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ORIGINAL ARTICLE

High Fat Diet Exacerbates Neuroinflammation in an Animal Model of Multiple Sclerosis by Activation of the Renin Angiotensin System

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Abstract Epidemiological studies suggest a positive correlation between the incidence and severity of multiple sclerosis (MS) and the intake of fatty acids. It remains to be clarified whether high fat diet (HFD) indeed can exacerbate the disease pathology associated with MS and what the underlying mechanisms are. In this study, we determined the influence of HFD on the severity and pathology of experimental autoimmune encephalomyelitis (EAE), an animal model of MS. Mice were fed either normal diet (ND) or HFD and subsequently induced with EAE. Immunohistochemical staining and real-time PCR were used to determine immune cell infiltration and inflammatory mediators in the central nervous system (CNS). Our data show that HFD increases immune cell infiltration and inflammatory mediator production in the CNS and thereby aggravates EAE. Moreover, our data demonstrate that activation of the renin angiotensin system (RAS) is associated with the HFD-mediated effects on EAE severity. These results show that HFD exacerbates an autoreactive immune response within the CNS. This indicates that diets containing excess fat have a significant influence on neuroinflammation in EAE, which may have important implications for the treatment and prevention of neuroinflammatory disorders.

Keywords Experimental autoimmune encephalomyelitis · Renin angiotensin system · Inflammation · High fat diet

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Introduction

Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), characterized by demyelination, focal T cell and macrophage infiltration, axonal injury and loss of neurological function (Hellings et al. 2002). MS develops in individuals that are genetically susceptible and requires additional exposure to environmental factors (Bar-Or et al. 1999; Hellings et al. 2002; Aranami and Yamamura 2008).

Nutrition has been suggested to play a role in the initiation and pathogenesis of MS and its animal model experimental autoimmune encephalomyelitis (EAE). For instance, a long term large consumption of saturated animal fat is associated with an increased frequency of MS and a more severe EAE course (Swank et al. 1952; Wright et al. 1965; Thompson 1966; Alter et al. 1974; Lauer 1997; Zhang et al. 2000; Schwarz and Leweling 2005; Thompson 2008; Winer et al. 2009). Similarly, a large number of other dietary compounds like sweets, alcohol, coffee and tea is associated with MS incidence (Antonovsky et al. 1965; Berr et al. 1989; Sepcic

et al. 1993; Tola et al. 1994). In contrast, caloric restriction and dietary molecules such as polyphenols, carotenoids, vitamin D and niacin are able to counteract inflammation in MS and improve disease symptoms (Munger et al. 2004; Rao and Rao 2007; Piccio et al. 2008; Smolders et al. 2008; Mehta et al. 2009). Interestingly, disturbances in cholesterol metabolism have also been linked to enhanced progression of MS and EAE (Diestel et al. 2003; Teunissen et al. 2003; Mueller et al. 2008; Wheeler et al. 2008).

High fat diet (HFD) has been reported to impact CNS homeostasis and to induce a CNS inflammatory response. For example, HFD induces brain inflammation and oxidative stress and decreases the expression of brain-derived neurotrophic growth factor (BDNF), thereby decreasing neuronal plasticity, cognitive function and learning (Wu et al. 2003; Zhang et al. 2005; Milanski et al. 2009; White et al. 2009; Morrison et al. 2010; Pistell et al. 2010; Uranga et al. 2010). In addition, obese mice show a decreased amount of myelin and marked changes in the composition of myelin, indicating that myelination in the CNS is affected by dietary compounds (Sena et al. 1985; Bruce-Keller et al. 2009; Winer et al. 2009). Thus HFD may exacerbate an ongoing neuroinflammatory response in diseases such as MS. However, the underlying mechanisms involved in HFD mediated effects on neuroinflammation are largely unidentified.

Interestingly, HFD is known to induce elevated oxysterol levels that are reported to activate the brain renin angiotensin system (RAS) (Mateos et al. 2011), making the RAS a candidate mechanism for diet mediated effects on the neuroimmune system. In MS and EAE the RAS plays a crucial disease promoting role (Stegbauer et al. 2009). RAS is a major endocrine system playing an important role in the regulation of blood pressure and body fluid homeostasis (Lanz et al. 2010). It is well established that the CNS contains its own RAS which has important biological and neurological functions, like the processing of sensory information and the regulation of emotional responses, learning and memory (von Bohlen und Halbach and Albrecht 2006; Grobe et al. 2008; Mateos et al. 2011). In addition to its role in neurological processes, the RAS has been implicated in inflammatory responses, such as the regulation of immune cell infiltration, the modulation of antigen presenting cells, the suppression of regulatory T cells (Tregs) and the induction of T helper (TH) 1 and TH17 cells (Suzuki et al. 2003; Platten et al. 2009). Furthermore, the RAS is upregulated in the spinal cord of EAE mice and in brain lesions of MS patients and blocking the RAS ameliorates EAE (Platten et al. 2009; Stegbauer et al. 2009).

