



Pulse waveform analysis on temporal changes in ocular blood flow due to caffeine intake: a comparative study between habitual and non-habitual groups

Aishah Ismail¹ · Mehwish S. Bhatti¹ · Ibrahima Faye¹ · Cheng Kai Lu¹ · Augustinus Laude^{1,2,3} · Tong Boon Tang¹ 

Received: 30 March 2018 / Revised: 23 May 2018 / Accepted: 29 May 2018 / Published online: 6 June 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Purpose To evaluate and compare the temporal changes in pulse waveform parameters of ocular blood flow (OBF) between non-habitual and habitual groups due to caffeine intake.

Method This study was conducted on 19 healthy subjects (non-habitual 8; habitual 11), non-smoking and between 21 and 30 years of age. Using laser speckle flowgraphy (LSFG), three areas of optical nerve head were analyzed which are vessel, tissue, and overall, each with ten pulse waveform parameters, namely mean blur rate (MBR), fluctuation, skew, blowout score (BOS), blowout time (BOT), rising rate, falling rate, flow acceleration index (FAI), acceleration time index (ATI), and resistive index (RI). Two-way mixed ANOVA was used to determine the difference between every two groups where $p < 0.05$ is considered significant.

Result There were significant differences between the two groups in several ocular pulse waveform parameters, namely MBR (overall, vessel, tissue), BOT (overall), rising rate (overall), and falling rate (vessel), all with $p < 0.05$. In addition, the ocular pulse waveform parameters, i.e., MBR (overall), skew (tissue), and BOT (tissue) showed significant temporal changes within the non-habitual group, but not within the habitual group. The temporal changes in parameters MBR (vessel, tissue), skew (overall, vessel), BOT (overall, vessel), rising rate (overall), falling rate (overall, vessel), and FAI (tissue) were significant for both groups (habitual and non-habitual) in response to caffeine intake.

Conclusion The experiment results demonstrated caffeine does modulate OBF significantly and response differently in non-habitual and habitual groups. Among all ten parameters, MBR and BOT were identified as the suitable biomarkers to differentiate between the two groups.

Keywords Pulse waveform · Laser speckle flowgraphy · Effect of caffeine · Habitual and non-habitual · Ocular blood flow

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00417-018-4030-9>) contains supplementary material, which is available to authorized users.

✉ Tong Boon Tang
tongboon.tang@utp.edu.my

¹ Centre for Intelligent Signal and Imaging Research (CISIR), Universiti Teknologi PETRONAS, Bandar Seri Iskandar, Perak, Malaysia

² Tan Tock Seng Hospital, National Healthcare Group Eye Institute, Jalan Tan Tock Seng, Singapore, Singapore

³ Lee Kong Chian School of Medicine and School of Materials Science and Engineering, Nanyang Technological University, Singapore, Singapore

Introduction

Caffeine is associated to promote alertness, attention, and task-switching [1]. It is estimated that an average cup of coffee contains 100 mg of caffeine. Approximately, 240 ml of brewed coffee contains 72 to 130 mg of caffeine while a shot of espresso contains 58 to 76 mg of caffeine. Caffeine is known for its effect on blood flow in the body. Caffeine increases blood vessel resistance and decreases blood flow in the human optical nerve head (ONH) [2] and there is a significant vasoconstriction response of the retinal vessel diameter 1 h after an intake of 200 mg caffeine in young healthy subjects [3]. There were studies on tolerance of caffeine among habitual consumers and comparison between those with different levels of caffeine consumption [4]. However, the difference in ocular blood flow (OBF) changes as a result of caffeine intake between non-habitual and habitual consumers is not known.

Laser speckle flowgraphy (LSFG) is a non-invasive method to quantify the velocity of blood flow in the ONH without the need of using any pupil dilation agent [5, 6]. The LSFG uses laser speckle phenomenon, an interference event where coherent light sources such as lasers are scattered by a diffusing surface, thus producing speckle pattern, which can be described statistically. In accordance with the movement of blood cells in the tissue, the structure of the speckle pattern varies according to blood flow velocity. The mean blur rate (MBR) represents the index of the blood velocity. Hence, the greater the movement of red blood cells, the higher rate of MBR. The LSFG device captures MBR images at 30 frames per second for a period of 4 s and its embedded software synchronizes the images with cardiac cycles. The averaged MBR of a heartbeat are displayed as heartbeat map and provides pulse waveform parameters [5].

The aim of this study is to evaluate the effects of caffeine on OBF between non-habitual and habitual caffeine consumers. Caffeine capsules were used to induce changes of OBF. To our best knowledge, this is the first comparative study about how ocular blood flow pulse waveform parameters differ in time series between habitual and non-habitual caffeine consumers.

Methods

Subjects

Healthy subjects (age 21–30) with no medical condition or history were recruited. Habitual coffee consumption was defined as daily coffee consumption, and non-habitual coffee consumption was defined as non-daily coffee consumption [7]. In this study, we defined habitual caffeine consumers as those who consumed more than three cups of coffee per day and non-habitual as those who do not take coffee or tea beverages at all. Exclusion criteria include any physical, mental, and eye diseases; intake of medications; smokers; obesity; and known hypersensitivity to caffeine. In total, 19 subjects were recruited; 8 subjects were classified as non-habitual consumers and 11 subjects were classified as habitual consumers. All subjects were briefed about the purpose, processes, and risks before the start of the study.

Study protocol

The subjects were instructed not to consume any alcohol, energy, and carbonated drinks 1 week before (for the habitual group) and abstained from caffeinated beverages and vigorous physical activities 24 h before the experiment. This is to ensure washout period from previous caffeine consumption [8]. Next, subjects were instructed to have at least 6 h of sleep and female subjects confirmed not to be on a menstrual cycle. Subjects were allowed to have breakfast but with only plain water 2 h before the experiment. All experiments were carried out at the same time each day to avoid diurnal variation effect. All laboratory sessions started at 10 a.m. The session took place in a dark, quiet room with an ambient temperature maintained at 24 °C. Upon arrival, each subject was given 15-min rest in the room to maintain normal blood pressure. Blood pressure and several LSFG readings were taken 10 min before the experiment started. The session then started by giving two capsules of caffeine, equivalent in total to 180 mg of caffeine together with 200 ml of mineral water and LSFG measurements were taken immediately (marked as time point t1, 0 min). LSFG measurements were taken every 20 min for 2 h as shown in Fig. 1.

