Monitoring of Caffeine Consumption Effect on Skin Blood Properties by Diffuse Reflectance Spectroscopy

Matija MILANIC[1,2], Rok HREN[1,3,4], Jost STERGAR[1,2], Urban SIMONCIC[1,2]

[1]Faculty of Mathematics and Physics, University of Ljubljana, Ljubljana, Republic of Slovenia, [2]Jožef Stefan Institute, Ljubljana, Republic of Slovenia, [3]Institute of Mathematics, Physics, and Mechanics, Ljubljana, Republic of Slovenia, [4]Syreon Research Institute, Budapest, Hungary

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Summary

Caffeine is the most widely consumed psychoactive substance worldwide, affecting numerous tissues and organs, with notable impacts on the central nervous system, heart, and blood vessels. The effect of caffeine on vascular smooth muscle cells is an initial transient contraction followed by significant vasodilatation. In this study we investigate the use of diffuse reflectance spectroscopy (DRS) for monitoring of vascular changes in human skin induced by caffeine consumption. DRS spectra were recorded on volar sides of the forearms of eight healthy volunteers at time intervals of 0, 30, 60, 120, and 180 min after consumption of caffeine, while one subject served as a negative control. Analytical diffusion approximation solutions for diffuse reflectance from three-layer structures were used to assess skin composition (e.g. dermal blood volume fraction and oxygen saturation) by fitting these solutions to experimental data. The results demonstrate that cutaneous vasodynamics induced by caffeine consumption can be monitored by DRS, while changes in the control subject not consuming caffeine were insignificant.

Key words

Caffeine consumption • Diffuse reflectance spectra • Inverse diffusion approximation

Corresponding author

M. Milanic, Faculty of Mathematics and Physics, University of Ljubljana, Jadranska 19, Ljubljana, Republic of Slovenia. E-mail: matija.milanic@fmf.uni-lj.si

Introduction

Caffeine is the psychoactive substance most widely consumed globally, and in contradistinction to many other psychoactive substances, it remains legal and unregulated worldwide. It can be found in various sources, such as coffee, tea, soft drinks, chocolate, and many medications. On average, adults consume approximately 2.4 mg of caffeine per kilogram of body weight per day [1]. Upon consumption, caffeine is rapidly and completely absorbed from the intestinal tract. The maximum caffeine blood concentration is observed in 30-45 min when subjects are fasting and is delayed in presence of food ingestion. The average metabolic half-life in humans ranges from 2.5 to 4.5 h [1]. The large inter-individual differences in caffeine blood concentration following the administration of an equal dose can mainly be attributed to variations in individual metabolism.

Caffeine acts on the cells by different mechanisms involving a wide range of molecules, thus affecting multiple tissues and organs, including the central nervous system, heart and blood vessels [1]. One notable effect is a general increase in sympathetic tonus, resulting in elevated blood pressure. The vessels are mainly affected through mechanisms on the endothelial and vascular smooth muscle cells (VSMC). Direct effect of caffeine on VSMC initially leads to transient vasoconstriction, triggered by an increased level of cytoplasmic Ca^{2+}; later, caffeine induces significant vasodilatation through various mechanisms, including interactions with the myosin-actin complex, inhibiting muscle contraction. Regular caffeine consumption results in a certain tolerance to caffeine. The action of caffeine on small vessels is depicted in Figure 1.

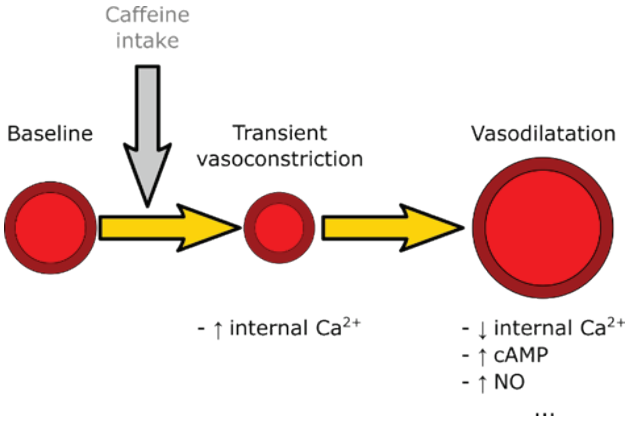

Fig. 1. A scheme of direct caffeine effect on skin vessels. Caffeine initially increases internal concentration of Ca^{2+} facilitating the contraction of the myosin-actin complex. Later on, vasodilatation appears due to decreased cytosolic Ca^{2+}, increased cAMP and NO, all decreasing activity of the myosin-actin complex [1].

The purpose of this study is to explore the feasibility of diffuse reflectance spectroscopy (DRS) to observe and monitor skin vascular changes induced by caffeine consumption. A positive outcome of this investigation would demonstrate that DRS could serve as a direct and noninvasive method for monitoring the actions of vasoactive substances, such as antihypertensive drugs.

