Gingival blood flow response upon thermal stimulation: comparison between young and middle-aged healthy men

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Objective: To observe differences in gingival blood flow indices between young and middle-aged men after the application of thermal stimulation.

Methods: Maxillary anterior gingival blood flow at rest was measured in 25 healthy men with no differences in blood pressure (young group: 21−33 years old, n = 12; middle-aged group: 55−65 years old, n = 13) using laser speckle flowgraphy. Pulse waveform analysis was performed to calculate blood flow indices, including gingival vascular conductance, falling rate, and blowout time (BOT). Thermal stimulus was applied to the gingiva at 40°C for 1 minute to increase blood flow, and changes in blood flow indices were measured for 2 minutes after stimulation.

Results: There were no differences in gingival vascular conductance, BOT, and falling rate between the groups at rest. Upon the application of localized thermal stimulus, gingival blood flow and vascular conductance increased for 1 minute after heating in both groups. A decrease (P < 0.05) in BOT, index for the persistence of high blood flow, was observed in the middle-aged group upon temporary vasodilation due to thermal stimulus but not in the young group.

Conclusion: The ability to maintain gingival blood flow may decrease with age.

Key words: aging, blood flow, gingiva, laser speckle flowgraphy, thermal stimulation

Introduction

Gingival inflammation, a pathological state of periodontal disease, is correlated with changes to gingival blood vessel structures and hemodynamics.1−3 Therefore, gingival hemodynamics are essential for assessing oral health. The number of patients with periodontal disease increases with age,4 suggesting that age-related changes in gingival hemodynamics may contribute to the progression of periodontal disease. However, the changes in human gingival blood flow with age have not been thoroughly studied because of a lack of suitable measurement methods for blood flow in the human gingiva in vivo.5 We recently reported about a type of laser speckle flowgraphy (LSFG) technique that can be used to accurately measure a wide range of human gingival blood flow values and allow interindividual comparisons.6 Measurements of blood flow in the maxillary anterior gingiva of 124 healthy men aged 22−69 years using LSFG to examine gingival vascular conductance and blood flow parameters showed that the ability to maintain blood flow decreased in elderly subjects, suggesting that gingival blood circulation may deteriorate with age. These changes in gingival blood flow indices were correlated with increases in systolic blood pressure (SBP) and pulse pressure (PP), which in turn indicated the progression of large arterial stiffness.7 This suggests that stiffening of the aorta may be involved in the deterioration of gingival microcirculation. However, the specific mechanisms remain unclear.

Therefore, in the present study, we measured maxillary anterior gingival blood flow in 25 healthy Japanese men who showed no differences in at-rest blood pressure, grouped according to age (young group: 21−33 years, n = 12; middle-aged group: 55−65 years, n = 13). We then examined whether or not there were age-related differences in the responses of gingival hemodynamics to local thermal stimulation of the maxillary anterior gingiva.

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Materials and Methods

Subjects
The study was approved by the ethics committee of Kao Corporation, Tokyo, Japan (S058-170721) and was conducted according to the Declaration of Helsinki ethical principles. The study was comprised of 25 healthy Japanese men between the ages of 21 and 65. Written informed consent was obtained from all the subjects. Prior to enrolment in the study, blood pressure measurements and oral examinations were performed. Participants with age-related blood pressure increases, individuals with an SBP of 140 mmHg or higher or diastolic blood pressure (DBP) of 90 mmHg or higher, were excluded from the study. Based on the oral examinations, individuals currently undergoing treatment at medical institutions or dental clinics, and/or those with serious wounds in the oral cavity, acute or severe gingival inflammation, spontaneous bleeding, pus discharge from periodontal pockets, and/or tooth mobility, were also excluded from the study. The selected individuals were divided into 2 groups according to age (young group: 21 – 33 years old, n = 12; middle-age group: 55 – 65 years old, n = 13), and their periodontal status was recorded.

