of smooth muscle cells or deposition of extracellular matrix elements, such as fibrillar collagens. Our data suggests that, in this particular treatment paradigm, proliferation of smooth muscle cells may be the dominant factor. We observed an increase in mRNA levels of mKi-67 (a cell proliferation marker) in rats fed a HF diet, which was prevented by Canr treatment. Additional studies are required to verify that the increased Ki67 expression occurs in the smooth muscle cells and not in immune cells or in the endothelium. In agreement with our findings, MR activation by aldosterone was shown to induce proliferation of rat aortic smooth muscle cells *in vitro*, a process prevented by the MR antagonist eplerenone [28]. Further, we previously reported that Canr treatment prevented wall hypertrophy in mesenteric resistance arteries from rats fed a HF diet [48]. We did not observe an increase in MCA stiffness suggesting that there is no evident alteration in the extracellular matrix elements in the MCA wall, this is in keeping with the lack of a change in MMP mRNA expression between the HF and Control-NC rats.

Despite the increase in wall thickness we did not observe increased myogenic tone generation. This is in contrast to findings in obese Zucker rats where adult rats (14-16 weeks of age) show increased myogenic tone [49]. Interestingly the 5-HT mediated constriction was elevated in the HF fed rats, this could not be improved by Canr. In fact, there was a trend towards an increase in the sensitivity of the MCA to 5-HT in the Canr treated rats. Several studies suggest that aldosterone itself has vasoactive properties that result in either contraction or dilation depending on the duration of the aldosterone treatment and the status of the vascular endothelium [42]. When we assessed the 5-HT mediated contraction the arteries had been in aldosterone free buffer for at least an hour, therefore, it is unlikely that direct rapid / non-genomic effects of aldosterone on the vasculature are responsible for the

increased contraction observed here. However, we cannot rule this possibility out completely. Further studies are required to assess the importance of these findings to the regulation of cerebral blood flow in the overweight rats.

We used a combination of techniques to assess the possible impact of the observed artery remodeling on cerebral homeostasis and function. Recent studies in spontaneously hypertensive rats have shown that demyelination is associated with cerebral hypoperfusion and dementia development [31]. As hypothesized, we observed increased demyelination in the rats fed the HF diet in both brain regions studied. This brain injury was associated with a reduction in the expression of DC, a marker of new and immature neurons. Reduced DC expression indicates impaired neurogenesis and DC expression is reduced in other models of dementia [12]. Demyelination was reduced by MR antagonism and the expression of DC was increased. This finding suggests that prevention of cerebral artery remodeling may improve cerebral perfusion potentially reducing white matter damage and development of dementia. The HF diet alone did not affect synaptophysin mRNA expression but MR antagonism increased the expression. Synaptophysin regulates synapse formation [70], and increased expression may be indicative of improved synaptic formation or plasticity. Synapse loss has been observed in several forms of dementia [8]. The hypothesis that MR antagonists can prevent dementia development is not without precedent. A study using wildtype and type 2 diabetic mice showed that spironolactone improved special memory assessed by the Morris water maze [64]. Conversely, studies in hypertensive patients show that elevated aldosterone levels are a risk factor for dementia development [77]. Although we propose that cerebral hypoperfussion is the causative factor in obesity associated cognitive decline it is important to recognize that other mechanisms are possible. The MR is expressed in macrophages, and MR antagonism and myeloid cell specific MR knockout alters the polarity of these cells to produce an anti-inflammatory / wound healing phenotype [20]. Recent studies suggest that the switch to a wound healing phenotype induced by knocking the MR out in myeloid cells reduces the clinical symptoms and neuroinflammation in a mouse model of multiple sclerosis, a demyelinating condition [44]. In keeping with the idea that macrophages may be important in obesity associated neurovascular diseases, a recent study showed that low fat feeding with caloric restriction reduced the expression of phagocytic markers and inflammatory microglial activation [79]. Our results suggest there could be fewer macrophages associated with the cerebral arteries in the rats treated with Canr. This could be an important determinant of artery remodeling, our own previous studies have shown that macrophage depletion improved cerebral artery structure in a rat model of essential hypertension [53]. Recent studies form Faraco *et al* have linked cerebral perivascular macrophages to cognitive decline in mouse models of hypertension [17]. Additional studies will be required to assess the role of macrophages in the cerebral artery remodeling and the associated changes in myelination and neuron and synapse formation.

