Determination of the Effect of Having Energy Drinks by Analyzing Blood Perfusion Signal

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***Abstract*—In this work, we evaluate the effect of having energy drinks using a laser Doppler Flowmetry technique by analyzing the blood perfusion signal before and after having energy drinks on healthy human subjects. After having energy drinks, it is observed that the amplitude of blood perfusion signal increases. Further analyzing the blood perfusion signal we have tried to determine the effect of having energy drinks on human subjects. The oscillations extended over a wide frequency scale and their periods varied in time. Within the frequency range studied, 0.0095–1.6 Hz, five characteristic oscillations were revealed, arising from both local and central regulatory mechanisms, e.g. endothelial/metabolic activity, sympathetic nerve activity, vascular myogenic activity, respiratory activity, and heart activity. We observed a significant change in metabolic and sympathetic nerve activity after having energy drinks. The frequency component relating metabolic and sympathetic nerve activity increase around 300% and 140% respectively. It is also observed a moderate change in myogenic and heart activity as the relative and absolute amplitude of the oscillations of this frequency interval increased around 80% and very little or all most no change in respiratory activity in response to having energy drinks.**

***Index Terms*—acqKnowledge software, blood perfusion signal, energy drinks, LDF**

I. Introduction

Laser Doppler Flowmetry (LDF) is an established and reliable method for measurement of blood perfusion in microvascular research. Periodic oscillations in the microvasculature are detected by the noninvasive technique of the LDF. The spectral analysis of the LDF signal from the human forearm skin has revealed five characteristic frequencies [1]-[3]. In addition to the cardiac and respiratory rhythms around 1 and 0.3 Hz, respectively [2]-[5], three frequencies have been detected in the regions around 0.1, 0.04, and 0.01 Hz in human skin [1]-[3]. It is suggested that periodic oscillations with a frequency of around 0.1 Hz (a-waves) reflect intrinsic smooth muscle (myogenic) activity of blood vessels [2], [4]-[9], whereas the frequency around 0.04 Hz (b-waves) represents neurogenic stimulation of resistance vessels [7]. Golenhofen suggested that oscillations of around 0.01 Hz (minute-rhythm) resulted from changes in the metabolism of the perfused tissue [10]. The different spectral components are thought to modulate vascular smooth muscle cell activity. This results in a specific level of vascular tone, which in combination with the rheological properties and the active dilator activity, determines vascular resistance.

Since the 1990s, energy drinks (ED) have gone from being the latest craze and fad to a permanent fixture in our culture. An energy drink is a beverage that contains some form of legal stimulant and/or vitamins which are supposed to give consumers a short term boost in energy. Make a mental note that while the Food and Drug Administration (FDA) is allowing companies to sell and market their energy drink products, there is still very little research that has been done on them. It is suspected that the FDA allows them to be added simply because they do not pose any immediate danger to energy drink consumers [11].

The aim of the study is to determine the microvascular changes in the periodic oscillations of cutaneous blood perfusion after having energy drinks using laser Doppler flowmetry Technique. We hypothesized that having energy drinks changes in microvascular control mechanisms of the skin would result in differences in the spectral components and their corresponding amplitudes.

II. Materials and Methods

A. Subjects

Twelve healthy, male subjects between 24 and 32 years old were enrolled. Mean ±SD (range) for age, weight, height, and Body Mass Index (BMI) were given in Table I. The subjects had not taken any medication during the week prior to the study. None of the subjects were smokers and they refrained from alcohol and caffeine containing drinks at least 4 hours prior to the study or had a history of cardiovascular disease or other illness. Exclusion criteria included: obesity (BMI>30 kg/m^2),

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underweight (BMI <18.5 kg/m^2) and history of cardiovascular disease or other illness. After being informed of the study design, they gave their written consent. The study was approved by the local Ethics Committee.

TABLE I
DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS

Parameter	Value (N=12)[a]
Age (yrs)	23.33±2.87
Weight (kgs)	63.67±10.61
Height (cms)	165.89±8.03
BMI (kg/m^2)	22.27±3.30

[a] Values are Mean ±SD.

B. *Energy drinks:*

An energy drink is a beverage that contains some form of legal stimulant and/or vitamins which are meant to give consumers a short term boost in energy [11]. The "Magical" ingredients of these drinks have one thing in common: they all contain a lot of sugar and/or caffeine. These could be considered the "active ingredients".