Material and Methods

Eight healthy volunteers (four females and four males) were enrolled in the study. These volunteers, who were employees of the University or the Research Institute, provided detailed information about their age, gender, weight, lifestyle, and current health status through a questionnaire. The age range of the volunteers was between 26 and 37 years old and their weight varied from 53 to 104 kg. None of the volunteers were smokers, but five of them reported being regular coffee drinkers, consuming an average of 1-2 cups of coffee per day. One volunteer was identified as a heavy coffee drinker drinking approximately 6 cups of coffee daily. Based on the information gathered from the questionnaire all volunteers were determined to be healthy at the time of the study. These individuals underwent regular health checks by a dedicated medical doctor as a part of their employment at the University or the Research Institute. Prior to the study, informed written consent was obtained from each volunteer, and the study was conducted in accordance with the Declaration of Helsinki.

The initial measurement aimed to validate the use of the DRS technique. To achieve this, DRS measurements were conducted before, during, and after the cuff test on the left upper arm. The purpose of the cuff test was to actively change oxygenation and blood volume fraction in the limb. The cuff was set on upper arm and inflated to the pressure of 200 mm Hg for five minutes preventing any blood flow to the lower part of the arm. The cuff test DRS was measured 5 min after the inflation, and 2 min after the cuff was removed (after).

The main measurements involved caffeine consumption. The volunteers ate their last meal 2-3 h before the measurement, did not consume any caffeine, alcohol or did not smoke. They rested at least 1 h before measurement and did not perform physical activities. The volunteers consumed 3×45 ml of solution containing 300 mg of caffeine derived from guarana (Guarana natural energy, Medex, Slovenia). Measurements were performed before and 30 min, 1 h, 2 h, 3 h after the caffeine intake. These intervals were selected to correspond to typical time intervals of serum caffeine peak and half-concentration as mentioned in the Introduction.

DRS were measured using an integrating sphere (ISP-REF by Ocean Optics, Dunedin, FL) and a spectrometer (USB4000, Ocean Optics) in the 400-1000 nm spectral range. Spectral response was calibrated using a Spectralon© white standard (Labsphere, North Sutton, NH). 100 subsequently acquired spectra (at 15 ms integration time) were averaged to improve the signal-to-noise ratio resulting in 2.5 s measurement in total.

In addition to the caffeine consumption measurement, the same protocol was performed on one volunteer not consuming any caffeine. These measurements served as the reference measurements.

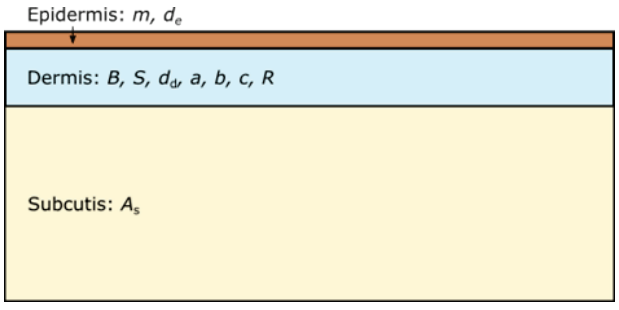

Fig. 2. A scheme of the layered skin model composed of epidermis, dermis and subcutis. Legend: m – melanin volume fraction, d_e – epidermis thickness, B – blood volume fraction, S – oxygen saturation, d_d – dermis thickness, a – scattering amplitude, b – scattering coefficient, c – beta-carotene concentration, R – effective vessel radius, A_s – subcutaneous scattering multiplier.

The measured DRS in the 480-700 nm spectral region were analyzed by the tree-layer diffuse approximation (DA) method including the Delta-Eddington source approximation [2]. The three-layer DA model was selected since it provided needed robustness regarding both blood volume fraction and oxygen saturation determination. The DA method was selected over the more accurate Monte Carlo method because the former is computationally less demanding, which is a significant advantage when performing an inverse light-transport analysis. Human skin was modeled by three optically homogeneous layers, representing the epidermis, dermis, and subcutis (Fig. 2). Each layer was characterized by a specific set of optical properties. The determined skin parameters were melanin volume fraction m in epidermis, thickness of epidermis d_e and dermis d_d, dermal blood volume fraction B and oxygen saturation S, and beta-carotene concentration. Thickness of the subcutaneous layer was infinite inherently to the DA model. Additionally, reduced scattering coefficient function parameters of dermis were determined to include possible variations of the skin scattering [3].

$$\mu_s' = a \cdot \left(\frac{\lambda}{500\,\mathrm{nm}} \right)^{-b} \quad (1)$$

The reduced scattering coefficients of epidermis and dermis were equal to avoid introducing additional parameters into the fitting procedure.

The absorption coefficient of the dermis $\mu_{a,d}$ was calculated as a weighted sum of the blood $\mu_{a,b}$, the average baseline value for skin $\mu_{a,0}$=0.25 cm^{-1} and absorption coefficient of lumirubin [4] $\mu_{a,l}$ for each wavelength according to Jacques [3]:

$$\mu_{a,d} = \mu_{a,b} + \mu_{a,0} + \mu_{a,l} \quad (2)$$

The baseline absorption was selected according to Bjorgan *et al*. The blood absorption coefficient was calculated as a linear combination of the oxygenated $\mu_{a,oxy}$ [5] and deoxygenated whole blood $\mu_{a,dxy}$ [5], weighted by the oxygen saturation level S, blood volume fraction B and vessel correction factor C:

$$\mu_{a,b} = C \cdot B \cdot (S \cdot \mu_{a,oxy} + (1-S) \cdot \mu_{a,dxy}) \quad (3)$$

The vessel correction factor C accounts for the effect of blood packing into discrete vessels and was calculated as [6]:

$$C = \frac{1 - \exp(2R\mu_{a,b})}{2R\mu_{a,b}} \quad (4)$$

where R corresponds to the effective vessel radius.

