Journal of Oral Biosciences 62 (2020) 327–335

Contents lists available at ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Original Article

Hypertriglyceridemia-induced brain-derived neurotrophic factor in rat submandibular glands

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ARTICLE INFO

Article history:
Received 30 July 2020
Received in revised form
25 August 2020
Accepted 27 August 2020
Available online 12 September 2020

Keywords:
Brain-derived neurotrophic factor
Hypertriglyceridemia
Plasma
Saliva
Salivary gland

ABSTRACT

Objectives: Salivary glands produce brain-derived neurotrophic factor (BDNF), which increases plasma BDNF content. Salivary BDNF influences the hippocampus and enhances anxiety-like behaviors. Dyslipidemia affects the brain, promoting depression and anxiety-like behaviors. This study was performed to investigate whether hypertriglyceridemia influences salivary BDNF expression.
Methods: Hypertriglyceridemia was induced in rats by high-fat diet intake for 10 weeks. BDNF protein levels in the saliva and submandibular glands were measured using enzyme-linked immunosorbent assay (ELISA). *Bdnf* mRNA levels in the submandibular gland were determined using real-time polymerase chain reaction.
Results: A hypertriglyceridemia rat model was established. Body weight did not differ between the control and hypertriglyceridemia groups. *Bdnf* mRNA and protein expression was increased in the submandibular gland in the hypertriglyceridemia group compared to the control group. BDNF expression was also significantly increased in the saliva of the hypertriglyceridemia group.
Conclusions: This is first study to show that hypertriglyceridemia induces BDNF expression in the rat submandibular gland and suggests that salivary BDNF is associated with lipid metabolism.

1. Introduction

Dyslipidemia is a state in which levels of low-density lipoprotein (LDL) cholesterol and triglycerides in the blood are higher than normal, or the levels of high-density lipoprotein (HDL) cholesterol are decreased [1]. Lipids in the blood are primarily derived exogenously through the diet or through synthesis in the liver. Although cholesterol is a necessary nutrient, prolonged high levels of cholesterol can cause arteriosclerosis and increase the risk of cerebral infarction or myocardial infarction [1]. Dyslipidemia not only causes abnormalities in the circulatory system but also affects the brain, increasing depression-like and anxiety-like behaviors [2]. Additionally, experiments in rats fed a high-fat diet showed that dyslipidemia is likely involved in diet-induced and/or obesity-induced cognitive decline [3]. Thus, dyslipidemia affects various organs and diseases.

Brain-derived neurotrophic factor (BDNF) is a very important molecule involved in the maintenance and transmission of nerve cells and synaptic plasticity, among other functions [4,5]. We previously reported that acute or chronic immobilization stress loading increases BDNF levels in the salivary glands and blood [6,7].

Abbreviations: BDNF, brain-derived neurotrophic factor; ELISA, enzyme-linked immunosorbent assay; PCR, polymerase chain reaction; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; H&E, hematoxylin-eosin; TrkB, tyrosine receptor kinase B.

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https://doi.org/10.1016/j.job.2020.08.003
1349-0079/

Increased BDNF in the salivary glands is correlated with a slight increase in the total amount of BDNF in the hippocampus [8]. Under stressful conditions, BDNF in the hippocampus is decreased, but salivary gland-produced BDNF may compensate for this in the hippocampus [9].

The role of BDNF has been widely examined in nerve cells of the central nervous system. In recent years, novel roles for BDNF outside of the nervous system have attracted attention [10], particularly its relationship with lipid metabolism [11,12]. However, the relationship between dyslipidemia and BDNF in the salivary glands is not well understood. In this study, we examined whether environmental changes such as dyslipidemia affect BDNF expression in the salivary glands.

2. Materials and methods

2.1. Animals

A total of 12 male Sprague–Dawley rats, aged 3 weeks (Japan SLC, Shizuoka, Japan), were housed in groups (3 per cage; cage dimensions, 260 × 380 × 180 mm) under pathogen-free conditions with controlled temperature, humidity (22 ± 3 °C, 55 ± 3%), and lighting (12-h:12-h light: dark cycle), and free access to food and water. After a 2–3 day acclimation period, the rats were randomly divided into a normal diet group ($n = 12$) and high-fat diet group ($n = 12$) and fed *ad libitum* for 10 weeks. The normal diet proportions were 54% carbohydrate, 20% protein, and 4.5% fat (MF; ORIENTAL YEAST Co., Ltd., Tokyo, Japan) [13]. The high-fat diet consisted of 58% lard (*wt/wt*), 30% fish powder, 10% skim milk, and a 2% vitamin and mineral mixture, equivalent to 7.5% carbohydrate, 24.5% protein, and 60% fat (F2WTD; ORIENTAL YEAST Co., Ltd.) [13]. All experiments were performed using a 10-week high-fat diet group and control group, unless otherwise indicated. Six rats from each group were used for histological analysis (hematoxylin-eosin, Oil red O, and immunohistochemical staining). Tissues from the remaining six rats were analyzed by real-time polymerase chain reaction (real-time PCR) and enzyme-linked immunosorbent assay (ELISA). To avoid diurnal variations in hormone expression that may affect cytokine production, we conducted all experiments between 13:00 and 16:00 h. All experimental procedures used in this study were reviewed and approved by the Ethics Committee for Animal Experiments of Kanagawa Dental University (approval number 2019–006) and performed in accordance with the Guidelines for Animal Experimentation of Kanagawa Dental University and ARRIVE guidelines for reporting animal research.

