EFFECT OF HIGH BLOOD CORTISOL CONCENTRATION ON THE SPECTROSCOPICALLY DETERMINED SECOND-ARY STRUCTURE OF PROTEINS AND LIPID BALANCE ON THE EXAMPLE OF ELITE WOMEN VOLLEYBALL PLAYERS

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Abstract

Cortisol is a stress hormone that plays a crucial role in the balance between phospholipids and lipids levels. In consequence, it affects the secondary structure of proteins. Currently cortisol concentration in serum is determined by a biochemical analysis. A new optical method to estimate the stress level is proposed in this work. Infrared and Raman spectroscopies were used to determine the quantitative and qualitative changes in lipids and proteins fractions in the function of cortisol concentration in 49 samples of serum collected from volleyball players at various stages of preparation for the competition. With the cortisol level increase, a decrease of structures related to PO^{2-} phospholipid groups and amides III and I bonds was noticed in the Raman spectra. The differences in absorbance visible in the FTIR spectra, were not statistically significant. However, the calculated absorbance ratios between the peaks related to vibrations of the lipid functional groups and amide I show, that with the increase of cortisol concentration, the increase of the amount of lipids and the decrease of the amount of proteins was observed in transmission spectra. Pearson correlation test presented positive correlations between phospholipid and protein levels and between cortisol concentration and phospholipids in transmittance spectra. Negative correlations between cortisol concentration, protein and phospholipid levels were observed in the Raman spectra. Obtained results showed, that using Raman spectroscopy the effect of increased cortisol concentration on proteins, phospholipids and lipids is directly visible. in the case of FTIR spectroscopy, one had to calculate the ratios between CH_2 and CH_3 lipids vibrations (2957 cm^{-1}, 2920 cm^{-1}, 2872 cm^{-1}) and amide I (1654 cm^{-1}), to show differences in the cortisol concentration in serum. These results suggest, that Raman spectroscopy is a more promising technique to obtain an information about cortisol levels in serum.

Keywords*: cortisol; stress level; FTIR; Raman spectroscopy; proteins; serum; lipids; volleyball players*

Introduction

Both control and precise estimate of the stress level are of significant importance for a wide range of various professions. They are essential for appropriately planned and precisely conducted training of professional athletes, as well as firefighters, certain types of military troops, police departments, etc. Without a doubt, the level of stress can influence the quality and efficiency of a professional activity. While for athletes a sports competition is just a moment in their performance, for other highly specialized professionals, such as medical staff involved in life-saving activities, stress is present every day. Cortisol is one of the stress hormones belonging to the hormones of the glucocorticoids group. This hormone is responsible for preparing the body to physical and mental stress. Oxidative stress resulting from physical exertion in elite athletes can take various forms (Martinovi et al.,

2009). Depending on the level of physical effort or competition, changes in the concentration of hormones in the body may also vary, but they always occur in female volleyball players' bodies (Filaire et al., 1998; Edwards et al., 2013; Roli et al., 2018). The higher the level of sports advancement of the female players, the bigger changes in cortisol levels can be observed, which proves that the body responds to the level of training load (Dziembowska et al., 2019). The very precise mechanism of cortisol activity is not fully explained because at the cellular level it has got an anabolic character (stabilization of lysosomal membranes and reduction of the activity of protein-digesting enzymes), while in relation to muscles it has a catabolic character (it stimulates, inter alia, myostatin synthesis) (Crewther et al., 2011; Cupps & Fauci, 1982). For this purpose, it affects the metabolism of proteins, carbohydrates and fats (Stachowicz & Lebiedzińska, 2016).

Long-term stress first results in a hyperactivation of the sympathetic nervous system. As a next step, the level of corticotropin releasing hormone (CRH) increases, followed by adrenocorticotropic hormone (ACTH) secretion. The secretion of ACTH causes the secretion of stress hormone, such as cortisol, from the adrenal cortex (Heinrichs et al., 1999). The high level of cortisol affects the lipid balance, especially between cholesterol, triglycerides and phospholipids (Sashin & Humuslu, 2004; Breen et al., 2008). It was shown that with the increase of cortisol concentration, the total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) fractions also increase (Maduka et al., 2015; Misra et al., 2008).

