RESEARCH

Telmisartan prevents development of obesity and normalizes hypothalamic lipid droplets

Elias Rawish^{1,2,3}, Laura Nickel^{1,2,3}, Franziska Schuster^{1,3}, Ines Stölting¹, Alex Frydrychowicz⁴, Kathrin Saar^{5,6}, Norbert Hübner^{5,6}, Alaa Othman^{3,7}, Lars Kuerschner⁸ and Walter Raasch^{1,2,3}

¹Institute of Experimental and Clinical Pharmacology and Toxicology, University of Lübeck, Lübeck, Germany

²DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Lübeck, Germany

³CBBM (Center of Brain, Behavior and Metabolism), Lübeck, Germany

⁴Department of Radiology and Nuclear Medicine, University-Hospital Schleswig Holstein and University of Lübeck, Lübeck, Germany

⁵Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin-Buch, Germany

⁶DZHK (German Centre for Cardiovascular Research), partner site Berlin, Berlin, Germany

⁷Institute for Clinical Chemistry, University Hospital Zürich, Zurich, Switzerland

⁸Life and Medical Sciences Institute (LIMES), University of Bonn, Bonn, Germany

Correspondence should be addressed to W Raasch: walter.raasch@uni-luebeck.de

Abstract

The AT₁ receptor blocker telmisartan (TEL) prevents diet-induced obesity. Hypothalamic lipid metabolism is functionally important for energy homeostasis, as a surplus of lipids induces an inflammatory response in the hypothalamus, thus promoting the development of central leptin resistance. However, it is unclear as to whether TEL treatment affects the lipid status in the hypothalamus. C57BL/6N mice were fed with chow (CON_{chow}) or high-fat diet (CON_{HFD}). HFD-fed mice were gavaged with TEL (8 mg/kg/day, 12 weeks, TEL_{HFD}). Mice were phenotyped regarding weight gain, energy homeostasis, and glucose control. Hypothalamic lipid droplets were analyzed by fluorescence microscopy. Lipidomics were assessed by performing liquid chromatography-mass spectrometry in plasma and hypothalami. Adipokines were investigated using immunosorbent assays. Glial fibrillary acidic protein (GFAP) was determined by Western blotting and immunohistochemical imaging. We found that body weight, energy homeostasis, and glucose control of TEL-treated mice remained normal while CON_{HFD} became obese. Hypothalamic ceramide and triglyceride levels as well as alkylne oleate distribution were normalized in TEL_{HFD}. The lipid droplet signal in the tanycyte layer was higher in CON_{HFD} than in CON_{chow} and returned to normal under TEL_{HFD} conditions. High hypothalamic levels of GFAP protein indicate astrogliosis of CON_{HFD} mice while normalized GFAP, TNF α , and IL1 α levels of TEL_{HFD} mice suggest that TEL prevents hypothalamic inflammation. In conclusion, TEL has anti-obese efficacy and prevented lipid accumulation and lipotoxicity, which is accompanied by an anti-inflammatory effect in the murine hypothalamus. Our findings support the notion that a brain-related mechanism is involved in TEL-induced weight loss.

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Introduction

Within the past few years, angiotensin II receptor (type 1) blockers (ARBs) have been shown to prevent the development of obesity in rodent models (He *et al.* 2010, Muller-Fielitz *et al.* 2012). Weight loss was observed both when telmisartan (TEL) was administered daily via the oral route to rats with fully diet-induced obesity in a treatment setting (Muller-Fielitz *et al.* 2014) and in patients during irbesartan therapy (Kintscher *et al.* 2007). These antiobese effects were only observed after giving high dosages of the drug and were found to be class effects and independent both of blood pressure reduction and of peroxisome proliferator-activated receptor gamma (PPAR γ) activation (Muller-Fielitz *et al.* 2011, 2012, 2014, Schuster *et al.* 2018).

Among other mechanisms, the antiobese effect of ARBs is dependent on intact leptin signaling. Leptin is a regulatory peptide, which is secreted by adipocytes as an afferent signal in a negative feedback loop, in order to induce anorexia after acting at its receptors in the brain (Friedman 2016). However, human obesity is not associated with an absence of leptin but rather with resistance to leptin activity (Konner & Bruning 2012). We recently showed that (a) homozygous Zucker rats (which represent a genetic animal model for leptin resistance due to a one-point mutation causing an amino acid substitution in the extracellular domain of the leptin receptor) remain obese despite ARB treatment, whereas heterozygous controls lose weight (Muller-Fielitz *et al.* 2011), (b) TEL treatment restores leptin sensitivity (Muller-Fielitz *et al.* 2015), and (c) TEL prevents diet-induced obesity and preserves leptin transport across the blood–brain barrier (BBB) in mice fed a high-fat diet (HFD) (Schuster *et al.* 2018). In addition to preventing obesity and restoring leptin sensitivity, TEL treatment normalized plasma levels of triglycerides (TGs), while TGs were markedly enhanced in control rats, which became obese and leptin resistant as a result of HFD feeding (Muller-Fielitz *et al.* 2014). This observation is in accordance with the notion of Banks, who asserted that high levels of circulating triglycerides have functional impact on the development of both peripheral and central leptin resistance and obesity by impairing leptin penetration through the BBB and leptin signaling in the brain (Banks *et al.* 2018).

In addition to peripheral alterations in TG concentrations, cerebral lipid levels have also been accused of altering food intake as prolonged intraventricular (i.c.v) infusion of mono-unsaturated oleic acid (OA) causes a decrease in food intake (Obici *et al.* 2002), whereas i.c.v infusion of saturated

palmitic acid (PA) induces central leptin resistance, leading to an increase in food intake (Cheng *et al.* 2015). However, diminished total fatty acid levels were unexpectedly found in the hypothalami of rodents on a HFD despite having higher serum levels than lean controls (Le Foll *et al.* 2014). Using alkyne fatty acid tracers (Thiele *et al.* 2012) and click chemistry, which is based on a copper-catalyzed azide-alkyne cycloaddition to detect alkyne lipids (Kolb *et al.* 2001), Le Foll's finding was recently related to tanycytes, hypothalamic cells facing the third ventricle and playing a gatekeeper role in lipid processing (Hofmann *et al.* 2017). Thus, hypothalamic ketone body and neutral lipid synthesis as well as lipid droplets (LDs) in tanycytes are increased, possibly shielding unsaturated oleic acid from astrocytes (Hofmann *et al.* 2017). It was further discussed that tanycytes may act as a metabolic sink for surplus neutral lipids under obesity conditions where systemic fatty acids are abundant (Hofmann *et al.* 2017). In addition to lipid metabolism alterations under obesity conditions, tanycytes have functional importance in regulating metabolism for central leptin transport and further signaling in an extracellular signal-regulated kinase (ERK)-dependent manner (Balland *et al.* 2014) as well as for glucose sensing via an ATP-sensitive K channel-dependent pathway (Frayling *et al.* 2011). Although the exact physiological mechanisms underlying glucose-induced responses in tanycytes are not fully understood yet, studies indicate that lactate, as a metabolic substrate of glucose captured in tanycytes, is released by monocarboxylate transporters (MCTs) (Cortes-Campos *et al.* 2011). Lactate flux is suggested to be used by nearby neurons in the arcuate nucleus (ARC) to detect changes in glucose levels in order to regulate feeding behavior (Cortes-Campos *et al.* 2013). Accordingly, inhibiting MCT-1 or glucose transporter 2 (GLUT2) in tanycytes impairs the neuronal response to fasting and glucose administration accompanied by altered feeding behavior (Elizondo-Vega *et al.* 2016, Barahona *et al.* 2018). Hence, recent data emphasize that metabolic coupling between tanycytes, astrocytes, and neurons in hypothalamic areas is associated with regulation of feeding behavior.