The goal of our study is to determine the influence of HFD on EAE severity and pathology and to elucidate the involvement of the RAS. Here we show that HFD increases immune cell infiltration, oxidative stress and pro-inflammatory cytokines in the spinal cord, thereby exacerbating EAE. Moreover, this was paralleled by an increase in peripheral inflammation and an upregulation of the RAS, indicating that the RAS is responsible for the HFD-mediated effects on EAE severity.

Material and methods

Mice and diets

Female C57BL/6J OlaHSD mice were purchased from Harlan (Horst, The Netherlands) at 8 weeks of age and acclimated to our facility for 2 weeks. Animals were housed under specific pathogen-free conditions in an accredited animal facility. Mice were randomized according to weight and at 10 weeks of age, mice were fed either normal rodent chow (ND; Harlan) ($N=10$) or a Western-type diet ($N=10$), containing 21 % fat and 0.004 % cholesterol (HFD; Harlan), starting 2 weeks before EAE induction until the end of the experiment. Animals had ad libitum access to food and water throughout the study. All animal procedures were in accordance with the EU Directive 2010/63/EU and were approved by the Hasselt University ethics committee for animal experiments.

EAE induction

Mice were immunized subcutaneously with 200 μg myelin oligodendrocyte glycoprotein (MOG_{35-55}) emulsified in 100 μl complete Freund's adjuvant (CFA) containing 4 mg/ml Mycobacterium tuberculosis (H37RA strain) according to manufacturer's guidelines (Hooke Laboratories, Lawrence, USA). Within 2 h and after 22–26 h, mice were intraperitoneally injected with 0.1 ml pertussis toxin. Mice were weighed and evaluated for neurological signs of the disease on a daily basis. EAE was scored according to a scale: 0= no clinical symptoms, 1=limp tail, 2=limp tail and weakness of hind legs, 3=limp tail and complete paralysis of hind legs, 4= limp tail, complete hind leg and partial front leg paralysis, 5= tetra paralysis or mouse is dead. Mice were sacrificed at day 27 post immunization.

Captopril treatment

Both EAE mice on ND and HFD were treated with captopril or left on normal drinking water. Captopril (Sigma, Bornem, Belgium) was dissolved in drinking water at a concentration of 0.2 mg/ml and replaced every 24 h. The daily dosage was estimated at 30 mg/kg assuming a daily fluid intake of 5 ml (Bachmanov et al. 2002). Captopril treatment started 2 weeks before EAE induction and lasted until the end of the experiment.

Immunohistochemistry

Mice were perfused with ringer solution, containing 8.6 g NaCl, 0.3 g KCl, 0.33 g $CaCl_2$, 0.17 g $NaHCO_3$, 2.5 g

$NaNO_2$, heparin and 10 mg phenolred. Next, mice brain and spinal cord tissue were isolated. Half of each tissue was frozen in tissuetek at −80 °C. Spinal cord tissue, of which sections of 5 μm were sliced with the cryostate, was used for immunohistochemistry. 6 sections per mice were stained for each mouse and 5 mice per group were used. Primary antibodies used were rat anti-mouse F4/80 (1:100; AbD Serotec, Düsseldorf, Germany) and rat anti-mouse CD3 (1:150; AbD Serotec). As a secondary antibody, goat anti-rat Alexa Fluor®555 (1:400; Life technologies, Merelbeke, Belgium) was used. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; 1:2500; Sigma). Stained sections were visualized using the Nikon Eclipse 80i fluorescence microscope (Nikon, Kingston, UK). Immune cells were quantified using NIS Elements BR4 software (Nikon).

RNA extraction and real-time quantitative PCR (RT-PCR)

Mice were perfused with ringer solution, containing 8.6 g NaCl, 0.3 g KCl, 0.33 g $CaCl_2$, 0.17 g $NaHCO_3$, 2.5 g $NaNO_2$, heparin and 10 mg phenolred. Next, mice peripheral blood mononuclear cells (PBMCs), brain and spinal cord tissue was isolated. Half of each tissue was snap frozen in liquid nitrogen and subsequently stored at −80 °C preceding RT-PCR analysis. Total RNA was prepared using the RNeasy lipid tissue kit (Qiagen, Venlo, The Netherlands), according to manufacturer's instructions. The RNA concentration and quality were determined with a NanoDrop spectrophotometer (Isogen Life Science, De Meern, The Netherlands). RNA was converted to cDNA using the reverse transcription system (Promega, Leiden, The Netherlands). The cDNA synthesis mix contained RNA supplemented with $MgCl_2$ (25 mM), RTase buffer (10×), dNTP mixture (10 mM), RNasin (20–40 U/Tl; 2500U), AMV RTase (20 U/Tl) and Oligo(dt) primer. The reverse transcription reaction was performed at 42 °C for 60 min, 95 °C for 5 min and 4 °C for 5 min, using the iCYCLER (biorad, Nazareth-Eke, Belgium).