There are several limitations of the current study that should be addressed. Firstly we studied only male rats, it is not clear at present that artery remodeling occurs in females rats fed the same high fat diet, we have however previously shown that MR antagonists do not affect hypertensive artery remodeling in female rats [59]. A second limitation is that we did not fully characterize the impact of HF-induced cerebral artery remodeling, and prevention by MR antagonism, on cerebral perfusion and function. Future studies assessing cognitive

function and *in vivo* localized hemodynamic regulation are warranted to understand the consequences of chronic intake of HF food on cerebral blood flow and neurovascular coupling.

In summary, feeding rats a HF diet from 3 weeks of age until early adulthood induces inward hypertrophic remodeling of the MCA, and increases expression of markers of smooth muscle cell proliferation and vascular inflammation. The artery remodeling was associated with neuronal demyelination and a reduction in neurogenesis. All these effects of HF diet were prevented by MR antagonism, thus presenting the possibility that MR antagonists may be an important therapeutic intervention of obese patients at risk of dementia development.

Perspective

Childhood obesity increases cardiovascular risk in adults, through mechanisms that are poorly understood. Obesity is a risk factor for cerebral vascular diseases, such as ischemic strokes and dementia, and the risk increases linearly to increases in body mass. This increased risk could be linked to the hyperaldosteronism observed in obese patients. The present study suggests that MR antagonists, such as spironolactone and eplerenone, are good candidates as therapies in obese patients at risk of dementia development.

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List of Abbreviations

5-HT	5-hydroxytriptamine
%HI	percent hemisphere infarcted
Canr	canrenoic acid
CC	corpus calosum
EC	external capsule
EDTA	ethylene diamine tetraacetic acid
EGTA	ethylene glycol tetraacetic acid
HF	high-fat
ICAM-1	intercellular adhesion molecule-1
MCA	middle cerebral artery
MCAO	middle cerebral artery occlusion

MR	mineralocorticoid receptor
NC	normal rat chow
qRT-PCR	quantitative real-time polymerase chain reaction

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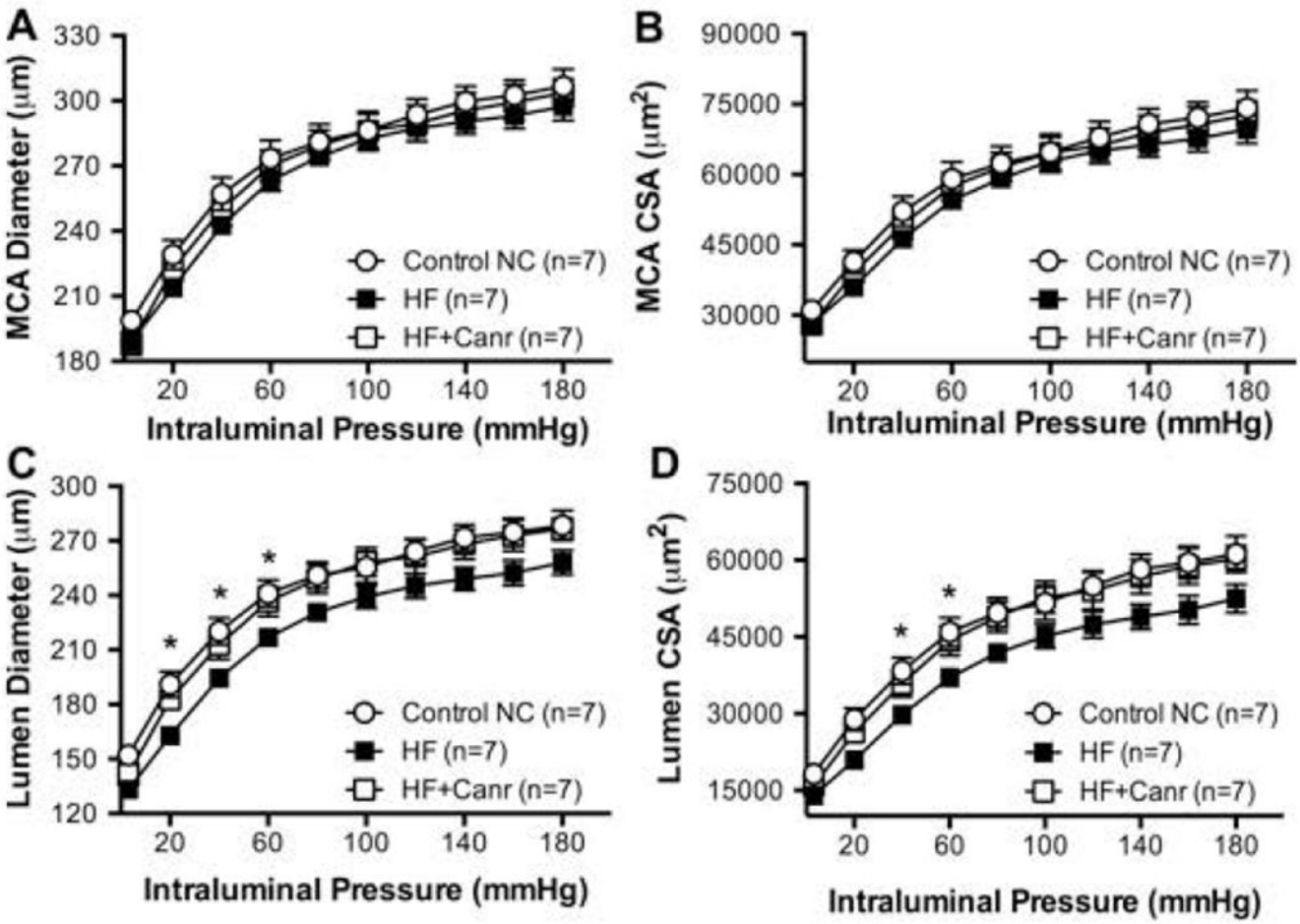