The absorption coefficient of epidermis was calculated as:

$$\mu_{a,e} = m \cdot \mu_{a,m} + \mu_{a,0} \quad (5)$$

with m representing melanin volume fraction in epidermis, and $\mu_{a,m}$ pure melanin absorption calculated as [7]:

$$\mu_{a,m} = 519\,\mathrm{cm}^{-1} \cdot \left(\frac{\lambda}{500\,\mathrm{nm}} \right)^{-3.53} \quad (6)$$

The subcutaneous reduced scattering coefficient was calculated as [8]:

$$\mu_{s,s}' = 1.05 \cdot 10^3 \cdot \left(\frac{\lambda}{\mathrm{nm}} \right)^{-0.68} \quad (7)$$

while the subcutaneous absorption was set to zero since it is negligible in the 480-700 nm spectral range [9]. Figure 3 shows absorption coefficients of pure melanin $\mu_{a,m}$ [3], oxy-hemoglobin $\mu_{a,oxy}$ [5], deoxy-hemoglobin $\mu_{a,dxy}$ [5], and beta-carotene [10].

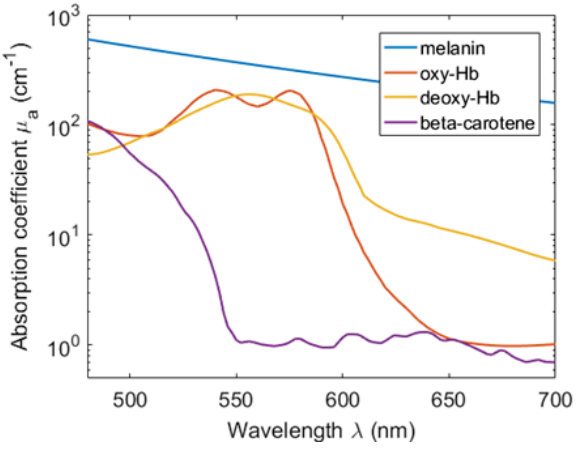

Fig. 3. Absorption coefficients of pure melanin, oxy-, deoxy-hemoglobin and beta-carotene used in the study. Beta-carotene absorption is in units (cm^{-1} mM^{-1}).

More information about the inverse DA model and optical properties can be found in Naglic *et al.* [11]. The whole measured DRS dataset (i.e. different measu-

rement times) of a volunteer was fitted simultaneously, keeping the time-independent parameters (*m, d_e, d_d, c, R, A_s*) identical for all spectra and allowing separate fitting of the time-dependent parameters (*B, S, a,* and *b*).

The DA model served as a model function in the fitting procedure. The model function output were the diffuse reflectances at different wavelengths in the spectral range 480-700 nm with 1 nm resolution. The fitting was performed by objective multi-dimensional minimization of the residual norm between the measured and the computed DRS set. The nonlinear least-squares algorithm implemented as a function *lsqnonlin* in Matlab Optimization Toolbox (Matlab 2016a, Mathworks Inc., Massachusetts, USA) was used. The covariance matrix was calculated as the inverse of the Hessian matrix $H=J^TJ$, J representing the Jacobian matrix of the fitting procedure. The variances of the fitted parameters were then obtained as diagonal elements of the covariance matrix and are presented in Results as standard deviations.

Results

The results from the initial validation using the cuff test are depicted in Figure 4. The measured spectra showed a good fit for the baseline (Fig. 3a, *blue line*), hypoxia (*red line*) and hyper-perfusion (*yellow line*). The analysis of the fit results (Fig. 4b) reveals a blood volume fraction of 1±0.1 % for the baseline which agrees well with the commonly reported values for the skin. During the cuff test, there is an elevated blood volume fraction of 2.2±0.2 % due to hypoxic vasodilatation. After the test, the blood volume fraction is 2.3±0.2 %, which is attributed to the reactive hyper-perfusion and consequent vasodilatation. Also the oxygen saturation (Fig. 4c) features the expected trends of 22±3 % at the baseline, 2±3 % during the hypoxia, and significantly elevated saturation of 67±3 % during the hyper-perfusion due to the fresh artery blood flow in the skin. The presented values of *B* and *S* are not absolute values due to limitations of the DA model. For example, the extremely low saturation during hypoxia is a result of these limitations. Nevertheless, the observed trends are consistent with existing literature reports [12], indicating reliability and validity of our findings.

Other parameters determined by fitting the initial validation spectra are presented in Table 1. The obtained values agree well with the reported values in the literature [3] and our other studies [11,13].