2.2. Saliva, blood, and tissue sample collection

After the 10-week period, all rats were deeply anesthetized by inhalation of 1–2% isoflurane and injected intraperitoneally (i.p.) with 1 mg/kg, pilocarpine-HCl (Sanpilo 1%; Santen Pharmaceutical Co., Ltd., Osaka, Japan) for saliva collection. Saliva secreted into the oral cavity during each 1-min period following the injection of the above stimulants was carefully collected into capillaries for 15 min (ringcaps; Hirschmann Laborgerate GmbH & Co. KG, Eberstadt, Germany) [14]. Next, the rats were exsanguinated by cardiac puncture under general anesthesia. Blood samples were collected and allowed to coagulate for 10 min at 22 °C before centrifugation to obtain plasma samples [15,16]. Plasma was used for blood biochemistry tests (triglyceride (TG), HDL cholesterol (HDL), LDL cholesterol (LDL), total cholesterol, and blood glucose). At the same time, tissue samples were collected, including samples from the submandibular gland, liver, adrenal gland, pancreas, kidney, and inguinal region fat, as previously described [15,17]. Aliquots of saliva, plasma, and tissue samples were immediately stored at −80 °C until use.

2.3. Histological analysis

Rats were sacrificed under deep anesthesia as described above between 13:00 and 16:00 h; rats in the experimental groups were sacrificed immediately after salivary sampling and blood collection, as described above. Resected rat liver and submandibular gland tissue samples were fixed in 10% buffered formaldehyde (pH 7.4) for 24 h and embedded in paraffin, after which serial 3-μm sections were cut and stained with hematoxylin-eosin (H&E) and processed for immunohistochemistry analysis. Frozen liver and submandibular gland sections were stained with Oil red O to reveal the presence or absence of intracellular lipids. Histological changes in the liver and submandibular gland were evaluated by H&E and Oil red O staining under a light microscope (B × 41, Olympus, Tokyo, Japan).

2.4. RNA extraction and cDNA synthesis isolation

Total RNA was isolated from the tissue samples using ISOGEN reagent (Nippon Gene, Toyama, Japan) according to the manufacturer's instructions. The RNA product was resuspended in 20 μL of diethyl pyrocarbonate-treated water. The quality of the RNA was judged from the pattern of ribosomal RNA after electrophoresis through a 1.5% agarose gel containing ethidium bromide, visualized by UV illumination. RNA concentrations were determined by measuring the absorbance at 260 nm with a SmartSpec Plus spectrophotometer (Bio-Rad, Hercules, CA, USA). RNA was stored at −80 °C until use. Total RNA was reverse-transcribed at 50 °C for 30 min, 99 °C for 5 min, and 58 °C for 5 min using a single-strand cDNA synthesis kit (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions [18]. Following the reverse transcriptase reaction, cDNA products were stored at −20 °C until use.

2.5. Real-time PCR analysis

Real-time PCR was performed using a LightCycler 480 system (Roche) according to the manufacturer's instructions [19]. Reactions were performed in a 20 μL volume (0.3 mM of each primer and 4 mM $MgCl_2$). Reaction mixtures containing Taq DNA polymerase, nucleotides, and buffer were prepared with LightCycler-DNA Master SYBR Green I mix (Roche Diagnostics). Oligonucleotide primers designed to amplify rat *Bdnf* were specific for the coding region of exon 5. The *Bdnf*-specific primer sequences were 5′-CAGGGGCATAGACAAAAG-3′ (forward) and 5′-CTTCCCCTTTTAATGGTC-3′ (reverse) (PCR product: 167 bp) and were designed and synthesized by Nippon Gene Laboratory [9]. Real-time PCR to amplify the rat β-actin (*Actb*) housekeeping gene was performed using a LightCycler Primer/Probe set, 5′-CCTGTATGCCTCTGGTCGTA-3′ (forward) and 5′-CCATCTCTTGCTCGAAGTCT-3′ (reverse) (PCR product: 260 bp) according to the manufacturer's instructions (Nihon Gene Research Labs, Inc., Sendai, Japan). Denaturation was performed at 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 10 s. Melting analysis and agarose gel electrophoresis were performed to confirm the specificity of the PCR products obtained using each primer pair. Gene expression is presented as the ratio of the copy number of *Bdnf* mRNA to *Actb* mRNA for each sample, as described previously [8].

2.6. BDNF ELISA

Tissue samples (submandibular gland, liver, adrenal gland, pancreas, kidney, inguinal region fat) were homogenized in ice cold lysis buffer composed of 137 mM NaCl, 20 mM Tris–HCl (pH 8.0), 1% NP40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 mg/mL aprotinin, 1 μg/mL leupeptin, and 0.5 mM sodium vanadate. The tissue homogenate solutions were centrifuged at 14,000×*g* for 15 min at 4 °C. Total protein concentrations were determined using the Bradford method with absorbance readings measured at 595 nm on a SmartSpec Plus spectrophotometer (Bio-Rad). The concentration of BDNF in the submandibular gland, liver, adrenal gland, pancreas, kidney, inguinal region fat, and saliva samples were quantified by sandwich ELISA (CYT306, Merck Millipore, Billerica, MA, USA), according to the manufacturer's instructions [20,21]. All assays were performed in F-bottom 96-well plates (Nunc, Roskilde, Denmark). BDNF standards and samples were incubated at 4 °C, overnight. Tertiary antibodies were conjugated to horseradish peroxidase and color was developed with tetramethylbenzidine and measured at 450 nm using the iMark Microplate Reader (Bio-Rad). All samples were tested twice, and the mean was calculated. Cross-reactivity to related neurotrophins (NGF, NT-3, and NT-4) was less than 3%. Intra- and inter-assay coefficients of variation were 3.7% and 8.5%, respectively. Concentrations of BDNF were determined by creating a calibration curve (standard curve) and calculating concentrations based on this calibration curve and the absorbance of the samples.