Importantly, changes in the concentration of these three lipids affect the liquidity of membranes, and, consequently, changes in the membrane transport occur (Maes et al, 1995). However, other results showed that cortisol level does not affect the lipid balance (Christiansen et al., 2007). Sports training, especially endurance training, affects the hormonal and lipid balance (Fikenzer et al., 2018). The demand for the differentiation of ingredients, such as amino acids, protein, creatine and caffeine, macro- and microelements is very important for modern athletes. Therefore, the study of the correlation between the cortisol level and lipid balance and, consequently, changes in the secondary structure of proteins should be conducted specifically in athletes.

Fourier-transform infrared (FTIR) and Raman spectroscopy are the techniques that provide information about the chemical compositions of measured samples. Consequently, quantitative and qualitative data concerning the vibrations corresponding to the functional groups from phospholipids, proteins and lipids can be obtained with these techniques. In both spectroscopic techniques, two major distinct regions originating from lipid vibrations can be identified. The high wavenumber spectral range between 2800 cm^{-1} and 3100 cm^{-1} corresponds to C-H stretching vibrations that mainly originate from hydrocarbon chains, while the low wavenumber range between 1090 cm^{-1} and 1240 cm^{-1} corresponds to the PO^{2-} groups from phospholipids (Derenne et al, 2014; Czamara et al., 2015). Thanks to the high sensitivity of FTIR and Raman spectroscopy, spectral signatures characteristics for each lipid class give an opportunity to use these techniques to determine fine structure details. Consequently, lipid phase and orientation studies in liposomal and monolayer systems are possible due to FTIR and Raman spectroscopy (Derenne et al., 2013; Wolkers, 2013). High intensity structures in FTIR spectrum corresponding to amide I bonds provide very accurate information on the secondary structure of proteins (Arunkumar et al., 2019).

The aim of this study is to determine the structural changes in the secondary structure of proteins and in the lipid balance caused by different cortisol concentration in serum. For this purpose, medical laboratory service was used to determine cortisol levels in serum. FTIR and Raman spectroscopy were applied to determine the differences in the value of absorbance and Raman intensities of individual peaks in measured spectra corresponding to phospholipid, protein and lipid functional groups. Finally, only the qualitative results will be shown in this study.

Methods

Materials

All experimental protocols and methods used in this study were confirmed by the ethics committees (protocol number 3/11/2017) obtained from Institutional Review Board at Rzeszow University. All methods were carried out in accordance with relevant guidelines and regulations.

The research was carried out on the members of one of the best professional club teams that have played for three seasons (2019 – 2021) in the professional volleyball league in Poland and the European Cups. All 13 elite women volleyball players represented a very high sport level (1 player - the highest world level, 5 players - high international level, 3 players - the national team level, 4 - high level of league games). The number of completed training sessions was 240 h. The 10-week pre-games workout during which the research was conducted resulted in reaching the training effects as previously planned. All players improved their strength, speed, endurance and coordination indicators. No injuries that would have interrupted the players' training cycle occurred during the preparation time. After the preparatory period, all the participants improved their cardiovascular and respiratory parameters of maximal oxygen consumption (VO2). There was an increase in the mean values of VO2 (ml/kg per min) in the group of female competitors from 41,40 ml/kg

per min to 45,18 ml/kg per min. Changes in the body composition parameters that were discovered after the analyzed preparatory training period included a decrease in fat mass and an increase in muscle mass with slight changes in body weight. From each study participant, 5 mL of whole blood was drawn into an EDTA tube. The table with cortisol concentrations per training phase is presented below, Table 1.

Table 1. Cortisol concentrations per training phase for each player.