RT-PCR was conducted on a steponeplus detection system (Applied biosystems, California, USA). Universal cycling conditions were used: 10 min at 95 °C, 40 cycles of 15 s at 95 °C and 60s at 60 °C. The reaction mixture contained SYBR green master mix (Applied biosystems), 10 μM forward and reverse primers, RNase free water and 5 ng/μl cDNA template in a total reaction volume of 10 μl. Normalization of RT-PCR data was done by determination of the most stable reference genes, as previously described (Vandesompele et al. 2002). GeNorm software determined hydroxymethylbilane synthase (HMBS) and beta actin (ACTB) as the most stable reference genes in CNS tissue and cyclophilin A (CYCA) and TATA box binding protein (TBP) in peripheral blood mononuclear cells (PBMCs). Relative quantitation of gene expression was accomplished by using the comparative Ct method. Primers used for RT-PCR are shown in supplementary table 1.

Determination of cholesterol and oxysterol levels

Mouse whole brain and plasma samples were spun in a speed vacuum dryer (12 mbar) (Savant AES 1000) for 24 h to relate individual sterol concentrations to their dry weight. The sterols were extracted from the dried tissue by placing them in a 2 ml mixture of chloroform-methanol (2:1) for 24 h at 4 °C. Gas chromatography/mass spectrometry (GC/MS) was used to determine cholesterol and oxysterol levels, as described previously (Ledesma et al. 2003; Grimm et al. 2005).

Statistical analysis

Results were statistically analyzed with Graph Pad Instant version 5.01. To test for normality, d'Agostino and Pearson omnibus normality test was used. Normally distributed data sets were analyzed by means of ANOVA (3 or more groups) or two-tailed unpaired student *T*-test (2 groups). The Kruskal-Wallis (3 or more groups) or Mann–Whitney analysis (2 groups) was used for non parametric data sets. $*P<0,05$; $**P<0,01$ and $***P<0,001$.

Results

HFD exacerbates EAE

To determine the impact of HFD on the pathogenesis of EAE, mice were fed either ND or HFD and immunized with MOG two weeks afterwards. Mice had comparable weights prior to the start of HFD (data not shown). HFD led to a significant increase in body weight of EAE mice compared to EAE mice on ND (mean (ND): 19.1 +/− 0.22 g vs mean (HFD): 21.4 +/− 0.33 g $P<0.05$). In both groups, weights dropped immediately prior to clinical onset. However, weights of mice on HFD remained higher throughout the experiment (Fig. 1a). HFD significantly exacerbated EAE disease severity compared to mice on ND, while disease onset was not significantly affected. The mean disease score, starting from disease onset, was 1.97 +/− 0.11 for the ND mice compared to 2.59 +/− 0.17 for the HFD mice (Fig. 1b) (area under the curve (ND): 34.64 +/− 1.2 vs area under the curve (HFD): 50.1 +/− 1.7; $P<0.05$).

HFD increases immune cell infiltration and inflammation in the CNS

EAE is featured by the infiltration of peripheral immune cells into the CNS leading to a local inflammatory response. To elucidate whether this process is modulated by HFD, the accumulation of macrophages (F4/80) and T cells (CD3) of EAE mice on ND and HFD was assessed by immunohistochemistry (at 27 days post immunization). EAE mice fed with

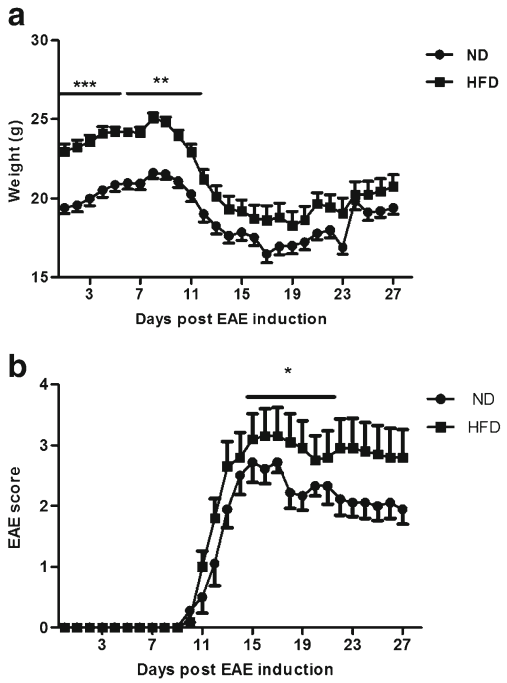

Fig. 1 HFD increases EAE disease severity. Weight (**a**) and clinical score (**b**) of WT mice on ND ($N=10$) and HFD ($N=10$) after induction of EAE. Data are expressed as mean ± standard error of the mean. Data of two representative experiments are shown

HFD showed significantly more T cell and macrophage infiltration in their spinal cord compared to ND mice (Fig. 2a–f) (ND: 122 +/− 18.51 cells/mm^2 vs HFD: 679 +/− 57.09 cells/mm^2 and ND: 107 +/− 11.48 cells/mm^2 vs HFD: 1143 +/− 251.42 cells/mm^2 respectively $P<0.01$). This indicates that HFD boosts neuroinflammation by increasing immune cell infiltration into the CNS.