Hypothalamic inflammatory processes have been shown to play a crucial role in the development of diet-induced obesity (Valdearcos *et al.* 2015). Although the exact cellular mechanisms remain incompletely understood, previous evidence suggests that the inflammatory response is mediated by interactions between neurons and glial cells such as astrocytes and tanycytes (de Git & Adan 2015). Dietary fat increases the content of several lipids, such as saturated acyl-CoA

species in the hypothalamus, activating the inhibitor of the kappa B kinase-beta (IKK β) pathway, which can induce central leptin and insulin resistance (Posey *et al.* 2009). Thus, hypothalamic lipotoxicity, based on an excessive accumulation of some lipid species causing metabolic alterations, is strongly assumed to be a key mechanism in the development of obesity (Gonzalez-Garcia *et al.* 2017).

Considering these findings, the aim of the present study was to investigate whether TEL treatment can normalize hypothalamic fatty acid metabolism and LD accumulation in the tanycyte region in mice despite HFD feeding while the amount of LD is high in obese but not in lean controls. We further asked whether TEL treatment can alter the hypothalamic lipidome of HFD-fed mice, using mass spectrometry technique to focus on expected lipid accumulation and lipotoxicity due to HFD. Finally, we determined whether TEL treatment could normalize hypothalamic inflammation in HFD-fed mice by measuring various inflammatory parameters via immunosorbent assays and microarray technique and determining glial fibrillary acidic protein (GFAP) via immunohistochemical and Western blot analyses. Indeed, astrogliosis in the hypothalamic region was identified to serve as a hallmark of CNS injury and low-grade inflammation occurring in obesity in both rodents and humans, while it is largely reversible after becoming lean by returning to regular food (Jastroch *et al.* 2014). Thus, we show here that weight-reducing doses of TEL prevented lipid accumulation and lipotoxicity, which is accompanied by an anti-inflammatory effect in the murine hypothalamus.

Materials and methods

Reagents

Alkyne oleate was synthesized as described (Thiele *et al.* 2012). DAPI (4',6-diamidino-2-phenylindole) and general laboratory chemicals were obtained from Sigma-Aldrich. Lipid-free BSA was purchased from AppliChem (Darmstadt, Germany). LD540 (4,4-difluoro-2,3,5,6-bis-tetramethylene-4-bora-3a,4a-diaza-s-indacene) was synthesized as described previously (Spandl *et al.* 2009).

Animals

All animal care and experimental procedures were performed in accordance with the NIH guidelines for the care and use of laboratory animals and were approved

by the animal ethics committee of the local regulatory authority (Ministerium für Energiewende, Landwirtschaft, Umwelt und ländliche Räume des Bundeslandes Schleswig-Holstein). The results of all studies involving animals are reported in accordance with the ARRIVE guidelines. Male C57BL/6N mice (from Charles River Laboratories) aged 6–8 weeks were kept in individually ventilated cages in groups of two to five individuals at 23°C under a 12 h/12 h dark (18:00 h–06:00 h)/light (06:00 h–18:00 h) cycle. All animals were habituated to laboratory conditions for at least 10 days before experiments were started. All animals had free access to water. A total of 99 mice were included in the two parts of the study (part 1: $n=39$, part 2: $n=60$). Functional studies and hypothalamic lipid analyses were mainly performed in part 1, while gene expression was exclusively determined in part 2.

Study protocol (part 1)

For the workflow of the first part of the study, see Supplementary Fig. 1 (see section on [supplementary materials](#) given at the end of the article). The group size was assessed by a power analysis (alpha value=0.017, power 80%) to be at least $n=13$ in each group by expecting a weight difference of 8 ± 6 g after TEL treatment. Two mice died of an unexpected anesthetic accident during MRI analyses. All animals were monitored daily by visual inspection and weighing. Mice received one of these two diets: HFD (EF acc. D12492 (I) mod. from ssniff®, Soest, Germany) with 24.0 MJ/kg or normal fat diet (NFD, EF acc. D12450B mod. from ssniff®, Soest, Germany) with 18.0 MJ/kg. Once a day, mice received TEL (8 mg/kg_{bw}) or vehicle by oral gavage in a volume of 5 μ L/g body weight. TEL dosages were evaluated recently (Muller-Fielitz *et al.* 2012) and have been confirmed in numerous studies both in rats (Miesel *et al.* 2012, Muller-Fielitz *et al.* 2014, 2015, Schuchard *et al.* 2015, Winkler *et al.* 2016, Gustaityte *et al.* 2018) and in mice (Schuster *et al.* 2018, Dapper *et al.* 2019) to reveal antiobese effects. For administration, TEL was suspended in 10% gum arabic (Carl Roth GmbH, Karlsruhe, Germany), resulting in a suspension of 1.6 mg/mL TEL. Mice were allocated by block randomization to the following groups: NFD and vehicle treatment (CON_{chow}), HFD and vehicle treatment (CON_{HFD}), and HFD and TEL treatment (TEL_{HFD}). Mice were phenotyped according to the following approach: at w6, energy expenditure (EE), respiratory exchange rate (RER), locomotion, and drinking and feeding behavior were determined in a subgroup of mice for 5 days by indirect calorimetry using the PhenoMaster System™

(TSE, Bad Homburg, Germany) (Schuster *et al.* 2018). Data were analyzed separately for light and dark periods (only considering a core period of 8 h for each period) and averaged for the 5 days. At w7, insulin sensitivity was determined by performing an insulin tolerance test (ITT) as recently described (Schuster *et al.* 2018, Dapper *et al.* 2019). To validate leptin sensitivity, a leptin resistance test (LRT) was performed at w8 (Dapper *et al.* 2019). Details of the ITT and LRT are specified in the Supplementary materials. At w10, fat mass was assessed by using MRI technique (Schuchard *et al.* 2015, Winkler *et al.* 2016). At w11, tail blood was withdrawn after a 6-h fasting period to determine plasma lipids. At w13, mice were killed, whereupon organs and blood were removed. As lipid droplet signal has already been demonstrated to be higher in tanycytes of obese than in lean mice by investigating only three animals each group (Hofmann *et al.* 2017), we performed hypothalamic lipid as well as immunosorbent and immunohistochemical analyses in a subset of five animals of each group here, as described below.