2.7. Tissue preparation for BDNF immunohistochemistry analysis

Immunohistochemical analysis was performed using the Simple stain MAX-PO (Nichirei, Tokyo, Japan). Slides were pre-incubated in 3% H_2O_2 for 5 min. The sections were then incubated with an anti-human BDNF monoclonal antibody (1:100, MAB248, Techne, Minneapolis, MN, USA) for 1 h at 22 °C, as described previously [22,23]. After washing with PBS, the sections were incubated with the secondary antibody, horseradish peroxidase-labeled anti-rabbit IgG with amino acid polymer (Nichirei), for 30 min at 22 °C. Color was developed using 0.02% 3,3′-diaminobenzidine-tetrahydrochloride containing 0.0003% H_2O_2 in Tris-buffered saline for 5 min, followed by counterstaining with hematoxylin. For negative control experiments, non-immunized rabbit or mouse IgG was used instead of the primary antibody. To determine the binding specificity, a competitive assay was also conducted using recombinant BDNF (R&D Systems, Inc., Minneapolis, MN, USA).

2.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism (v. 6.05.; GraphPad, Inc., San Diego, CA, USA). Values are reported as the mean ± standard deviation. Data were analyzed using Welch's-*t* test. *P* values < 0.05 were considered statistically significant. Statistical analysis was performed under the advice of Prof. Ayumi Shintani (Department of Clinical Epidemiology and Biostatistics, Osaka University Graduate School of Medicine).

3. Results

3.1. Body weight, total food intake, and total energy

There were no differences in the mean body weight between the normal diet and high fat diet-fed groups at the end of the experiment in 13-week-old rats (mean final body weight, control: 426.90 ± 34.41 g; dyslipidemia: 444.32 ± 63.22 g; Fig. 1A). Total food intake in the hypertriglyceridemia group was significantly lower than that in the control group (mean total food intake, control: 1231.22 ± 111.76 g; hypertriglyceridemia group: 822.18 ± 119.01 g, $P < 0.0001$; Fig. 1B). Total energy ingestion was significantly lower in the hypertriglyceridemia group than in the control group (mean total energy, control: 18493.539 ± 1678.675 kJ; hypertriglyceridemia group: 15507.431 ± 2244.741 kJ, $P < 0.05$; Fig. 1C).

3.2. Blood biochemistry

To evaluate the effect of normal or high-fat diet on blood biochemistry parameters, we measured the concentrations of triglyceride (TG), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol, and blood glucose in the plasma. The hypertriglyceridemia had significantly higher TG, HDL, LDL, and total cholesterol concentrations in the plasma compared to the control group ($P < 0.01$; Fig. 2A). Blood glucose did not differ between the control group and hypertriglyceridemia group ($p > 0.05$; Fig. 2A).

3.3. Histological changes in the liver

To evaluate the histological changes in liver tissues following the consumption of different diets, we performed a morphological examination. As shown in Fig. 2B, both H&E and Oil red O staining revealed a greater induction of hepatocyte ballooning, inflammation, and hepatic steatosis in the hypertriglyceridemia group compared to the control group.

3.4. Histological changes in the submandibular gland

To evaluate histological changes in the submandibular gland tissue after the consumption of the different diets, we measured the wet weight of the submandibular gland and observed tissue morphology. No significant differences were observed in the right and left side wet weight of the submandibular glands between the control group and hypertriglyceridemia group (Fig. 3A). As shown in Fig. 3B, based on H&E and Oil red O staining, the submandibular gland tissue showed no characteristic changes in the acinar cells, mucous cells, myoepithelial cells, and duct cells in the hypertriglyceridemia group.

3.5. Bdnf mRNA expression in rat submandibular gland tissue

Bdnf mRNA expression in the submandibular gland was significantly higher in the hypertriglyceridemia group (0.005057091 ± 0.000718292) than in the control group (0.000876459 ± 0.000186429) ($P < 0.0001$; Fig. 4A).

3.6. Expression of BDNF protein in the submandibular gland, saliva, and plasma

BDNF levels in the submandibular gland tissue were significantly increased in the hypertriglyceridemia group (78.15 ± 3.10 pg/mg) compared to the control group (7.40 ± 0.29 pg/mg, $P < 0.0001$; Fig. 4B). Saliva BDNF was significantly elevated in the hypertriglyceridemia group (19.94 ± 4.63 pg/mL) compared with the control group (5.25 ± 1.85 pg/mL, $P < 0.001$; Fig. 4B).

3.7. BDNF immunohistochemistry in the submandibular gland

Submandibular gland tissue from the control group showed little to no expression of BDNF in various duct-type cells (white arrows in Fig. 5A), acinar cells and myoepithelial cells (white arrowheads in Fig. 5A). Intense BDNF expression was observed in