To determine whether the increase in immune cells in the CNS after HFD coincides with an increased expression of pro-inflammatory mediators, RT-PCR was performed. EAE mice on HFD exhibit a significantly increased expression of inflammatory cytokines IL1β, IL6 and interferon gamma (IFNγ) in their spinal cord (Fig. 3). In parallel, there was a 4-fold increased expression of inducible nitric oxide synthase (iNOS) in the spinal cord of HFD mice compared to ND mice, indicating increased levels of oxidative stress. Additionally, in HFD but not in ND mice, there was a significant upregulation of the C-C chemokine receptor type 5 (CCR5).

To determine whether the observed effects directly result from an altered cholesterol metabolism within the CNS, the expression of several genes involved in cholesterol metabolism, such as 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGCR), liver X receptor (LXRα), ATP binding cassette transporter 1 (ABCA1) and apolipoprotein E (APOE), was assessed by RT-PCR. However, HFD had no significant influence on the expression of these genes involved in cholesterol metabolism compared to EAE mice on ND (Fig. 4).

HFD upregulates the RAS

To determine whether activation of the RAS underlies the exacerbation of CNS inflammation after HFD, the expression of genes involved in the RAS was quantified (Fig. 5a). The expression of angiotensin converting enzyme (ACE) and angiotensinogen (AGT) was found to be significantly increased in brain tissue from EAE mice on HFD compared to that of EAE mice on ND. No changes were observed in angiotensin II type 1 receptor (AT1R) gene expression.

To determine whether induction of the RAS is indeed responsible for the HFD mediated effects on EAE, mice on ND and HFD were treated with the ACE blocker captopril and immunized with MOG 2 weeks afterwards. Captopril treatment significantly ameliorated EAE in both the ND (AUC (ND): 39.03 +/− 0.66 vs AUC (ND+Capto): 8.95 +/− 1.84 $P<0.001$) and HFD (AUC (HFD): 55.67 +/− 1.53 vs AUC (HFD+Capto): 12.94 +/− 1.97 $P<0.001$) mice (Fig. 5b). Initially, the captopril treated ND mice showed a delayed disease onset compared to HFD mice on captopril treatment. However, during the chronic stages of EAE, disease scores did not differ between ND and HFD mice. These data indicate that the RAS is responsible for the HFD induced worsening of EAE during the chronic disease stage (Fig. 5b).

Oxysterols are known to activate the RAS. To determine whether the HFD mediated upregulation of the RAS is accomplished through oxysterols, oxysterol levels in ND and HFD EAE mice were determined in plasma and brain. HFD lead to increased plasma, but not brain, cholesterol levels compared to ND (Fig. 6b,d). However, we observed no significant differences in the levels of 24S hydroxycholesterol (24(S)OHC), 27 hydroxycholesterol (27OHC) and 7 alpha hydroxycholesterol (7αOHC) between EAE mice on ND and mice on HFD (Fig. 6a,c).

HFD induces systemic inflammation

HFD is known to induce a systemic pro-inflammatory response. As enhanced systemic inflammation may boost neuroinflammation, we determined the levels of several inflammatory mediators with RT-PCR in PBMCs isolated from EAE mice on ND and HFD. EAE mice on HFD showed a significant increase in the expression of iNOS and IL6 (Fig. 7) and a decreased expression of TNFα and TGFβ in their PBMCs compared to mice on ND.

To elucidate whether activation of the RAS is responsible for the increased systemic and CNS inflammation, we compared the levels of several inflammatory cytokines in PBMCs and spinal cord tissue isolated from EAE mice on ND and HFD with or without captopril treatment. Captopril treatment did not significantly decrease the gene expression levels of inflammatory mediators in PBMCs of mice on ND or HFD. In contrast, captopril treatment significantly decreased the expression of