Study protocol (part 2)

For the workflow of the second part of the study, see Supplementary Fig. 2. In a second cohort of animals consisting of 60 mice (20 mice per group), we further aimed to investigate whether hypothalamic gene expression is altered in response to feeding and TEL. The group size was assessed by a power analysis (alpha value=0.008, power 80%) to be at least $n=20$ in each group by expecting genetic differences of $15\pm12\%$ of hypothalamic (an)orexigenic peptides (Muller-Fielitz *et al.* 2014, 2015, Schuchard *et al.* 2015). Feeding and treatment were analogous to the aforementioned approach without performing any functional characterization, except for body weight. After 6 weeks, animals were killed and hypothalami were removed for microarray analysis following tissue homogenization by using a Bullet Blender® and the Bullet Blender® PINK Bead Lysis Kit (both Next Advance, Troy, USA) and then isolating RNA by using the NucleoSpin® RNA Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The RNA was processed and hybridized on the Affymetrix Mouse Gene 2.0 Array (Thermo Fisher Scientific) according to the manufacturer's protocol (Schuster *et al.* 2018).

Preparation of brain slices

For lipid analysis, brain slices (bregma level -1.4 to -1.9 , 250 μm thickness, vibratome) were prepared immediately

following decapitation and incubated with 50 mM alkyne oleate according to a previously described protocol (Hofmann *et al.* 2017); for detailed information, see Supplementary materials. For immunohistochemistry, mice were transcardially perfused with 25 mL of PBS and then with 25 mL of 4% paraformaldehyde (PFA) in PBS at a flow rate of 20 mL/min. Slices (bregma level -1.7 to -1.8 , 50 μm thickness, vibratome) were fixed, adding 4% PFA to the PBS for 10 min before washing with PBS.

Hypothalamic lipid analyses

Hypothalamic LDs containing neutral lipids were (semi-) quantified in brain slices by staining LDs with LD540, a lipophilic dye developed for imaging of lipid droplets (Spandl *et al.* 2009), and the micrographs were then quantified (see below). Hypothalamic neutral glycerolipids as well as phosphoglycero-, sphingo-, and acylcarnitine lipids were analyzed by liquid chromatography-mass spectrometry (LC-MS, see Supplementary materials). Hypothalamic fatty acid metabolism was analyzed in the slices using tracer alkyne oleate and detection by copper(I)-catalyzed azide-alkyne cycloaddition, CuAAC, commonly termed click chemistry (Hofmann *et al.* 2017). This method combines high sensitivity with fast sample turnover and is free of radioactivity (Thiele *et al.* 2012). Alkyne oleate was localized by fluorescence microscopy using a biotinylated azide reporter (Sigma-Aldrich, product no. 762024) and streptavidine-Alexa 488 conjugate (BioLegend, San Diego, USA) as described previously (Hofmann *et al.* 2014). Cellular alkyne oleate metabolites were characterized by reacting hypothalamic lipid extracts with 3-azido-7-hydroxycoumarin (Basclick, Neuried, Germany, product no. BCFA-047) before performing thin-layer chromatography (TLC) as described previously (Thiele *et al.* 2012).

Microscopy

Epifluorescence microscopy was performed using a Zeiss Observer. Z1 microscope (Carl Zeiss) equipped with a Fluor 40x (1.30 NA) objective and a Photometrics Coolsnap K4 camera (Teledyne Photometrics, Tucson, USA). Optical sectioning was performed using the apotome mode, which also served to avoid stray light due to slice thickness. The light source was a Polychrome V 150 W Xe lamp (Till Photonics, Graefeling, Germany). All images were processed by employing the ZEN (Carl Zeiss) and Fiji (Schindelin *et al.* 2012) software. If appropriate, projections of z-stacks were calculated by summarizing corresponding

pixel values using Fiji software. For semi-quantitative analysis of immunostaining, all corresponding samples were processed identically in the same dish on the same day (details in Supplementary materials). Samples for lipid analysis were processed identically on different days. Identical acquisition settings were applied to record all corresponding images. Blinded semi-quantification was performed using Fiji software according to modified, previously described procedures (Hofmann *et al.* 2017).

Biochemical analysis

Blood glucose was measured using a commercial glucose sensor (Ascensia Elite XL and Elite Sensor, Bayer Vital GmbH, Leverkusen, Germany). Plasma concentrations of diverse adipocytokines were measured in 10 μ L of sample by immunosorbent assays according to the manufacturer's instructions using the Milliplex mouse metabolic magnetic bead panel kit MADKMAG-71K-07.mouse (interleukin-6 (IL6), insulin, leptin, MCP1 (monocyte chemoattractant protein-1), PAI1 (plasminogen activator inhibitor 1), resistin, TNF α (tumor necrosis factor- α)) and MHSTCMAG-70K-13 mouse high-sensitivity equipment (granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (INF γ), IL10, IL12, IL13, IL1a, IL2, IL4, IL5, IL7, IL6, MCP1, and TNF α ; all from Merck/Millipore). We also determined adipocytokines in the hypothalamic tissue samples. Thus, slices of hypothalami (bregma level -1.4 to -1.9 , 250 μ m thickness, vibratome) were homogenized in 120 μ L phosphate buffer (containing 0.2% Triton X-100). Then, 5 μ L of this homogenate were used for protein analysis for normalization according to the Bradford method (Sigma-Aldrich) and 10 μ L of tissue homogenate were mixed with 10 μ L saline and centrifuged (10 min, 1000g). Following this, 10 μ L of supernatant was used for immunosorbent assays. The lipid profiles in plasma and hypothalami were analyzed according to other published methods (Othman *et al.* 2012) with slight modifications (for detailed information, see Supplementary materials).

GFAP immunohistochemistry (IHC)

For IHC analyses, fixed slices (see above) were transferred to 24-well culture plates containing PBS. Blocking buffer (1% BSA, 0.1% Triton X-100 in PBS) was added for 1 h before incubation with primary antibody GFAP (Synaptic systems, Göttingen, Germany, product no. 173004, 1:500) in blocking buffer at 4°C overnight. PBS was used

for washing, incubation with secondary antibody (goat anti-guinea pig A488, Invitrogen, Thermo Fisher Scientific, product no. A-11073, 1:500), and DAPI (1:100) staining before mounting.