The experiment was performed in a laboratory with the room temperature of 25°C and humidity of 50%. Participants were prohibited from eating or drinking anything other than water and performing any oral hygiene activity, such as brushing, 30 minutes before the measurement. After entering the laboratory, participants were equipped with a non-invasive continuous sphygmomanometer and an electrocardiograph to begin measuring their blood pressure and heart rate (HR). After 15 minutes of acclimation, the participant's gingival blood flow was measured in a sitting position. A plastic tube with circulating warm water kept at a surface temperature of 40°C was placed into contact with the maxillary anterior gingiva for 1 minute. After 1 minute, the tube was

Figure 1. Typical analysis of gingival blood flow in a 37-year-old participant
A. Gingival blood flow was measured in the region of the maxillary anterior gingiva enclosed by the white line (approximately 240 mm × 40 mm; more than 30,000 pixels). B. A heartbeat map superimposed on a gingival image. The red area indicates high MBR, whereas the blue area indicates low MBR (excluding teeth). C. Pulses were extracted from heartbeat-induced fluctuations in the blood flow. D. A representative standardized pulse waveform. MBR, mean blur rate
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removed, and the gingival blood flow was measured a second time.

**Gingival blood flow measurement by LSFG**
Gingival blood flow was measured using the previously described LSFG method. Briefly, the participant's head was fixed with a chin rest to reduce artefacts. The mouth was opened using a mouth opening instrument, and blood flow in the maxillary anterior gingiva was measured at a distance of 80 mm. The measurement at rest was taken at 30 fps for 30 seconds, and the post-heating measurement was taken 30 seconds after the thermal stimulus at 30 fps for 2 minutes.

**Gingival blood flow analysis**
The analyzed region was set to the maxillary anterior gingiva (Figure 1A). The mean blur rate (MBR), an indicator of gingival tissue blood flow used for LSFG (Figure 1B), was obtained as previously reported. A higher MBR corresponds to improved or faster blood flow. Heartbeats were extracted from heartbeat-induced blood flow fluctuations (Figure 1C), and the standardized average pulse waveforms per heartbeat (the pulse waveforms) were assessed (Figure 1D).

**Gingival blood flow parameters**
Gingival vascular conductance was calculated by dividing the gingival MBR by the mean blood pressure (MBP). The 4 pulse waveform parameters, flow acceleration index (FAI), acceleration time index (ATI), falling rate, and blowout time (BOT) were calculated from the pulse waveforms obtained by LSFG (Figure 2). These indices showed the maximum acceleration rate of blood flow rise during the heartbeat, the peak position of the pulse

![A. Flow acceleration index (FAI)](image)

\[ FAI = \text{Max} \left( \Delta MBR_t \right) \]

![B. Acceleration time index (ATI)](image)

\[ ATI = C \times \left( \frac{P}{F} \right) \]

![C. Falling rate](image)

\[ \text{Falling rate} = C \times \left( \frac{S_F}{S_{all}} \right) \]

![D. Blowout time (BOT)](image)

\[ BOT = C \times \left( \frac{W}{F} \right) \]

*Figure 2.* Characteristics of pulse waveform parameters.

A. The flow acceleration index (FAI) indicates the maximal blood flow increase per frame (1/30 s) within a single heartbeat. B. The acceleration time index (ATI) indicates the peak position of blood flow and is derived from the ratio of the time before reaching the peak to a single heartbeat. C. The falling rate is derived from the falling area of the waveform and indicates serial changes in the rate of decrease in blood flow. The falling rate is defined as the ratio of the area above the curve (SF) relative to the entire area (Sall) after the peak. D. Blowout time (BOT) is an indicator of the persistence of high blood flow and represents the time at which the wave maintains more than half of the mean of the maximum and minimum MBR during a beat.
waveform, serial changes in the rate of the decrease in blood flow, and the persistence of high blood flow, respectively (Figure 2).

Measurement of basal physiological values and oral indices
SBP, DBP, MBP, and PP were measured with a non-invasive continuous sphygmomanometer (Finometer MIDI; Finapres Medical Systems BV, Enschede, Netherlands). Values were converted to brachial blood pressure. In addition, HR and electrocardiogram measurements were taken with a memory HR monitor (LRR-03; GMS, Tokyo, Japan). The participants’ body mass index (BMI) values were calculated from their heights and body weights. Probing pocket depth (PPD) and gingival index (GI), an index for gingival inflammation, were measured using a periodontal probe following the Oral Health Surveys: Basic Method.