Figure 1.

HF treatment caused inward remodeling of the MCA in rats. Chronic HF treatment did not change MCA outer diameter (A) and the MCA cross-sectional area (CSA, B). There was a small reduction in the MCA lumen diameter (C) and lumen CSA (D), particularly at lower intraluminal pressures. MCAs were bathed in warm (37°C), oxygenated (95% O_2) Ca^{2+}-free PSS supplemented with 2mmol/L EGTA and 10μmol/L SNP. MCAs were allowed to equilibrate for 5 minutes at each intraluminal pressure before measurement was performed. *$p<0.05$, statistically different from Control NC and HF+Canr, two-way ANOVA with a Tukey's post-test for multiple comparisons.

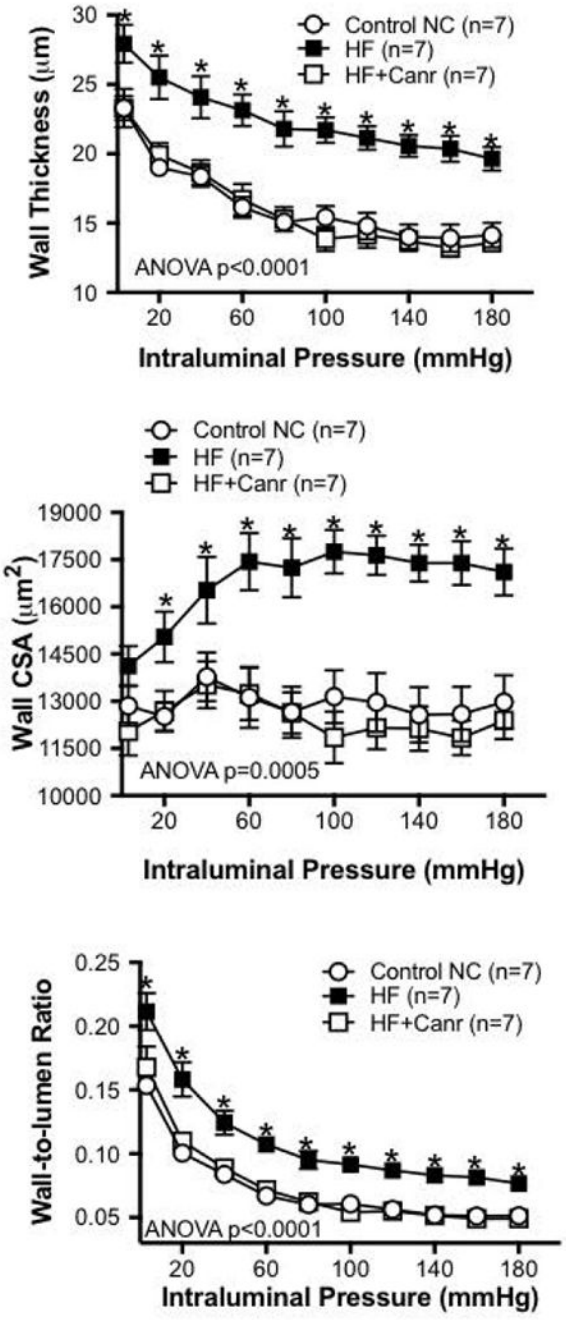

Figure 2.