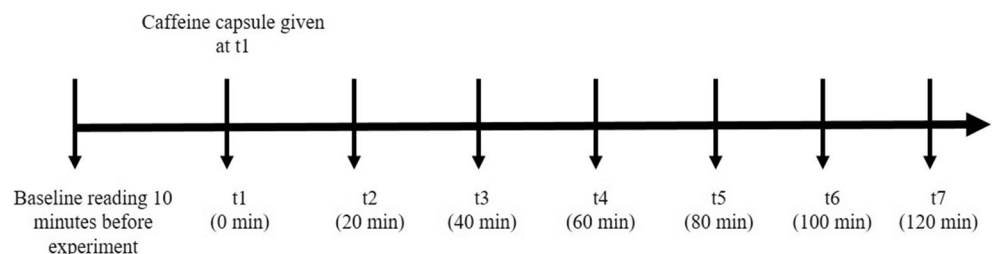
Caffeine capsule

Caffeine capsule used in this study is 250 mg guarana seed extract capsule (GNC Live Well Pharmacy, Malaysia)—vegetable capsule contained 36% (90 mg) of caffeine per capsule. Guarana capsule is a natural source of caffeine, which has the invigorating properties to promote stamina and physical performance [9]. The reason for using caffeine capsules is to avoid bias and to standardize the actual amount of caffeine as brewed coffee does not have a standardized amount of caffeine. Besides, the taste and add-ins such as sugar and dairy may vary according to the subjects.

Laser speckle flowgraphy measurements

Each subject was sat comfortably with his/her head positioned on a chin rest. The laser speckle flowgraphy system LSFG-NAVI (Softcare Co., Ltd., Japan) was used to measure the ocular blood flow. The LSFG-NAVI system was adjusted according to eye position. The subject's head was then covered over with a lightweight dark scarf. LSFG measurements were taken in a dark room and were performed only on the right eyes for all subjects.

Fig. 1 Time points of LSFG measurements for 2 h with 20-min interval



Full ONH was fit to be captured within the frame and the focus was adjusted by looking at the live-capture image. The focus of the image was adjusted during the real-time image to eliminate any gap which otherwise appeared as horizontal dark lines [10]. Each LSFG measurement was taken over a period of 4 s and three consecutive recordings were acquired at each time point. LSFG analyzer software (LSFG analyzer Version 3.1.58; Softcare Co., Ltd., Japan) automatically detected the beginning and the end of each cardiac cycle which were recorded within the 4-s period and identical phases of cardiac cycle images were normalized to one image sequence representing a complete cardiac cycle. Average of signal intensity over the complete cardiac cycle was calculated producing a composite map, which represented the distribution of mean blood flow in one complete cardiac cycle in ONH [11]. An ellipse rubber band was manually set on ONH regions of interest, and pulse waveform parameters were extracted based on selected region of interest. Identical size and position of rubber bands were set for all subsequent images of the same subject. Through vessel segmentation function, pulse waveform parameters in vessel and tissue regions within the ONH area were determined. These were performed through digital cross-section analysis where it differentiated between visible vessels and tissue region within the ONH interest region. The pulse waveform parameter values of three separate regions were obtained: the overall (mean of all values), vessel, and tissue of the ONH (Fig. 2). The description and the calculation of each pulse waveform were described in detailed by Fukami [12].

Statistical analysis

Statistical analysis was performed using SPSS statistical software version 23. The data were observed to follow normal distribution and outliers were removed. Two-way mixed ANOVA was conducted to determine the significant difference between groups at different measuring time points. This was then confirmed with one-way repeated measure (or Friedman's test) when normality is violated for each group of pulse waveform separately. Each

pulse waveform was compared based on (i) between-subject factor (non-habitual and habitual groups) and (ii) within-subject factor (time points). In this study, $p < 0.05$ is considered to be significantly different [13]. For the graph, all values were averaged and normalized according to the baseline reading for each group separately to permit comparisons between groups.

Result

The analyses were based on the average of three captured images of 19 eyes (8 non-habitual and 11 habitual). The mean scores and standard deviation of each pulse waveform parameters were summarized for each region respectively (see supplementary table (Table S1, overall region; Table S2, vessel region; and Table S3, tissue region)). Graphs comparing two groups for each pulse waveform and region are presented in Figs. 3, 4, 5, 6, and 7.

Difference between non-habitual and habitual groups

The ocular pulse waveforms were analyzed separately based on the three defined regions and the following ten waveform parameters, namely mean blur rate (MBR), fluctuation, skew, blowout score (BOS), blowout time (BOT), rising rate, falling rate, flow acceleration index (FAI), acceleration time index (ATI), and resistive index (RI). Among all, several parameters that demonstrated significant difference between two groups are MBR (overall, vessel, and tissue regions), BOT (overall region), rising rate (overall region), and falling rate (vessel region), all with $p < 0.05$. All p values are summarized in Table 1.

Significant response to caffeine observed only in the non-habitual group

We identified several pulse waveform parameters that showed significant changes only in the non-habitual group in response to caffeine intake, i.e., MBR (overall), skew (tissue), BOS (tissue),