Western blot analysis of GFAP

Equal amounts of proteins (50 μ g in 40 μ L) were separated on 7.5% SDS-polyacrylamide gels. Equal loading was confirmed by incubating the membranes with actin mouse antibodies (Merck-Millipore, product no. MAB 1501, 1:5000). Target proteins were detected by applying a guinea pig antibody specific for GFAP (Synaptic Systems, Göttingen, Germany, product no. 173004, 1:2500). Immunocomplexes were then labeled with the corresponding horseradish peroxidase-conjugated anti-mouse or anti-guinea pig IgGs (Dako) and semi-quantitatively detected by enhanced chemiluminescence (West Dura substrate, Fisher Scientific) using a high-resolution CCD (charge-coupled device) camera (ChemiDoc system and Quantity One software, Bio-Rad).

Calculations and statistics

Data and statistical analysis comply with the recommendations for experimental design and analysis in pharmacology. In graphs and tables, data are expressed as means $\pm$ s.d. Food intake was determined by the Phenomaster System and consumed energy was calculated using the energy density of the special diets (24 kJ/g and 18 kJ/g). In order to quantify the total effect over the observation period in response to ITT, areas under the curves (AUCs) were calculated for each individual animal based on their plasma glucose delta values. $C_{\min}$ values were also calculated considering delta values. All data were checked for outliers using the GraphPad® outlier calculator (<http://graphpad.com>) and tested for Gaussian distribution and variance homogeneity (GraphPad Prism 8). Strain differences were evaluated by 2-way ANOVA, followed by Bonferroni's multiple comparison post-test to prove treatment effects. A two-way or one-way ANOVA was only performed when Gaussian distribution and variance homogeneity were detected. Bonferroni's multiple comparison *post hoc* test was only performed if P was <0.05 . Welch's correction for ANOVA was considered when variance inhomogeneity between groups was detected. Kruskal–Wallis test with Dunn's multiple comparison test was used when values were not distributed in a Gaussian fashion. Correlation analyses were performed

by two-tailed Pearson test. Signals gained from microarray experiments were normalized by Affymetrix Expression Console Software and both log values and fold-changes were calculated. These data were statistically examined by Affymetrix Transcriptome Analysis Console Software (Thermo Fisher Scientific).

Results

Effects on growth, energy homeostasis, and plasma levels of lipids and adipokines

Initial body weight did not differ between groups (CON_{chow} 23.3 ± 1.4 g; CON_{HFD} 22.8 ± 1.4 g; TEL_{HFD} 23.2 ± 1.8 g; $F=0.4$, $P=0.68$). HFD-fed mice developed obesity as both final body weight and fat mass clearly exceeded those of chow-fed controls. TEL treatment normalized weight gain and fat mass despite HFD feeding (Fig. 1A and B). Accordingly, organ weights were enhanced when mice were fed with HFD but remained at or even below chow levels when simultaneously treated with TEL (Fig. 1C, D and E). Similar findings on body weight in response to HFD feeding and TEL treatment were also observed in the 2nd group of mice, which was used for microarray analyses of inflammation parameters (Supplementary Fig. 3).

The increase in both plasma leptin and body weight already suggested that leptin resistance had developed in HFD-fed mice (Fig. 1F and H). The findings that hypothalamic leptin content was almost undetectable (Fig. 1G and H) in HFD-fed mice and that body weight in LRT was not reduced compared to chow-fed controls (Fig. 1I) further indicate that HFD-fed controls developed leptin resistance. In contrast, plasma leptin, hypothalamic leptin content, and body weight gain upon leptin challenge stayed at normal chow levels as a result of TEL treatment despite HFD feeding (Fig. 1F, G, H and I). Following immunosorbent analyses, the plasma concentration of PAI1 was higher in CON_{HFD} than in CON_{chow} but normalized in TEL_{HFD} mice, while the levels of IL4 and IL6 were higher in both CON_{HFD} and TEL_{HFD} mice. IL5 levels were higher in TEL_{HFD} than in CON_{chow} mice. IL13 was similar in all groups (Table 1). IL1 α and IL10 were below the detection limit in most samples of all groups. Lipids in plasma were determined by LC-MS. In particular, ceramides, cholesteryl esters, phosphatidylcholines, phosphatidylethanolamines, and sphingomyelins were higher in both CON_{HFD} and TEL_{HFD} mice, with the exception that ceramides were normalized to chow levels by TEL (Table 2 and Supplementary Fig. 4).

Obesity of HFD-fed mice was related more to lower energy expenditure and locomotion (Fig. 2A and E) than to a heightened energy intake, which was, at least in the dark phase, even slightly reduced (Fig. 2C). Upon TEL, energy expenditure was higher, whereas energy intake was lower than in HFD-fed controls in the dark periods (Fig. 2A and C). Due to high-fat feeding, RER varied in HFD controls and TEL-treated mice between 0.75 and 0.8, thus indicating fat metabolism, while RER was almost 1.0 in chow-fed controls, prompting utilization of carbohydrates (Fig. 2B). Water intake was reduced by HFD feeding but increased above chow levels as a result of TEL treatment (Fig. 2D).

Effects on glucose homeostasis

While plasma glucose was higher in CON_{HFD} and TEL_{HFD} than in CON_{chow} mice, TEL normalized plasma insulin, suggesting that insulin sensitivity was not impaired as much as in CON_{HFD} mice (Fig. 3A and B). This conclusion is supported by the observation that glucose utilization was better in TEL_{HFD} than in CON_{HFD} as the AUC and the C_{min} levels in response to the ITT were higher (Fig. 3C, D and E).

Effects on hypothalamic lipids

The signal of the neutral lipid containing LD was quantified by epifluorescence microscopy as exemplarily illustrated in Fig. 4A. The LD signal in tanycytes was ten-fold higher in CON_{HFD} than in CON_{chow} and declined to normal in TEL_{HFD} mice (Fig. 4B). Hypothalamic levels of the neutral lipids, triglycerides, and ceramides (Cer) (particularly TG(56:5), TG(56:6), TG(56:7), TG(58:8) and Cer(d36:1) (d18:1/18:0)) were higher in CON_{HFD} than in CON_{chow} and normalized in TEL_{HFD} mice (Fig. 4C). Hypothalamic content of other lipids, including free fatty acids, did not differ between groups with the highest levels of phosphatidylcholine and phosphatidylethanolamine and the lowest levels of diacylglycerol (Supplementary Figs 5 and 6). Analysis of hypothalamic acute lipid metabolism in brain slices showed a lower signal of alkyne oleate-derived lipids in the ARC of CON_{HFD} than in CON_{chow} mice, while no difference was observed in the tanocyte layer. TEL again normalized alkyne oleate signal distribution (Fig. 5A and B). Despite these changes in intrahypothalamic alkyne lipid localization, no alterations in the metabolic fate of the alkyne oleate tracer was observed between the groups (Fig. 5C).