MR is involved in the hypertrophic growth of the MCA wall in obese rats. Chronic HF treatment caused a dramatic hypertrophy of the MCA wall, observed as an increase in the wall thickness (A) and the wall CSA (B). The wall hypertrophy was prevented in HF rats that received the MR antagonist Canr. As consequence of the increase in wall thickness, the wall-to-lumen ratio of MCAs from rats fed HF alone was higher than rats fed Control NC or HF+Canr (C), suggesting an inward hypertrophic remodeling. MCAs were bathed in warm (37°C), oxygenated (95% O_2) Ca^{2+}-free PSS supplemented with 2mmol/L EGTA and

10μmol/L SNP. MCAs were allowed to equilibrate for 5 minutes at each intraluminal pressure before measurement was performed. *$p<0.001$, statistically different from Control NC; $^{\alpha}p<0.001$, statistically different from HF+Canr, two-way ANOVA with a Tukey's post-test for multiple comparisons.

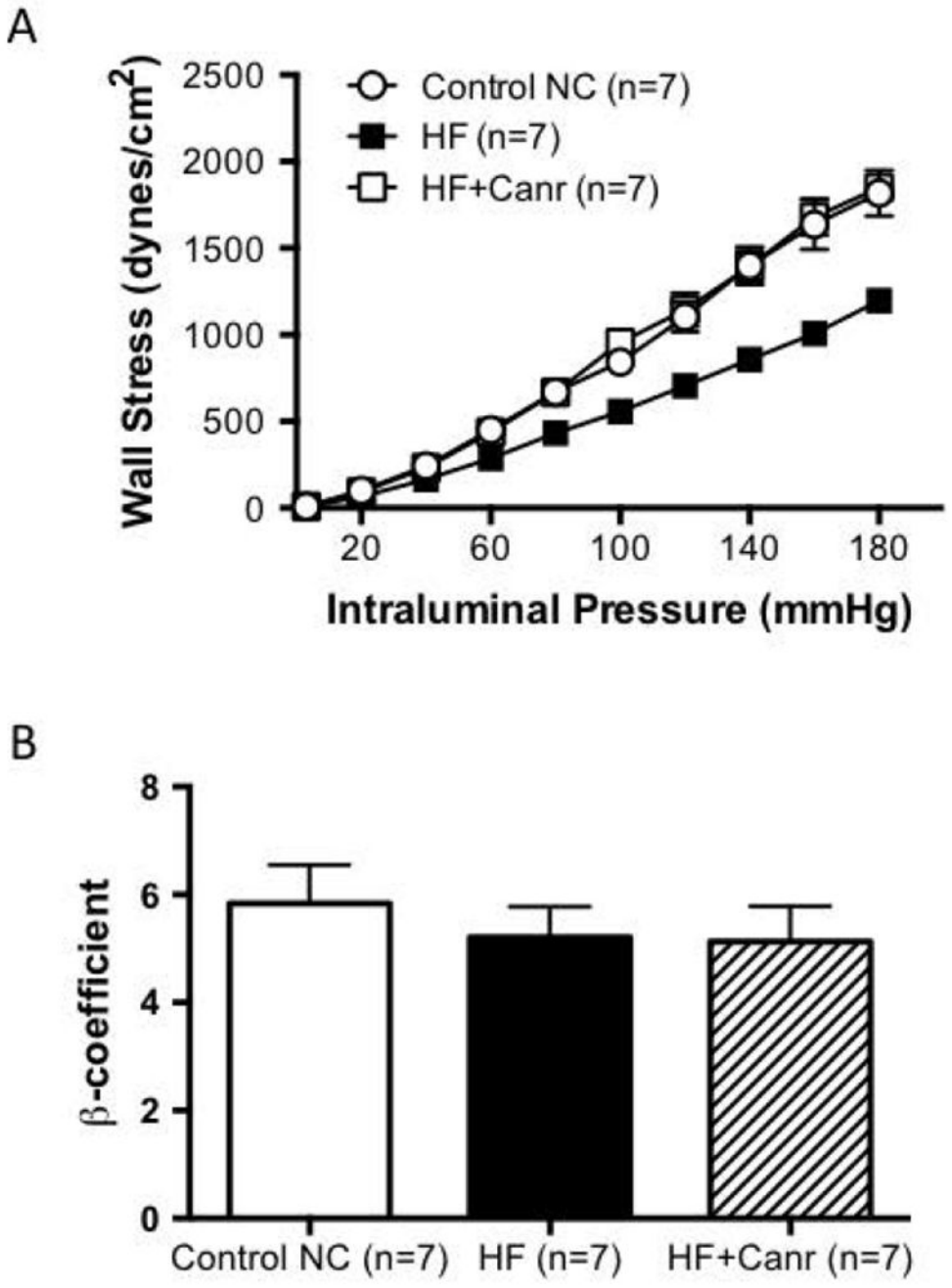

Figure 3.

MR antagonism did not change mechanical properties of the MCA. There was a reduction in wall stress in MCAs from rats fed a HF diet alone when compared to rats fed Control NC or HF+Canr, possibly a consequence of the increase in wall thickness (A). The stiffness (B) of the MCA were unchanged by treatments. MCAs were bathed in warm (37°C), oxygenated (95% O_2) Ca^{2+}-free PSS supplemented with 2mmol/L EGTA and 10μmol/L SNP. MCAs were allowed to equilibrate for 5 minutes at each intraluminal pressure before measurement was performed. *p<0.001, statistically different from Control NC; $^{\alpha}$p<0.001, statistically

different from HF+Canr, two-way ANOVA with a Tukey's post-test for multiple comparisons.

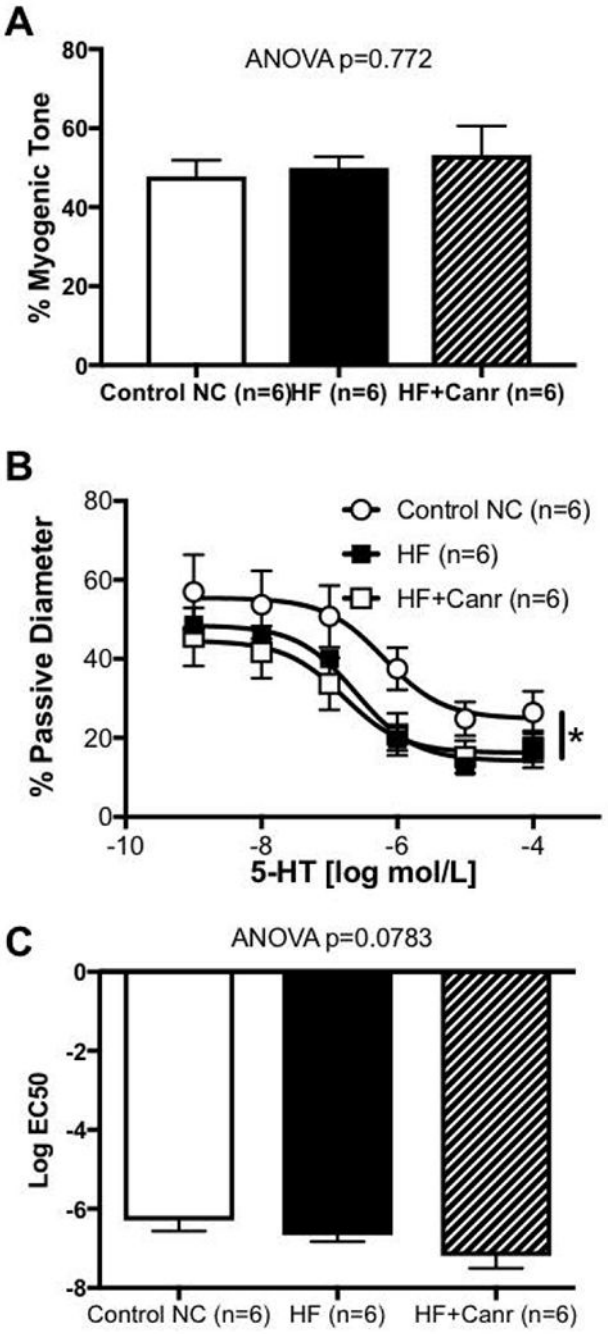

Figure 4.