In this experiment we have used drinks of serving size of 250ml/can which contains caffeine 62.5mg/250ml can, 27 gm sugar per 250ml can and other ingredients e.g. water, acidity regulators: citric acid and sodium citrate, carbon dioxide, taurine (0.4%), flavor, inositol (0.02%), colours: E 150d, riboflavin, enriching substances: vitamins (niacin, panthotenic acid, vitamin B6, vitamin B12).

C. *Experimental setup*

The study was performed in a quiet room with the temperature kept at 22℃ (21-23). The subjects were resting in the supine position throughout the whole experimental period. LDF measurements were performed with the Laser Doppler Flowmetry module, LDF100C (Biopac, USA), a commercially available instrument was used for the display of the LDF signal and a wide range of fiber-optic based probes, TSD140 series (Biopac, USA) in order to access the tissue, double-sided adhesive rings (ADD200 series, Biopac, USA) were used to attach surface type probes to tissue.

D. *Testing Procedure*

Food intake was restricted to a light meal 2 hours prior to the test. LDF measurements were carried out in a room in which the temperature was maintained constant at 22℃ (21-23) with the subjects in a supine position. At least 20 minutes were allowed for acclimatization before the LDF measurements were performed on the skin of the middle finger tip. Blood perfusion in skin (forearm on middle finger tip) was measured immediately before and after the consumption of energy drinks. In this experiment we have recorded the blood perfusion signal about 10 minutes before having energy drinks and 20 minutes after having energy drinks. Each participant had an initial visit to the experimental laboratory, for a physical examination and a medical history assessment.

E. *Laser Doppler Flowmetry*

Laser Doppler Flowmetry provides a semi quantitative as-assessment of microvascular blood perfusion, which is expressed in Blood Perfusion Unit (BPU), which is a relative unit, defined using a carefully controlled motility standard comprising a suspension of latex spheres undergoing Brownian motion. LDF measurements from the skin reflect blood flow in capillaries, arterioles, venules, and dermal vascular plexa. They also reflect a small nutritive and a large thermoregulatory aspect of perfusion [12]. The LDF technique offers substantial advantages over other methods in the measurement of microvascular blood perfusion. This technique provides promise and opportunity to adapt the methodology in various fields of research, for example, in cerebral monitoring (stroke, injury), transplantation surgery (skin grafts, free flaps), vital organ monitoring (organ viability), tumor vascular research (angiogenesis) and peripheral vascular research (diabetes). Studies have shown that it is both highly sensitive and responsive to local blood perfusion and is also versatile and easy to use for continuous monitoring [13], [14].

The principle of laser Doppler flowmetry technique is shown in Fig. 1. Low power laser light is used to illuminate tissue using a fiber optic; the light is scattered by the static tissue structures and moving blood cells; the moving blood cell imparts a Doppler Shift; an adjacent fiber detects light returned from the tissue; this light contains Doppler shifted and unshifted light; the signal is processed to extract the signal related to the moving red blood cells.

F. *Spectral analysis of skin LDF signal*

Spectral analysis of skin LDF signal was performed by means of Biopac AcqKnowledge software. The frequency

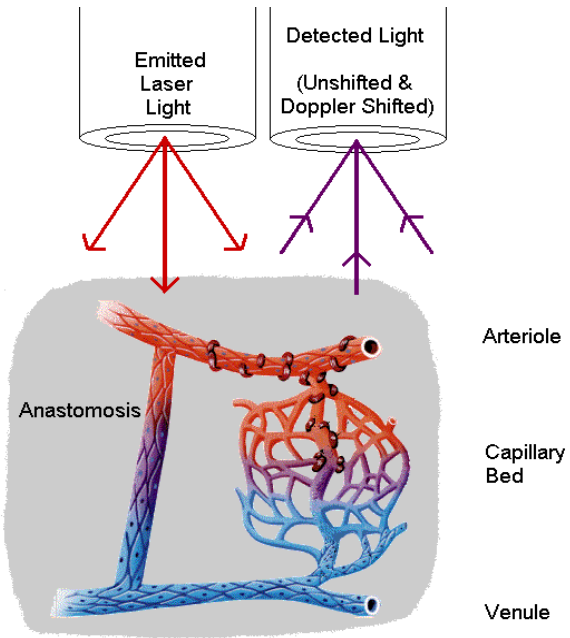

Figure 1. Laser Doppler Flowmetry technique [15].