Figure 1

Growth of mice upon chow (CON_{chow}) or HFD feeding plus vehicle treatment (CON_{HFD}) and HFD feeding plus TEL treatment (TEL_{HFD} , 8 mg/kg/day). (A) Gain in body weight ($F^W = 63.0$, $P < 0.0001$); (B) Fat mass ($F^W = 15.5$, $P = 0.0071$); (C) Left ventricular weight ($F = 89.3$, $P < 0.0001$); (D) Liver weight ($F^W = 9.3$, $P = 0.0017$); (E) Kidney weight ($F = 24.9$, $P < 0.0001$); (F) Plasma leptin ($F = 49.1$, $P < 0.0001$); (G) Leptin content in hypothalamus ($F^W = 6.8$, $P = 0.041$); (H) Correlation between final body weight and leptin levels in plasma (left Y-axis, dots, $r = 0.763$, $r = 0.447$) or hypothalamus (right Y-axis, triangles; $r = -0.814$, $P = 0.394$); (I) Change in body weight after 4 days in leptin resistance test (LRT, $F^W = 3.3$, $P = 0.05$). Means $\pm$ s.e.; Statistical differences between groups were calculated by ANOVA followed by Bonferroni's multiple comparison test or Welch ANOVA test (indicated by F^W) followed by Games–Howell's multiple comparisons test in case of variance inhomogeneity. * $P < 0.05$ vs CON_{chow} ; † $P < 0.05$ vs CON_{HFD} .

Effects on hypothalamic inflammation

GFAP immunofluorescence signal was three times higher in the ARC of CON_{HFD} than in CON_{chow} mice but returned to normal levels when HFD-fed mice were simultaneously

treated with TEL. Moreover, the GFAP signal positively correlated with body weight (Fig. 6A). Western blot analysis confirmed the immunohistochemical results (Fig. 6B). Using immunosorbent assays, hypothalamic cytokine levels were quantified in a small subgroup of

Table 1 Adipokines in plasma of mice upon chow (CON_{chow}) or HFD feeding plus vehicle treatment (CON_{HFD}) and HFD feeding plus TEL treatment (TEL_{HFD}, 8 mg/kg/day).

Plasma adipokine	CON _{chow}	CON _{HFD}	TEL _{HFD}	
Resistin (ng/L)	1.50 ± 0.29 (12)	2.9 ± 0.91 (10) ^a	2.69 ± 0.85 (13) ^a	FW = 19.9, <i>P</i> < 0.0001
Pai1 (ng/L)	1.13 ± 0.32 (12)	1.89 ± 0.25 (10) ^a	1.13 ± 0.18 (13) ^b	FW = 3.9, <i>P</i> = 0.038
Il4 (ng/L)	0.31 ± 0.57 (11)	2.87 ± 2.7 (9) ^a	3.02 ± 3.13 (10) ^a	H = 8.4, <i>P</i> = 0.015
Il5 (ng/L)	19.5 ± 22.4 (11)	39.91 ± 28.69 (9)	74.6 ± 74.1 (11) ^a	H = 6.9, <i>P</i> = 0.038
Il6 (ng/L)	n.d. (11)	22.6 ± 26.2 (9)	24.7 ± 40.5 (11)	H = 8.3, <i>P</i> = 0.016
Il13 (ng/L)	39.2 ± 10.9 (9)	63.5 ± 39.5 (9)	43.1 ± 14.5 (8)	F = 2.2, <i>P</i> = 0.331

Means ± s.d.; ^a*P* < 0.05 vs CON_{chow}; ^b*P* < 0.05 vs CON_{HFD} following ANOVA (F) and Bonferroni's Multiple Comparison Test or Welch ANOVA test (indicated by F_w) followed by Games–Howell's multiple comparisons test or Kruskal–Wallis test (indicated by H) and Dunn's multiple comparisons test. The number sizes are enclosed in brackets. IL-6 levels were not detectable (n.d.) in CON_{chow}.

each treatment group. Following ANOVA analysis, TNF α was found to be slightly higher in HFD- than in chow-fed mice. This trend was also observed for IL1 α and IL13. However, it should be considered that the number of samples here is critically low for reliable, robust findings (Fig. 6C). Using the gene array technique, we analyzed hypothalamic expression of 34,471 genes and found that expression levels of 1736 hypothalamic genes differed between CON_{chow} and CON_{HFD} mice, while 301 of these 1736 genes were again normalized by TEL. While gene expression of GFAP, Il1a, Il6, Il13, and TNF was not influenced either by HFD feeding or by TEL treatment, expression levels of neurotrophin 5 and cytochrome c oxidase subunit 5B (mitochondrial) were reduced in CON_{HFD} and those of neurotrophin 5 were normalized in TEL_{HFD} mice (Table 3). In contrast, expression levels of peroxiredoxin 3, SerpinA3N, Cxcl12 (C-X-C motif chemokine 12), and follistatin-like 1 were higher in CON_{HFD} and again normalized in TEL_{HFD} mice (Table 3). Expression of components of the renin angiotensin II system (RAS) was similar between different groups with the exception that expression of ACE was higher in CON_{chow} and CON_{HFD} than in TEL_{HFD} and that of AT₂ receptor was lower in CON_{HFD} than in TEL_{HFD} (Supplementary Fig. 7).

Discussion

Confirming our previous findings (Muller-Fielitz *et al.* 2012, 2014, 2015, Schuchard *et al.* 2015, Winkler *et al.* 2016, Schuster *et al.* 2018), chronic TEL treatment of HFD-fed mice attenuated an increase in body weight, decreased food intake, and normalized leptin sensitivity, locomotion, energy expenditure, and glucose utilization to CON_{chow} levels in the current study as well.

As the aim of inhibiting AT₁ receptors is not primarily to regulate food intake, the question arises as to why chronic ARB treatment has this efficacy. Based on our previous work, we assume that, in particular, CNS-dependent mechanisms may be involved as TEL markedly reduced weight gain and energy intake in rodents fed with HFD but only to a minor extent in transgenic rodents with a brain-specific deficiency in angiotensinogen (Winkler *et al.* 2016). Confirming the notion of an underlying central mechanism, the hypothalamic expression of the orexigenic neuropeptide MCH (melanin-concentrating hormone) was diminished in lean controls but not in obese Zucker rats in response to chronic ARB treatment (Muller-Fielitz *et al.* 2011). In this context, the expression levels of neurotrophin 5,

Table 2 Lipids in plasma of mice upon chow (CON_{chow}) or HFD feeding plus vehicle treatment (CON_{HFD}) and HFD feeding plus TEL treatment (TEL_{HFD}, 8 mg/kg/day).