	Phase 1	**Phase 2**	**Phase 3**	**Phase 4**
Player 1	26.7	19.2	17.4	16.0
Player 2	30.9	24.1	23.1	22.6
Player 3	21.1	18.3	16.3	14.6
Player 4	23.0	14.6	15.5	9.9
Player 5	27.9	19.1	15.2	12.7
Player 6	23.3	16.1	13.7	14.5
Player 7	29.8	24.8	20.9	21.6
Player 8	17.6	12.5	13.7	13.4
Player 9	33.7	17.5	11.5	19.0
Player 10	36.5	23.1	25.5	22.8
Player 11	24.1		18.1	19.2
Player 12		20.3	15.3	
Player 13	10.7	18.9	16.3	13.6

Collection and processing of blood samples

A total number of 49 samples was collected (four times during a 10 week training cycle) in the mornings (from 7:00 to 8:30 AM) from the athletes (before breakfast), in the preparation for their following league season. The intervals between samples collection were set at 3 weeks. Blood samples were processed at the Centre for Innovative Research in Medical and Natural Sciences on the same day they were collected. The samples were first centrifuged at 1000 x g at 4 °C for 5 minutes in the collection tube, then serum was removed and stored at −80 °C until further analysis.

Methods

Cortisol concentration

Cortisol testing was performed with Abbott's Cortisol Reagent Kit. Alinity and Cortisol is a microparticle chemiluminescent marker (CMIA) immunochemical assay for the quantification of cortisol in human serum, serum or urine on the Alinity and Abbott Analyzer. The defined measuring interval (the range of values expressed in µg/dL (nmol/L) that meets the acceptable requirements for linearity, imprecision and systematic error (for the Alinity and Cortisol assay ranges from 1.0 to 59.8 µg/dL (27.6 to 1649.9 nmol /L). The tests were performed on blood serum collected in the morning to clot activator 4ml tubes from Vacutest Kima.

Spectroscopic techniques

In this study we divided all samples into four groups with different range of the cortisol concentration: (i) 27-30 µg/dL; (ii) 17-19 µg/dL; (iii) µg/dL; 14-16 µg/dL and (iv) 10-13 µg/dL. Directly before the analysis, all serum samples were thawed and subsequently measured by Raman and FTIR spectroscopies. The samples were vortexed for 20 sec prior to the measurement and the serum was collected from the bottom of the tube. All measurements were performed in triplicates according to the following procedure. For each sample, the same volume of serum was obtained. Thus, from 3x49 measurements 147 spectra were collected and the 3 spectra from each sample were averaged using the OPUS software.

Raman spectroscopy

The serum samples were investigated using a Nicolet NXR 9650 spectrometer equipped with an Nd:YAG laser (1064 nm) and a germanium detector. Measurements were performed in the range of 150 to 3700 cm^{-1} with a laser power of 1.0W. Unfocused laser beam of a diameter of approximately 100 µm was used, the spectral resolution was equal to 8 cm^{-1} and 64 scans were performed. All obtained Raman spectra were normalized using the vector normalization. Baseline correction was also performed.

FTIR spectroscopy

In this study we used Vertex 70 spectrometer to obtain FTIR spectra of serum. Attenuated Total Reflectance (ATR) technique was used, where the ATR had a diamond crystal; after measurements the crystal was cleaned using ethanol. The measurements were done in all IR average region (400 cm^{-1} – 4000 cm^{-1}). Each sample was scanned 64 times using 2 cm^{-1} spectral resolution. All obtained spectra were analyzed using OPUS 7.0 software, where baseline correction and normalisation was applied.

Statistical analysis

To obtain information about statistical significance between differences of FTIR absorbance and Raman intensity of analyzed peaks in four groups of samples with different cortisol concentration ranges, average value of the absorbance and intensity was calculated. It was performed using spectra obtaining for the all samples assigned to a given group. The results were presented as means ± SEM (the standard error of the mean). To determine the negative and positive

correlation between phospholipids, lipids, proteins vibrations and cortisol concentration, the Pearson's test with the $p < 0.001$ value was calculated using Past 3.0 software.