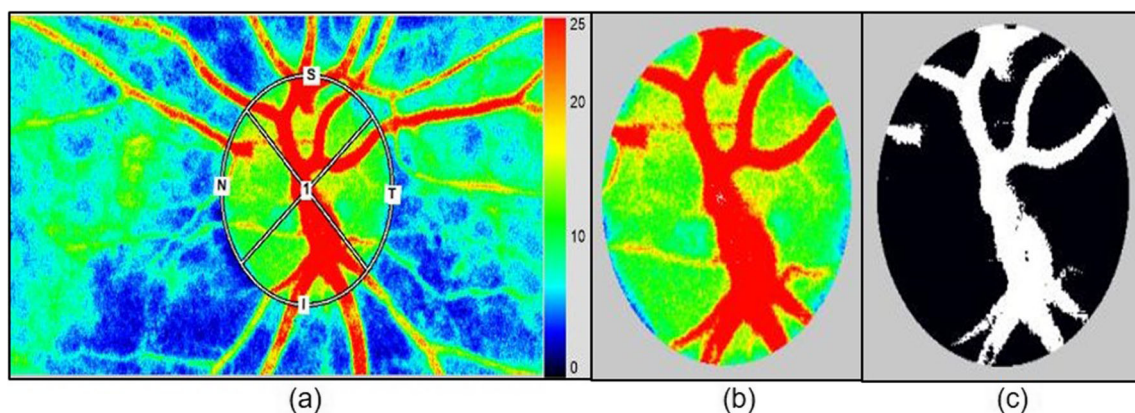
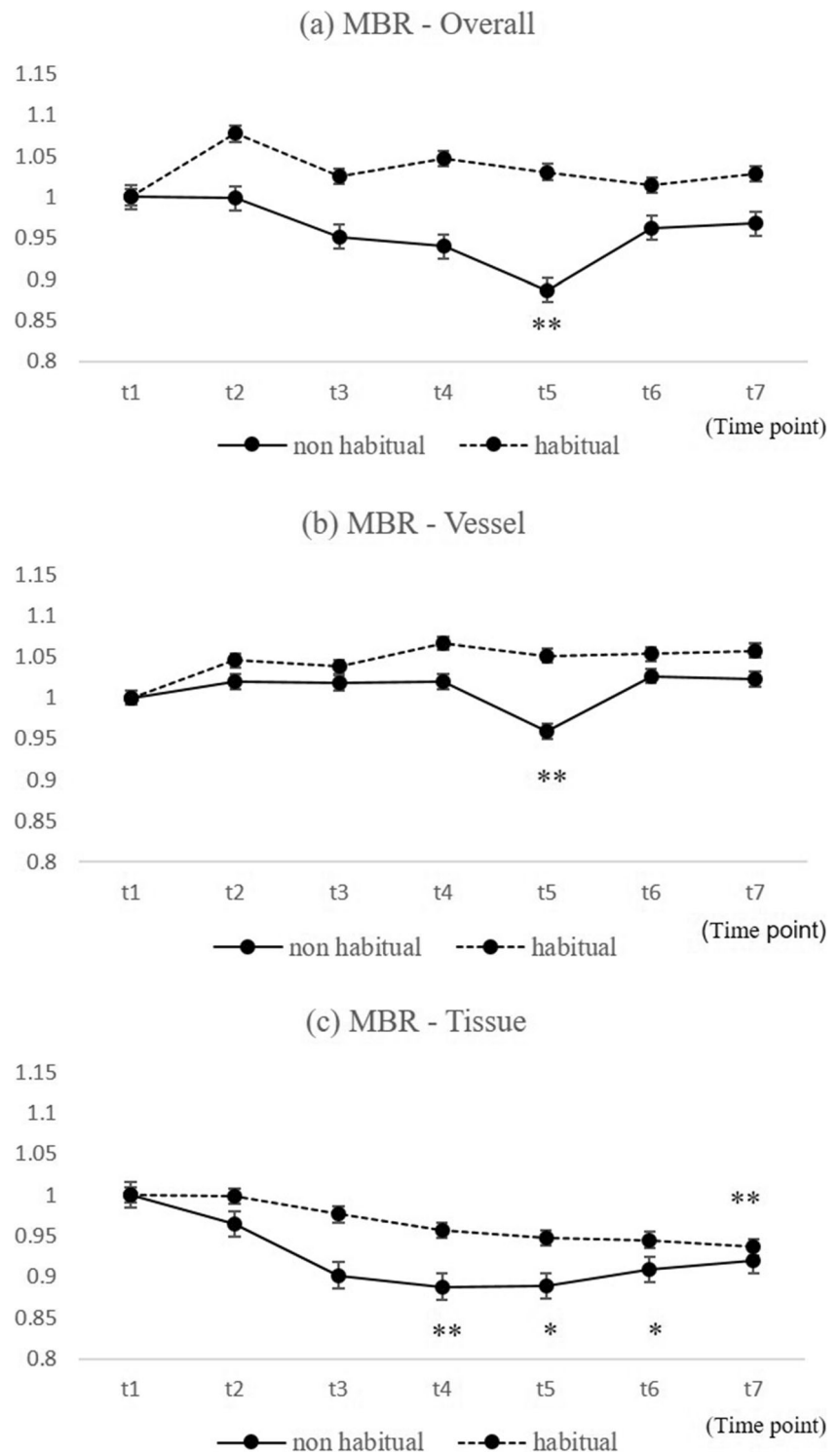


Fig. 2 Definition of ONH region of interest. **a** ONH in the whole frame, region of interest was selected using ellipse rubber band and extracted out. **b** ONH region of interest, defined as overall ONH. **c** Vessel

segmentation of ONH region into vessel area (white) and tissue area (black) within rubber band. A set of 10 pulse waveform parameters from each of three region (overall, vessel, and tissue) were extracted out

Fig. 3 MBR at **a** overall region, **b** vessel region, and **c** tissue region. Vertical bars represent the standard deviation of means and asterisks represent statistically significant difference at the respective time points (* $p < 0.05$ and ** $p < 0.01$)