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spectrum of the same LDF signal is calculated by Fourier transform. The spectrum is obtained by averaging spectra calculated from a window of 10 minutes moved along the signal around 2 minutes. Following recent studies [16]-[20], the frequency interval studied (from 0.009 to 1.6 Hz) was divided into five subintervals: 0.009–0.02 Hz (endothelial activity), 0.02–0.06 Hz (sympathetic activity), 0.06–0.2 Hz (vascular myogenic activity), 0.2–0.6 Hz (respiratory activity), and 0.6–1.6 Hz (heart activity). The spectral density for total spectrum and for each frequency interval was measured before and after the consumption of energy drinks.

III. Results

A. *Recording of LDF signal*

A typical laser Doppler flowmetry recording of human forearm on the middle finger tip at normal condition is shown in Fig. 2 for 20 seconds, which oscillate around 700 BPU whereas Fig. 3 shows the LDF recording at the same position after having energy drinks. It is seen that the amplitude of the LDF perfusion signal has been increased after consumption of energy drinks showing an increased level of oscillation around 1100 BPU.

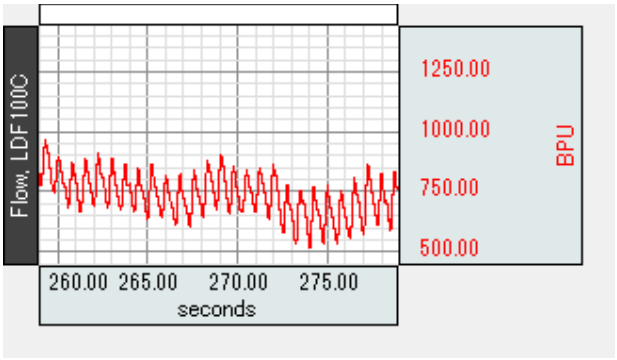

Figure 2. LDF recording at normal condition (before having ED).

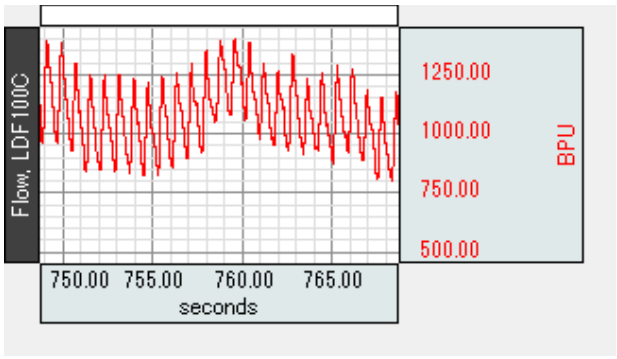

Figure 3. LDF recording after having ED.

Blood perfusion signal of a typical subject both before and after the consumption of energy drinks for are shown in Fig. 4 and Fig. 5 respectively. In Fig. 4 it is observed that the blood perfusion signal (BPS) is oscillating around 700 BPU. The level of BPS fluctuates irregularly, there are several peaks at 1.74, 2.10, 2.79, 3.98, 6.77 minutes, whose values are around the maximum amplitude of LDF

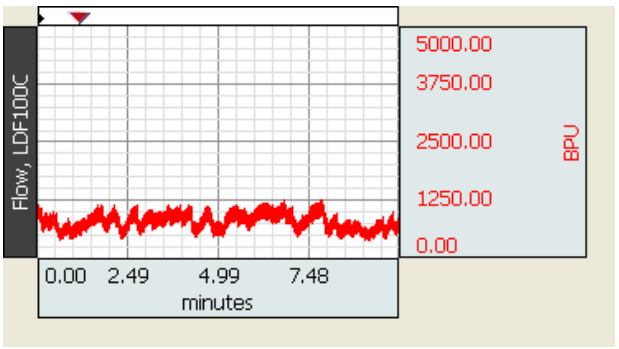

Figure 4. Typical LDF recording at normal condition during the

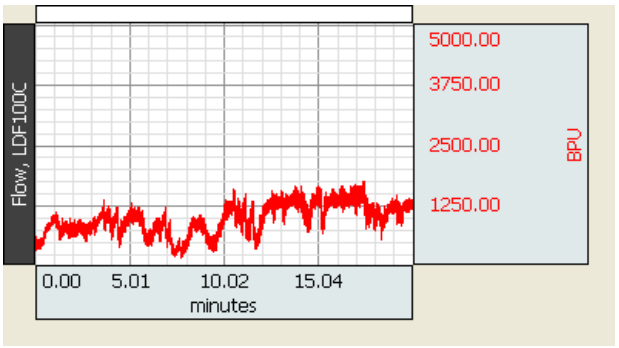

Figure 5. Typical LDF recording after having ED during the experimental period.