MR antagonism increased constriction to 5-hydroxytriptamine (5-HT) in MCAs from obese rats. Obesity did not change spontaneous myogenic tone generation in MCAs (A). Rats fed HF+Canr showed enhanced constriction to 5-HT then rats fed Control NC or HF alone (B). HF feeding had no effect on the log EC50 for 5-HT (C). MCAs were bathed in warm (37°C), oxygenated (95% O_2) PSS and allowed to equilibrate for 10 minutes at each 5-HT concentration before measurement was performed. *p<0.001, statistically different from

Control NC and HF alone, two-way ANOVA with a Tukey's post-test for multiple comparisons.

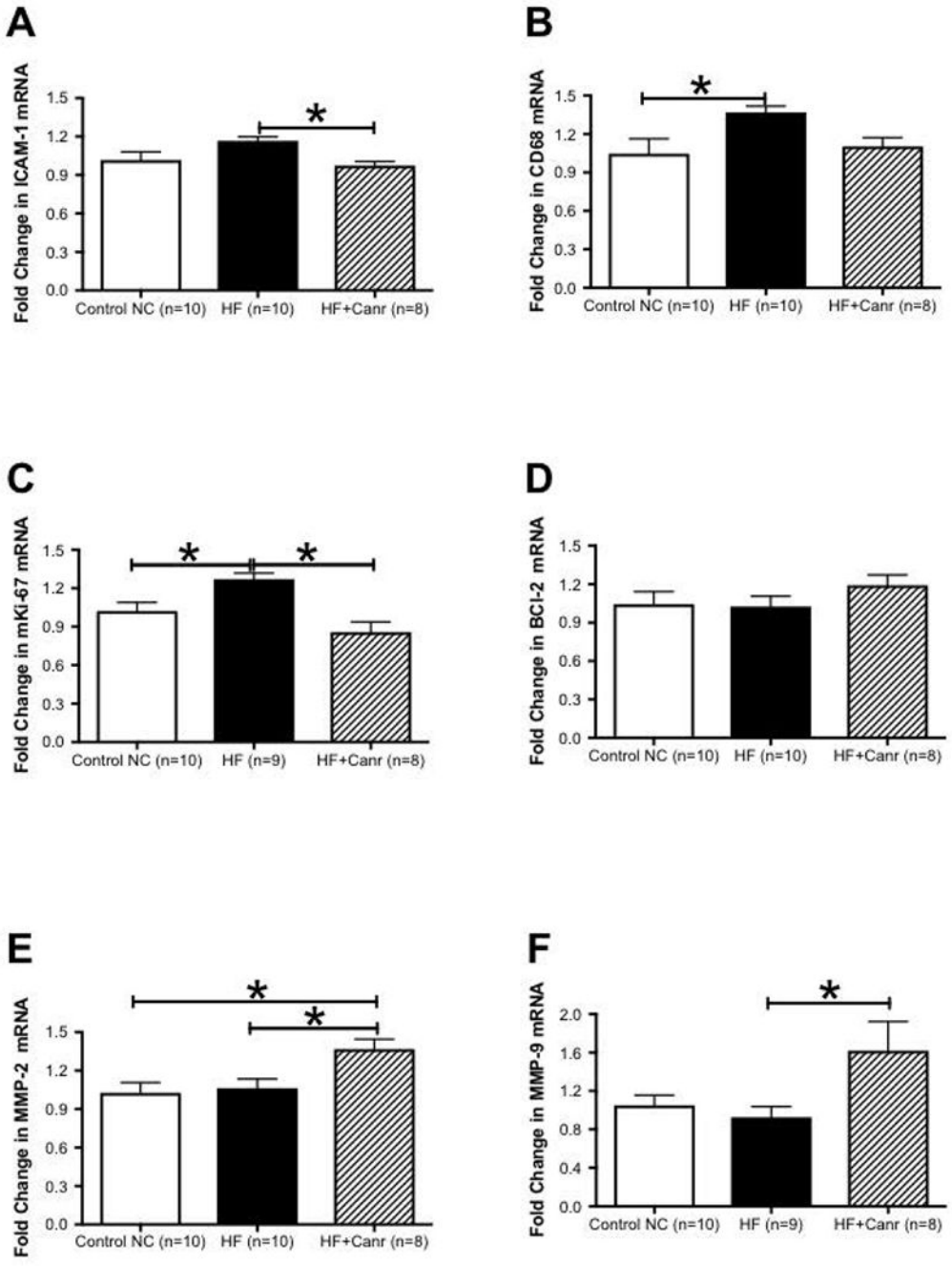

Figure 5.