Lipid (μ M)	CON _{chow} (n = 13)	CON _{HFD} (n = 11)	TEL _{HFD} (n = 12)	
Ceramide	0.72 ± 0.16	1.41 ± 0.38 ^a	0.88 ± 0.06 ^{a,b}	FW = 15.4 <i>P</i> = 0.0002
Cholesterol ester	4020 ± 611	5429 ± 1527 ^a	6054 ± 1213 ^a	H = 18.9, <i>P</i> < 0.0001
Lysophosphatidyl ethanolamine	20.8 ± 5.3	25.0 ± 3.2	20.3 ± 3.8 ^b	F = 4.2, <i>P</i> = 0.023
Lysophosphatidylglycerol	0.56 ± 0.34	0.34 ± 0.06	0.15 ± 0.04 ^a	F = 11.9, <i>P</i> = 0.0001
Phosphatidylcholine	457 ± 57	622 ± 68 ^a	559 ± 80 ^a	F = 17.1, <i>P</i> < 0.0001
Phosphatidylethanolamine	8.9 ± 2.6	13.7 ± 4.1 ^a	14.3 ± 2.2 ^a	F = 11.5, <i>P</i> = 0.0002
Sphingomyelin	82.6 ± 8.9	108.9 ± 26.7 ^a	123.5 ± 18.4 ^a	F = 25.9, <i>P</i> < 0.0001
Triglyceride	155 ± 57	129 ± 21	108 ± 22 ^a	FW = 4.9, <i>P</i> = 0.015

Means ± s.d.; ^a*P* < 0.05 vs CON_{chow}; ^b*P* < 0.05 vs CON_{HFD} following ANOVA (F) and Bonferroni's Multiple Comparison Test or Welch ANOVA test (indicated by F_w) followed by Games–Howell's multiple comparisons test or Kruskal–Wallis test (indicated by H) and Dunn's multiple comparisons test.

Figure 2

Energy homeostasis of mice upon chow (CON_{chow}) or HFD feeding plus vehicle treatment (CON_{HFD}) and HFD feeding plus TEL treatment (TEL_{HFD}, 8 mg/kg/day). (A) Energy expenditure, light ($F = 1.0$, $P = 0.36$); dark ($F = 6.5$, $P = 0.004$); (B) RER; light ($F = 50.2$, $P < 0.0001$); dark ($F^W = 640$, $P < 0.0001$); (C) Energy intake, light ($F = 11.0$, $P = 0.0003$), dark ($F = 41.2$, $P < 0.0001$); (D) Water intake, light ($F = 26.7$, $P < 0.0001$), dark ($F = 53.6$, $P < 0.0001$); (E) Locomotion, light ($F = 0.9$, $P = 0.42$), dark ($F = 4.2$, $P = 0.026$). Means $\pm$ s.d.; Statistical differences between groups were calculated by ANOVA followed by Bonferroni's multiple comparison test or Welch ANOVA test (indicated by F^W) followed by Games-Howell's multiple comparisons test in case of variance inhomogeneity. * $P < 0.05$ vs CON_{chow}; † $P < 0.05$ vs CON_{HFD}.

Figure 3

Glucose control of mice upon chow (CON_{chow}) or HFD feeding plus vehicle treatment (CON_{HFD}) and HFD feeding plus TEL treatment (TEL_{HFD}, 8 mg/kg/day). (A) Baseline glucose (ANOVA: F^W 11.9, $P = 0.0004$); (B) Baseline insulin (ANOVA: F 7.6, $P = 0.0021$); (C) Glucose response upon ITT (2-ANOVA; time: F 128.4, $P < 0.0001$; treatment: F 2.0, $P = 0.16$; interaction: time: F 3.3, $P = 0.0007$); (D) AUC of glucose response upon ITT (ANOVA: F 6.0, $P = 0.0065$); (E) C_{min} of glucose response upon ITT (ANOVA: F 4.8, $P = 0.015$); Means $\pm$ s.d.; Statistical differences between groups were calculated by ANOVA followed by Bonferroni's multiple comparison test or Welch ANOVA test (indicated by F^W) followed by Games-Howell's multiple comparisons test in case of variance inhomogeneity. * $P < 0.05$ vs CON_{chow}; † $P < 0.05$ vs CON_{HFD}.

Figure 4

Neutral lipid analysis via fluorescence microscopy and by LC-MS in hypothalamic brain slices of mice upon chow (CON_{chow}) or HFD feeding plus vehicle treatment (CON_{HFD}) and HFD feeding plus TEL treatment (TEL_{HFD}, 8 mg/kg/day). (A) Illustration of the tanyocyte layer at the third ventricle and staining of lipid droplets (LD) by LD540 for blinded quantification. LD signals in the tanyocyte layer were quantified by employing a threshold value to eliminate signal noise. (B) Quantification of LD signal ($n = 4$ each group, F 64.0, $P < 0.0001$; ANOVA followed by Bonferroni's multiple comparison test) and exemplary micrographs showing LD540 (orange) and DAPI (blue) signals (upper panel) or LD540 only (lower panel). (C) Hypothalamic concentrations of ceramides (lipid: F = 309.9, $P < 0.0001$; treatment: F = 2.8, $P = 0.064$; interaction: F = 2.1, $P = 0.0133$) and triglycerides (two-way-ANOVA upon LC-MS measurement followed by Bonferroni's multiple comparison test, lipid: F = 41.7, $P < 0.0001$; treatment: F = 15.6, $P < 0.0001$; interaction: F = 0.7, $P = 0.831$). Means $\pm$ s.d.; * $P < 0.05$ vs CON_{chow}; † $P < 0.05$ vs CON_{HFD}.

