44 - DIFFERENTIATION OF RUNNERS ATHLETES IN THE METABOLIC ADAPTATION TO THE BRUCE ERGOSPIROMETRY TEST: AN APPROACH BY DRIFTS CONSIDERING GENDER

VITOR ESTEVAM SEVERO, GUILHERME GÖRGEN DA ROCHA,
MIRIAM BEATRIS RECKZIEGEL, HILDEGARD HEDWIG POHL, VALERIANO ANTONIO CORBELLINI
UNIVERSIDADE DE SANTA CRUZ DO SUL, SANTA CRUZ DO SUL, RS, BRASIL
valer@unisc.br

INTRODUCTION

Infrared spectroscopy Fourier Transform (FT-IR) has emerged in recent years as an analytical method with a variety of applications both in the biomedical (ELLIS, GOODACRE 2006; SHAW; MANTSCH 2006; WANG; MIZAIKOFF 2008) and in the sports area (DESGORCES; TESTA; PETIBOIS 2008; BENEZZEDDINE-BOUSSAIDI et. al. 2008). Its great advantage as an analytical technique is supported in the fact that it is a tool that does not require specific reagents for different analytes, and that, due to the possibility of automation allows analysis of large numbers of samples. Another great advantage of this technique is the use of small sample volumes making collections minimally invasive to athletes and\or patients (PETIBOIS; DÉLÉRIS 2003b).

The FT-IR is a technique used for analysis of organic components and it is known as a vibrational spectroscopy since the spectrum originates from the transitions between vibrational energy levels of a covalent bond of a molecule. Based on these principles, defined spectral ranges associated with known molecular chemical functions (fatty acids, proteins, amino acids, sugars, etc.) can be used to compare structural information or to determine molecular concentrations between sets of biological samples (Longas , et al., 2011).

In this context, the aim of this study was to evaluate the application of infrared spectroscopy of whole blood to differentiate metabolic adaptations and performance of athletes in the Bruce ergospirometry test, regarding gender.

MATERIALS AND METHODS

The methodology consisted of a cross-sectional descriptive analytical study, with quantitative methodological design. The sample was composed of a group of 34 runners (12 in 2008, 14 in 2009 and 8 in 2010), from the UNISC Athletics Team of different modalities (sprinters, middle-distance runners and distance runners), six males. These athletes voluntarily joined the research, by word of consent. This study is part of the project "Correlation between blood biochemical profile and performance of athletes in the Bruce ergospirometry test and in specific tests using infrared spectroscopy", proposed and approved by the Ethics Committee protocol 2146/08.

The subjects were recommended bland diet and rest in the 24 hours before the test, and initially submitted to anthropometric, blood pressure and resting heart rate evaluations. Then the athlete underwent ulnar vein puncture and it was performed stress test according to the Bruce protocol treadmill and gas analyzer TEEM 100, evaluating the cardiorespiratory performance from the volume of oxygen consumption (VO2), carbon dioxide (VCO2), respiratory quotient (RQ), heart rate (HR) and blood pressure (BP). During the test, heart rate was recorded every 20 seconds with digital frequency meter and blood pressure measured every 3 minutes with a mercury sphygmomanometer (Missouri). The exercise test was performed up to exhaustion or termination of the protocol with 3 min recovery (post-test). Blood samples (5 mL) were collected from the cubital fossa with vacutainer without anticoagulant in pre-test and post-test stages and from the fingertip with a lancet and pipette in the transition between two stages. Triplicate samples of 5 µL of blood were collected in eppendorf tubes with 150 mg of KBr (VETEC, spectroscopic grade) in stages pre and post-test and simplicates in transition between two stages. Blood samples were lyophilized (2h15min, 1x10-4 torr) and introduced in diffuse reflectance accessory with monochromatic light (PIKE Technologies, Madison, USA) attached to a Nicolet Magna 550 FTIR spectrometer (Thermo Nicolet Corporation, Madison, USA) or Infrared spectrophotometer brand/model Spectrum 400 FT-IR/FT-NIR Spectrometer (Perkin Elmer ®). The spectra were recorded between 4000 a 600 cm-1 with a spectral resolution of 4 cm-1 and 16 pulses of scanning.

In the analysis by infrared spectroscopy, the spectra were normalized (between 0 and 1,0) and stored in software OMNIC E.S.P version 7.0. The spectral data (absorbance intensity at a given frequency associated with specific bands, Table 1, for the medium spectra) for the three years were statistically analyzed after application of Student's t-test, according to the following categories:

- Male versus female gender to the pre-test stage;
- Male versus female gender to the post-test stage.

