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REGULAR ARTICLE

Regulation of tyrosine hydroxylase in periodontal fibroblasts and tissues by obesity-associated stimuli

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Abstract

Tyrosine hydroxylase (TH) catalyzes the rate-limiting step in the synthesis of catecholamines and has been connected to aggravated progression of periodontal disease under chronic stress. Obesity is known to increase the risk of periodontitis and adipokines have been suggested to be a pathomechanistic link. This study examines if obesity-associated stimuli have regulatory effects on TH levels in periodontal cells and tissues. Human periodontal ligament fibroblasts were cultured in the presence of leptin or visfatin for up to 2 days. Untreated cells served as control. TH regulation was analyzed by real-time PCR, immunocytochemistry and ELISA. TH gene expression in periodontal tissues of normal-weight and obese rodents was determined. Examination of gingival biopsies from rats and patients with and without periodontal disease was performed by real-time PCR or immunohistochemistry. For statistics, ANOVA and post hoc tests were applied ($p < 0.05$). In vitro, TH gene expression and protein levels were increased by leptin and visfatin. In vivo, TH gene expression was upregulated in periodontal tissues of obese rodents as compared to normal-weight animals. Additionally, increased TH gene expression was found in rat gingival biopsies with experimental periodontitis. Human gingival biopsies from sites of periodontitis confirmed the animal data by demonstrating elevated TH levels at periodontally diseased sites. This study provides original evidence that obesity-associated stimuli induce a TH upregulation in periodontal cells and tissues. Since TH levels were also increased at periodontitis sites, our in vitro and animal findings suggest that this enzyme could represent a pathomechanism whereby obesity contributes to periodontitis.

Keywords Tyrosine hydroxylase · Periodontal ligament · Periodontitis · Obesity · Adipokines

Introduction

Periodontitis is currently the most common chronic inflammatory disease, which may begin in childhood but has the highest occurrence in adults aged 30 years and older (*Slots* 2017). This complex infectious disease is characterized by the irreversible destruction of the periodontium caused by oral pathogenic microorganisms present in dental plaque and by the host immune response. The periodontium comprises the tooth-supporting tissues, i.e., gingiva, periodontal ligament (PDL), root cementum and alveolar bone. PDL fibroblasts, which play a major role in extracellular matrix synthesis and remodeling, are the dominant cell type in the PDL (*Marchesan* et al. 2011). The etiology of periodontitis is multifactorial and different cofactors, such as smoking, genetic factors and occlusal loading, are critical for the initiation and progression of the disease (*Tatakis and Kumar* 2005). Periodontitis is the primary reason for tooth loss in adult

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patients but pocket formation and resultant tooth loss are not the only consequences of untreated periodontitis (*Tatakis and Kumar* 2005; *Yucel-Lindberg and Båge* 2013). Multiple studies describe associations between periodontitis and a number of other diseases related to general health, such as diabetes mellitus, myocardial infarction and obesity (*Chaffee and Weston* 2010; *Chávarry* et al. 2009; *Chistiakov* et al. 2016; *Nibali* et al. 2013; *Suvan* et al. 2011).

Obesity is a chronic metabolic disease and one of the most prevalent public health problems, with a continuously increasing prevalence over the last decades (*Martinez-Herrera* et al. 2017). Obese patients, in addition to other systemic problems, have an increased risk of periodontitis (*Chaffee and Weston* 2010; *Martinez-Herrera* et al. 2017). Exact underlying mechanisms of this connection may not have been unraveled yet but adipokines seem to be an important pathomechanistic link (*Deschner* et al. 2014; *Nokhbehsaim* et al. 2014; *Nokhbehsaim* et al. 2013a, 2013b). Adipokines are synthesized and secreted by adipocytes and other cells, such as leukocytes, in the adipose tissue, which is considered to be a complex endocrine and highly active metabolic organ (*Adamczak and Wiecek* 2013; *Krysiak* et al. 2012; *Raucci* et al. 2013; van de *Voorde* et al. 2013). Leptin and visfatin, two of these bioactive molecules, are produced not only in adipose tissue but also locally in periodontal cells and tissues (*Damanaki* et al. 2014; *Deschner* et al. 2014; *Nogueira* et al. 2014; *Nokhbehsaim* et al. 2014; *Nokhbehsaim* et al. 2013a). Their plasma levels are increased in obese individuals (*Chang* et al. 2011; *Considine* et al. 1996). Leptin is an appetite suppressant and stimulates energy consumption but it also modulates the immune system and the inflammatory response (*Carbone* et al. 2012; La Cava 2012; *Krysiak* et al. 2012; *Paz-Filho* et al. 2012; *Procaccini* et al. 2012). Visfatin is a nicotinamide phosphoribosyltransferase and is important for cell metabolism. Moreover, this molecule induces production of inflammatory mediators and nuclear factor-kappaB activation (*Moschen* et al. 2007). Therefore, both adipokines influence not only metabolic regulation but also inflammatory and wound healing processes in the periodontium (*Conde* et al. 2011; *Nokhbehsaim* et al. 2014; Nokhbehsaim et al. 2013 b). However, the exact cellular mechanisms remain unclear.