Results and discussion

Using FTIR and Raman spectroscopy, we studied the applicability of these techniques to detect the changes in the secondary structure of the amount of proteins and lipids which were correlated with cortisol concentration in serum of volleyball players. In our research we studied the differences in the value of absorbance of individual functional groups and the frequency shift of the peaks. To determine the secondary structure of proteins we made a deconvolution of the amide I infrared (IR) region.

a) FTIR spectroscopy

First, the whole spectral range where the reflectivity measurements were performed. In Fig. 1, the characteristic FTIR peaks for phospholipid, protein and lipid vibrations in serum were visible. Two peaks at 1079 cm^{-1} and 1243 cm^{-1} corresponded to the symmetric and asymmetric stretching vibrations of -O-P=O (PO^{2-}) from phospholipids, respectively. Three characteristic peaks originating from protein vibrations were noticed at 1265 cm^{-1} , 1525 cm^{-1} and 1654 cm^{-1} (amide III, amide II, amide I, respectively). Furthermore, in Fig. 1a the peaks correspond to symmetric and asymmetric vibrations of CH_2 and CH_3 groups at 2872 cm^{-1} , 2920 cm^{-1} and 2957 cm , respectively (Hands, et al, 2014; Bonnier et al., 2014; Merrell, et al., 2004; Roche, et al., 2009; Finoulst et al., 2009; Petrich et al., 2009; Hughes et al., 2014).

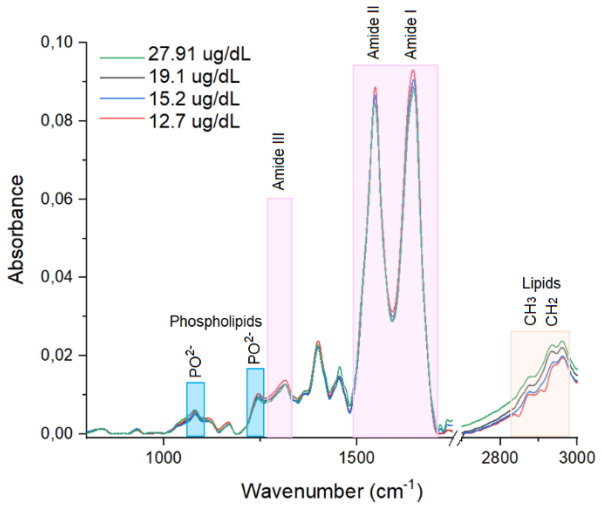

Figure 1. FTIR spectra of serum collected from women with various cortisol concentrations: 27.91 µg/dL (green spectrum); 19.1 µg/dL (black spectrum), 15.2 µg/dL (blue spectrum) and 12.7 µg/dL (red spectrum).

FTIR spectra showed enhanced absorbance of lipid group vibrations and reduced absorbance of amide bonds with increasing serum cortisol concentration, Fig. 1. However, the differences in the amide bonds, were visible only in the case of two smallest cortisol concentrations. While the differences in the absorbance of CH_2 and CH_3 lipids functional groups were visible between all analyzed samples. The smallest differences in the absorbance were visible in IR region corresponding to phospholipid vibrations. However, we observed that the intensity of asymmetric stretching vibrations of PO^{2-} was the highest in serum of women with 12.7 µg/dL cortisol concentration (red spectrum). Importantly, obtained spectra showed, that the observed differences between analyzed samples were very small. Therefore, to show the statistical significance of these differences, an average absorbance values of each analyzed peaks presented in FTIR spectra were calculated and the result is presented in Figure 2.

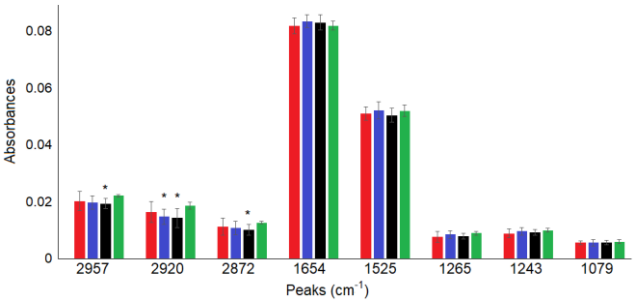

Figure 2. Average absorbance of peaks measured by a FTIR spectroscopy for selected cortisol concentration ranges: 10-13 µg/dL (red); 14-16 µg/dL (blue), 17-19 µg/dL (black) and 27-30 µg/dL (green). Significant differences in the absorbance in comparison with 27-30 µg/dL; 17-19 µg/dL; µg/dL; 14-16 µg/dL; 10-13 µg/dL, were marked by "*"; "^"; "#" and "&", respectively.