Statistical analysis
Statistical analyses were conducted using SPSS Ver. 23 (IBM, Armonk, NY, USA). The participants’ basal physiological values and gingival blood flow parameters are expressed as means ± standard errors. For comparisons of basal physiological values, periodontal status, and gingival blood flow parameters between the two age groups, two-sample t-tests were performed. For comparisons of each parameter before and after thermal stimulation, paired t-tests were performed. Values of P < 0.05 were considered statistically significant.

Results

Systemic basal physiological values and periodontal status
Table 1 shows the characteristics and gingival blood flow parameters in both age groups. The BMI in the middle-aged group was significantly higher than that in the young group (P < 0.05). There were no significant differences in SBP, DBP, MBP, PP, HR, PPD, or GI between the groups. All the participants had no or only mild gingival inflammation, and the mean GI did not exceed 2.0 for any participant.

Gingival blood flow under resting conditions
There were no significant differences in gingival MBR, vascular conductance (MBR/MBP), FAI, BOT, or falling rate between the groups. In contrast, ATI was significantly higher in the middle-aged group than that in the young group (P < 0.01, Table 1).

Changes in MBP, HR, gingival MBR, and vascular conductance (MBR/MBP) during thermal stimulation
Table 2 shows the MBR, HR, and gingival blood flow parameters for both age groups at rest and at 30 seconds and 2 minutes after discontinuation of localized heating. Typical changes over time in MBP, HR, gingival MBR, and gingival vascular conductance (MBR/MBP) in the groups before and after localized thermal stimulus to the gingiva and changes in the average values of each group are shown in Figures 3 and 4, respectively. MBP did not change after localized stimulation was applied to the gingiva, whereas HR temporarily decreased immediately after placing the heated plastic tube on the gingiva (P < 0.05). In both groups, gingival MBR and vascular conductance (MBR/MBP) significantly increased upon thermal stimulation of the maxillary anterior gingiva when compared with their pre-stimulus values (At rest: P < 0.01, Table 2, Figure 4). Gingival MBR was significantly higher than its value at rest for approximately 1 minute after the localized heating of the gingiva was discontinued in both groups with no significant differences between the groups. Neither were there any significant differences between the groups regarding how high their gingival MBR and vascular conductance (MBR/MBP) values had increased in response to the thermal stimulation.

Changes in pulse waveforms of gingival blood flow before and after thermal stimulation
Figure 5 shows the mean pulse waveforms of gingival blood flow for the 2 groups at rest and at 30 seconds and 2 minutes after discontinuation of the localised thermal stimulus to the gingiva. In both groups, the amplitude of the waveform temporarily increased, and the time to reach peak blood flow became shorter in response to thermal stimulation. However, 2 minutes after thermal stimulation was discontinued, the pulse waveform returned to almost the same shape as that at rest (Figure 5). To compare the pulse waveforms of gingival blood flow at rest and at 30 seconds after discontinuation of the thermal stimulus in detail, standardized pulse waveforms of gingival blood flow in both groups were evaluated (Figure 6). In the young group, there were no significant changes in either the increase or decrease of gingival blood flow induced by the heartbeat before or after thermal stimulation.