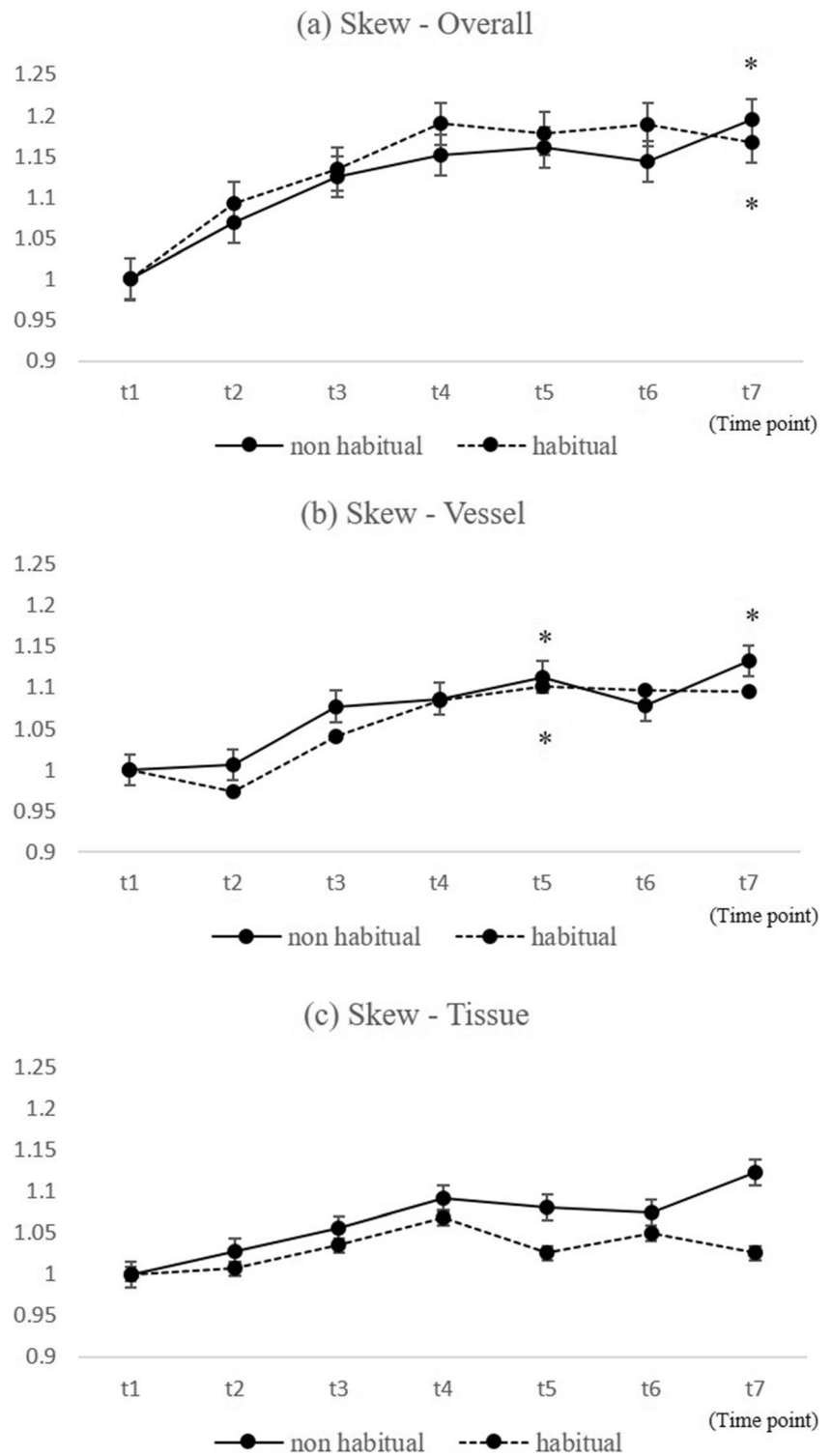


and BOT (tissue). MBR for overall region showed a significant ($p = 0.001$) response at t5, 80 min after caffeine consumption. The skew in tissue region also showed significant difference ($p = 0.013$) between groups at multiple time points; the BOT in tissue region showed significant decrease within group ($p = 0.005$), and between groups at different time points ($p = 0.007$). Differences in p value between the groups are summarized in Table 2.

Significant response to caffeine intake regardless of being habitual or non-habitual

The following are pulse waveform parameters which have responded significantly to caffeine ingestion by both groups: the MBR in vessel region (non-habitual group, $p = 0.01$; habitual group, $p = 0.024$) and tissue region (non-habitual, $p = 0.000$

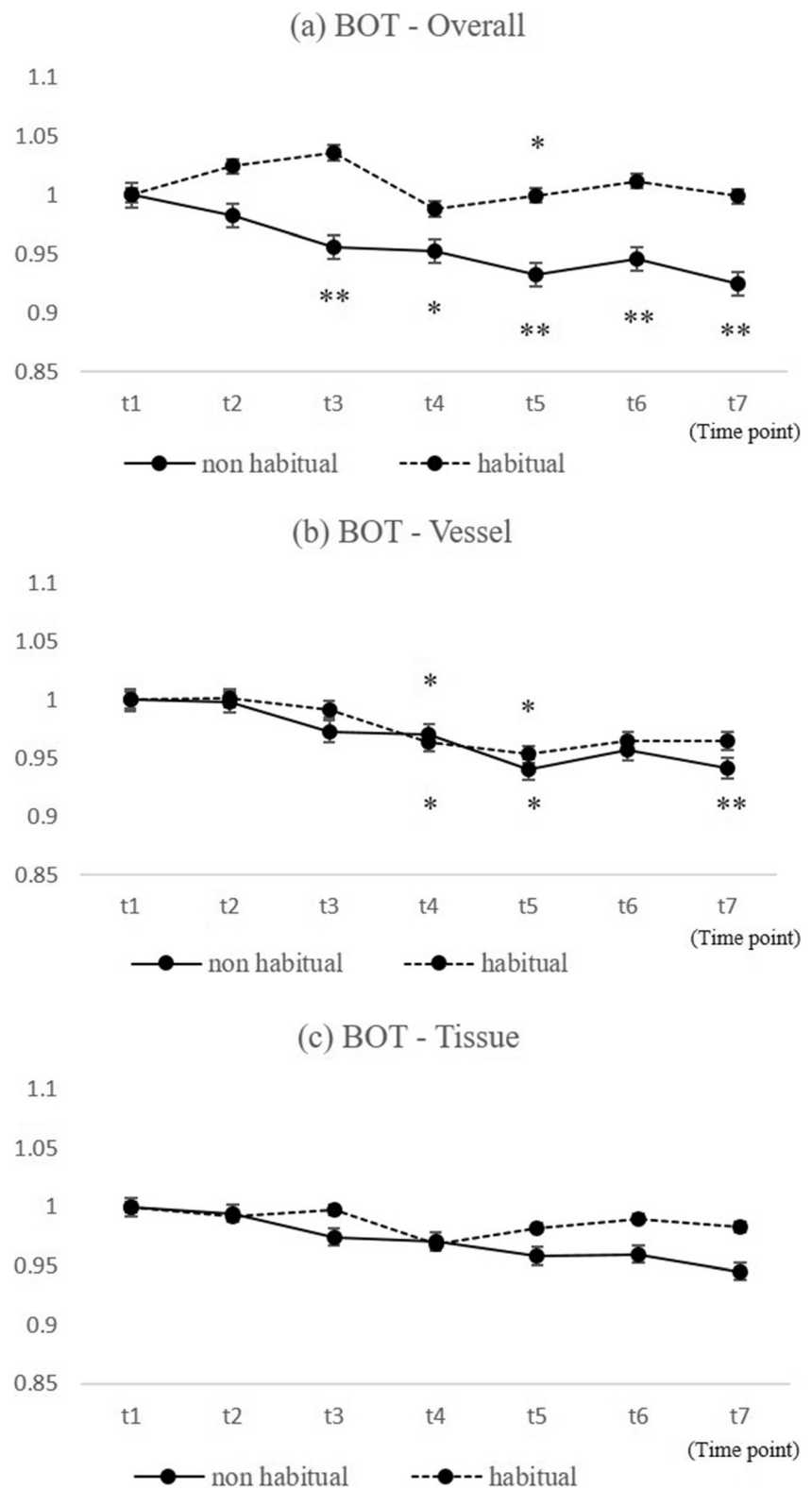
Fig. 4 SKEW at **a** overall region, **b** vessel region, and **c** tissue region. Vertical bars represent the standard deviation of means and asterisks represent statistically significant difference at the respective time points (* $p < 0.05$ and ** $p < 0.01$)