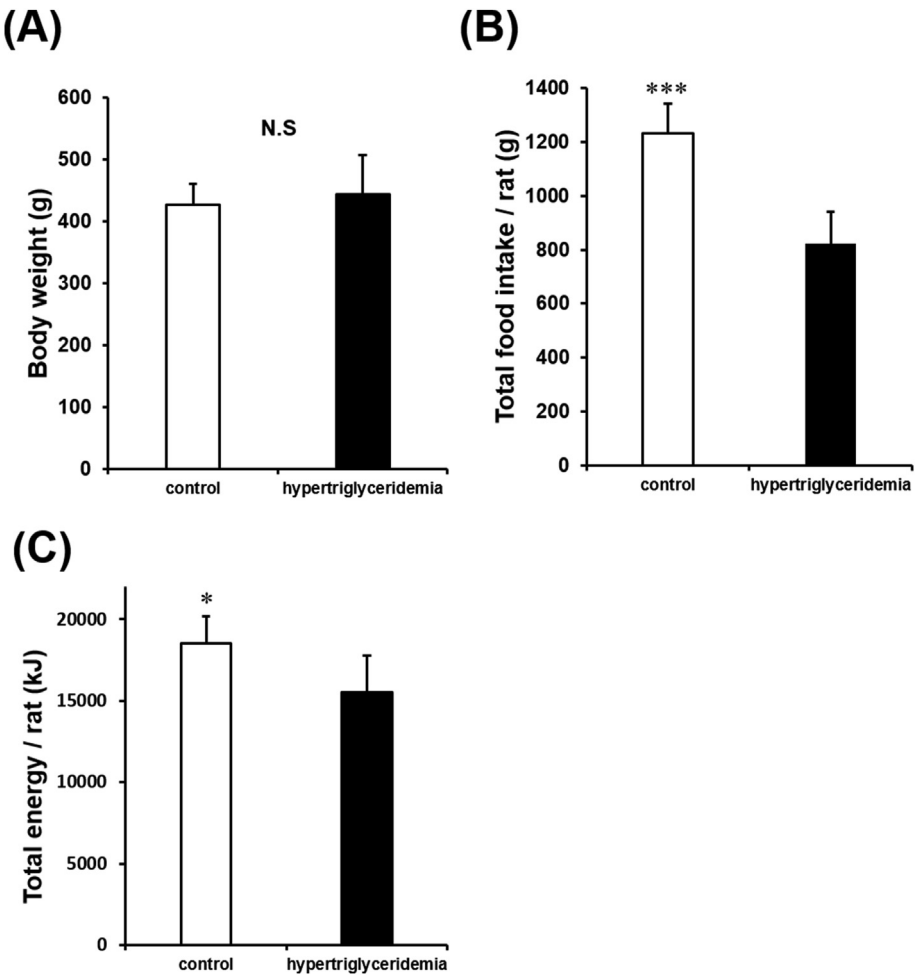

Fig. 1. Body weight and total food intake in the control and hypertriglyceridemia groups. (A) There was no difference in body weight between the hypertriglyceridemia group and control group at 13 weeks of age (B) Total food intake was significantly different between the control and hypertriglyceridemia groups after 10 weeks (C) Total energy ingestion was significantly different between the control and hypertriglyceridemia groups. Values are the mean ± standard deviation ($n = 6$); *$p < 0.05$, ***$p < 0.0001$ by Welch's-t test. N.S: not significant.

various duct-type cells from the hypertriglyceridemia group (black arrows in Fig. 5B); however, BDNF expression was not consistently observed in acinar cells or myoepithelial cells in the hypertriglyceridemia group (black arrowheads in Fig. 5B).

3.8. Expression of BDNF protein in various organs

BDNF protein expression was measured in the liver, adrenal gland, pancreas, kidney, and inguinal region fat in the control group and hypertriglyceridemia group (Fig. 6). The hypertriglyceridemia group had significantly lower BDNF expression in the liver and adrenal gland tissue compared to the control group. There were no differences in BDNF protein levels between the control group and hypertriglyceridemia group in the pancreas, kidney, and inguinal region fat.

4. Discussion

In this study, TG, HDL, LDL, and total cholesterol were elevated in the blood of rats in the hypertriglyceridemia group compared to the controls. Furthermore, because there was no difference in glucose between the control group and hypertriglyceridemia group, we established a rat model with abnormal lipids without abnormal sugars. This is because the high fat diet feed does not contain sugar. Moreover, in the hypertriglyceridemia group, the rats consumed smaller amounts of food, and their body weight did not significantly increase compared to the control group. Similar findings were observed in dyslipidemia model rats produced using the same diet [24]. There are many reports stating that rodents fed a high-fat diet have a significantly higher final body weight than those fed a low-fat diet [25–27]. However, in this study, there was no difference in body weight, likely due to differences in breeding environments and conditions [25–27]. In most previous studies, rats were individually housed [25–27]; however, in this study, three rats were housed per cage. In the breeding environment and conditions in this study, fighting among rats could have increased or decreased. In addition, the total energy ingestion was significantly lower in the rats in the high-fat diet compared to the normal diet, which is likely responsible for the lack of difference in the final body weights. Furthermore, because the fat tissue weight in the accessory testicle increases, lipid deposition is observed in the tissue [24]. Histological analysis of the liver clearly revealed fatty liver, indicating that various organs had lipid deposits. In the salivary gland, as adipose tissue increases, salivary gland function declines in aging rats [28]. However, lipid deposition in the submandibular gland was not observed with Oil red O staining in the model rats. There was no significant difference in the salivary gland wet