Tyrosine hydroxylase (TH) is a tetrahydrobiopterin-requiring, iron-containing monooxygenase and catalyzes the rate-limiting step in the synthesis of catecholamines, which is the conversion of L-tyrosine to L-dopa (*Nagatsu* 1995). Catecholamines are central and sympathetic neurotransmitters and adrenomedullary hormones, which are commonly known as stress hormones (*Boyanova* 2017). Under long-term stress conditions, TH protein has been found to be upregulated (*Nagatsu* 1995). Obesity is known to be a stressful condition in which sympathetic nerve activity has already been shown to play a major role (*Guarino* et al. 2017; *Guilherme* et al. 2017). Interestingly, a consistent increase of TH levels with adipose sympathetic nerve activity has already been described in mice and TH activity has been associated with obesity in bovine adrenal medullary cells (*Guilherme* et al. 2017; *Shibuya* et al. 2002). TH has also been shown to play a major role in the aggravated periodontal destruction under chronic stress (*Lu* et al. 2014). Its expression was demonstrated to be increased in periodontal tissues of rats with ligature-induced periodontitis and then further elevated by chronic stress correlating with an increased periodontal destruction (*Lu* et al. 2014).

However, the role and regulation of TH in the interactions of obesity and periodontal destruction have been neglected so far. In this study, we analyze if obesity-associated stimuli have regulatory effects on TH levels in periodontal cells and tissues. Our hypothesis is that adipokines and obesity induce an upregulation of TH levels in periodontal cells and tissues.

Material and methods

Culture and treatment of cells

After informed consent of the patient or legal guardian and approval of the Ethics Committee of the University of Bonn (#117/15), PDL fibroblasts from caries-free and periodontal healthy donors (mean age 18 years, min-max 11–42 years) were harvested. Cells were explanted from the middle third of the tooth root after extraction during orthodontic treatment and cultured in Dulbecco's modified essential medium (DMEM, Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal bovine serum (FBS, Invitrogen), 100 units penicillin and 100 µg/ml streptomycin (Invitrogen) at 37 °C in a humidified atmosphere of 5% CO_2 for 2 to 4 weeks (*Basdra and Komposch* 1997; *Mariotti and Cochran* 1990). Cells at third passage were phenotyped with known specific markers and subsequently used between third and fifth passages (*Basdra and Komposch* 1997; *Rath-Deschner* et al. 2009). The cells were seeded (5000 cells/cm^2) on culture plates and grown to 80% confluence and 1 day prior to the experiments, FBS concentration was reduced to 1%. Physiological concentrations of the obesity-associated molecules leptin (1–10 ng/ml; R&D Systems, Minneapolis, MN, USA) and visfatin (30–300 ng/ml; Biomol, Hamburg, Germany) were used for fibroblast stimulation in vitro (*Nokhbehsaim* et al. 2014; *Nokhbehsaim* et al. 2013a, 2013b; *Pradeep* et al. 2011; *Raghavendra* et al. 2012; *Zimmermann* et al. 2013). Untreated cells served as control.

Analysis of gene expression

After exposure to leptin or visfatin or no treatment for 24 h, RNA of PDL fibroblasts ($n = 9$ replicates derived from three donors) was obtained with a commercially available RNA extraction kit (RNeasy Protect Minikit, Qiagen, Hilden,

Germany) and transcribed to cDNA with the iScript Select cDNA Synthesis Kit (Bio-Rad Laboratories, Munich, Germany). Gene expression of TH was determined by real-time PCR in an iCycler iQ detection system (Bio-Rad Laboratories) with a 25-μl reaction mixture consisting of 1 μl of cDNA, 2.5 μl respective QuantiTect Primer Assay (Qiagen), 12.5 μl QuantiTect SYBR Green Master Mix (Qiagen) and 9 μl of nuclease-free water. The applied protocol followed the manufacturer's instructions and comprised a heating phase at 95 °C for 5 min to activate the enzyme, 40 cycles including a denaturation step at 95 °C for 10 s and a combined annealing/extension step at 60 °C for 30 s per cycle. Glyceraldehyde-3-phosphate dehydrogenase served as a housekeeping gene for normalization.

Analysis of protein levels

For immunocytochemistry, PDL fibroblasts of three donors were grown to 80% confluence on glass coverslips (Carl Roth, Karlsruhe, Germany) and either treated with leptin (3 ng/ml) or visfatin (100 ng/ml) or left untreated for 24 h. Cells were fixed in 4% paraformaldehyde (Sigma-Aldrich, Munich, Germany) at pH 7.4 and room temperature (RT) for 10 min. Permeabilization was carried out with 0.1% Triton X-100 (Sigma-Aldrich) for 5 min. Incubation with serum block (Dako, Hamburg, Germany) for 20 min prevented nonspecific background staining and was followed by application of rabbit polyclonal antibody TH (5 μg/ml; R&D Systems) at 4 °C overnight. Subsequently, the cells were treated with secondary antibody goat anti-rabbit IgG-HRP (Dako) for 45 min. An exposition to DAB chromogen (Thermo Fisher Scientific, Waltham, MA, USA) for 10 min at RT in the dark made the antibody binding visible. Multiple washing steps with PBS were carried out throughout the procedure (Invitrogen). Counterstaining with Mayer's hematoxylin (Merck Eurolab, Dietikon, Switzerland) for 1 min finished the process and the coverslips were mounted with DePex mounting medium (Serva Electrophoresis, Heidelberg, Germany) on microscope slides. An Axioplan 2 imaging microscope (Carl Zeiss, Jena, Germany) was used for taking standardized photomicrographs with an AxioCam MRc camera (Carl Zeiss) and AxioVision 4.7 software (Carl Zeiss). Images of one representative donor are shown.