The average values of absorbance showed that statistically significant differences in intensity occur only in peaks corresponding to CH_2 and CH_3 lipids functional groups, Figure 2. Moreover, these significant differences were visible only between volleyball players in cortisol concentration between 27-30 µg/dL and 17-19 µg/dL, as well as 14-16 µg/dL. It means, that using FTIR spectra it is unlikely to distinguish serum samples with different levels of cortisol. However, given that cortisol affects lipids and proteins, the ratio of peaks 2957 cm^{-1}/1654 cm^{-1}, 2920 cm^{-1}/1654 cm^{-1}, 2872 cm^{-1}/1654 cm^{-1}, were calculated, Figure 3.

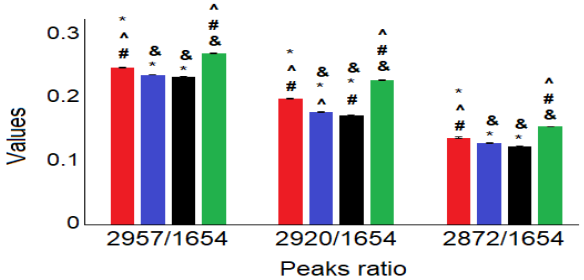

Figure 3. Values of ratio between peaks corresponding to CH_2 and CH_3 lipids vibrations (2957 cm^{-1}, 2920 cm^{-1}, 2872 cm^{-1}) and amide I (1654 cm^{-1}) obtained from FTIR spectra of serum with different cortisol concentration: 10-13 µg/dL (red); 14-16 µg/dL (blue), 17-19 µg/dL (black) and 27-30 µg/dL (green). Significant differences in the absorbance in comparison with 27-30 µg/dL; 17-19 µg/dL; µg/dL; 14-16 µg/dL; 10-13 µg/dL, were marked by "*"; "^"; "#" and "&", respectively.

Figure 3 showed, that the highest values of ratio between CH_2 and CH_3 lipids vibrations (2957 cm^{-1}, 2920 cm^{-1}, 2872 cm^{-1}) and amide I vibrations (1654 cm^{-1}) in the samples with the greatest cortisol concentration, was presented. These differences were significant in comparison with other analyzed cortisol concentration ranges, which suggest that using values of ratio of individuals peaks it is possible to show high cortisol concentration using FTIR spectroscopy. Furthermore, using values of ratio between CH_2 and CH_3 lipids vibrations and amide I bonds at 2957 cm^{-1}, 2920 cm^{-1}, 2872 cm^{-1}, 1654 cm^{-1} wavenumbers, respectively, it may be possible to show the smallest cortisol concentration (10-13 µg/dL). In the case of samples with cortisol concentration between 14-16 µg/dL and 17-19 µg/dL, only using value of ration between wavenumbers at 2920 cm^{-1} and 1654 cm^{-1}, these concentration of analyzed hormone could be show. However, as it was visible in Figure 3, the differences in the peaks ratios between the samples with cortisol concentration (i) 10-13 µg/dL, (ii) 14-16 µg/dL, (iii) 17-19 µg/dL were very small. Therefore, we suggest, that larger number of samples should be analyzed to show, if obtained results in this experiment will be still significant.

b) *Raman spectroscopy*

In the Raman spectra presented in Fig. 4, Raman shifts at 1032 cm^{-1} and 1240 cm^{-1} originating from the vibrations of phosphorate groups of phospholipids were noticed (Cheng et al., 2005). Amide III, amide II and amide I vibrations were placed at 1266 cm^{-1}, 1544 cm^{-1} and 1655 cm^{-1} (Dukor, 2006; Huang et al., 2003; Stone et al., 2004; Stone et al., 2002). Amide vibrations are the most prevalent structures found in organic molecules and various biomolecules e.g. peptides, proteins, DNA, and RNA. The unique chemical structure of amide groups allows them to enter into chemical reactions with a very large number of compounds. The double character of the amide CO-N bond is responsible for this. Consequently, amide I is analyzed in spectra using stretching vibrations of CO and N-H bonds. While, amide II and III bonds are mixtures of the stretching vibrations of C-N and H-N-C bonds. Symmetric and asymmetric vibrations of CH_2 and CH_3 groups were noticed between 2800 cm^{-1} and 2950 cm^{-1} (Kline et al., 1997).