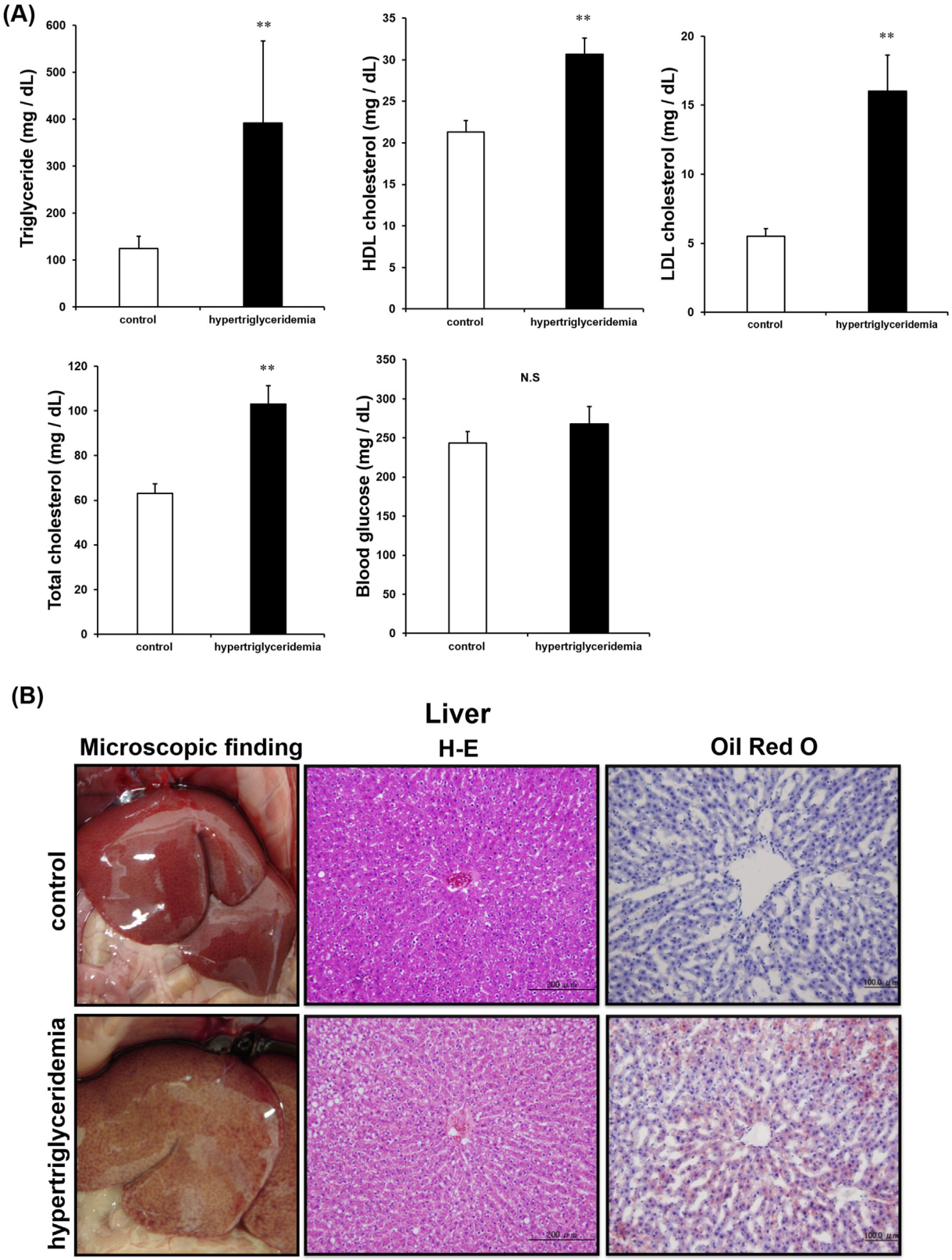

Fig. 2. Blood biochemistry and liver histological examination in the control and hypertriglyceridemia groups. (A) The hypertriglyceridemia group had significantly higher triglycerides, HDL cholesterol, LDL cholesterol, and total cholesterol in the plasma compared to the control group. There was no significant difference in the blood glucose concentration. Values are the mean ± standard deviation ($n = 6$); $^{**}p < 0.001$ by Welch's-t test. N.S: not significant (B) Representative images of the rat from the control and hypertriglyceridemia groups. Micrographs, H&E staining (scale bar = 200 μm), and Oil red O staining (scale bar = 100 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