Moreover, protein amounts of TH in lysates of PDL fibroblast ($n = 9$ replicates derived from three donors) were quantified by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Abbexa, Cambridge, UK). Cells were stimulated with proinflammatory adipokines leptin and visfatin for 24 h and 48 h. Manufacturer's instructions were executed precisely and absorbance was measured with a microplate reader (PowerWave x, BioTek Instruments, Winooski, VT, USA) at 450 nm. Data were normalized by BCA values (Pierce™ BCA Protein Assay Kit; Thermo Fisher Scientific).

Diet-induced obesity models

The animal experiments were conducted according to the ethical standards of the University of Bonn (AZ 84-02.04.2012.A131/84-02.04.2016.A202 and AZ 87-51.04.2010.A394). All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Eight male C57BL/6 mice were acquired (Charles River, Sulzfeld, Germany) and kept in a temperature (23 ± 1 °C)- and humidity (40–65%)-controlled environment with a 12-h dark-light cycle and food and water ad libitum. The four animals of the experimental group were given a normal diet for 6 weeks and, subsequently, a high-fat diet (HFD; D12492, ssniff Spezialdiäten) (*Damanaki* et al. 2018a). The four animals of the control group received a normal diet (D12450B, ssniff Spezialdiäten, Germany) during the entire study. All animals were sacrificed at week 19 and their jaws were harvested and hemisected for further analysis including RNA extraction and real-time PCR (see "Analysis of gene expression" section).

Ten male Wistar rats (Charles River, Sulzfeld, Germany) were purchased and housed in a temperature (21 °C)- and humidity (35%)-controlled environment with a 12-h dark-light cycle. Food and water were given ad libitum. The test group consisted of five animals, which were first fed a normal diet until week 4 and subsequently received a high-fat (25.1% fat), high-sucrose (28.8% sucrose) diet (HFSD, sniff, Germany) until week 15. The five animals of the control group were kept at a normal diet for the entire 15 weeks. Animal weight was measured and documented weekly (*Damanaki* et al. 2018b). At week 15, all animals were sacrificed for further analyses. The jaws were dissected and the gingival tissues were harvested for RNA extraction and real-time PCR (see "Analysis of gene expression" section).

Experimental periodontitis model

Animal experiments were carried out with consent of the Ethical Committee on Animal Experimentation (protocol number: 23/2012) from the School of Dentistry at Araraquara, University Estadual Paulista (UNESP) and in accordance with the recommendations of the ARRIVE guidelines. TH gene expression was studied in gingival biopsies of rats with ligature-induced periodontitis and compared to healthy controls (*Nogueira* et al. 2017). Male adult Holtzman rats (average weight 300 g) were kept under controlled conditions (22–25 °C, 12-h light/dark cycle, standard laboratory diet and water ad libitum) and housed in plastic cages in the animal facilities of the School of Dentistry at Araraquara. Eight rats were randomly assigned into two

groups: control (sham-operated) and ligature-induced periodontal disease. All animals were anesthetized with intramuscular injections of ketamine chlorhydrate 10% (0.08 ml/100-g body weight) and xylazine chlorhydrate 2% (0.04 ml/100-g body weight). Periodontitis was induced by cotton ligatures located at the cervical area of both maxillary first molars, fixed with a mesial knot and left in place for 6 days. After the assigned time, the animals were sacrificed by an anesthetic overdose. Maxillary jaws and gingival tissues around maxillary first molars were collected and further analyzed.

Human biopsies

After approval of the Ethics Committee of the University of Bonn was granted (#043/11) and patients gave written and informed consent, biopsies of three periodontally healthy donors as well as biopsies of inflamed tissue of periodontally diseased sites of three diagnosed periodontitis patients were harvested in the Department of Oral Surgery of the University of Bonn (*Damanaki* et al. 2014; *Memmert* et al. 2018; *Memmert* et al. 2017). The patients were examined by specialists from the Clinic of Dental Medicine and categorized as healthy when gingival index (GI) = 0 (no clinical inflammation), probing pocket depth (PD) ≤ 3 mm and neither clinical nor radiographic bone loss was determined. Patients of the periodontitis group presented with sites of GI > 1 and PD ≥ 5 mm and clinical as well as radiographic bone loss ≥ 3 mm. Smokers and patients taking any kind of medication or patients suffering from systemic diseases were excluded. Healthy human gingival specimens were obtained during wisdom teeth removal or tooth extractions for orthodontic reasons. Patients were diagnosed as periodontally healthy by the criteria described above. Inflamed periodontal tissue was obtained during tooth extractions for periodontal reasons at the site of periodontal destruction, which was classified as mentioned above. The amount of tissue and the removal site varied between the samples because due to ethical reasons, only tissue was taken that would have otherwise been discarded.