Although in the middle-aged group, the increase in blood flow, induced by the heartbeat, became rapid after thermal stimulation, the outflow of blood also became rapid after reaching its peak; and the time at which MBR was 50% or more was reduced (Figure 6). And while no significant differences were noted between the groups in terms of changes in gingival MBR or vascular...
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Table 1. Participant characteristics and gingival blood flow parameters in the young and middle-aged groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Young (n = 12)</th>
<th>Middle-aged (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>20.9 ± 0.6</td>
<td>22.7 ± 0.5*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>106.6 ± 2.9</td>
<td>110.5 ± 3.7*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>63.2 ± 2.0</td>
<td>61.5 ± 2.0*</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>80.8 ± 2.3</td>
<td>80.9 ± 2.6*</td>
</tr>
<tr>
<td>PP (mmHg)</td>
<td>43.5 ± 1.9</td>
<td>49.0 ± 2.1*</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>67.7 ± 4.1</td>
<td>62.9 ± 2.6</td>
</tr>
<tr>
<td>PPD (mm)</td>
<td>1.8 ± 0.06</td>
<td>1.87 ± 0.04</td>
</tr>
<tr>
<td>GI</td>
<td>0.87 ± 0.05</td>
<td>0.78 ± 0.09</td>
</tr>
<tr>
<td>MBR (au)</td>
<td>862.7 ± 59.1</td>
<td>887.4 ± 55.2</td>
</tr>
<tr>
<td>MBR/MBP (au/mmHg)</td>
<td>10.8 ± 0.8</td>
<td>11.0 ± 0.7*</td>
</tr>
<tr>
<td>FAI (au)</td>
<td>64.1 ± 8.9</td>
<td>53.8 ± 7.0</td>
</tr>
<tr>
<td>ATI</td>
<td>29.1 ± 1.3</td>
<td>34.6 ± 1.3**</td>
</tr>
<tr>
<td>Falling rate</td>
<td>12.3 ± 0.2</td>
<td>12.3 ± 0.3</td>
</tr>
<tr>
<td>BOT</td>
<td>55.3 ± 0.9</td>
<td>53.9 ± 1.3</td>
</tr>
</tbody>
</table>

*The blood pressure data for the middle-aged group show the average for 12 participants because the blood pressure sensor for 1 participant deviated during measurement.

Mean ± standard error

*P < 0.05, **P < 0.01 vs. the young group (t-tests)

au, arbitrary unit

Table 2. Changes in gingival blood flow before and after thermal stimulus in the young and middle-aged groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Young (n = 12)</th>
<th>Middle-aged (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At rest</td>
<td>30 s after heating</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>80.8 ± 2.3</td>
<td>81.0 ± 2.6</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>67.7 ± 4.1</td>
<td>68.7 ± 4.7</td>
</tr>
<tr>
<td>MBR (au)</td>
<td>10.8 ± 0.8</td>
<td>13.8 ± 1.1**</td>
</tr>
<tr>
<td>FAI (au)</td>
<td>64.1 ± 8.9</td>
<td>101.5 ± 14.1**</td>
</tr>
<tr>
<td>ATI</td>
<td>29.1 ± 1.3</td>
<td>27.9 ± 1.1</td>
</tr>
<tr>
<td>Falling rate</td>
<td>12.3 ± 0.2</td>
<td>11.8 ± 0.3</td>
</tr>
<tr>
<td>BOT</td>
<td>55.3 ± 0.9</td>
<td>55.9 ± 2.4</td>
</tr>
</tbody>
</table>

*MBP data for the middle-aged group are the average for 12 participants because the blood pressure sensor for 1 participant deviated during measurement.

Mean ± standard error

*P < 0.05, **P < 0.01 vs. At rest (paired t-test)

†P < 0.05, ‡P < 0.01 vs. The young group (t-test)

MBP, mean blood pressure; HR, heartbeat; MBR, mean blur rate; FAI, flow acceleration index; ATI, acceleration time index; BOT, blowout time
conductance (MBR/MBP) before and after thermal stimulation, some differences were observed in the shapes of the pulse waveforms after thermal stimulation. Based on these results, gingival blood flow parameters related to blood inflow, outflow, and persistence were compared before and after thermal stimulation (Table 2). In both groups, FAI temporarily increased in response to heating the gingiva (P < 0.01, Table 2). Although there were no changes in the ATI, falling rate, or the BOT either before or after the thermal stimulus in the young group, BOT decreased in the middle-aged group compared with its pre-stimulus value (At rest: P < 0.05). In addition, although there were no differences in the falling rates at rest between the two groups. The falling rate after heating was significantly higher in the middle-aged group than that in the young group (P < 0.05).

**Discussion**

*The relation between blood pressure and gingival blood flow at rest*

In a previous report of gingival blood flow measurement using LSFG under resting conditions, we showed that gingival vascular conductance (MBR/MBP) and BOT decreased with age, whereas the ATI and falling rate increased. These changes correlated with age-related increases in SBP and PP, suggesting that age-related elevations in SBP and PP may have caused these changes in gingival blood flow parameters. These results suggested that the participants’ resting blood pressure influences gingival blood flow at rest. Therefore, in this study, a comparison of gingival blood flow was conducted between younger and middle-aged participants, with no

![Figure 3](image-url)
differences in normal blood pressure among the groups.