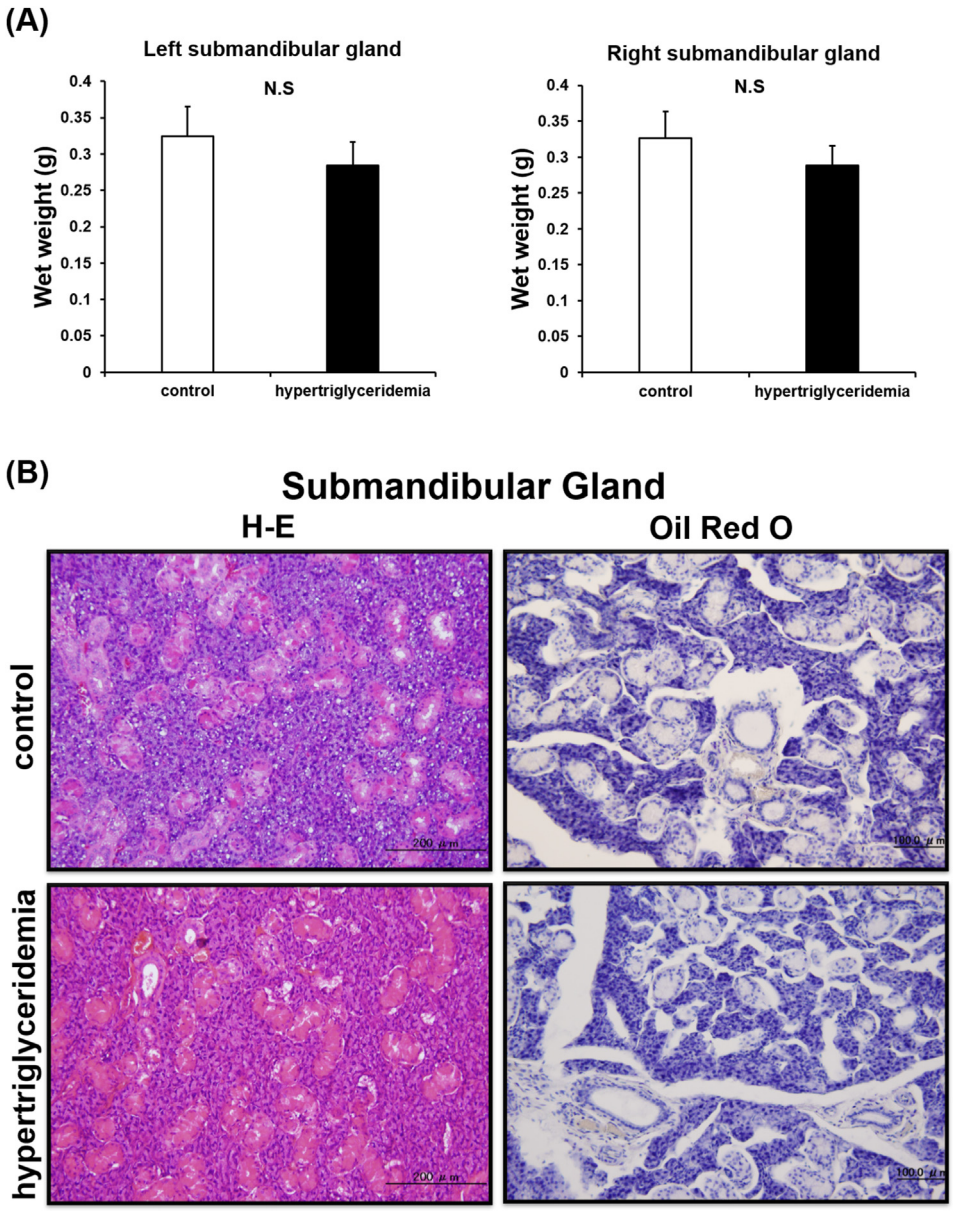

Fig. 3. Submandibular gland wet weight and histological examination from the control group and the hypertriglyceridemia group. (A) There was no significant difference in the wet weight of the submandibular gland on both the right and left side in the hypertriglyceridemia group. Values are the mean ± standard deviation ($n = 6$). N.S: not significant (B) Representative images of the submandibular glands from rats in the control group and the hypertriglyceridemia group. H&E staining (scale bar = 200 μm), and Oil red O staining (scale bar = 100 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

weight between the control group and hypertriglyceridemia group. Additionally, in our model, macroscopic and microscopic changes were not observed in the salivary glands. However, Matczuk et al. reported that TGs were increased in the submandibular glands of rats fed similar diets; thus, further studies are needed to determine how fats such as TG are involved in salivary gland function [29].

The BDNF protein concentration in the submandibular gland tissue and the BDNF mRNA expression in the submandibular gland were significantly increased in the hypertriglyceridemia group compared to the control group. Particularly, BDNF levels were very low in the control group. BDNF expression in the rat salivary glands is generally very low under non-stress conditions [18]. Here, high-fat diet increased BDNF production in the salivary glands. No previous studies have shown that BDNF is increased in the salivary glands of rats with hypertriglyceridemia. The mechanism by which the high-fat diet increases salivary gland BDNF is unclear. Numerous factors contribute to increased BDNF, such as mastication [9], mild physical exercise [30], dietary restrictions [31], obesity [32], and some foods [33,34]. Additionally, adding different fatty acids to a high-fat diet changes the blood BDNF concentration [35]. In rats, blood BDNF concentration is associated with the salivary glands [22], and lipids likely increase salivary gland BDNF.

As BDNF in the salivary glands increased, BDNF in the saliva also increased in the high-fat diet group, as expected. Saruta et al. reported that BDNF in the salivary glands is transported to the brain [8]. Hayashi et al. also reported that when lactoferrin was administered in the lower tongue, it transited to the brain, where it increased the total amount of lactoferrin [36]. The lower tongue is also a route of drug administration and is therefore a systemic