recording (1250 BPU). There is no significant change in the peaks of LDF recording at normal condition. After having energy drinks, the maximum amplitude of LDF recording is about 1750 BPU at 17.55 minutes. Between the beginning and the ending of the LDF recording, there are several peaks (in 1.02, 5.01, 7.00, 10.50, 15.04, 17.55 minutes) whose values are increasing in nature with time. There is a significant change in the peaks of LDF recording basically after 10 minutes of having energy drinks than the initial period. After having energy drink, we can see that up-to 10 minutes the maximum amplitude of LDF recording is around 1250 BPU which can be considered as normal condition. The action of having energy drinks is observed appreciably after several minutes (around 10 minutes) and we observed a significant increase in blood flow.

B. *Histogram of the recorded Blood Perfusion Signal*

Fig. 6 shows the histogram of BPS of human forearm on the middle finger tip at normal condition of fig. 4 whereas fig. 7 shows the histogram of BPS at the same time duration after having energy drinks when the level of BPS signal increased as shown in fig. 5 for time duration 10 minutes to 20 minutes. In normal condition, the maximum hits (around 110000) of LDF recording occur at 806.12 to 894.12 BPU and maximum value of attains at 1158 BPU. After having energy drinks, the maximum hits (around 200000) of BPS occur between 1192 to1331 BPU and maximum value of BPS attains around 1600 BPU. Before having energy drinks most of the hit occurs between 453 to 1070 BPU and in case of

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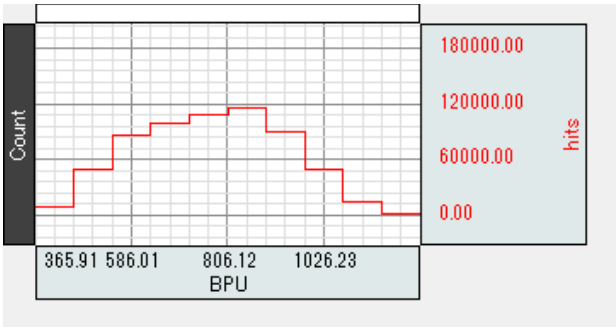

Figure 6. Histogram of LDF signal at normal condition

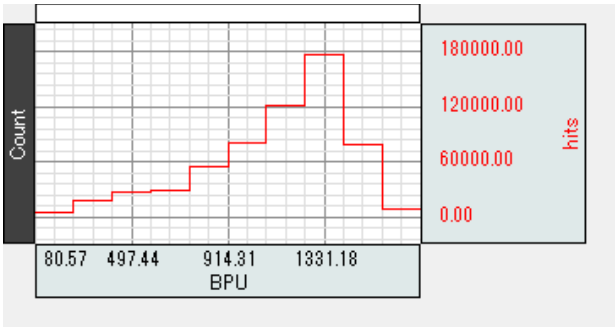

Figure 7. Histogram of LDF signal after having ED.

after having energy drinks most of the hit occurs between 1053 to 1470 BPU. It is clearly observed that there is a significant increase in the value of BPS after having energy drinks.

C. *Frequency analysis*

The spectral amplitude of blood perfusion signal before and after having energy drinks are shown in Fig. 8 and Fig. 9 respectively. Recent studies [16]-[20], the frequency interval studied (from 0.009 to 1.6 Hz) was divided into five subintervals: 0.009–0.02 Hz (endothelial activity), 0.02–0.06 Hz (sympathetic activity), 0.06–0.2 Hz (vascular myogenic activity), 0.2–0.6 Hz (respiratory activity), and 0.6–1.6 Hz (heart activity). Periodic oscillations with peak amplitudes of around 1, 0.3, 0.1, 0.04, and 0.01 Hz were demonstrated in the LDF signal. The peaks were determined by a numerical procedure for detection of local maxima in the fast Fourier transform. Total frequency spectrum is shown from 0.009 to 1.6 Hz.