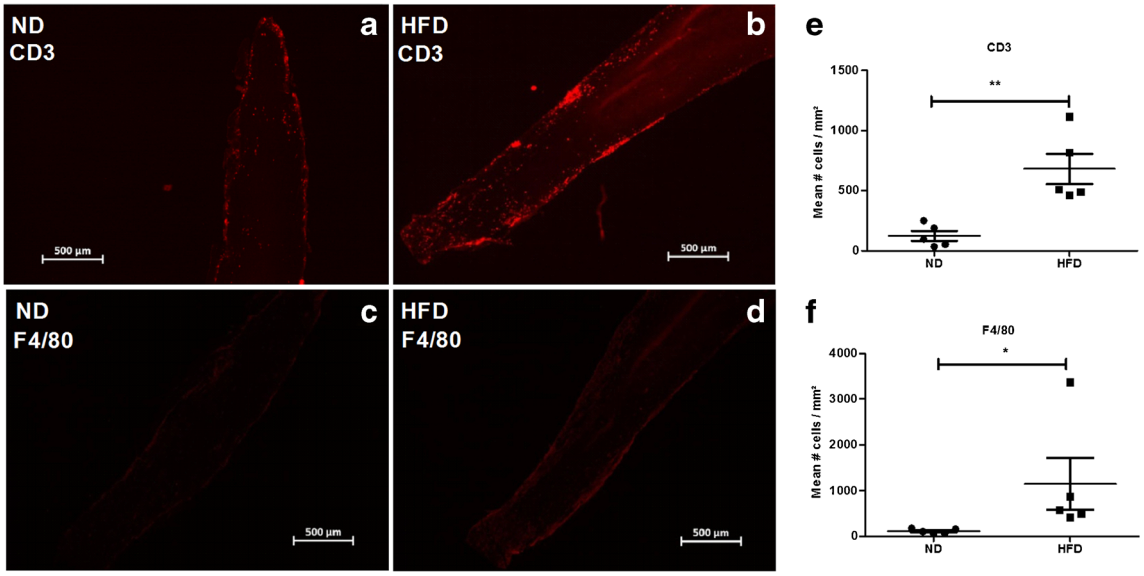

Fig. 2 HFD increases macrophage and T cell infiltration into the CNS. Immunohistochemical sections of spinal cord tissue from mice with EAE on ND (**a**, **c**) or HFD (**b**, **d**) on day 27 post immunization stained with primary rat anti-mouse CD3 antibodies (**a**, **b**, **e**) or rat anti-mouse F4/80 antibodies (**c**, **d**, **f**). Six representative sections were stained for each mouse. $N=5$

TGFβ, TNFα, IL6, GM-CSF and IL1β in the CNS of mice on ND and TNFα in the CNS of mice on HFD (Fig. 8). Interestingly, captopril treatment increased the expression of IL1β in PBMCs from mice on HFD, indicating that captopril may also have pro-inflammatory effects. These results indicate that HFD mediated RAS induction mainly affects the CNS inflammatory response and does not boost the immune response in peripheral systems.

Discussion

In this study we provide evidence that the expression of essential RAS components is increased in brains of EAE animals on HFD, which coincides with an increased immune cell infiltration and inflammation in the CNS. These findings strongly suggest that HFD affects lesion progression during neuroinflammatory diseases such as MS.

Induction of the RAS will have multiple consequences that lead to an enhancement of CNS inflammation. AngII increases vascular permeability by the release of prostaglandins and vascular endothelial cell growth factor and the rearrangement of cytoskeletal proteins. Furthermore, AngII contributes to the recruitment of inflammatory cells into the tissue through the induction of adhesion molecules and chemokines by resident cells (Suzuki et al. 2003). Interestingly, we observed an increased expression of CCR5 and an increased infiltration of macrophages and T cells into the spinal cord of EAE mice on HFD compared to mice on ND. Consistent with our findings,

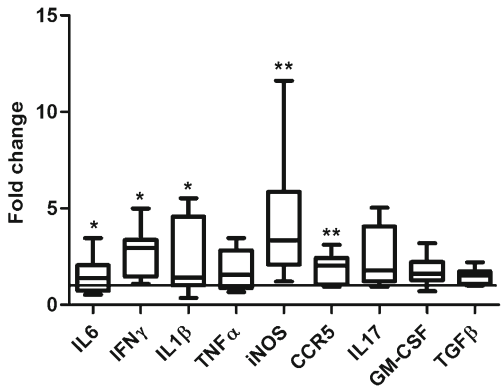

Fig. 3 HFD increases inflammation in the CNS. The expression of the inflammatory genes IL6, IFNγ, IL1β, TNFα, iNOS, CCR5, IL17, TGF and GM-CSF was measured with RT-PCR in spinal cord tissue from mice with EAE on ND (*flat line*) or HFD at day 27 post immunization. Expression was normalized using HMBS and ACTB and converted to fold change values using the $2^{-\Delta\Delta CT}$ method. $N=5$