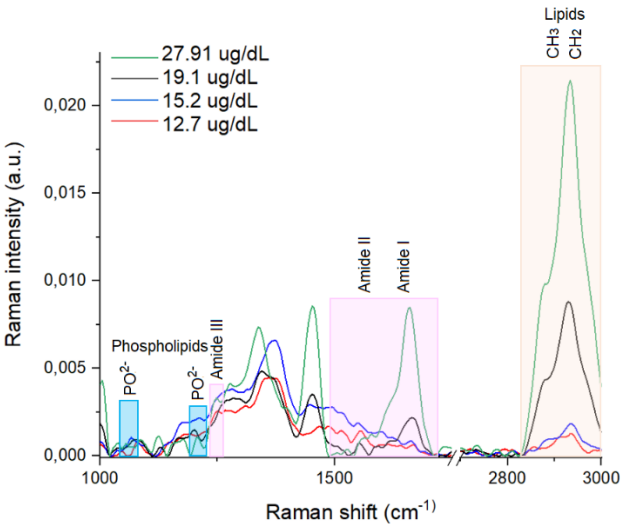

Figure 4. Raman spectra of serum collected from women with various cortisol concentrations: 27.91 µg/dL (green spectrum); 19.1 µg/dL (black spectrum), 15.2 µg/dL (blue spectrum) and 12.7 µg/dL (red spectrum).

The structures in Raman spectra, resulting from the lipid vibrations are the most sensitive to a cortisol level and their intensity increases with an increasing cortisol content in plasma. The intensity of structures related to amide vibrations also increases with an increasing cortisol level, but observed changes in this case are less spectacular. These observation was different for the spectrum characteristic for women with 15.2 µg/dL cortisol concentration. It could be caused by individual features, but also by the changes in the female body during the menstrual cycle, which also affects the entire hormonal balance. Therefore, male animals are used in most medical experiments in animal models. However, in order to be able to accurately determine the cause of such a visible difference in the Raman spectra, it is necessary to perform another experiment in which serum from women is collected at a different stage of the cycle.

The differences in the Raman intensities between analyzed groups were clearly show in the Raman spectra, however, to obtain statistical information, the average values of Raman intensities of peaks presented in Figure 4 were calculated and presented in Figure 5.

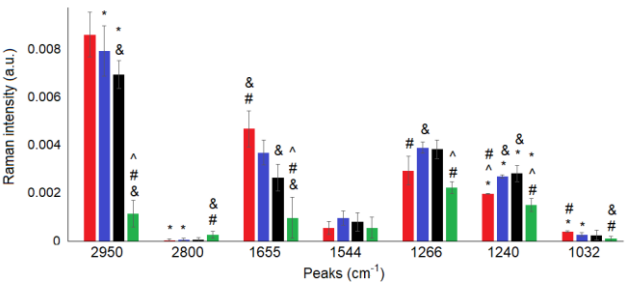

Figure 5. Average Raman intensities of peaks measured by a Ramna spectroscopy for selected

cortisol concentration ranges: 10-13 μg/dL (red); 14-16 μg/dL (blue), 17-19 μg/dL (black) and 27-30 μg/dL (green), where * vs. 27-30 μg/dL; ^ vs. 17-19 μg/dL; # vs. 14-16 μg/dL; & vs. 10-13 μg/dL.