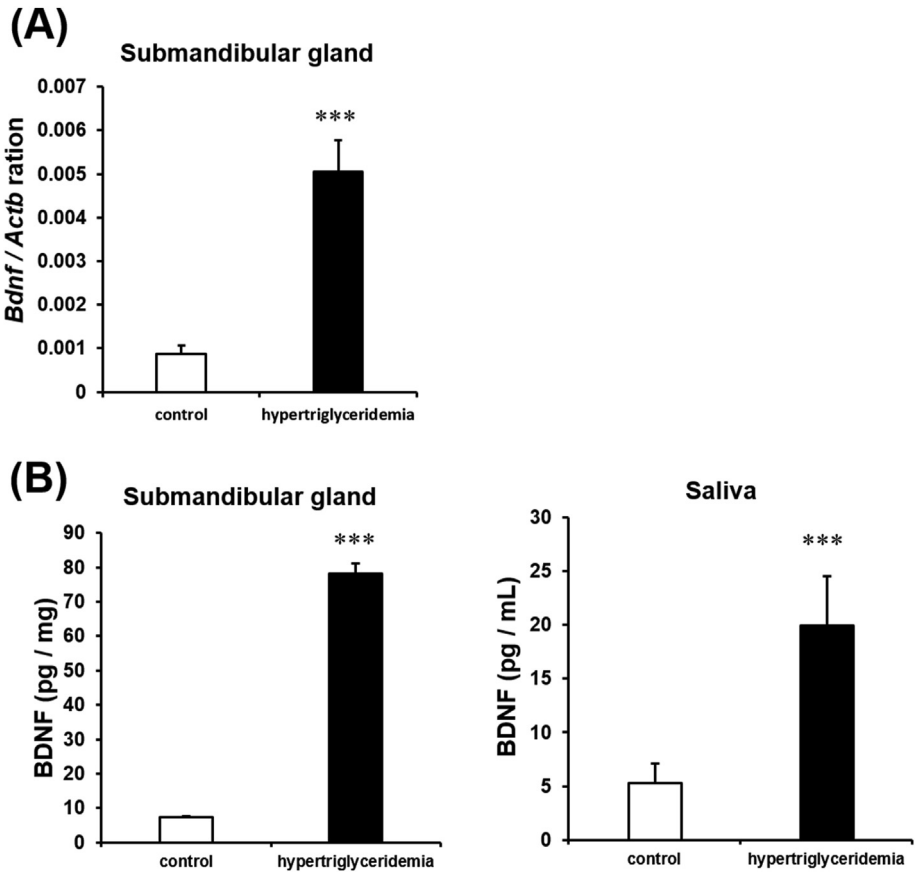

Fig. 4. Effect of diet-induced changes on BDNF expression and concentration in the submandibular gland and saliva. (A) Real-time-PCR analysis of *Bdnf* mRNA expression in the submandibular gland. In the hypertriglyceridemia group, *Bdnf* expression was significantly increased compared to in the control group (B) ELISA showing the protein concentration of BDNF in the submandibular gland and saliva. In the hypertriglyceridemia group, the BDNF protein concentration was significantly increased in the submandibular gland and saliva compared to in the control group. Values are the mean ± standard deviation ($n = 6$); ***$p < 0.0001$ by Welch's-t test.

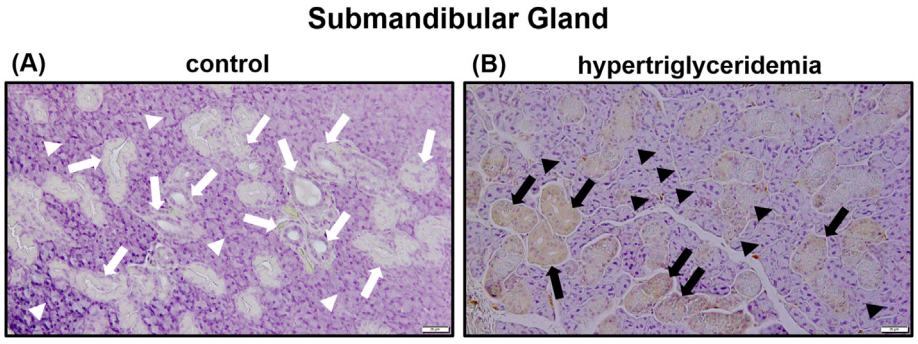

Fig. 5. BDNF immunohistochemistry analysis in submandibular glands of control and hypertriglyceridemia groups. Photomicrographs show the immunohistochemical localization of BDNF protein, identified with an anti-BDNF monoclonal antibody in paraffin-embedded sections of submandibular gland from (A) control group and (B) hypertriglyceridemia group. Control group showed little to no expression of BDNF in various duct-type cells (white arrows (A)) and acinar cells or myoepithelial cells (white arrowheads (A)) in the submandibular gland tissues. BDNF protein was observed in duct cells (black arrows (B)), and there was no obvious BDNF expression in acinar cells or myoepithelial cells (black arrowheads (B)) in the hypertriglyceridemia group. Scale bars = 20 μm.

transition route. Based on this, BDNF in the saliva may also transit to the rat brain. Toriya and colleagues showed that when the BDNF receptor tyrosine receptor kinase B (TrkB) in the paraventricular nucleus was suppressed, food intake increased and weight increased accordingly [37]; it is unclear where the BDNF was injected, although TrkB is expressed in the paraventricular nucleus [37]. Compared to the control group, rats in the hypertriglyceridemia group had a lower food intake, suggesting that BDNF may be involved in a feeding suppression mechanism in the central nervous system. Salivary gland BDNF may mediate this mechanism. However, it has also been reported that leptin, which has an anti-feeding effect, is increased in dyslipidemia models [38]. Leptin and BDNF have been shown to be correlate, but act through different molecular mechanisms [39].

One limitation of this study is that we did not investigate the expression and localization of TrkB, a receptor for BDNF. In a previous study, we reported that TrkB was not expressed in the salivary glands or peri-oral tissues of rats [4,17]. Therefore, it is very