Tissues were fixed in 4% phosphate-buffered paraformaldehyde (Sigma-Aldrich) for 48 h. With hydrating and dehydrating steps in an ascending ethanol series (AppliChem, Darmstadt, Germany), tissues were prepared for paraffin embedding (McCormick Scientific, Richmond, IL, USA). Serial sections of 3-μm thickness were cut. These slices were dried on microscope slides (Carl Roth) overnight at 37 °C. For the immunostaining process, the thin tissue slices were deparaffinized, rehydrated and rinsed with TBS (TRIS und NaCl, MP Biomedicals, Illkirch, France; Merck, Darmstadt, Germany) for 10 min. Next, endogenous peroxidase was blocked with 0.3% methanol (AppliChem)/H_2O_2 (Merck) solution for 10 min. Serum block (Dako) for 20 min was applied, to avoid nonspecific antigen binding. After the two blocking steps, rabbit polyclonal antibody to TH (2 μg/ml; R&D Systems) was administered overnight in a humid chamber at 4 °C. Secondary antibody goat anti-rabbit IgG-HRP secondary antibody (Dako) was then applied at RT for 30 min followed by DAB chromogen (Thermo Fisher Scientific) incubation for 10 min. Counterstaining with Mayer's hematoxylin (Merck) for 1 min was followed by dehydrating and coverslipping the biopsies. Standardized pictures were taken with an AxioCam MRc camera (Carl Zeiss) at an Axioskop 2 microscope (Carl Zeiss).

Statistical analysis

For statistics, IBM SPSS Statistics software (Version 22, IBM SPSS, Chicago, IL, USA) was used. Mean values were computed. All experiments were performed in triplicate and reproduced at least twice. For statistical comparisons between the two groups, parametric (Student's *t* test) and nonparametric (Mann-Whitney *U* test) tests were used. ANOVA followed by the post hoc Dunnett's test was used for multiple comparisons in order to calculate significance. Differences between groups were considered significant at $p < 0.05$. Immunohistochemistry and immunocytochemistry were semiquantitatively evaluated based on nuclear and cytoplasmic immunoreactivity against TH primary antibody.

Results

Regulation of TH expression by adipokines

First, we analyzed TH regulation in fibroblasts exposed or not to adipokines. Treatment of PDL fibroblasts with either leptin or visfatin for 24 h resulted in a significantly increased TH gene expression (Fig. 1a). Exposure of the PDL cells to leptin led to a strong upregulation by 78.9-fold while visfatin induced an upregulation by 9.1-fold. Furthermore, stimulatory effects were dose dependent (Fig. 1b, c).

The upregulation of TH by adipokine stimulation on transcriptional levels was also observed at protein level. As analyzed by immunocytochemistry, exposure to leptin or visfatin for 1 day led to an increased immunoreactivity against TH, as compared to untreated cells. TH protein was shown to be distributed throughout the cell cytoplasm of stimulated PDL fibroblasts (Fig. 2a–c). Furthermore, significantly elevated TH protein levels were found in lysed cells after 1 day of leptin stimulation and 2 days of visfatin stimulation as compared to control fibroblasts as determined by the ELISA test (Fig. 2d, e). Therefore, TH expression was significantly induced by the two adipokines on the transcriptional as well as on the protein level.