Higher intensity of peak at 2950 cm^{-1} in the serum with 17-19 μg/dL; # vs. 14-16 μg/dL; & vs. 10-13 μg/dL cortisol concentration was observed in comparison with samples with 27-30 μg/dL hormone level. When we compered samples with 17-19 μg/dL and 10-13 μg/dL cortisol concentration, higher intensity of peak corresponding to CH_3 groups in the second group was noticed. The highest intensity of peak at 2800 cm^{-1} was observed in the samples with 27-30 μg/dL cortisol concentration and these result was statistically significant in comparison with groups with 10-13 μg/dL and 14-16 μg/dL cortisol level. The smallest amount of amide I in the group with the highest cortisol concentration was noticed. Furthermore, the differences in the case of peak at 1655 cm^{-1} between analyzed groups, were significant. The intensity of amide I peak increases when cortisol concentration decreases. Also in the case of amide III vibrations, the smallest intensity was observed in the group of samples with the highest cortisol level and the differences between analyzed groups were statistically significant. Similar trend was observed for peaks corresponding to phospholipids vibrations.

As we showed, changes in the cortisol concentration caused chanes in the amount of proteins, lipids and phospholipids functional groups, which were visible as a changes in the Raman intensities and ratio betweeen individual peaks in the case of FTIR spectra. To show correlation between analyzed chemical compounds and cortisol concentration, Pearson correlation test was performed, Table 2.

Table 2. Pearson correlation test between the concentration of cortisol in the serum and the surface area of peaks originating from phospholipids, proteins and lipids determined from FTIR and Raman spectra. $p < 0.001$.

FTIR spectroscopy – area of peaks corresponding to functional groups from:				
	phospholipids	proteins	lipids	cortisol concentration
phospholipids	1.00	0.95	-0.71	0.70
proteins	0.95	1.00	-0.89	0.10
lipids	-0.71	-0.89	1.00	0.87
cortisol concentration	0.70	0.10	0.87	1.00
Raman spectroscopy – area of peaks corresponding to functional groups from:				
	phospholipids	proteins	lipids	cortisol concentration
phospholipids	1.00	0.48	-	-0.57
proteins	0.48	1.00	-0.80	-0.90
lipids	-	-0.80	1.00	0.76
cortisol concentration	-0.57	-0.90	0.76	1.00

Pearson correlation test (Table 1) presented positive and negative correlations between the properties of analyzed samples calculated from FTIR and Raman spectra. Positive correlation means that when the amount of measured factors increases, also the second factor increases, while negative correlation means that if we notice an increase of some factor, the amount of the next factor decreases. From the FTIR spectra, positive correlations between phospholipids and proteins and cortisol concentration and phospholipids, proteins and lipids, respectively, were identified. Negative correlation between lipids and phospholipids was visible from FTIR data. A strong negative correlation between lipids and proteins was observed in FTIR spectra. In Raman data also positive and negative correlations were obtained. Positive correlation between phospholipids and proteins and between cortisol concentration and lipids were visible, while for cortisol concentration and proteins, as well as phospholipids, negative correlations were found. Lack of a correlation between proteins and lipids was noticed from the Raman spectra.

In this study, two complementary optical techniques, FTIR and Raman spectroscopies were used to study the qualitative and quantitative chemical changes in phospholipids, proteins and lipidsin serum of elite volleyball players induced by different cortisol concentration.

Raman data show (Figs. 4, 5) a decrease of the Raman intensity of peaks corresponding to PO^{2-} groups from phospholipid when the cortisol level in serum increased. Similar results for protein vibrations (amide I, amide III) was visible in Raman

spectra, and moreover, in the Raman spectra a frequency shift corresponding to amides bonds was noted, which means, that structure of proteins was changed. Figs. 2, 4 show that with the increase of cortisol concentration, increase of CH_2 and CH_3 lipid vibrations was detected. Furthermore, the highest values of ratio between CH_2 and CH_3 lipids vibrations (2957 cm^{-1}, 2920 cm^{-1}, 2872 cm^{-1}) and amide I vibrations (1654 cm^{-1}) in the samples with the greatest cortisol concentration in the group of samples with the highest cortisol concentration also showed, that an increases of lipids functional groups and decreases of amides vibrations were correlated with values of cortisol concentration, Figure 3. Indeed, stress hormones, such as cortisol, play a very important role in the balance between protein and lipids fractions in the human body (Annoncia & Marino, 2016). These hormones cause the increase of the cholesterol level simultaneously decreasing the level of phospholipids, which was observed in our Raman spectra, as shown in Figs. 3, 4 and Pearson correlation test in Table 1. Cortisol causes significant changes in the lipid balance, especially between phospholipid, cholesterol and triglyceride fractions. Stress can cause changes in the phospholipid structure, such as shortening their carbon chain, leading to the creation of a new kind of phospholipid (Roy et al., 2010). Furthermore, the balance between phospholipids and cholesterol plays a crucial role in the fluidity of the cell membrane and it negatively affects the structure of the membrane and proteins transport (Larsen, 2013).