Figure 5

Hypothalamic fatty acid metabolism in slices of mice upon chow (CON_{chow}) or HFD feeding plus vehicle treatment (CON_{HFD}) and HFD feeding plus TEL treatment (TEL_{HFD}, 8 mg/kg/day). Analysis of hypothalamic alkyne lipid distribution by fluorescence microscopy. Exemplary micrographs (A) showing alkyne oleate (green) and DAPI (blue) signals and ROIs (left panel) for ARC (large box) and tanycyte layer (small box). (B) Micrographs were quantified and signals in ARC ($F = 64.0$, $P < 0.0001$) or tanycyte layer ($F = 2.5$, $P = 0.136$) plotted after ANOVA and Bonferroni's multiple comparison test. (C) Analysis of alkyne oleate metabolism in hypothalamic slices of mice upon chow (CON_{chow}) or HFD feeding plus vehicle treatment (CON_{HFD}) and HFD feeding plus TEL treatment (TEL_{HFD}, 8 mg/kg/day) by thin-layer chromatography (TLC); aCE, alkyne cholesteroles; aDAG, alkyne diacylglycerol; aTAG, alkyne triacylglycerol; aOL, alkyne oleate; aPL alkyne phospholipids; two-way ANOVA indicated differences in composition of lipids ($F = 1233$, $P < 0.0001$) without differing between the groups.

a natural ligand for trkB (tropomyosin receptor kinase B) that suppresses both appetite and body weight and increases lipolysis in murine models of obesity (Tsao *et al.* 2008), were interestingly found here to be reduced in CON_{HFD} but normalized in TEL_{HFD} mice. This finding provides further evidence that the hypothalamic neurocircuits for regulating energy intake are affected by TEL. In contrast, downregulation of hypothalamic expression of AT₁ receptors is less involved, as demonstrated here and elsewhere (Muller-Fielitz *et al.* 2011, Schuchard *et al.* 2015, Winkler *et al.* 2016). The pleiotropic potency of ARBs

to stimulate PPAR γ is likely not related to the observed anorectic effects (Muller-Fielitz *et al.* 2012, Schuster *et al.* 2018). As TEL-treated rats preferred chow and the less well-tasting cafeteria diet compared to nontreated controls (Muller-Fielitz *et al.* 2012, 2015, Schuchard *et al.* 2015, Winkler *et al.* 2016), the anti-hedonic behavior may also need to be considered, especially as central RAS regulates reward via the dopaminergic or serotonergic systems (Voigt *et al.* 2008, Narayanaswami *et al.* 2013). We finally investigated whether the potency of ARBs to reduce the drive of the hypothalamic–pituitary–adrenal (HPA) axis contributes to a decrease in food intake. However, we recently demonstrated that stress-induced eating of high-calorie, good-tasting food only plays a minor role or none at all (Gustaityte *et al.* 2018). As outlined in the introduction, the antiobese effect of ARBs is dependent on intact leptin signaling and leptin penetration across the BBB. Considering the major role of hypothalamic lipid metabolism in the physiological regulation of energy balance (Gonzalez-Garcia *et al.* 2017), we hypothesized here that improved hypothalamic lipid metabolism represented a potential effect of reconstituting leptin sensitivity due to TEL treatment. As mentioned, tanycytes have been shown to be a conduit for circulating leptin into the hypothalamus (Muller *et al.* 2010). Blood-borne leptin is taken up by tanycytes in the median eminence and then released into the mediobasal hypothalamus containing the ARC, a process that is disrupted in mice with diet-induced obesity (Balland *et al.* 2014). Taken together with further findings showing that HFD feeding triggers neurogenic activity in median eminence tanycytes (Lee *et al.* 2012), tanycytes might be functionally important for metabolic sensing. Although studies demonstrating an active pathophysiological role of tanycytes in lipid sensing and energy balance control are still lacking, recent evidence suggests that tanycytes play a role in restricting passage of unsaturated fatty acids into the hypothalamus of HFD-fed mice (Hofmann *et al.* 2017).

Hence, the primary aim of the present study was to investigate the effects of chronic TEL treatment on the hypothalamic lipid profile. To this end, we employed an *in vitro* system of acute brain-slice cultures incubated with a state-of-the-art alkyne oleate tracer that highly matches natural oleate in structure and biological behavior (Gaebler *et al.* 2013). Based on this technique we were able to track lipid distribution by fluorescence microscopy and lipid metabolism by TLC analysis. Furthermore, we analyzed cellular lipid droplets, a major site of neutral lipid metabolism and storage (Kuerschner *et al.* 2008, Walther & Farese 2012) by fluorescence microscopy.

Figure 6

Markers of inflammation in hypothalami of mice upon chow (CON_{chow}) or HFD feeding plus vehicle treatment (CON_{HFD}) and HFD feeding plus TEL treatment (TEL_{HFD}, 8 mg/kg/day). (A) Exemplary immunofluorescence micrographs of GFAP expression in the ARC used for signal quantification ($F_W = 6.3$, $p = 0.027$) and in correlation to body weight; (B) Western blot analysis of GFAP expression ($F_W = 4.6$, $p = 0.036$); (C) Hypothalamic levels of cytokines (TNFα: $F = 4.2$, $P = 0.042$, IL1α: $F = 4.1$, $P = 0.07$, IL13: $F = 2.3$, $P = 0.21$) determined by immunosorbent assays. Means $\pm$ s.d.; Statistical differences between groups were calculated by ANOVA followed by Bonferroni's Multiple Comparison Test or by Welsh ANOVA test (indicated by F_W) followed by Games–Howell's multiple comparisons test in case of variance inhomogeneity. * $P < 0.05$ vs CON_{chow}; † $P < 0.05$ vs CON_{HFD}.

While confirming Hofmann's findings regarding obese CON_{HFD} mice (Hofmann *et al.* 2017), the current study newly demonstrates that TEL treatment not only re-establishes that unsaturated oleic acid labels hypothalamic astrocytes under HFD conditions but also

that the LD content of tanycytes that increases as a result of HFD is normalized. Apart from neutral glycerolipids, LDs also contain sterol esters and sphingolipids (Senkal *et al.* 2017). Such a lipid pattern was confirmed in our LC-MS analyses as levels of TGs and ceramides increased

Table 3 Expression of various genes in hypothalami of mice upon chow (CON_{chow}) or HFD feeding plus vehicle treatment (CON_{HFD}) and HFD feeding plus TEL treatment (TEL_{HFD}, 8 mg/kg/day).