Tabela – Bandas e respectivos movimentos vibracionais utilizados para descrição dos espectros de reflectância difusa no infravermelho com Transformada de Fourier de sangue total.

N°	Spectral region (cm^{-1})	Attribution
1	3700-3400	νO-H (stO-H) = axial stretching of hydroxyl group
2	3400-3110	νN-H (stN-H) = axial stretching of amines group
3	3110-3000	νC=C-H (stC=C-H) = axial stretching C-H of vinyl group
4	2990-2950	ν_{as}C-H (st_{as}C-H) = asymmetric axial stretching C-H of methyl group
5	2950-2890	ν_{as}C-H (st_{as}C-H) = asymmetric axial stretching C-H of methylene group
6	2890-2860	ν_sC-H (st_sC-H) = symmetric axial stretching C-H of methyl group
7	2860-2840	ν_sC-H (st_sC-H) = symmetric axial stretching C-H of methylene group
8	1800-1760	νC=O (stC=O) = axial stretching of carbonyl group of carboxylic acids
9	1760-1590	νC=O (stC=O) = axial stretching of carbonyl group, amide I
10	1590-1490	δN-H ($_{ang}$N-H) = Angular deformation of amide group, amide II, tyrosine band
11	1490-1430	νC-CH_3 (stC-CH_3) = asymmetric axial stretching C-H of methylene group
12	1430-1350	ν_sC=O (st $_s$C=O) = symmetric axial stretching of carbonyl group of carboxylate group
13	1310-1240	amide III
14	1240-1220	ν_{as}P=O (st $_{as}$P=O) = symmetric axial stretching of >PO $_2^-$ group of phosphodiester
15	1200-1140	νC-O (stC-O) = axial stretching and angular deformation of group C -O-C, C-O, C-O-P of carbohydrates and derivatives phosphorylated
16	1140-1120	νC-O (stC-O) = axial stretching and angular deformation of group C -O-C, C-O, C-O-P of carbohydrates and derivatives phosphorylated
17	1120-1090	νC-O (stC-O) = axial stretching and angular deformation of group C -O-C, C-O, C-O-P of carbohydrates and derivatives phosphorylated
18	1090-1000	νC-O (stC-O) = axial stretching and angular deformation of group C -O-C, C-O, C-O-P of carbohydrates and derivatives phosphorylated

SOURCE: NAUMANN, 2000.

RESULTS

The data regarding the athletes evaluated are presented in Table 2. Besides the identification of athletes, the table includes the year of assessment, the athlete's sport, age in years and anthropometric data such as weight and height synthesized in the form of BMI (body mass index).

Table 2 – Data of athletes submitted to the Bruce protocol performed in 2008, 2009 and 2010.

	Nº	Modality	Age (years)	BMI ($kg.m^{-2}$)
		Male Athletes		
2008	**01**	Distance Runner	27	21,40
	02	Sprinter (100 a 200m)	17	25,14
	03	Sprinter (200 a 400m)	21	25,02
	04	Distance Runner	15	19,25
	10	Sprinter (100 a 200m)	17	22,03
	12	Distance Runner	28	23,22
2009	**01**	Distance Runner	16	18,13
	02	Sprinter (100 e 200 m)	18	22,12
	03	Race Walking	19	20,88
	04	Distance Runner	16	19,04
	05	Distance Runner	29	22,62
	08	Distance Runner	29	22,04
	11	Distance Runner	14	21,84
2010	**01**	Distance Runner	17	21,08
	02	Sprinter (100 e 200 m)	30	22,36
	03	Race Walking	20	22,34
	04	Distance Runner	19	21,93
	05	Distance Runner	29	23,02
	06	400 m	19	23,07
	?(SD)		21,05(5,59)	21,92(1,78)
		Female Athletes		
2008	**05**	Distance Runner 3.000m w obstacles	19	20,00
	06	Sprinter (400m)	18	20,62
	07	Long and triple jumps	18	19,77
	08	Middle-distance Runner	17	17,07
	09	Distance Runner	15	18,52
	11	Shot put	18	26,30
2009	**06**	400 m	19	21,32
	07	Long and triple jumps	20	21,12
	09	Distance Runner	16	18,9
	10	Shot put	19	28,2
	12	Middle-distance Runner	19	16,60
	13	Race Walking	21	22,31
	14	Distance Runner	34	19,71
2010	**07**	Long and triple jumps	35	20,11
	08	Distance Runner	19	16,44
	?(SD)		20,47(5,89)	20,47(3,26)

LEGEND: BMI: Body mass index. M = male; F = female. ȳ = mean; SD = standard-deviation.