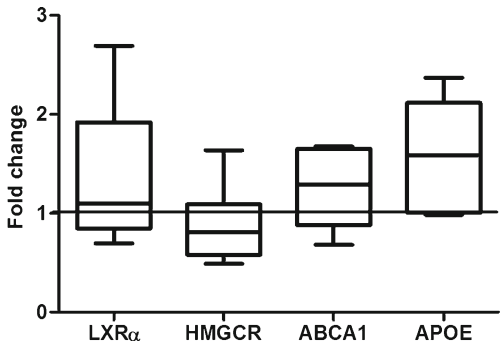

Fig. 4 HFD has no significant influence on genes involved in cholesterol metabolism in the CNS. The expression of the cholesterol metabolism related genes LXRα, HMGCR, ABCA1 and APOE was measured with RT-PCR on spinal cord tissue from mice with EAE on ND (*flat line*) or HFD at day 27 post immunization. The expression was normalized using the most stable housekeeping genes (HMBS and ACTB) and converted to fold change values using the $2^{-\Delta\Delta CT}$ method. $N=5$

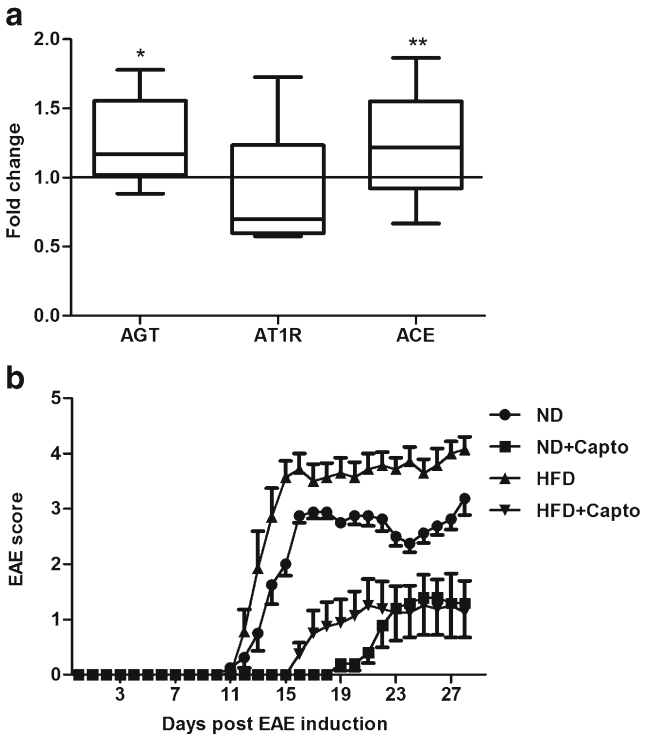

Fig. 5 HFD upregulates the RAS. **a** The expression of the RAS related genes ACE, AT1R and AGT was measured with RT-PCR on brain tissue from mice with EAE on ND (*flat line*) or HFD at day 27 post immunization. The expression was normalized using the most stable housekeeping genes (HMBS and ACTB) and converted to fold change values using the $2^{-\Delta\Delta CT}$ method. $N=5$. **b** Clinical score of mice on ND ($N=10$) and HFD ($N=10$) with or without captopril (capto) treatment after induction of EAE. Data are expressed as mean ± standard error of the mean

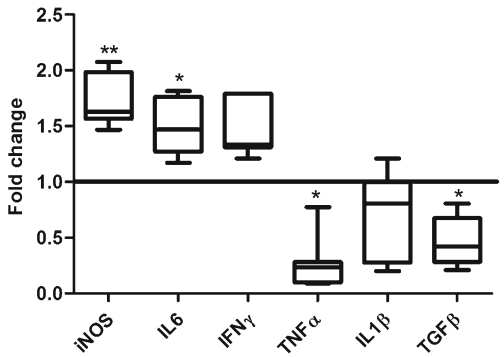

Fig. 7 HFD increases peripheral inflammation. The expression of the inflammatory genes IL6, IFNγ, IL1β, TNFα, iNOS and TGF was measured with RT-PCR in PBMCs from mice with EAE on ND (*flat line*) or HFD at day 28 post immunization. Expression was normalized using CYCA and TBP and converted to fold change values using the $2^{-\Delta\Delta CT}$ method. $N=7$

Lavin et al. reported that in healthy mice HFD leads to an increased macrophage infiltration into the brain, which was correlated with an induced expression of macrophage chemoattractant molecule CCL2 (Lavin et al. 2011). Furthermore, EAE animals and MS patients exhibit an upregulation of the RAS and RAS blockage ameliorates EAE by decreasing immune cell infiltration into the CNS (Stegbauer et al. 2009). Collectively, these findings indicate that HFD promotes infiltration of immune cells into the brain.

We show a disease aggravation upon HFD in a MOG induced chronic EAE model in C57BL/6J mice. This is in line with a previous study performed by Winer et al., showing that diet induced obesity mice more rapidly progress to a more severe EAE (Winer et al. 2009). In contrast, Piccio et al.