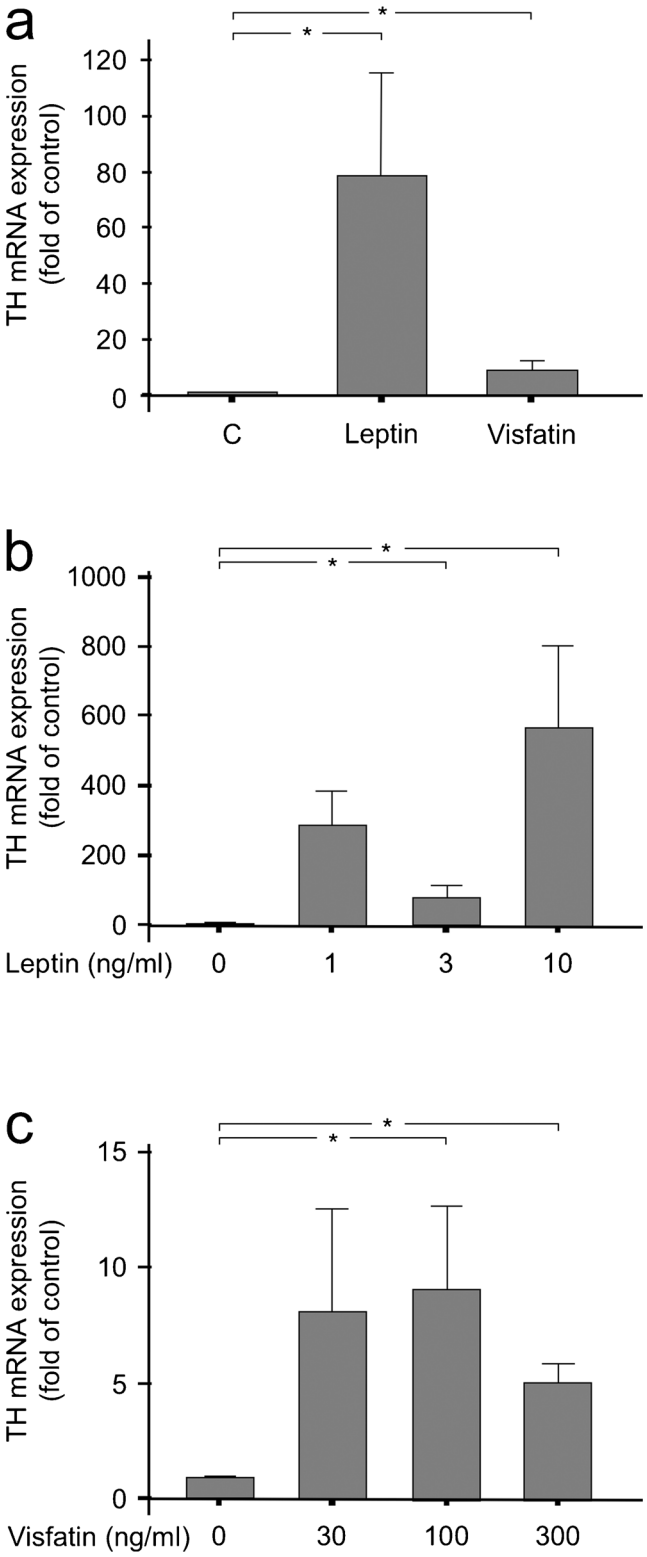

Fig. 1 **a** Effects of leptin (3 ng/ml) or visfatin (100 ng/ml) on TH gene expression in PDL fibroblasts at 1 day. Mean ± SEM ($n = 9$); *significant ($p < 0.05$) difference between groups. **b**, **c** Dose response of TH gene expression to varying concentrations of leptin (1–10 ng/ml) or visfatin (30–300 ng/ml) in PDL fibroblasts at 1 day. Untreated cells served as control. Mean ± SEM ($n = 9$); *significant ($p < 0.05$) difference between groups

Regulation of TH expression by diet-induced obesity

In order to evaluate TH regulation in a more complex situation, diet-induced obesity models were used. TH gene expression changes in periodontal tissues of obese mice and rats were compared to TH gene expression changes in normal-weight controls. In both species, mice and rats, a significant upregulation of TH gene expression in gingival tissues of obese animals as compared to control animals was found (Fig. 3a, b). These results show that obesity leads to an elevated TH gene expression in periodontal tissues of the affected rodents.

Regulation of TH expression by periodontitis

Furthermore, TH regulation was analyzed in inflamed periodontal tissues. To determine TH expression in periodontal destruction, an animal model with ligature-induced periodontitis was used. TH expression was increased in tissue biopsies of periodontally diseased rats as compared to control animals (Fig. 3 c). To further expand and complete our findings, we finally studied TH expression in human biopsies. Immunohistochemistry was performed on human periodontal tissues of inflamed and healthy sites and inflamed sites showed enhanced TH protein levels in fibroblasts of the gingival lamina propria (indicated by arrows), in cells of the inflammatory infiltrate and in endothelial as well as in epithelial cells (Fig. 3 d–f).

Discussion

The results of the present study suggest that TH levels are increased after challenge with the obesity-associated molecules leptin and visfatin in human PDL fibroblasts. TH was also elevated in periodontal tissues of obese rodents as compared to healthy control animals. Therefore, our in vitro and in vivo study provides original evidence that obesity-associated stimuli induce an upregulation of TH in periodontal cells and tissues. Moreover, periodontally diseased animals as well as human periodontitis patients showed a TH upregulation in periodontal tissues.

In the present study, TH levels were increased after leptin and visfatin stimulation and these results were consistent at transcriptional and protein levels. Leptin-induced changes of TH levels have been described in adrenal medullary cells, where an increased activity of TH was observed after leptin treatment (*Shibuya* et al. 2002). But to our knowledge, our study is the first one linking increased levels of TH with visfatin stimulation of cells. As both stimulants are proinflammatory adipokines, similar effects could have been expected. Future studies should unravel the exact mechanisms by which adipokines increase TH expression in PDL fibroblasts.