at t4, $p = 0.05$; t5, $p = 0.05$; t6, $p = 0.027$; and habitual, $p = 0.001$) shows significant difference; the skew in overall region (non-habitual, $p = 0.0004$; habitual, $p = 0.002$ —Friedman, $p = 0.01$) and vessel region (non-habitual, $p = 0.024$; habitual, $p = 0.015$) both demonstrates significant increase; the BOT in overall (non-habitual, $p = 0.0005$; habitual, $p = 0.015$) and vessel

regions (non-habitual, $p = 0.004$; habitual, $p = 0.025$) demonstrates significant decrease; the rising rate in overall region showed significant increase (non-habitual, $p = 0.0021$; habitual, $p = 0.001$) and temporal response showed significant difference between groups at time point t1, $p = 0.000$; t2, $p = 0.002$; and t3, $p = 0.013$; the falling rates in overall (non-habitual, $p = 0.025$;

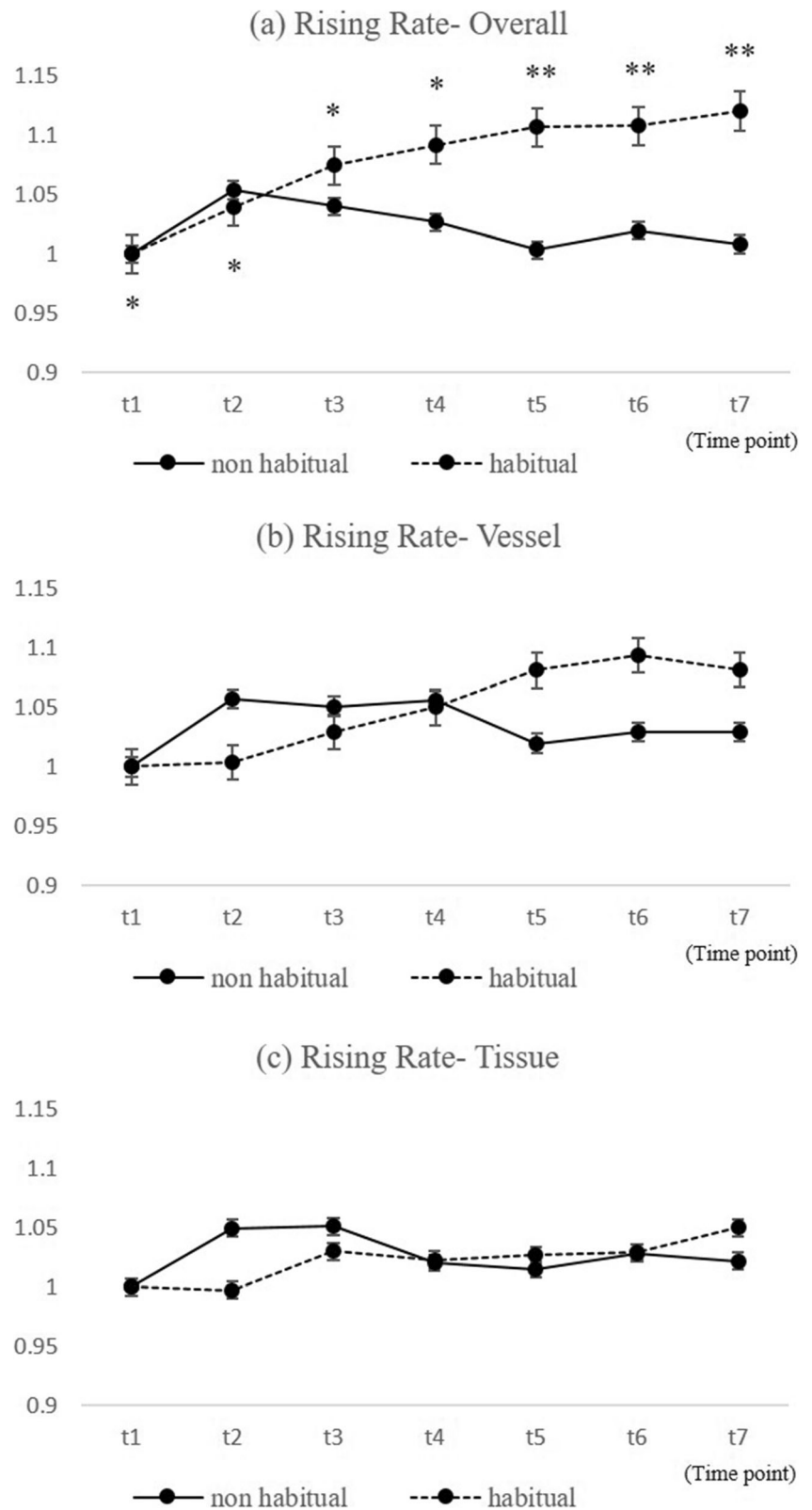
Fig. 5 BOT at **a** overall region, **b** vessel region, and **c** tissue region. Vertical bars represent the standard deviation of means and asterisks represent statistically significant difference at the respective time points (* $p < 0.05$ and ** $p < 0.01$)



habitual, $p = 0.002$) and vessel regions (non-habitual-vessel, $p = 0.007$; habitual vessel, $p = 0.007$) both demonstrate significant increase and temporal response showed significant difference in mean at different time points, $p = 0.000$; the FAI in tissue region

(non-habitual-tissue, $p = 0.000$; habitual, $p = 0.047$) and temporal response showed significant difference in mean at different time points, $p = 0.000$. p values reporting significant difference within groups at each region are summarized in Table 3.