In participants in the middle-aged group, whose blood pressure was similar to that in participants in the young group, there were no significant differences in gingival MBR, gingival vascular conductance (MBR/MBP), BOT, and falling rate compared to the young group. These results were consistent with the hypothesis that the participants' blood pressure strongly influenced these three parameters. ATI was significantly higher in the middle-aged group than in the young group, as was also evidenced in our previous report, suggesting that blood inflow to the gingival tissue may be slower in middle-aged individuals. Because there were no differences in systemic hemodynamic parameters between the two groups, it is likely that the differences in ATI occurred because of other age-related changes in the peripheral gingival tissue.

Changes in gingival blood flow caused by thermal stimulation

Many studies have revealed that blood flow increases with the application of thermal stimulation, electrical stimulation, capsaicin, or nitric oxide (NO) in human and animal gingiva. In those studies, however, age-

**Figure 4.** Time-course changes in MBP, HR, gingival MBR, and vascular conductance (MBR/MBP) in A, the young group and B, the middle-aged group before and after localized thermal stimulation to the gingiva (i.e., heating). MBP (A1,B1), HR (A2,B2), gingival MBR (A3,B3), and vascular conductance (MBR/MBP) (A4,B4).

*P < 0.05 vs. At rest (paired t-test)

Young group (n = 12), middle-aged group (MBP: n = 12; HR: n = 13; MBR: n = 12; MBR/MBP: n = 11). MBP data for the middle-aged group are the average for 12 participants because the blood pressure sensor for 1 participant deviated during measurement. MBR data for the middle-aged group are the average for 12 participants because another participant in this group moved his face upon measuring gingival blood flow after thermal application, making it impossible to measure gingival blood flow for more than 1 minute after the stimulation. Therefore, MBR/MBP data for the middle-aged group are the average for 11 participants.
Figure 5. Mean pulse waveforms for a single heartbeat before and after thermal stimulation in A, the young group and B, the middle-aged group. Time was plotted on the horizontal axis with the duration of 1 heartbeat set as 100, and blood flow (MBR [au]) was plotted on the vertical axis (means ± standard errors).

Figure 6. Mean pulse waveforms for a single heartbeat at rest and at 30 seconds after heating in A, the young group and B, the middle-aged group. Time was plotted on the horizontal axis with the duration of 1 heartbeat set as 100. Blood flow (MBR [%]) was plotted on the vertical axis using 0 and 100 as the minimum and maximum values of the mean pulse waveforms, respectively (means ± standard errors). In B’, the middle-aged group, reductions in the blood flow after reaching the peak became faster at 30 seconds after heating and the duration of high blood flow was shorter compared with that at rest. In A’, the young group, reductions in the blood flow after reaching the peak did not change after the thermal stimulus.
related changes in responsiveness during stimulation were not evaluated. In forehead skin, which is dominated by parasympathetic vasodilator fibers in addition to sympathetic fibers, similar to the gingiva, the reactivity of the skin blood vessels to cooling stimuli is reported to decrease with age. Therefore, in addition to measuring blood flow at rest, a comparison of changes in responsiveness upon simple stimulation to the gingival blood vessels was performed. A method was selected in which the gingival area was locally heated for a short time with a plastic tube kept at a constant temperature with warm water, which could be performed without difficulty and posed a very low physical burden on the participant. By heating the maxillary anterior gingiva for 1 minute at 40°C, a temporary increase in blood flow was confirmed in both groups without any significant changes in blood pressure, indicating that this increase in blood flow was caused by the temporary peripheral vasodilation in the gingiva. The HR decrease during thermal stimulation suggested that this vasodilation occurred via activation of parasympathetic nerves. Moreover, because warming at 40°C did not cause pain, and the temperature was lower than the threshold for the activation of the transient receptor potential cation channel subfamily V member 1, also known as the capsaicin receptor (<43°C), nociceptive C-fibers were not likely to be involved in this vasodilation in the gingiva.