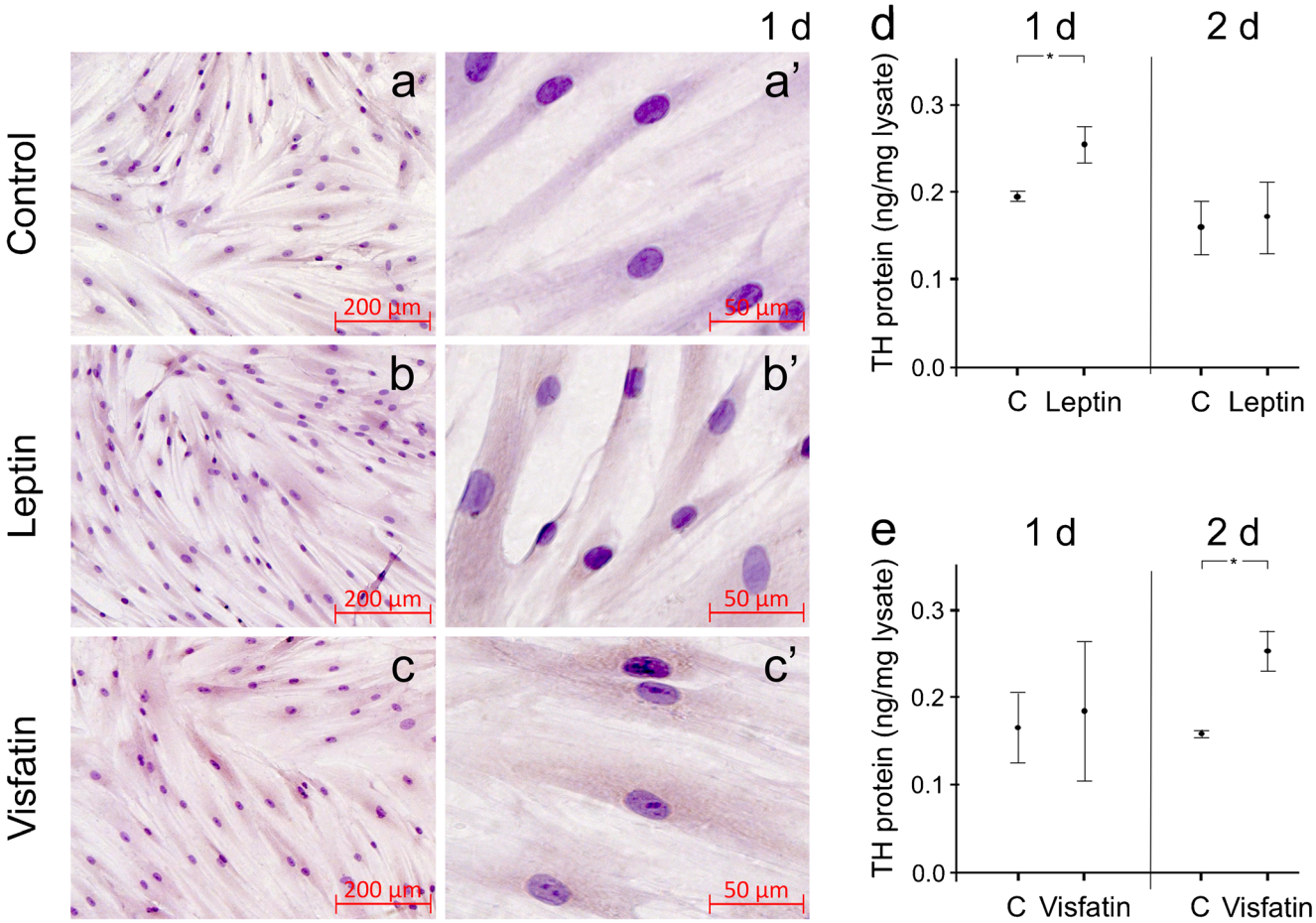

Fig. 2 (a–c) Immunocytochemistry analysis of TH protein levels in PDL fibroblasts after incubation with leptin (3 ng/ml; b, b') or visfatin (100 ng/ml; c, c') for 1 day. Untreated cells served as control (a, a'). PDL fibroblasts of three donors were analyzed and images of one representative donor are shown. **d**, **e** TH protein levels in lysed PDL fibroblasts after stimulation with leptin (3 ng/ml; **d**) or visfatin (100 ng/ml; **e**) for 1 day and 2 days as analyzed by ELISA. Untreated cells served as control. Mean ± SEM ($n = 9$); *significant ($p < 0.05$) difference between groups

The stimulus excited by both adipokines was dose dependent. As plasma levels of both adipokines are increased in obese individuals as compared to healthy controls, the dose dependency of TH expression further supports our findings that TH might also be elevated in obese individuals as compared to healthy controls (*Chang* et al. 2011; *Considine* et al. 1996; *Taşkesen* et al. 2012). But future studies are needed to analyze TH levels in periodontal tissues of obese individuals.

In order to study the TH gene expression in the more complex in vivo setting, animal models were used. As expected, TH expression was significantly increased in gingival tissues of obese mice and rats as compared to control animals. The stimulation of neuronal signaling by adipocytes in mature mice has already been under investigation. It was shown that obese mice display elevated TH levels in inguinal white adipose tissue (*Guilherme* et al. 2017).

Elevations of TH levels have already been related to periodontitis (*Lu* et al. 2014). In the study of Lu and coworkers, the effect of chronic stress on periodontal destruction was examined. It was found that chronic stress accelerated the destruction of the periodontium by TH upregulation and subsequently the activation of the sympathetic nervous system. The sympathetic nervous system impacts immune responses, which result in accelerated periodontal destruction. Our results support and expand the findings of Lu and colleagues as we showed TH regulation in human PDL fibroblasts on gene expression and protein levels. Moreover, we were able to extrapolate our in vivo data from rat gingival biopsies to human periodontitis patients. Our study demonstrated that fibroblasts of the gingiva propria, cells of the inflammatory infiltrate and endothelial as well as epithelial cells contribute to the elevated TH levels in periodontal inflammation. However, future studies are needed to further elucidate the complex effects of TH in periodontal cells and tissues.