Fig. 6 Rising rate at **a** overall region, **b** vessel region, and **c** tissue region. Vertical bars represent the standard deviation of means and asterisks represent statistically significant difference at the respective time points (* $p < 0.05$ and ** $p < 0.01$)

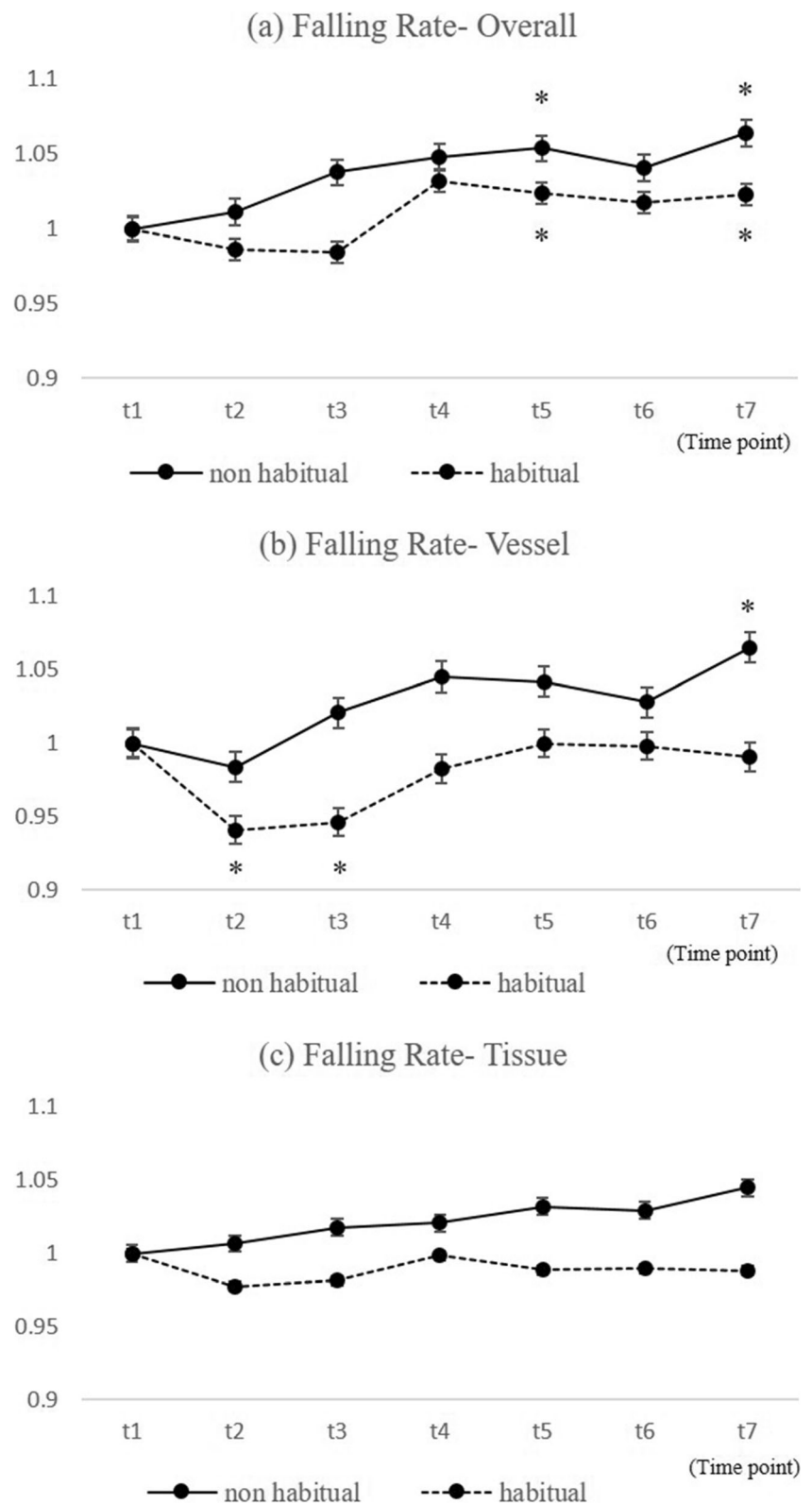


Discussion

Our experimental results found that the pulse waveform parameters differing significantly between the two groups were

MBR in all three different regions, BOT in overall region, rising rate in overall region, and falling rate in vessel region. Primarily, in this study, the index of blood velocity (i.e., MBR) demonstrated significant difference between these two groups

Fig. 7 Falling rate at **a** overall region, **b** vessel region, and **c** tissue region. Vertical bars represent the standard deviation of means and asterisks represent statistically significant difference at the respective time points (* $p < 0.05$ and ** $p < 0.01$)



in all three regions. Of overall region, the non-habitual group showed highly significant irregular temporal pattern of MBR, while no similar change was observed in the habitual group. Moreover, the non-habitual group had significant changes

observed in several pulse waveform parameters, namely MBR (overall), skew (tissue), BOS (tissue), and BOT (tissue).