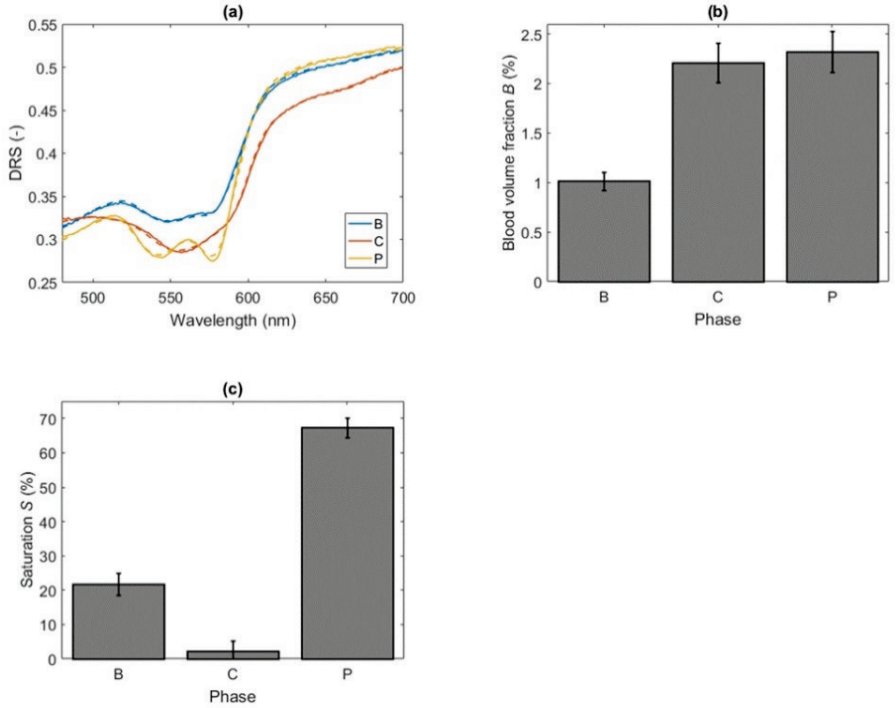

Fig. 4. Analysis of the initial DRS measurements on the volar forearm of a volunteer before – B, during – C, and after – P the cuff test. (**a**) Measured spectra (solid line) and corresponding fits (dashed line). (**b**) Determined blood volume fraction B and (**c**) blood oxygenation S during the cuff-test phases. Error bars represent estimated errors of the fit.

Table 1. Determined values of common physiological and morphological parameters for the initial validation of the procedure.

Parameter	*m* (%)	d_e (mm)	d_d (mm)	*c* (μM)	*R* (μm)	A_s (-)
Value	1.1 ± 0.1	0.09 ± 0.01	1.47 ± 0.15	3.5 ± 0.5	15	1.5 ± 0.9

m – melanin volume fraction, d_e – epidermis thickness, d_d – dermis thickness, *c* – bilirubin concentration, *R* – effective vessel diameter, A_s – subcutis scattering coefficient amplitude.

After the initial validation, the reference measurement on the volunteer not consuming caffeine was analyzed. The maximum variation between the maximum and minimum measured spectral value 0.8 % was present at λ=570 nm (Fig. 5a) between the 0.5 h and 2 h spectra, while in general it was much smaller. Similarly to the cuff test, the obtained fits agree well with the measurements. The average determined B was 1.3 % with standard deviation 0.2 %, all the determined values being within the confidence interval of the fit, demonstrating relatively good stability regarding the blood volume fraction. S features high initial saturation (39±3 %) followed by relatively stable values between 28±3 % and 31±3 %. The initial increased saturation is also evident in the spectrum at 0 h time (pronounced camel-back), which is most likely due to the incomplete rest of the volunteer before the first measurement. The saturation became almost constant at later times.

The impact of caffeine consumption on volunteer 1 is presented in Figure 6. Volunteer 1, a 26-year-old female weighing 57 kg, did not consume coffee prior to the study. The effect of the caffeine consumption is evident (Fig. 6a): the difference between the maximum (at 0.5 h) and minimum (at 3 h) observed DRS value at 570 nm is 4 %, which is five times larger than observed in the reference measurement. For comparison, the cuff test measurement (light blue line) featuring low B and S is included. B follows the expected trends according to the physiological mechanisms presented in Introduction. After 0.5 h a significant vasoconstriction is present, B being 68 % of the baseline B. Afterwards, B progressively increases exceeding the baseline value at 3 h by 9 % due to the vasodilatation. S initially decreases to 63 % of the baseline value at 0.5 h time, which is due to the reduced blood flow and simultaneous increased oxygen consumption. At 3 h S increases back to the baseline value due to the vasodilatation and increased blood perfusion bringing fresh blood. As expected, the cuff test results in B are well above all other B values (81 % above the baseline), and S decreases to 1 %, which agrees with the validation part of the study.

An example of a coffee drinking subject is presented in Figure 7. Volunteer 2 was a 33-year-old male weighing 83 kg and consuming 2 cups of coffee daily, which corresponds to an approximate caffeine intake of 100 mg. The difference between the maximum (0 h) and minimum (3 h) at 570 nm is 3 %, which is similar as for volunteer 1. Here, B initially remains almost at the same level as the baseline, but it increases by 45 % after 3 h. This trend also agrees well with the vascular physiology of caffeine, since the initial vasoconstriction phase is only transient and commonly not observable at all, while the subsequent vasodilatation phase should be observed in all subjects [1]. The oxygen saturation shows an initial significant decrease to 72 % of the baseline, followed by a gradual increase reaching 83 % at 3 h. In agreement with the above results, much higher B and smaller S are present for the cuff test. However, the fit of the cuff-test spectrum was not ideal, failing to fit the minimum at 557 nm which is due to the limitations of the three-layer skin model.