	Signal (log 2)			Fold change (P-value)		
	CON _{chow}	CON _{HFD}	TEL _{HFD}	CON _{chow} vs CON _{HFD}	CON _{chow} vs TEL _{HFD}	CON _{HFD} vs TEL _{HFD}
GFAP	9.21	9.09	9.06	1.09 (0.958)	1.11 (0.149)	1.02 (0.205)
Il1alpha	3.72	3.77	3.70	0.97 (0.798)	1.02 (0.333)	1.05 (0.314)
Il6	3.02	2.93	2.95	1.06 (0.029)	1.05 (0.198)	0.98 (0.344)
Il13	5.29	5.22	5.27	1.05 (0.633)	1.02 (0.942)	0.97 (0.539)
TNFα	4.71	4.68	4.78	1.02 (0.528)	0.95 (0.637)	0.93 (0.326)
Interleukin 1 beta	3.44	3.27	3.50	1.13 (0.123)	0.96 (0.451)	0.83 (0.022)
Neurotrophin 5	4.74	4.44	4.66	1.23 (0.000)	1.06 (0.286)	0.84 (0.003)
Peroxiredoxin 3	8.21	8.48	8.35	0.79 (0.000)	0.90 (0.030)	1.10 (0.071)
SerpinA3N	9.17	9.64	9.12	0.62 (0.000)	1.03 (0.639)	1.43 (0.000)
Cxcl12	6.25	6.44	6.42	0.86 (0.001)	0.88 (0.025)	1.01 (0.615)
Follistatin-like 1	7.28	7.58	7.31	0.77 (0.001)	0.98 (0.943)	1.21 (0.002)
Cytochrome c oxidase subunit 5B (mitochondrial)	10.88	10.73	10.69	1.11 (0.002)	1.14 (0.000)	1.03 (0.428)

$n = 20$ each group.

in the hypothalami of CON_{HFD} mice but were normalized by TEL. It is worthy of note that LDs appear to play a crucial role in mitigating ER stress caused by excess of saturated fatty acids on HFD (Welte & Gould 2017). Recent evidence indicates that overnutrition causes ER stress by increasing ceramide levels in the hypothalamus, inducing insulin and leptin resistance (Contreras *et al.* 2014). Conversely, inhibiting central ceramide synthesis restores insulin signaling in the hypothalamus of obese Zucker rats (Campana *et al.* 2018). Accordingly, our observed boost in hypothalamic TGs and ceramides in CON_{HFD} mice and their normalization upon TEL treatment may indicate an influence of the drug on ER stress. Thus, this pathway must additionally be considered for maintaining insulin sensitivity under ARB treatment, although other pleiotropic effects such as PPAR γ -dependent or HPA-dependent mechanisms are well established (Kintscher *et al.* 2008, Miesel *et al.* 2012).

As the accumulation of unfolded or misfolded proteins due to ER stress can initiate inflammation (Zhang & Kaufman 2008), hypothalamic lipotoxicity and inflammation are inextricably linked with each other. Hypothalamic inflammation was shown to induce central leptin resistance associated with the development of obesity (De Souza *et al.* 2005). Remarkably, inflammation can be detected in the hypothalamus after only 1 day of HFD feeding and, therefore, before body weight has been gained, suggesting a cause in the pathophysiology of obesity (Thaler *et al.* 2012). Moreover, reduced leptin transition across the BBB of obese mice (serving as an indicator for leptin resistance) was also attributed to a reactive gliosis due to low-grade inflammation as described in the hypothalamus of obese rodents and humans (Thaler *et al.* 2012). Accordingly, HFD feeding was associated with an impairment in BBB structure due to astrocytic gliosis such that feeding behavior regulating POMC (pro-opiomelanocortin) and NPY (neuropeptide Y) neurons became less accessible to blood-borne metabolic signals (Horvath *et al.* 2010).

Considering all these findings, we further investigated the anti-inflammatory potential of TEL during HFD feeding. In accordance with the established knowledge that upregulation of GFAP serves as a common marker of astrogliosis (Filous & Silver 2016, Li *et al.* 2019), we have indeed confirmed previous findings (Lee *et al.* 2018) that hypothalamic inflammation and abnormal glucose metabolism develop in cases of overnutrition-induced obesity. Normalized GFAP protein levels and immunoreactivity in TEL_{HFD} mice may consequently indicate that astrogliosis can be prevented by blocking AT₁

receptors despite HFD feeding. This finding is furthermore in line with the reduction of TNF α and IL1 α levels in these animals. Both TNF α and IL1 α were observed to activate NF- κ B (nuclear factor kappa B) in cultured hypothalamic neurons (Jang *et al.* 2010), which can induce SOCS3 (suppressor of cytokine signaling 3) and PTP1B (protein tyrosine phosphatase 1B) expression and result in leptin resistance (de Git & Adan 2015). However, we did not detect an influence of either HFD feeding or TEL treatment on gene expression of GFAP, IL1 α , or TNF α . This may be attributable to post-transcriptional and translational protein degradation regulation. Hypothalamic inflammation as a result of obesity and its prevention by TEL is once more confirmed by our Affimetrix arrays as expression levels of SerpinA3N, follistatin-like 1, and Cxcl12, which all have been implicated in nutritionally mediated hypothalamic inflammation, were increased in CON_{HFD} and again normalized in TEL_{HFD}. With respect to tissue specificity, the anti-inflammatory effect of TEL is likely not a widespread feature. This notion is supported by the fact that TEL affects the levels of the pro- and anti-inflammatory cyto- and adipokines such as resistin, IL-4, and IL-5 predominately in CNS but not in plasma.

As recently identified, a lack of body weight gain despite HFD upon TEL treatment is partly mediated by an off-target mechanism via an ACE2/angiotensin-(1-7)/Mas-dependent pathway (Schuchard *et al.* 2015). Indeed, several studies support an anti-inflammatory effect of the ACE2/Ang-(1-7)/Mas axis (Simões e Silva *et al.* 2013), as i.c.v infusion of Ang-(1-7) was found to reduce NF- κ B activity in brain tissue after cerebral ischemia, which was associated with decreased expression levels of TNF- α and IL-1 β (Jiang *et al.* 2012). However, there is a lack of data concerning an Ang-(1-7) effect on hypothalamic lipid metabolism, although peripheral lipids are decreased by Ang-(1-7) (Moreira *et al.* 2017). In addition, several *in vitro* and *in vivo* studies have determined that TEL itself shows anti-(neuro) inflammatory potential (Saravanan *et al.* 2015, Xu *et al.* 2015, Li *et al.* 2016). Interestingly, a TEL-associated reduction in adipogenesis and body weight gain was blocked after PPAR- δ gene knockout (He *et al.* 2010). Considering that PPAR- δ is involved in controlling energy homeostasis by stimulating genes for fatty acid catabolism and is thought to protect against the neuronal effects of saturated fatty acids (Poon *et al.* 2015), a further potential mechanism of TEL's anti-obese effect becomes apparent.

In summary, our data show that TEL treatment normalizes hypothalamic ceramide levels, TG levels, and alkyne oleate distribution despite HFD. The abolished

accumulation of neutral lipids and lipotoxicity are further accompanied by an anti-inflammatory effect in the murine hypothalamus. These findings further add to the knowledge of the weight-regulating effects of TEL in the body (Supplementary Fig. 8).

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/JOE-19-0319>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

E R, L K, L N, F S, I S, A F, K S, N H, A O, and W R performed the research; E R, L K, and W R designed the research study; E R, A O and W R analyzed the data; and W R and E R wrote the paper.

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