In response to caffeine ingestion, several pulse waveform parameters reacted significantly in both the groups. Some of

Table 1 *p* value of the difference between groups at each region

Parameter	Overall	Vessel	Tissue
MBR	0.0210	0.0380	0.0400
BOT	0.0180	–	–
Rising rate	0.0020	–	–
Falling rate	–	0.0000	–

them were more significant in the non-habitual group while the rest in the habitual group; the earlier include MBR (vessel and tissue regions), skew (overall), BOT (overall), and FAI (tissue); the latter were rising rate and falling rate. We further observed skew (overall and vessel regions) in both groups was significantly increase in time; when examined closer, skew in the non-habitual group was higher significantly compared to the habitual group, indicating that blood flow would rise in speed faster in the non-habitual than in the habitual group in response to caffeine. When blood flow rises quickly, both the rising rate and the falling rate also increase and descend quickly.

BOT represents how long the MBR stays above the average value over its entire waveform. High BOT indicates that the blood flow volume in the vasculature is at a high level for a long time between heartbeats [14]. In this study, BOT (overall and vessel regions) was observed to decrease over time in both the groups. However, the non-habitual group showed highly significant decrease when compared to the habitual group, and the mean BOT graph of both groups as plotted in Fig. 5 shows the BOT level of the habitual group is higher. These results validate that vessel resistance in the habitual group is stronger compared to that in the non-habitual group most likely as a result of the adaptation to long-term caffeine consumption. They also support the suggestion that the BOT can serve as an important vascular resistance biomarker [15]. Vessel resistances are essential in maintaining vascular homeostasis, ensuring sufficient blood flow for nutrient delivery. In turn, impaired vessel resistance will provide important information on retinal and cerebrovascular diseases [16].

Our experimental results are consistent with the exaggerated effect on cerebral blood flow due to caffeine as observed in non-habitual consumers while no effect shown in habitual consumers [17]. Cerebral blood flow was found to decrease

Table 2 *p* value that is significant in non-habitual but not significant in habitual group

Region	Parameter	Non-habitual	Habitual
Overall	MBR	0.0010	0.2144
Tissue	Skew	0.0127	0.4883
	BOT	0.0050	0.3984

Table 3 *p* value that is significant in both groups

Region	Parameter	Non-habitual	Habitual
Overall	Skew	0.0004	0.0100
	BOT	0.0005	0.0150
	Rising rate	0.0210	0.0010
	Falling rate	0.0250	0.0020
Vessel	MBR	0.0100	0.0240
	Skew	0.0240	0.0150
	BOT	0.0040	0.0250
	Falling rate	0.0074	0.0071
Tissue	MBR	0.0000	0.0010
	FAI	0.0000	0.0470

in low and moderate caffeine consumers compared to high caffeine consumers. This can be explained where habitual caffeine consumers are known to adapt or tolerate the effect of caffeine on their bodies as long-term caffeine consumption promotes the additional build-up of adenosine receptors [18] to compensate the vasoconstriction effect due to caffeine intake [17]. Hence, it would take an extra dose of caffeine to exert the same effect as the non-habitual consumers. The study by Bell et al. [8] further showed that non-caffeine consumers benefit more from caffeine supplementation. Our experiment results demonstrated that the non-habitual group experienced a greater effect on OBF pulse waveform in comparison to the habitual group.

In this study, we excluded physiological factors such as gender, age, and BMI although they might affect MBR [11, 19]. For instance, smaller size individuals might have a greater effect of caffeine compared to larger size individuals. Furthermore, caffeine normally starts to take effect 15 min after ingestion; however, this varies depending on one's individual metabolism. Some response with an acute rise in blood pressure, while some do not see increase in their blood pressure [3, 20]. Besides, to avoid diurnal variation, this study was conducted at the same time, i.e., 10 a.m., as several reports showed significant diurnal variation in healthy subjects, from 9 a.m. to 12 p.m. [21–23]. This is because heart rate is strongly correlated with LSFG pulse waveform as the number of frames, 30 per seconds reflect the duration of heartbeat [5], and heart rate is known to be low in the morning and high in the evening [22]. Next, increase in sympathetic activity will constrict blood vessels and the level of sympathetic activity is usually high in the morning [12, 24].

Admittedly, this study presents several limitations. First, all subject were university students and finding non-habitual caffeine subjects was challenging, leading to limited sample size. As such, the sample size was too small for subdivision of gender, for instance. Next, we did not compare the readings between both eyes as we assumed them to experience the

same effect. Moreover, there was no comparison with other modalities to measure ocular blood flow and vascular changes such as optical coherence tomography.

In conclusion, despite the presence of variability in physiological factors such as gender, age, and BMI, this study demonstrates a significant microcirculation changes in ONH in terms of caffeine effects between habitual and non-habitual consumers. In addition, our results indicated pulse waveform parameters MBR and BOT might serve as important biomarkers to differentiate between non-habitual and habitual caffeine consumers.

Funding information The project was funded by the Ministry of Higher Education Malaysia under HICoE scheme to CISIR. The sponsor had no role in the design or conduct of the research.

Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

References

- McLellan TM, Caldwell JA, Lieberman HR (2016) A review of caffeine's effects on cognitive, physical and occupational performance. *Neurosci Biobehav Rev* 71:294–312. <https://doi.org/10.1016/j.neubiorev.2016.09.001>
- Okuno T, Sugiyama T, Tominaga M, Kojima S, Ikeda T (2002) Effects of caffeine on microcirculation of the human ocular fundus. *Jpn J Ophthalmol* 46(2):170–176
- Terai N, Spoerl E, Pillunat LE, Stodtmeister R (2012) The effect of caffeine on retinal vessel diameter in young healthy subjects. *Acta Ophthalmol* 90(7):e524–e528. <https://doi.org/10.1111/j.1755-3768.2012.02486.x>
- Kennedy DO, Haskell CF (2011) Cerebral blood flow and behavioural effects of caffeine in habitual and non-habitual consumers of caffeine: a near infrared spectroscopy study. *Biol Psychol* 86(3):298–306. <https://doi.org/10.1016/j.biopsycho.2010.12.010>
- Sugiyama T, Araie M, Riva CE, Schmetterer L, Orgul S (2010) Use of laser speckle flowgraphy in ocular blood flow research. *Acta Ophthalmol* 88(7):723–729. <https://doi.org/10.1111/j.1755-3768.2009.01586.x>
- Sato T, Sugawara J, Aizawa N, Iwama N, Takahashi F, Nakamura-Kurakata M, Saito M, Sugiyama T, Kunikata H, Nakazawa T, Yaegashi N (2017) Longitudinal changes of ocular blood flow using laser speckle flowgraphy during normal pregnancy. *PLoS One* 12(3):e0173127. <https://doi.org/10.1371/journal.pone.0173127>
- Zimmermann-Viehoff F, Thayer J, Koenig J, Herrmann C, Weber CS, Deter HC (2016) Short-term effects of espresso coffee on heart rate variability and blood pressure in habitual and non-habitual coffee consumers—a randomized crossover study. *Nutr Neurosci* 19(4):169–175. <https://doi.org/10.1179/1476830515Y.0000000018>
- Bell DG, McLellan TM (2002) Exercise endurance 1, 3, and 6 h after caffeine ingestion in caffeine users and nonusers. *J Appl Physiol* 93(4):1227–1234. <https://doi.org/10.1152/jappphysiol.00187.2002>
- Portella Rde L, Barcelos RP, da Rosa EJ, Ribeiro EE, da Cruz IB, Suleiman L, Soares FA (2013) Guarana (Paullinia cupana Kunth) effects on LDL oxidation in elderly people: an in vitro and in vivo study. *Lipids Health Dis* 12:12. <https://doi.org/10.1186/1476-511X-12-12>
- Aizawa N, Yokoyama Y, Chiba N, Omodaka K, Yasuda M, Otomo T, Nakamura M, Fuse N, Nakazawa T (2011) Reproducibility of retinal circulation measurements obtained using laser speckle flowgraphy-NAVI in patients with glaucoma. *Clin Ophthalmol* 5:1171–1176. <https://doi.org/10.2147/OPHT.S22093>
- Luft N, Wozniak PA, Aschinger GC, Fondi K, Bata AM, Werkmeister RM, Schmidl D, Witkowska KJ, Bolz M, Garhofer G, Schmetterer L (2016) Ocular blood flow measurements in healthy white subjects using laser speckle flowgraphy. *PLoS One* 11(12):e0168190. <https://doi.org/10.1371/journal.pone.0168190>
- Fukami M, Iwase T, Yamamoto K, Kaneko H, Yasuda S, Terasaki H (2017) Changes in retinal microcirculation after intravitreal ranibizumab injection in eyes with macular edema secondary to branch retinal vein occlusion. *Invest Ophthalmol Vis Sci* 58(2):1246–1255. <https://doi.org/10.1167/iovs.16-21115>
- Laerd S (2015) Two-way mixed ANOVA using SPSS statistics. Statistical tutorials and software guides. <https://statistics.laerd.com/premium/spss/twma/two-way-mixed-anova-in-spss-24.php>. Accessed 8 Jan 2018
- Tsuda S, Kunikata H, Shimura M, Aizawa N, Omodaka K, Shiga Y, Yasuda M, Yokoyama Y, Nakazawa T (2014) Pulse-waveform analysis of normal population using laser speckle flowgraphy. *Curr Eye Res* 39(12):1207–1215. <https://doi.org/10.3109/02713683.2014.905608>
- Enaida H, Nagata S, Takeda A, Nakao S, Ikeda Y, Ishibashi T (2016) Changes in chorioretinal blood flow velocity and cerebral blood flow after carotid endarterectomy. *Jpn J Ophthalmol* 60(6):459–465. <https://doi.org/10.1007/s10384-016-0472-y>
- Bettermann K, Slocomb J, Shivkumar V, Quillen D, Gardner TW, Lott ME (2017) Impaired retinal vasoreactivity: an early marker of stroke risk in diabetes. *J Neuroimaging : Official J Am Soc Neuroimaging* 27(1):78–84. <https://doi.org/10.1111/jon.12412>
- Addicott MA, Yang LL, Peiffer AM, Burnett JR, Burdette JH, Chen MY, Hayasaka S, Kraft RA, Maldjian JA, Laurienti PJ (2009) The effect of daily caffeine use on cerebral blood flow: how much caffeine can we tolerate? *Hum Brain Mapp* 30(10):3102–3114. <https://doi.org/10.1002/hbm.20732>
- Meredith SE, Juliano LM, Hughes JR, Griffiths RR (2013) Caffeine use disorder: a comprehensive review and research agenda. *J Caffeine Res* 3(3):114–130. <https://doi.org/10.1089/jcr.2013.0016>
- Yanagida K, Iwase T, Yamamoto K, Ra E, Kaneko H, Murotani K, Matsui S, Terasaki H (2015) Sex-related differences in ocular blood flow of healthy subjects using laser speckle flowgraphy. *Invest Ophthalmol Vis Sci* 56(8):4880–4890. <https://doi.org/10.1167/iovs.15-16567>
- Chandrasekaran S, Rochtchina E, Mitchell P (2005) Effects of caffeine on intraocular pressure: the Blue Mountains Eye Study. *J Glaucoma* 14(6):504–507
- Pemp B, Georgopoulos M, Vass C, Fuchsjäger-Mayrl G, Luksch A, Rainer G, Schmetterer L (2009) Diurnal fluctuation of ocular blood flow parameters in patients with primary open-angle glaucoma and healthy subjects. *Br J Ophthalmol* 93(4):486–491. <https://doi.org/10.1136/bjo.2008.148676>
- Fukami M, Iwase T, Yamamoto K, Ra E, Murotani K, Terasaki H (2017) Diurnal variation of pulse waveform parameters determined

- by laser speckle flowgraphy on the optic nerve head in healthy subjects. *Medicine* 96(44):e8312. <https://doi.org/10.1097/MD.00000000000008312>
23. Iwase T, Yamamoto K, Ra E, Murotani K, Matsui S, Terasaki H (2015) Diurnal variations in blood flow at optic nerve head and choroid in healthy eyes: diurnal variations in blood flow. *Medicine* 94(6):e519. <https://doi.org/10.1097/MD.0000000000000519>
24. Panza JA, Epstein SE, Quyyumi AA (1991) Circadian variation in vascular tone and its relation to alpha-sympathetic vasoconstrictor activity. *N Engl J Med* 325(14):986–990. <https://doi.org/10.1056/NEJM199110033251402>