Figure 8 presents the relative change of B compared to the baseline value B_0 at time 0 h for both the non-drinker and regular coffee drinker groups. For the non-drinkers, the general trend shows an increase in B (vasodilatation) at later times (i.e. 2 h), while significant variations in the trends are present among the individual subjects. Volunteer 1 demonstrates a significant decrease in B at 0.5 h, followed by a continuous and linear increase at later times. On the other hand, volunteers 3 and 4 show relatively stable values B close to B_0 until 2 h, followed by substantial increase and decrease to B_0 at 3 h. The group of regular coffee drinkers show more consistent trends in B. Generally, decrease of B is present at 1 h, followed by increase at 2 h, and gradual decrease to B_0 at later times. The exception is volunteer 2, who shows increased B at all time points. Yet, the actual variations of B within each group are individual and depend mostly on individual metabolism and weight. To further explore underlying physiological trends and minimize intersubject variances, an extension of the present study could involve adjusting the concentration of ingested caffeine according to the individual weight, instead of a uniform 300 mg caffeine dosage. However, the present protocol involving the equal dose for all volunteers simulate the common caffeine consumption where the amount of ingested coffee is measured in cups or mugs independent of individual consumer weight and gender. Thus, the present results shed light on the changes in skin oxygenation and blood volume under typical caffeine consumption circumstances.

The effect of caffeine consumption on the non-drinker and regular coffee drinker groups on S is presented in Figure 9. In both groups the main trend is significant decrease of S (up to 60 %). The only outlier is the volunteer 4 at 2 h, which might also be due to

an unknown physical activity (e.g. stair climbing due to remote location of the office).

To determine if any statistically relevant differences exist between the baseline *B* and *S* values and values at different times (Fig. 8 and Fig. 9), we conducted two sample *t*-test for unequal variances for the non-drinker and regular drinker groups. In the group of regular coffee drinkers, the t-tests for *B* yielded a $p<0.05$ at times 2 h and 3 h, and even at 1 h if volunteer 2 is removed from the dataset. In the non-drinker group, no statistically significant difference was observed. The lowest $p=0.11$ was obtained at 3 h time. On the other hand, for *S* both groups showed $p<0.05$ at 0.5 h and 1 h, and in the group of regular coffee drinkers also at 2 h. This simple statistical analysis shows that differences between the base values of *B* and *S* exist within each of the groups, indicating statistically significant increase in blood volume at later times and decrease of oxygenation especially at early times. The observed variance between the subjects was expected since it was reported in the literature [14]. To strengthen the relevance of the results of this pilot study, a larger number of volunteers (e.g. 50 in each group) would be needed, along with a comprehensive statistical analysis of the observed trends in these larger groups. However, based on the statistical test results and the diversity in age, gender, and weight among the volunteers involved, we can expect that the presented results are relevant.

In the experiment a relatively high quantity of caffeine was consumed (300 mg). To observe the effect of a lower concentration, one of the volunteers (2 years old female, non-drinker) consumed 100 mg of caffeine corresponding to approximately 2 cups of coffee. The results (Fig. 10) show similar trends to those obtained after consumption of 300 mg of caffeine. Specifically, minor vasoconstriction followed by vasodilatation and decreased oxygen saturation.