TABLE II
TYPICAL CHANGE IN THE SPECTRAL AMPLITUDE OF BLOOD PERFUSION SIGNAL

Frequency Band (Hz)	Before Having ED		After Having ED	
	Frequency of Peak Spectral amplitude (Hz)	Peak Spectral amplitude (BPU)	Frequency of Peak Spectral amplitude (Hz)	Peak Spectral amplitude (BPU)
0.0095 - 0.02	0.015	7.55	0.013	31.42
0.02 - 0.06	0.045	5.59	0.053	5.27
0.06 - 0.2	0.091	3.84	0.10	5.26
0.2 - 0.6	0.34	1.68	0.32	2.04
0.6 - 1.6	1.31	6.55	1.15	17.15

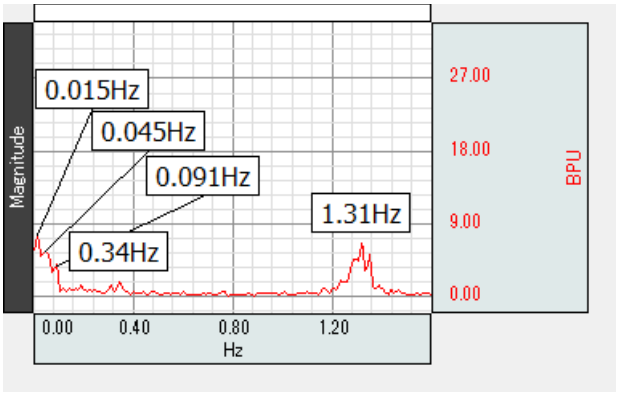

Figure 8. Spectral analysis of LDF signal at normal condition.

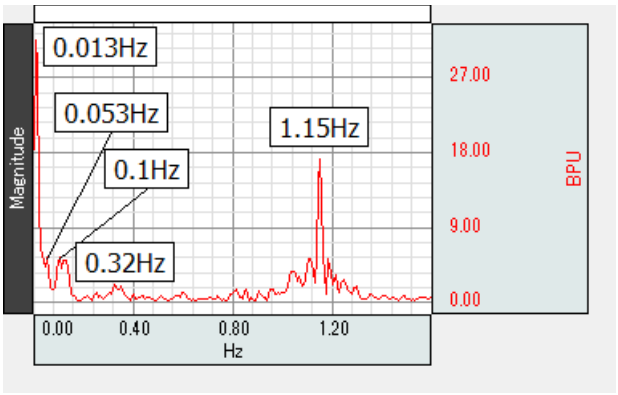

Figure 9. Spectral analysis of LDF signal after having ED.

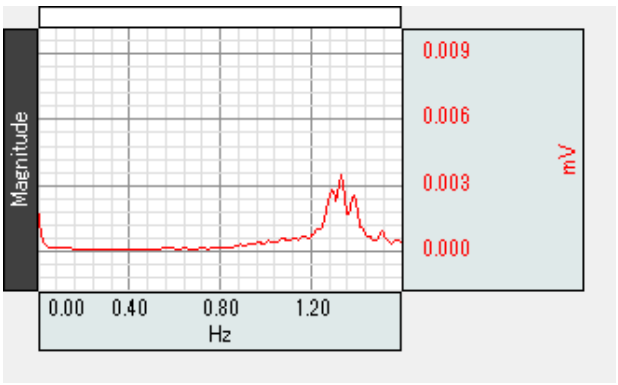

Figure 10. Spectral analysis of ECG signal at normal condition.

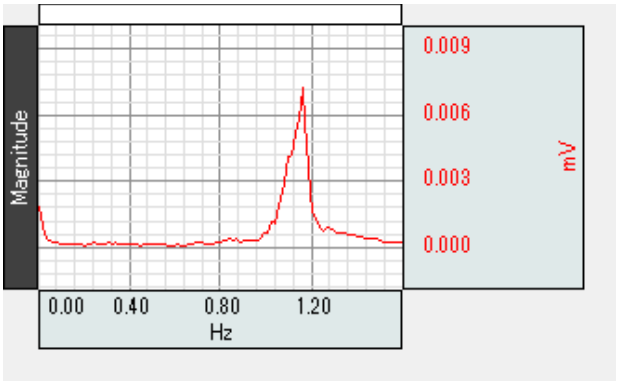

Figure 11. Spectral analysis of ECG signal after having ED.