Significant differences between stages were observed between male and female athletes before starting the Bruce ergospirometry test in the three years of collection. In 2008 the spectral ranges with these features were more extensive, including the regions 3075-2950 cm-1, 2910-2660 cm-1, 1750-1685 cm-1, 1590-1550 cm-1, 1530-1160 cm-1 and 1075-1065 cm-1. In 2009, spectral ranges with significant differences were observed in the regions 3370-3230 cm-1, 2390-2160 cm-1 and 1460-1445 cm-1. In 2010, the spectral ranges with $p < 0.05$ were the ones with 3225-3150 cm-1, 3140-3135 cm-1, 2960-2950 cm-1. Moreover, it can be seen that for the years 2008 and 2009 a range in common with the significant difference is the one that comprises a range from 1460 to 1445 cm-1. In comparison with 2008 and 2010, there are similarities in spectral ranges with $p < 0.05$ from 3060 to 2950 cm-1 and comparing 2009 and 2010 there are no spectral ranges with $p < 0.05$ in common.

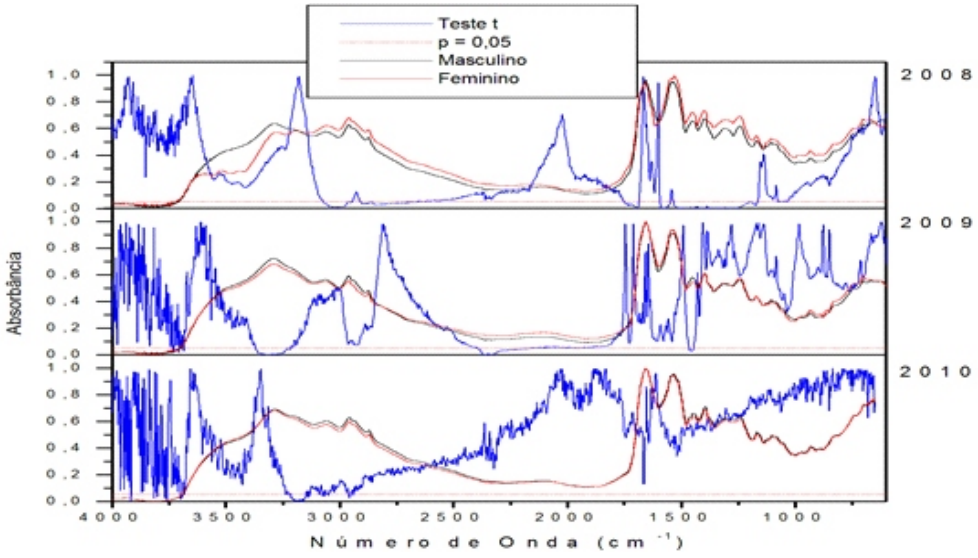

Figure 1: Medium spectra of diffuse reflectance infrared Fourier transform of whole blood from male and female

athletes in the resting stage (before Bruce test) with respective p-values according to the t-test, towards the frequency for the years 2008, 2009 and 2010.

Significant differences were also found between genders after the recovery phase of the ergospirometric test, in the three years of data collection, and in 2009 were broader the spectral ranges that showed significant differences. In 2008, the significant spectral ranges were from 2895 to 2375 cm-1 and 2290-2260 cm-1. In 2009, the spectral ranges with significant differences comprise the spectral range from 2460 to 1785 cm-1, 1630-1575 cm-1 and 1545 to 1540 cm-1. In 2010, the spectral ranges with $p < 0.05$ comprised 3685-3180 cm-1. In addition to that, it can be seen that for the years 2008 and 2009, a region with a significant difference in common is the range comprising 2290 to 2260 cm-1, comparing with 2008 and 2010/2009 and 2010 there are no similarities in regions with $p < 0.05$.

.