Stimulation with adipokines has been used in numerous studies for in vitro obesity research (*Damanaki* et al. 2014; *Nokhbehsaim* et al. 2013a, 2013b; *Nokhbehsaim* et al. 2014). Furthermore, leptin and visfatin have been linked to periodontitis and strongly suggested to be the pathomechanistic

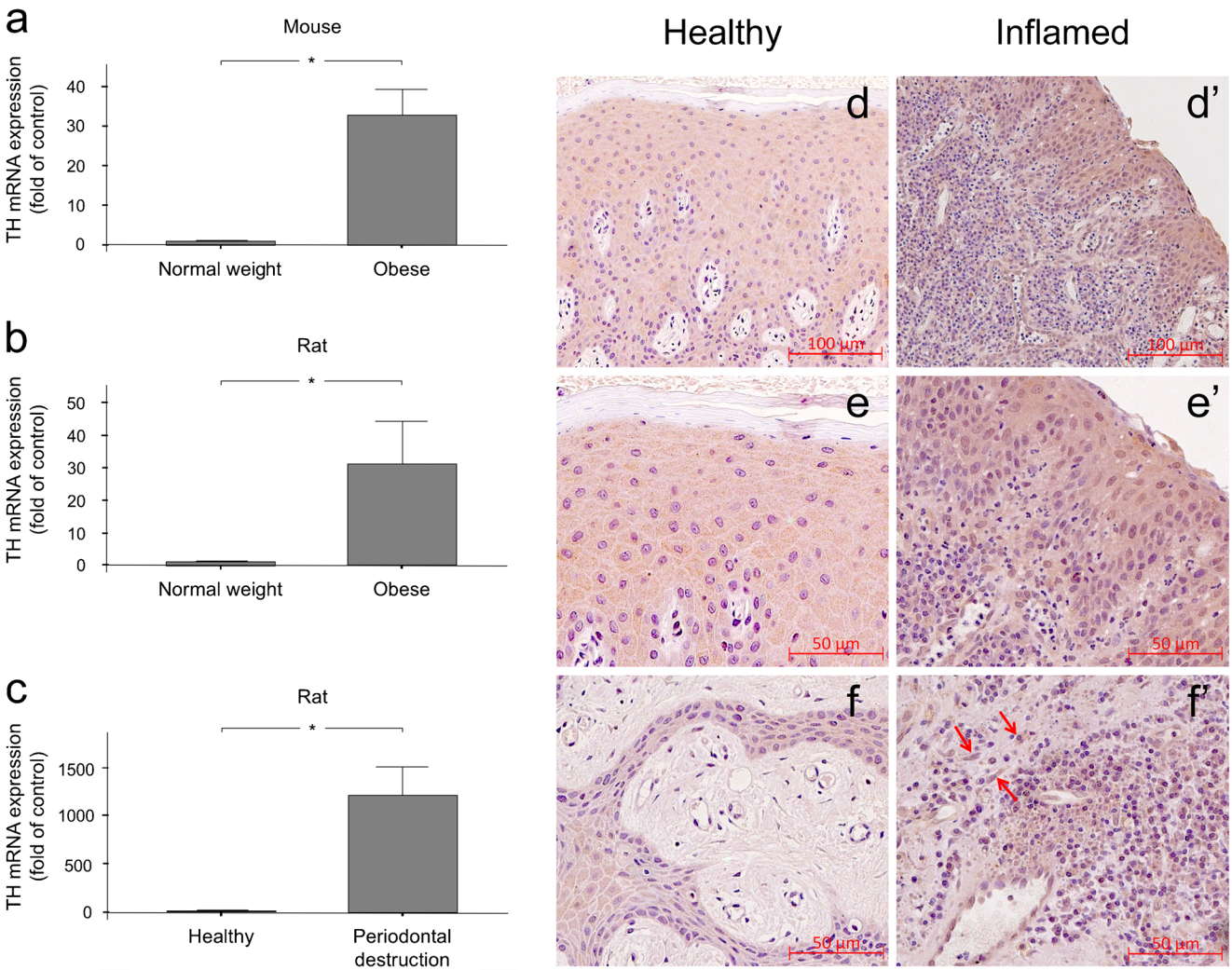

Fig. 3 **a**, **b** TH gene expression in periodontal tissues of obese mice and rats, as compared to normal-weight controls. Mean ± SEM ($n = 4$ mice/group; $n = 5$ rats/group); *significantly ($p < 0.05$) different from normal-weight controls. **c** TH gene expression in gingival biopsies of rats with ligature-induced periodontitis as compared to healthy controls. Mean ± SEM ($n = 4$ animals/groups); *significant ($p < 0.05$) difference between group. (d–f) TH protein levels in gingival biopsies of periodontally healthy (d–f) and periodontitis subjects (d'–f'), as analyzed by immunohistochemistry. Images in the *upper row* show an overview of the gingival biopsies (d, d'), whereas images of the *middle* and *lower rows* mainly display sections of the gingival epithelial tissue (e, e') or gingival subepithelial connective tissue (f, f') in a larger scale, respectively. Fibroblasts of the gingiva propria are indicated by *arrows*. Gingiva of three healthy donors and inflamed tissue of periodontally diseased sites of three diagnosed periodontitis patients were analyzed. Representative images of one donor per group are shown