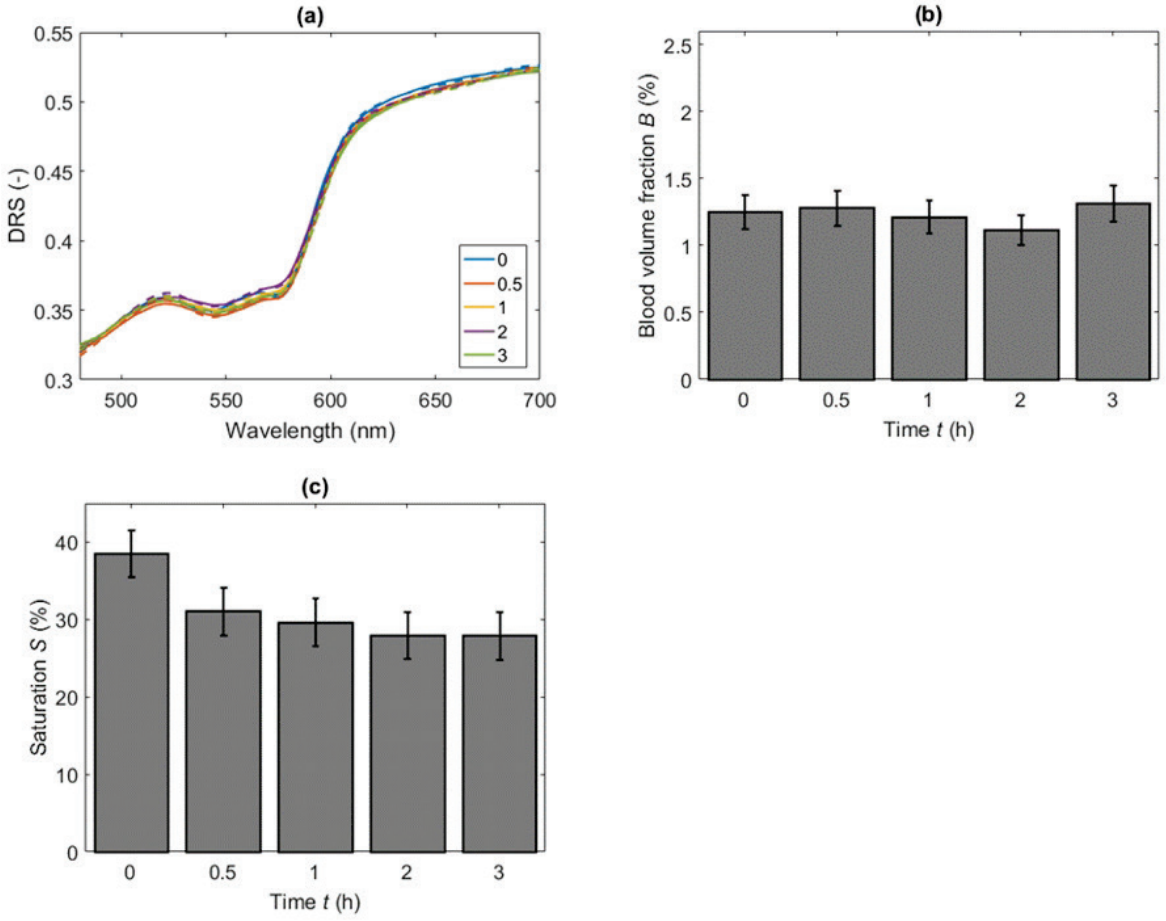

Fig. 5. Analysis of the reference DRS measured on the volar forearm of the volunteer not consuming caffeine. (**a**) Measured spectra (dashed line) and corresponding fits (solid line). (**b**) Determined blood volume fraction B and (**c**) blood oxygenation S at various time points 0-3 h. Error bars represent estimated errors of the fit.

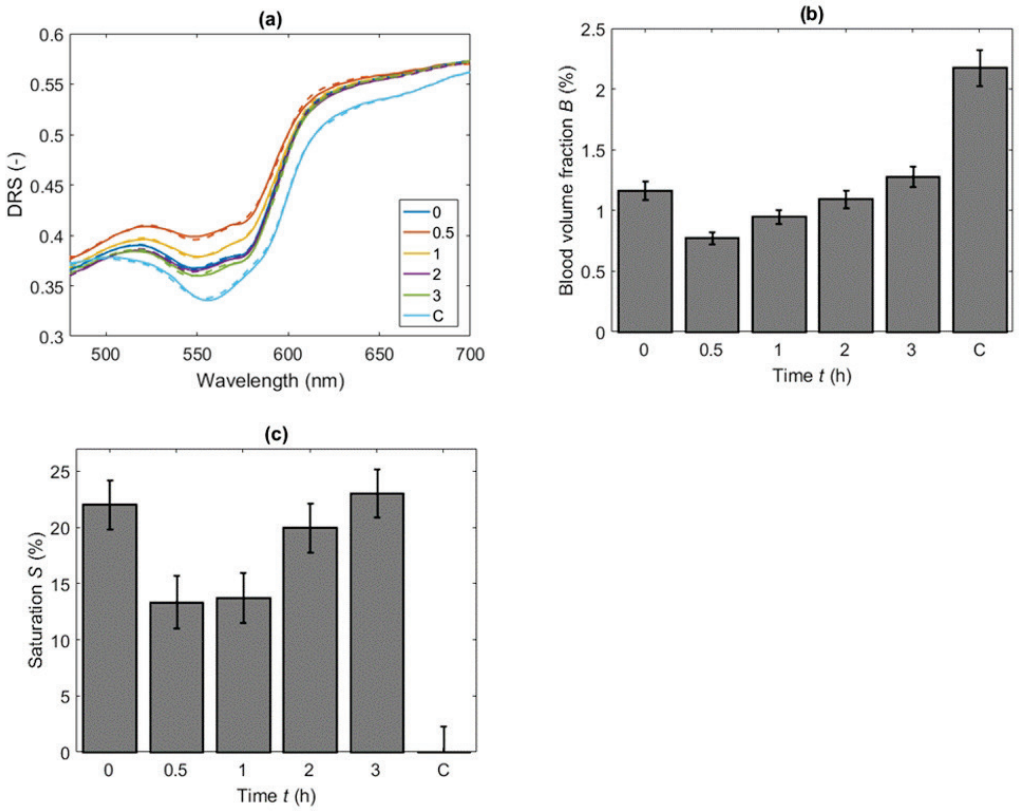

Fig. 6. Analysis of the DRS measured on the volar forearm of the volunteer 1 (coffee non-drinker) after consumption of 300 mg caffeine from guarana. (**a**) Measured spectra (solid line) and corresponding fits (dashed line). (**b**) Determined blood volume fraction *B* and (**c**) blood oxygenation *S* at various time points. Error bars represent estimated errors of the fit.