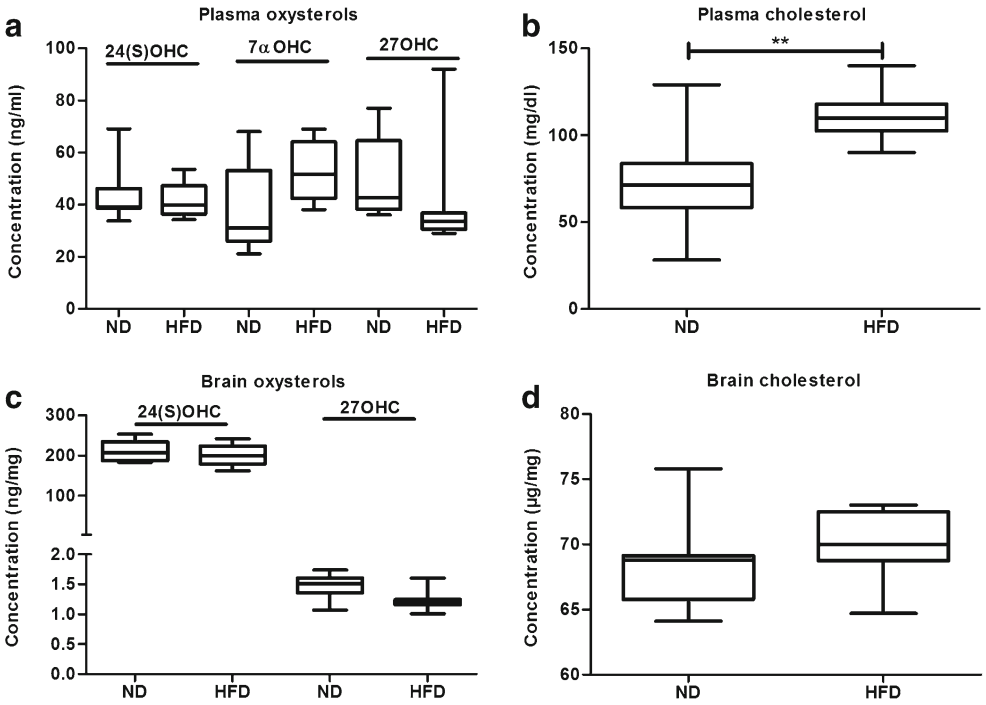

Fig. 6 Circulating and CNS oxysterols are not altered in EAE animals on HFD. The production of 24(S)OHC, 27OHC, 7αOHC and cholesterol was measured in blood and brain samples of EAE mice on ND or on HFD at day 28 post immunization. $N=7$