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The peak amplitude of each frequency band both before and after having energy drinks from 0.009 to 0.02 Hz; (II) from 0.02 to 0.06 Hz; (III) from 0.06 to 0.2 Hz; (IV) from 0.2 to 0.6 Hz; and (V) from 0.6 to 1.6 Hz, respectively have been listed in table II. In this case, we observed a significant change in metabolic and heart activity after having energy drinks. The frequency component relating metabolic and heart activity increase around 3 fold and 2 fold of its normal value. In normal condition the maximum magnitude of metabolic function (0.0095–0.02 Hz) is about 7.55 BPU (at 0.015 Hz) and heart function (0.6–1.6 Hz) is 6.55 BPU (at 1.35 Hz). After having energy drinks (as shown in Fig. 9), the peak spectral amplitude of metabolic activity is 31.42 BPU (at 0.013 Hz) and heart function is 17.15 BPU (at 1.15 Hz). The frequency relating to heart beat is decreased; it may be due the supine (relaxed) position of the subjects. It is also observed a moderate change in myogenic and respiratory activity. The peak spectral amplitude, relating to myogenetic activity attains 5.26 BPU from 3.84 BPU and relating to respiratory activity increases from 1.68 to 2.04 BPU.

Spectral analyses of ECG signal for assuring the frequency band of heart activity are shown in Fig. 10 and Fig. 11. The peak amplitude of heart activity occurs within the desired frequency range (0.6-1.6 Hz) both at before and after the consumption of energy drinks. The maximum heart activity has found around 1.2 Hz from ECG as well as LDF analysis.

D. Statistical analysis

TABLE III
AVERAGE CHANGE IN SPECTRAL AMPLITUDE OF VARIOUS FREQUENCY BAND OF BLOOD PERFUSION SIGNAL

Frequency Band (Hz)	Peak Spectral Amplitude (BPU) [b]		% Changes in Average Spectral Amplitude
	Before Having ED	After Having ED	
0.0095-0.02	6.1752±2.9118	24.8613±7.8995	302.59%
0.02-0.06	5.1877±2.9822	12.6214±6.7981	143.29%
0.06-0.2	3.2762±1.3426	6.0116±2.9685	83.49%
0.2-0.6	1.3532±0.3183	1.6671±0.4104	23.19%
0.6-1.6	8.6974±3.4637	16.0324±3.0303	84.33%

[b] Values are Mean ±SD.

The average peak spectral amplitude of different frequency bands of all subjects, before and after having ED, are shown in Table III. We observed a significant change in metabolic and sympathetic nerve activity after having energy drinks. The frequency component relating metabolic and sympathetic nerve activity increase around 300% and 140% respectively. It is also observed a moderate change in myogenic and heart activity as the relative and absolute amplitude of the oscillations of this frequency interval increased around 80% in response to having energy drinks. The respiratory activity is relatively less affected after having energy drinks, around 20% increase in spectral amplitude is observed.

IV. DISCUSSION

Frequency interval from 0.6 to 1.6 Hz (peak amplitude: around 1 Hz or 60 events per minute). The absolute amplitude of the oscillations around 1 Hz was increased around 80% after having energy drinks as compared to values at normal condition. Periodic oscillations of around 1 Hz in the skin are synchronous with the heart rate [2]-[5] and represent oscillatory changes of arteriolar diameter induced by pulsatile flow of the cardiac cycle. These oscillations also represent a correlation of flow to first-order pressure waves [21], [22]. The high absolute amplitude of these oscillations after having energy drinks probably reflects an increased stroke volume in response to consuming energy drinks. Also the frequency of the oscillations of around 1 Hz is decreased after having energy drinks may reflect the decrease in heart beat as the subjects go through long time in supine position.

Frequency interval from 0.2 to 0.6 Hz (peak amplitude: 0.3 Hz or 18 events per minute). The present study demonstrates around 20% increase of the peak spectral amplitude of the oscillations around 0.3 Hz of the cutaneous perfusion before and after exercise intake of energy drinks. Periodic oscillations of around 0.3 Hz are synchronous with respiration [2]-[5]. The respiratory-dependent oscillations are also correlated to the flow of second-order blood pressure waves in the arterial branch of the cutaneous circulatory system [21]. This rhythmic, second-order pressure wave oscillations can be explained by a coupling between the respiratory and circulatory system mediated by the autonomic nervous system and by respiratory-dependent, left cardiac preload alterations [22]. The little change in peak spectral amplitude may result from the relaxed position of subjects, as in supine or during rest, respiration depth i.e. depth of exhalation and inhalation increases. As the relative values of the amplitudes of the oscillations of around 0.3 Hz did not appreciably change in response to having energy drinks, we therefore conclude that the energy drinks have a relatively negligible effect on the respiratory system.