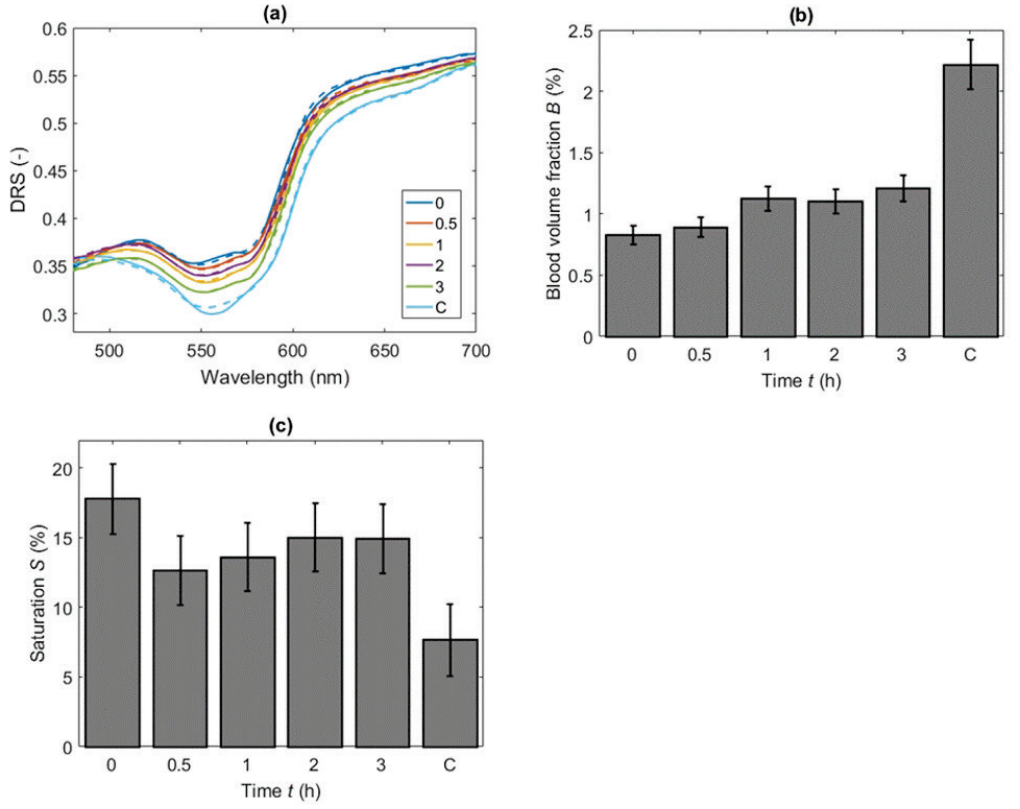

Fig. 7. Analysis of the DRS measured on the volar forearm of the volunteer 2 (coffee drinker) after consumption of 300 mg caffeine from guarana. (**a**) Measured spectra (dashed line) and corresponding fits (solid line). (**b**) Determined blood volume fraction *B* and (**c**) blood oxygenation *S* at various time points. Error bars represent estimated errors of the fit.

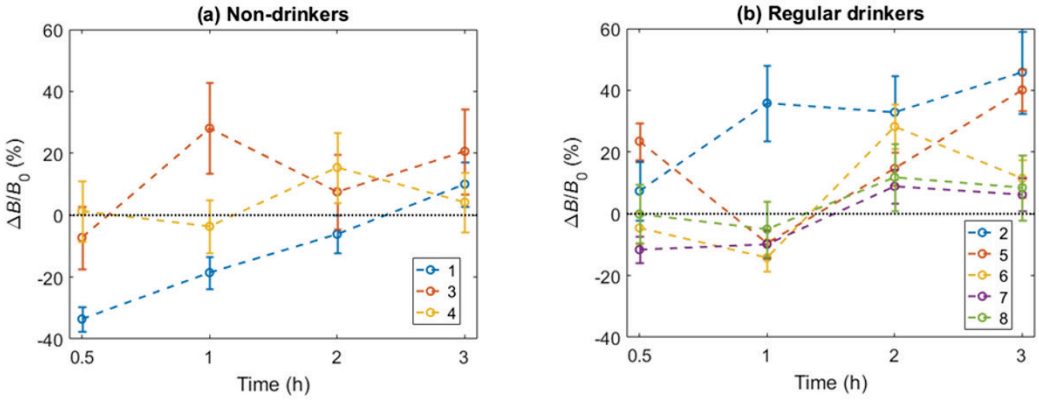

Fig. 8. Effect of caffeine consumption on skin *B* for (**a**) non-drinkers and (**b**) regular coffee drinkers.

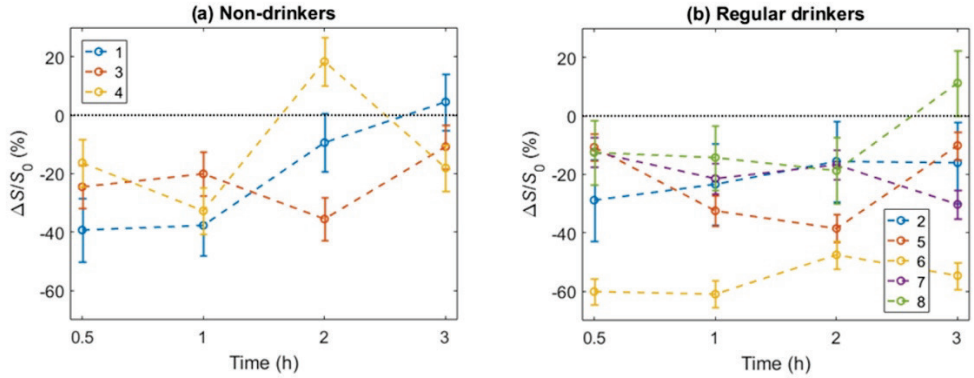

Fig. 9. Effect of caffeine consumption on skin *S* for (**a**) non-drinkers and (**b**) regular coffee drinkers.