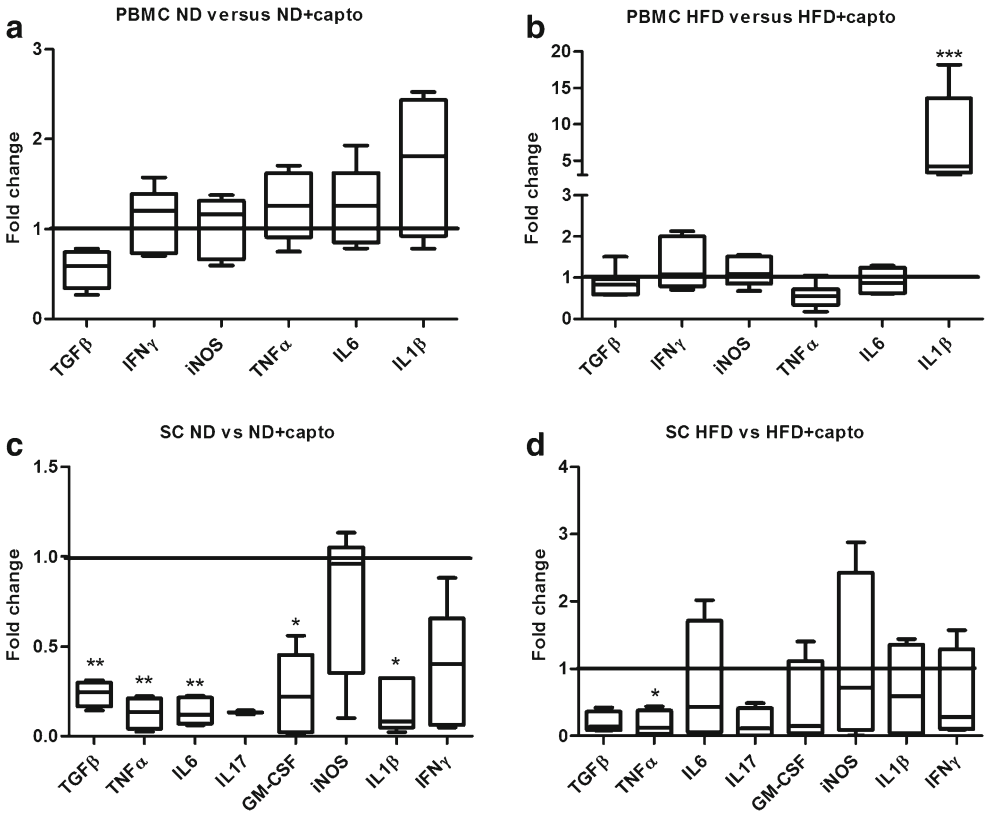

Fig. 8 Captopril attenuates the CNS inflammatory response and does not downregulate the immune response in peripheral systems. The expression of several inflammatory mediators was measured with RT-PCR in PBMCs and spinal cord tissues (SC) from mice on ND and HFD with or without (*flat line*) captopril treatment at day 28 post immunization. The expression was normalized using the most stable housekeeping genes (CYCA and TBP for PBMCs, HMBS and ACTB for spinal cord tissue) and converted to fold change values using the 2-ΔΔCT method. $N=4$

showed no influence of HFD on the disease course in a PLP induced relapsing-remitting EAE mouse model in SJL mice (Piccio et al. 2008). In the studies performed by Piccio and Winer, mice were pretreated with HFD for respectively 5 and 10 weeks to study the obesity related, long term effects of HFD on EAE. This obesity induced mouse model was generated by HFD containing 60 % fat and revealed an obesity mediated IL6-dependent pathway of TH17 bias in T cell priming and expansion resulting in a more severe EAE course (Winer et al. 2009). We pretreated mice for 2 weeks with a western type HFD containing 21 % fat before EAE induction and demonstrate that HFD containing less saturated fat also worsens the disease course. In line with this finding and in addition to an increased infiltration of immune cells into the CNS in response to HFD, we demonstrate that a shorter pretreatment with HFD increases the expression of pro-inflammatory mediators, such as IL6, IFNγ, IL1β and iNOS in spinal cord tissue of EAE mice on HFD compared to ND mice. Similarly, it was shown that activation of the CNS RAS during neuroinflammation in an animal model of hypertension and ischemia is responsible for an induction of iNOS expression in the CNS of these animals (Yamakawa et al. 2003; Saavedra 2011). Furthermore, the RAS is implicated in the induction of TH17 cells since blocking AngII suppressed autoreactive TH1 and TH17 cells and promoted antigen specific CD4+FoxP3+ Tregs during EAE (Platten et al. 2009). In line with this we found a trend towards an increased expression of the TH17 cytokines GM-CSF and IL17 in the spinal cord of EAE mice on HFD compared to ND.

Treatment with the ACE blocker captopril significantly lowered EAE disease severity. This is in concordance with previous studies showing that blocking the RAS with either renin, ACE or AT1R inhibitors ameliorated EAE by inducing Tregs and by inhibiting immune cell proliferation, TH1 and TH17 responses (Platten et al. 2009; Stegbauer et al. 2009). The suppression of disease severity by captopril was similar in both ND and HFD EAE mice, indicating that RAS activation is indeed responsible for the HFD induced exacerbation of EAE. However, at the early stage of EAE, other mechanisms might be involved, since HFD mice display an earlier disease onset compared to mice on ND despite RAS blockage. Notably, we show that HFD activates peripheral immune cells by inducing both iNOS and IL6 expression and by decreasing TGFβ expression in a RAS independent manner, suggesting that HFD increases peripheral inflammation at EAE onset. This may explain the earlier onset of EAE in captopril treated mice on HFD compared to captopril treated mice on ND. Previous studies in people with obesity showed elevated blood levels of inflammation associated markers, like acute-phase proteins, pro-inflammatory cytokines, chemokines, soluble adhesion molecules and prothrombotic mediators (Fernandez-Real and Pickup 2008; Kolb and Mandrup-Poulsen 2010; Calder et al. 2011). In contrast, we showed that HFD decreases the expression of TNFα in PBMCs, indicating that HFD may also have

anti-inflammatory effects in peripheral systems. Interestingly, our results show that captopril treatment suppresses CNS but not peripheral inflammation, indicating that the RAS is mainly involved in the regulation of the CNS inflammatory response upon HFD treatment and is not involved in the regulation of the peripheral immune response after HFD.

It was recently shown that mice on a cholesterol-enriched diet exhibit an LXR dependent upregulation of AGT in the CNS, which was mediated by induction of the oxysterols 27OHC and 24(S)OHC (Mateos et al. 2011). However, we observed no significant differences in plasma and brain 27OHC and 24(S)OHC concentrations between ND and HFD EAE mice.

To conclude, we show that HFD increases immune cell infiltration and inflammation in the spinal cord and thereby exacerbates EAE. Moreover, our data indicate the involvement of the RAS in the HFD-mediated effects on EAE severity. These findings may have important implications for the treatment and prevention of neuroinflammatory disorders such as MS and stress the importance of further studies into nutritional modulation of MS.

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Conflict of interest The authors declare that they have no conflict of interest.

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