link between obesity and periodontal destruction. Obesity is accompanied by elevated serum levels of leptin but it has also been reported that leptin is expressed in PDL cells (*Considine* et al. 1996; Deschner 2014; *Nokhbehsaim* et al. 2014). Leptin levels have been described to be decreased in gingival crevicular fluid of periodontitis patients as compared to healthy controls (*Gangadhar* et al. 2011; *Johnson and Serio* 2001; *Karthikeyan and Pradeep* 2007a, 2007b). In addition, its downregulatory effect on growth and transcription factors as well as matrix molecules was demonstrated (*Nokhbehsaim* et al. 2014). Interestingly, we found that leptin significantly upregulated TH gene expression and protein levels in PDL fibroblasts. Plasma visfatin levels are also increased in obese patients, while weight loss leads to a reduction (*Chang* et al. 2011; *Taşkesen* et al. 2012). Visfatin levels are elevated in crevicular fluid of periodontitis patients as compared to healthy controls (*Pradeep* et al. 2012; *Pradeep* et al. 2011; *Raghavendra* et al. 2012). Visfatin has also been shown to be locally produced by PDL fibroblasts and reported to contribute to periodontal inflammation and destruction (*Damanaki* et al. 2014; *Nogueira* et al. 2014; *Nokhbehsaim* et al. 2013a). In our study, we demonstrated that visfatin treatment of PDL fibroblasts led to significantly elevated TH levels. The in vitro model used in this study, however, can only be considered as a research tool with several limitations to the in vivo condition of obesity. Leptin and visfatin, for example,

are two important representatives of adipokines, which were connected to periodontal destruction by earlier studies (*Deschner* et al. 2014; *Nokhbehsaim* et al. 2013a). The effects of other adipokines such as adiponectin, resistin, or apelin on TH regulation in PDL fibroblasts are not taken into account in our in vitro setting and should be investigated in further studies. Furthermore, the effects of adipokines on additional periodontal cells like gingival cells, osteoblasts and cementoblasts should be investigated with a special focus on TH expression.

To examine TH regulation in periodontal tissues in a more complex in vivo setting, diet-induced obesity models of two different rodents were used. As expected, TH regulation was elevated in the gingival tissue of obese rodents. To extrapolate findings from rodents to humans, the applied animal models have to be cautiously validated for their suitability (*Kleinert* et al. 2018). In comparison to genetic animal models, which have also been used for obesity research, the high-fat diet-induced rodent models might be more appropriate for our research because there is usually no genetic mutation responsible for human obesity (*Damanaki* et al. 2018a; *Kleinert* et al. 2018; *Rosini* et al. 2012; *Speakman* et al. 2008). But the transferability of our results to humans still remains an issue to be considered.

Furthermore, a power analysis for the in vivo experiments could not be conducted due to a lack of preexisting data. To our knowledge, our study is the first one to assess TH in periodontal tissues by other means than immunohistochemistry. The material for our study was obtained from previously published studies (*Damanaki* et al. 2014; *Damanaki* et al. 2018a, 2018b; *Nogueira* et al. 2017). We reduced the probability of false-positive results by studying two different species. We were able to present data from rat and mouse diet-induced obesity models and we confirmed data of experimental periodontitis in rats by immunohistochemistry of inflamed and healthy human tissues.

Our study demonstrated that adipokines and obesity upregulate TH expression. Furthermore, we found that TH upregulation is associated with periodontitis. Therefore, TH could be considered as a pathomechanistic link whereby obesity contributes to periodontitis. Further studies should investigate the impact of TH on the catecholamine concentrations in periodontal tissues, the effect on the sympathetic nervous system and the resulting suppression of the immune response.

In summary, the present study provides novel insights into obesity-associated stimuli on periodontal cells and tissues. Leptin and visfatin as well as obesity in a diet-induced animal model caused an upregulation of TH. Since the TH levels were also increased at sites of periodontitis, our in vitro and animal findings suggest that this enzyme could represent a pathomechanism whereby obesity contributes to periodontitis.

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Compliance with ethical standards

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

Research involving human participants and/or animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee of the University of Bonn (#043/11) and with the 1964 Helsinki declaration and its later amendments.

All procedures of animal experiments as part of the diet-induced obesity models were performed in accordance with the ethical standards of the University of Bonn (AZ 84-02.04.2012.A131/84-02.04.2016.A202 and AZ 87-51.04.2010.A394). All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

All procedures of animal experiments as part of the experimental periodontitis model were performed in accordance with the ethical standards of the Ethical Committee on Animal Experimentation (protocol number: 23/2012) from the School of Dentistry at Araraquara, University Estadual Paulista (UNESP) and in accordance with the recommendations of the ARRIVE guidelines. All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Informed consent Informed consent was obtained from all individual participants included in the study.

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