Contrary to the report on the response in forehead skin, in the present study, we observed no significant differences between the two groups in terms of changes in gingival MBR and vascular conductance (MBR/MBP) before or after thermal stimulation. Systemic factors, such as hypertension, have been reported to lower cutaneous vasodilation by sympathetic vasodilator fibers. If a similar effect were to occur in the parasympathetic vasodilator fibers involved in gingival vasodilation, the fact that the two groups in the present study showed no significant differences in blood pressure could explain the lack of differences in responsiveness to the thermal stimulus. Moreover, the fact that the thermal stimulus in the present study was mild (40°C for 1 minute) might explain why there were no age-related differences in response to its application.

Changes in pulse waveforms of gingival blood flow caused by thermal stimulation

Significant increases in FAI after localized heating in the gingiva were observed in both groups, indicating that blood inflow to the gingival tissue became more rapid after the stimulus because of decreases in vascular resistivity due to peripheral vasodilation in the gingiva. Although no differences were observed in outflow parameters in the young group, BOT was temporarily decreased immediately after thermal stimulation in the middle-aged group, and the falling rate after stimulus was significantly higher in the middle-aged group than that in the young group. These results showed that during temporary vasodilation in the gingiva due to thermal stimulation, blood outflow from the gingival blood vessels in the middle-aged group became more rapid compared with that in the young group and at rest, indicating that the ability to maintain blood flow in the gingival vessels was reduced.

This decrease in the ability to maintain blood flow in the gingiva during localized thermal stimulation may be a result of the decreased extensibility of gingival arterioles and/or a decreased gingival vascular regulatory function. The length of the elastic fibers in gingival tissue decreases with age, leading to a decline in the elasticity of the gingiva. In addition, calcification in the arterioles of human dental pulp begins around the age of 40, together with a thickening of the intima and its elastic fibers. These reports suggest that an age-related hardening of arterioles and decrease in blood vessel extensibility may be occurring in the gingiva as well. The reduction in the ability to maintain blood flow to the gingiva after temporarily applying a thermal stimulus, as was observed in the middle-aged group, may be explained by the decrease in the extensibility of gingival blood vessels caused by temporary vasodilation. Nagashima et al. reported that the age-related decrease in the responsiveness of forehead cutaneous blood vessels to cooling stimuli is caused by the aging of vasoconstrictor and vasodilator nerves, such as decreases in responses of skin sympathetic nervous activity to stimuli, decreases in noradrenaline release from the nerve terminals of vasoconstrictive fibers, and decreases in the sensitivity of α receptors of cutaneous smooth muscles to noradrenaline, in advance of age-related histoanatomical changes in the cutaneous blood vessels. A reduction in NO producing activity has also been reported in studies of in vitro aging in cultured human umbilical vein endothelial cells. Therefore, production of NO in the gingiva, a vasorelaxant, and the reactivity of blood vessels to neurotransmitters are likely to decrease with age, and these neural factors may be involved in the decrease in blood flow persistence during temporary vasodilation by thermal stimuli.

This study had a couple of limitations. One was the relatively small sample population of volunteer participants, and the other was that only one heating stimulus of 40°C for 1 minute was tested. Therefore,
further research with different temperatures and heating durations is warranted to clarify the responses of gingival blood flow to thermal stimuli. Despite these limitations, using LSFG, gingival blood flow under thermal stimulation allowed us to accurately detect hemodynamic changes that could not be observed simply by measuring changes in blood flow volume. Moreover, focusing on blood-flow-persistence parameters, it was evidenced that, compared with younger individuals, middle-aged individuals, with almost no differences in at-rest gingival blood flow persistence, showed significant decreases in blood flow persistence.

In conclusion, when comparing middle-aged participants to younger participants with comparable at-rest blood pressures, we found that there were no decreases in indices such as gingival vascular conductance and blood flow persistence as measured by gingival blood flow at rest using LSFG. Upon application of localized thermal stimulation to the gingiva, the blood flow persistence in middle-aged participants significantly decreased compared with that in the younger participants. These results indicated that the ability to maintain gingival blood flow may decrease with age, which may affect the risk of developing periodontal disease.

Acknowledgments

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Conflicts of Interest: None

References