Frequency interval from 0.06 to 0.2 Hz (peak amplitude: 0.1 Hz or 6 events per minute). The main finding of the present study is an approximately 80% increase in the peak spectral amplitude of the periodic oscillations with a frequency of around 0.1 Hz of the cutaneous perfusion after consuming energy drinks. The oscillations around 0.1 Hz, also called a-waves [23], are suggested to be of local origin, representing the intrinsic myogenic activity of smooth muscle cells in resistance vessels [2], [6]-[9], [12], [24]. In these studies the rhythmicity was in the range of 4-10 events a minute, which corresponds well with the spontaneous activity recorded in micro- vascular smooth muscle cells [10]. Myogenic control as a possible mechanism for oscillatory changes of arteriolar diameter was suggested as early as 1964 by Folkow [25]. Meyer et al. and Schmidt et al. [8], [26] concluded that the vasomotion in terminal arterioles is the expression of an underlying pacemaker mechanism in smooth muscle cells. Increased absolute amplitude of the oscillations around 0.1 Hz indicates increased vasomotion induced by the intrinsic activity of vascular smooth muscle cells. They attributed the origin of this frequency to sympathetic activation. The proposed neurogenic origin of these oscillations in heart rate

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variability studies contrasts the regulatory mechanisms proposed in the studies of cutaneous blood flow, where the origin is suggested to be an intrinsic myogenic activity in the vessel wall, as mentioned above. We also demonstrated increased relative amplitude of the oscillations of around 0.1 Hz after having energy drinks. The fact that both the relative and absolute amplitude of the oscillations of this frequency interval increased in response to energy drinks indicates that the myogenic activity contributes relatively more to the regulation of blood perfusion after having energy drinks.

Frequency interval from 0.02 to 0.06 Hz (peak amplitude: 0.04 Hz or 2.5 events per minute). The periodic oscillations with peak amplitude around 0.04 Hz are called b-waves [23]. In the present study, the spectral amplitude of the oscillations of this frequency interval increase around 140% in response to consuming energy drinks. Oscillations with frequencies of around 1-3 events per minute were also observed by Colantuoni et al. [27] and were thought to be related to arteriolar vasomotion in the terminal vascular network. The same group also showed that slow waves with a frequency of around 1-3 per minute in rabbit skeletal muscle tissue, detected by LDF, are under neurogenic control since the waves disappeared after pharmacological nerve blockade [24]. In humans, Kastrup et al. [7] Demonstrated that b-waves disappeared completely after denervation, both after local and ganglionic nerve blockade and after sympathectomy. This implies that the energy drinks contributes relatively more to sympathetic nervous system, i.e. the activity of the sympathetic nervous system affect more after consumption of energy drinks.

Frequency interval from 0.009 to 0.02 Hz (peak amplitude: 0.01 Hz or 0.6 events per minute). Experimental studies using isolated blood vessels have shown that the period duration of the minute rhythm in muscle blood flow was 1 min, but for skin and liver blood flow 0.5 min [10]. Vasomotion with the frequency around 0.01 Hz in this study corresponds well with the minute-rhythm, which is proposed to represent metabolic processes in the perfuse tissue [10]. The proposed metabolic origin of this rhythm was based on the demonstration of a minute-rhythm oscillation in isolated metabolic systems, which led to the assumption that oscillations in cellular metabolism are important for the basic process of this rhythm in smooth muscle cells [10]. Our results demonstrate an approximately 300% increase in the absolute amplitude of the periodic oscillations with a frequency of around 0.01 Hz of the cutaneous perfusion after consuming energy drinks. These findings demonstrate that oscillations around 0.01 Hz may reflect an increase in metabolic activity in the skin in response to consuming energy drinks.

V. Conclusion

In this work we record the LDF signal in the supine position as the LDF probe is very sensitive to vibration or motion. But most of cases the consumer of energy drinks are young and they take it before various parties or young athletes, who take energy drinks before play to enhance their performance. To assess whether the energy drinks have any contribution to enhance their performance it is essential to study various physiological signals during their work or exercise.

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