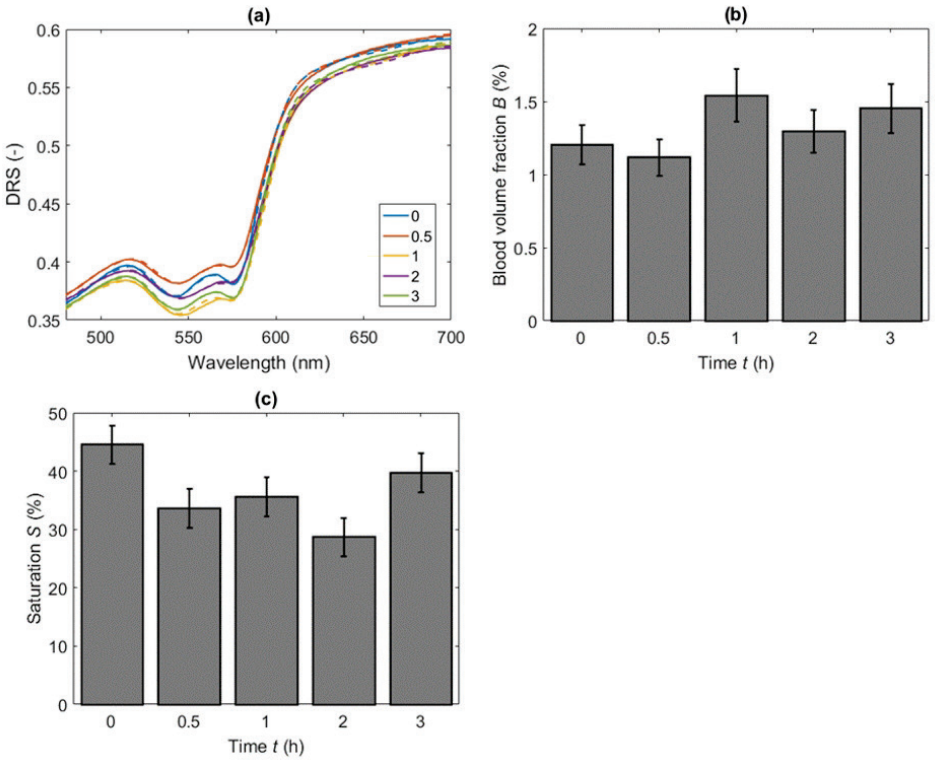

Fig. 10. Analysis of the DRS measured on the volar forearm of volunteer 3 (non-drinker) after consumption of 100 mg caffeine from guarana. (**a**) Measured spectra (dashed line) and corresponding fits (solid line). (**b**) Determined blood volume fraction *B* and (**c**) blood oxygenation *S* at various time points. Dashed lines present mean values of *B* and *S*.

Discussion

The findings of our study indicate that the impact of caffeine consumption on the human body can be detected and monitored by DRS applied to the forearm skin. The results show that there were negligible changes in spectra over time for the subject who did not consume any caffeine. However, significant variations corresponding to the constriction and dilation of blood vessels were observed in subjects who consumed caffeine.

In the group of non-drinkers, increase in blood volume fraction (vasodilatation) was typically observed at later time points, although only one volunteer showed an initial decrease (vasoconstriction). On the other hand, in the group of regular coffee drinkers, a decrease in blood volume fraction (vasoconstriction) was initially detected approximately one hour after caffeine consumption, followed by an increase at later time points. Both groups exhibited a general decrease in oxygen saturation.

These results highlight that DRS of the skin can provide information about the action of vasoactive substances. It also emphasizes the importance of considering consumption of vasoactive substances by human volunteers when performing DRS measurements since they can significantly affect the recorded spectra.

Our study shares some common ground with an animal study of Kmetova *et al.* [15], which also explored physiological responses to caffeine. While their focus lies on locomotor activity, our study concentrates on vascular dynamics, which highlights caffeine's multi-dimensional impact on physiological processes. Additionally, the study of Broz *et al.* [16] indicates how substances consumed for recreational purposes, like beer, can have observed changes in antioxidants and blood markers, thereby revealing systemic effects on the body. Another study [17] underscores caffeine's role in mitigating metabolic disorders and its effects on diverse physiological mechanisms. Although our study does not specifically explore the health aspects of caffeine, it contributes to the broader understanding of caffeine's multi-faceted effects on human physiology. Lastly, the study of Zhao *et al.* [18] examines tissue perfusion in preterm infants and considers circadian rhythms. While their study does not directly focus on caffeine and alcohol, our observation of temporal changes in skin vasodynamics following caffeine intake offers valuable insights into substance-induced effects on physiology over time.

Our research complements these studies by providing insights into the impact of caffeine on vascular dynamics through non-invasive DRS techniques. Together, these studies emphasize the need to comprehend the wide-ranging effects of commonly consumed substances on human health and physiology.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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