Chronic HF treatment increased mRNA for markers of vascular inflammation and proliferation in intracranial cerebral arteries, in a MR-dependent manner. mRNA expression of the inflammatory markers ICAM (intercellular adhesion molecule)-1 and CD68 (expressed by phagocytes) were increased in rats fed HF, and MR antagonism with Canr (HF +Canr) prevented their increase (A and B, respectively). Similarly, mRNA for the proliferation marker mKi-67 was increased in cerebral arteries from rats fed a HF diet, which was prevented by MR antagonism (C). No changes were observed in the apoptosis

marker Bcl-2 (D). HF feeding did not increase the mRNA expression of MMP-2 but a significant increase was observed in the HF-CANR group (E). A significant increase in MMP-9 mRNA expression was also observed in the HF-CANR group (F). mRNA expression was assessed by qRT-PCR using the $2^{-\Delta\Delta CT}$ method, 2-microglobulin was used for normalization of expression data. *$p<0.05$, one-way ANOVA with a Tukey's post-test for multiple comparisons * indicate groups with significant differences in the post-test.

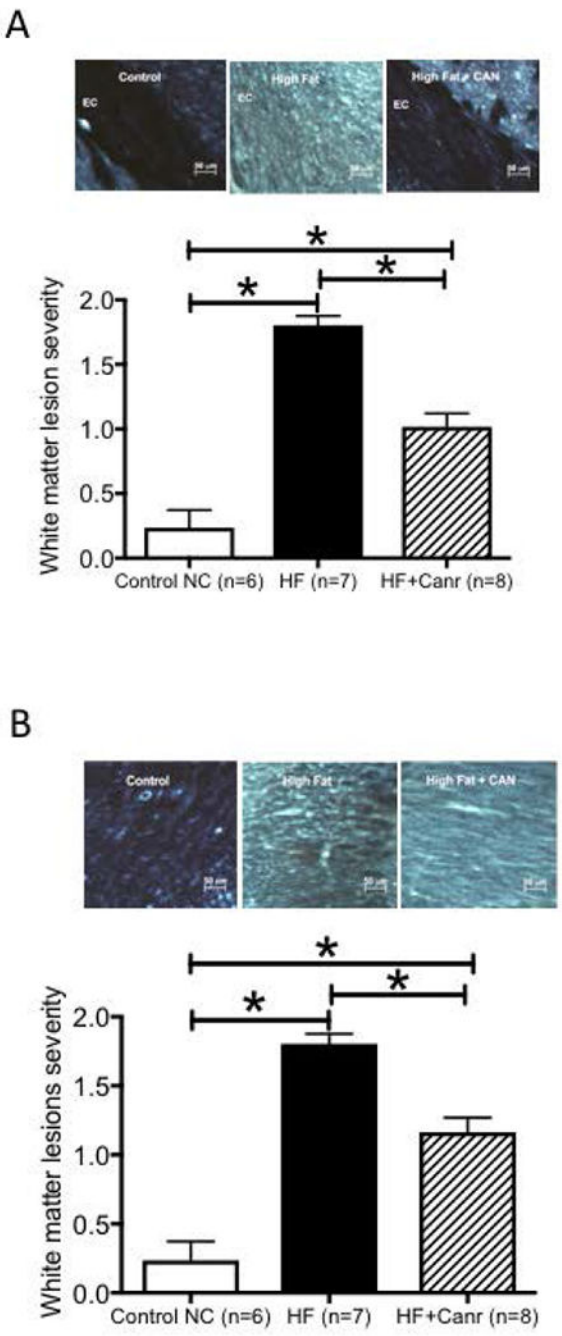

Figure 6.