Comparing the results of the serum collected from women with different cortisol concentration, (Fig. 4), a shift of the protein peak in the latter case towards higher wavenumbers along with the cortisol level increase was noticed. This shift may be caused by changes in the protein molecular structure, such as alterations in the amino acid composition (Larsen, 2013). Cortisol affects functioning of many body organs. It is responsible for regulating the increased protein synthesis in the liver and restricts their formation in muscles and skin cells (Larsen, 2013). Cortisol stimulates the synthesis of collagen and while the cortisol content remained on a high level, the collagen synthesis was being inhibited, which could have also influenced the secondary structure of the whole protein fraction (Papierska et al., 2008). Cortisol is also responsible for the immune system de-activation by reducing the secretion of pro-inflammatory cytokines that have a protein structure (Stachowicz & Lebiedzinska, 2016). Consequently, Raman spectra show, that a significant cortisol level dependent modifications of structures resulting from vibration of the lipid, amide and phospholipid groups.

The reference range for the morning cortisol level test is 150-250 ng/ml (Angeli et al, 1978; Huckelbridge et al., 2015), 507±20 nmol/L (Lippia, et al., 2009) or 5-25 μg/dL (Orsattiab, et al., 2008). In the conducted tests, the players obtained the following mean values in the group - Phase 1: 25.44 μg/dL (max. 36.6 μg/dL, min. 17.6 μg/dL), Phase 2: 19.04 μg/dL (max. 24.8 μg/dL, min.12.5 μg/dL), Phase 3: 17.11 μg/dL (max. 25.5 μg/dL, min. 11, 5 μg/dL), Phase 4: 16.65 μg dL (max. 22.8 μg/dL, min. 9.9 μg/dL). The noticeable average decrease in blood cortisol levels in subsequent studies in the preparatory period allows us to confirm the adaptability skills of elite woman volleyball players to stress factors - in the analysed case - training (Dziembowska, et al., 2019). The need for individualization/personal approach in the study of the physiological profile of elite woman volleyball players should also be emphasized.

Comparing the obtained results, it can be noticed that only in the case of Raman spectra, the significant differences in the lipids, amides and phospholipids groups were visible. Using FTIR spectroscopy in order to notice an increase in lipid functional groups and a simultaneous decrease in amide groups, it is necessary to calculate the ratio of peaks responsible for these biomolecules. Correlations between the concentration of phospholipids and cortisol concentration are visible only in the Raman spectra, while the differences in proteins - in the Raman spectra and partially in the FTIR spectra. This is due to different selection rules and physical laws that are responsible for the formation of the Raman and the infrared oscillation spectra (Hadjiivanov, et al., 2021). Finally, obtained results showed only qualitative changes. In next our works, we will try to create quantification model, which will which will give an unambiguous answer whether we can use spectroscopic methods to estimate changes in the amount of lipids, phospholipids and proteins caused by an increase or decrease in cortisol levels.

Conclusion

Considering the presented results, as well as the literature data, it can be stated that the cortisol affects the amount and structure of phospholipid, protein and lipid fraction. Results obtained from FTIR and Raman spectra well correlate with previous findings. Therefore, we think that optical techniques could thus become effective tools for estimating the concentration of cortisol in the blood and its influence on the structure of proteins and lipid balance. However, only Raman spectroscopy gives information about changes in all analyzed chemical compounds group, while in the FTIR spectra changes in lipids vibrations, which, as literature data shown, are strongly correlated with cortisol concentration, are visible. Moreover, obtained results showed only qualitative changes in analyzed groups of chemical compounds. For quantitative results, higher number of samples as well as model are clearly required in future research.

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