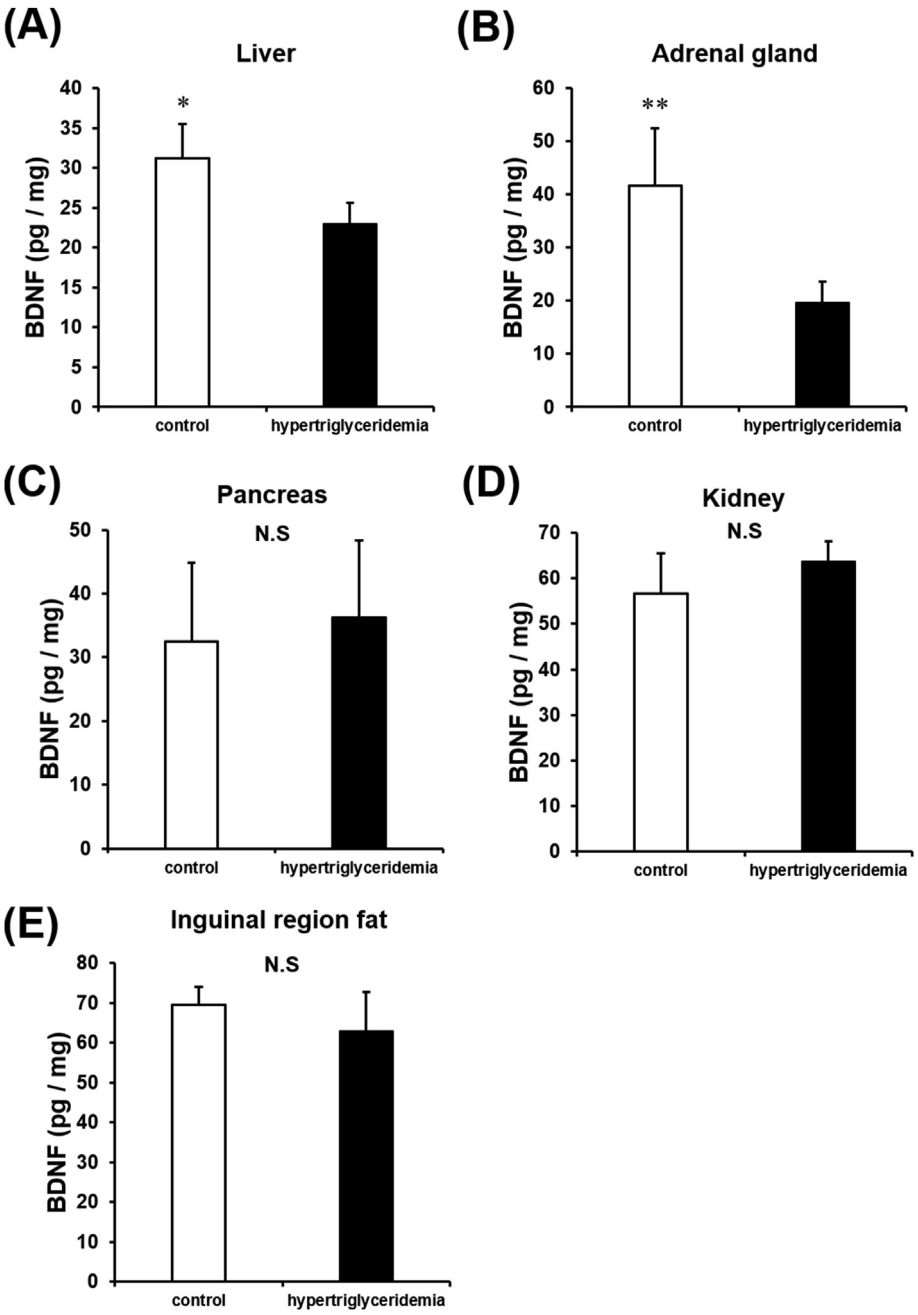

Fig. 6. Concentrations of BDNF protein in rat various organs. ELISA showing the protein concentration of BDNF in the liver, adrenal gland, pancreas, kidney, and inguinal region fat. In the hypertriglyceridemia group, BDNF protein was significantly decreased in the liver (A) and adrenal gland (B) compared to in the control group. The hypertriglyceridemia group showed no significant difference in the pancreas (C), kidney (D), and inguinal region fat (E). Values are the mean ± standard deviation ($n = 6$); *$p < 0.05$, **$p < 0.001$ by Welch's-*t* test. N.S: not significant.

important to examine the effects of TrkB on rats consuming a high-fat diet. Additionally, the distribution and increase/decrease of BDNF in systemic organs in the absence of salivary glands should be examined using the three major salivary glands resection model (sialoadenectomy). This may reveal the relationship between high-fat diet intake and increased salivary gland BDNF.

5. Conclusion

We determined that hypertriglyceridemia due to a high-fat diet increases *Bdnf* mRNA and protein levels in the rat submandibular gland tissue and increased salivary BDNF secretion. Further studies are needed to determine why consumption of a high-fat diet increases salivary gland tissue and salivary BDNF. Additionally, a comprehensive analysis focused on lipids in the salivary glands of the hypertriglyceridemia model and the factors enhancing BDNF production is needed. In this study, the detailed mechanism of how salivary gland BDNF is involved in feeding suppression is unknown and will be examined in our future studies.

Ethical statement

This study was approved by The Kanagawa Dental University Animal Care and Use Committee (approval number: 2019–006). No human samples were examined in this study.

Funding

This work was supported by the Kanagawa Dental University Research Fund (2019); Kakenhi Grant-in-Aid for Young Science (B) [grant number 26861582]; and Grant-in-Aid for Scientific Research (C) [grant number 19K10427] to J.S. from the Japan Society for the Promotion of Science.

CRediT authorship contribution statement

Takeo Kikuchi: Data collection, Formal analysis, Investigation, Writing - original draft. **Wakako Sakaguchi:** Data collection, Formal analysis, Investigation, Writing - original draft. **Juri Saruta:** Data collection, Investigation, Conceptualization, Methodology, Funding acquisition, Writing - original draft. **Yuko Yamamoto:** Investigation, Methodology, Visualization. **Masahiro To:** Resources, Supervision, Validation, Writing - review & editing. **Yuki Kurimoto:** Resources, Validation, Writing - review & editing. **Tomoko Shimizu:** Data curation, Writing - review & editing. **Keiichi Tsukinoki:** Conceptualization, Validation, Project administration, Writing - review & editing.

Conflicts of interest

No potential conflict of interest was reported by the authors.

Acknowledgments

The authors thank Professor Ayumi Shintani for statistical advice concerning this study. We would like to thank Editage (www.editage.com) for English language editing.

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