Chronic HF feeding resulted in neuronal demyelination and this effect was prevented by MR antagonism. Demyelination was assessed using Sudan black staining in the external capsule (EC, panel A) and the corpus calosum (CC, panel B). *p<0.05, one-way ANOVA with a Tukey's correction for multiple comparisons.

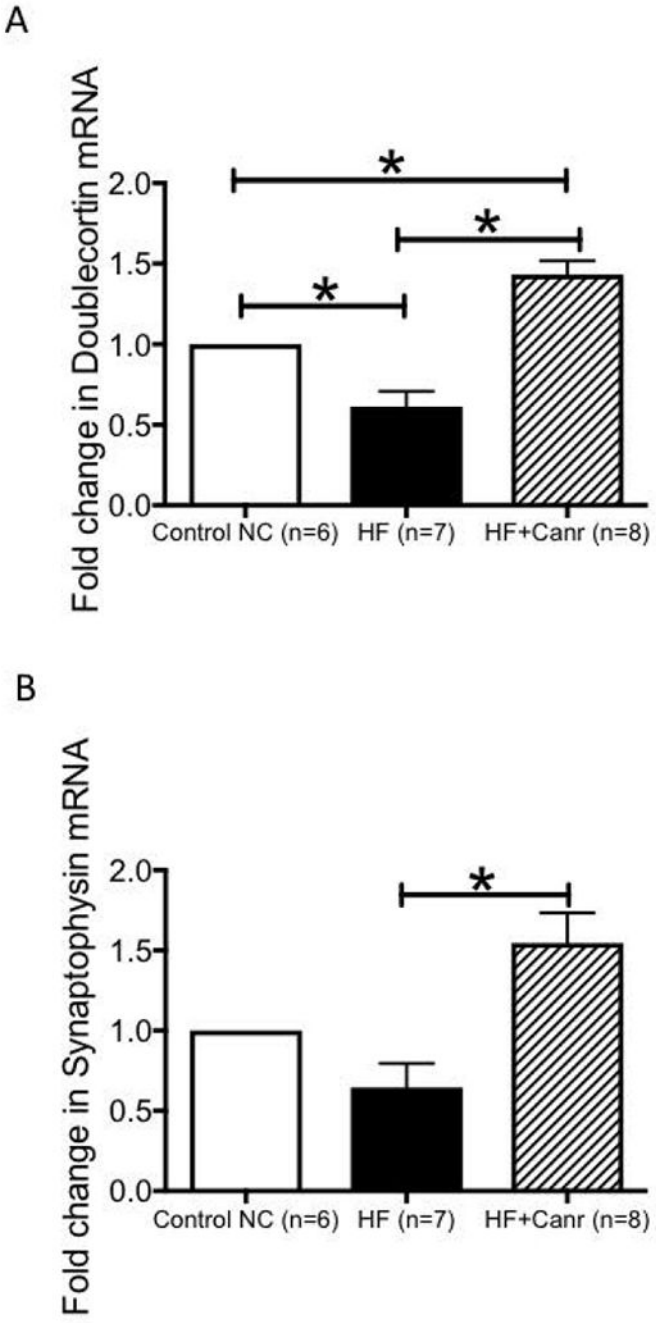

Figure 7.

Chronic HF feeding reduced doublecortin mRNA expression, and both doublecortin and synaptophysin mRNA is expression was increased by MR antagonism in HF rats. Doublecortin (A) and synaptophysin (B) mRNA expression was assessed by qRT-PCR. *p<0.05, one-way ANOVA with a Tukey's correction for multiple comparisons.

Table 1.

Physiological variables from rats fed control NC, HF alone and HF+Canr.

	Control NC	HF	HF+Canr
Body weight (g)	438±12	504±12*	467±11
Heart: bw	0.32±0.008	0.30±0.008	0.30±0.005
Kidneys: bw	0.63±0.015	0.51±0.015*	0.56±0.012
Abdominal fat: bw	1.08±0.11	2.88±0.11*	2.17±0.15
Insulin (ng/mL)	0.951±0.159	1.157±0.257	1.614±0.284
SBP	150±2	162±3*	165±4*
DBP	112±3	121±3*	123±4*

Data are means±SEM, n=8 for each experimental group. Bw = body weight; SBP = systolic blood pressure; DBP = diastolic blood pressure.

* significantly different from Control NC, $p<0.05$, one-way ANOVA with Tukey's correction for multiple comparisons.