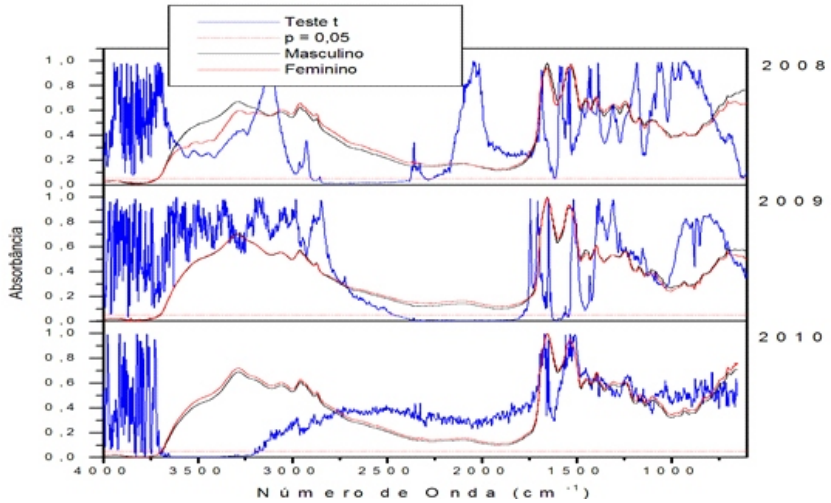

Figure 2: Medium spectra of diffuse reflectance infrared Fourier transform of whole blood from male and female athletes in the resting stage (post-test) with respective p-values according to the t-test, towards the frequency for the years 2008, 2009 and 2010.

Therefore, based on the spectra of athletes at rest, and comparing athletes, it is possible to notice many differences that show physiological characteristics already recorded in literature. In 2008, there was a significant difference between men and women in the regions 3050-2950 cm-1 and 2900-2700 cm-1 and 1750-1700 cm-1, related to the absorption of triglycerides, free fatty acids and cholesterol esters, higher among women. This behavior reflects a physiological characteristic of women for accumulating more fat than men and therefore may be an acceptable answer as differentiated adaptation during activity. Even on these spectral ranges is noted that in 2009 and 2010 there is evidence that with the increasing number of female representatives, these ranges could also prove significant (PETIBOIS et. al. 2001).

Also in the range from 1300 to 1200 cm-1 it there is a significant difference pointing out that women have a higher amount of carbohydrates in the pre-test compared to men.

At rest, however, not many differences were observed among athletes of both genders, unless the range indicating the presence of water, which showed that male athletes have a greater reduction of the presence of water in the blood, a possible explanation to the moving of liquid to the muscles and because of the greater amount of muscle in males, they exhibit this response. Besides this adaptation is noted in 2009, significant differences in the spectral range from 1650 to 1450 cm-1, which comprise proteins, indicating an increasing level in female athletes. However, these few differences lead us to say that in the post-test male athletes and female athletes have very similar characteristics, which means, in the pre-test the characteristics are quite different between the athletes and the post-test stage the physiological and biochemical adaptations tend to become similar.

CONCLUSION

From the data collected, it is possible to conclude that the Fourier Transform Infrared Spectroscopy (FT-IR) was shown to be sufficiently sensitive to evaluate the adaptation of athletes during the Bruce Ergospirometry Test, enabling the observation of particularities regarding the differences of genders.

Is is also important to mention the ease of use, speed, reliability combined with spectral sensitivity and a relatively small sample volume, which makes it a favorable technique for the area of exercise physiology and sports medicine.

REFERENCES

BENEZZEDDINE-BOUSSAIDI, L.; CAZORLA, G.; GUEZENNEC, C,-Y.; FONTAND, E.; BURNAT, P. Contrôle biologique de l'entraînement sportif par spectrométrie IR-TF Biological control of sport training by spectrometry FT-IR. Science & Sports, 23(6) 283–291, 2008.

DESGORCES, F.D.; TESTA, M.; PETIBOIS, C. Training-level induced changes in blood parameters response to on-water rowing races. Journal of Sports Science and Medicine 7, 425-430, 2008.

ELLIS D.I.; GOODACRE, R. Metabolic fingerprinting in disease diagnosis: biomedical applications of infrared and Raman. The Analyst, 131(8):875–885, 2006.

ESCANDAR, G. M.; DAMIANI, P.C.; GOICOECHEA, H.C.; OLIVIERI A.C. A review of multivariate calibration methods applied to biomedical analysis. Microchemical Journal, 82(1):29-42, 2006.

PETIBOIS, C.; CAZORLA, G.; CASSAIGNE, A.; DÉLÉRIS, G. Application of FT-IR spectrometry to determine the global metabolic adaptations to physical conditioning in sportsmen. Appied Spectroscopy, 56(10):1259-1267, 2002.

PETIBOIS, C.; CAZORLA, G.; GIN, H.; DÉLÉRIS, G. L'étiologie clinique du surentraînement au traversde l'examen sanguin : état des connaissances. La Revue de Médecine Interne,22(8):723-736, 2001.

PETIBOIS, C.; CAZORLA, G.; POORTMANS, J.R.; DÉLÉRIS, G. Biochemical aspects of overtraining in endurance

sports: the metabolism alteration process syndrome. Sports Medicine, 33(2):83-94, 2003.

PETIBOIS, C.; DÉLÉRIS, G. Stress-induced plasma volume change determined using FT-IR spectra. Applied Spectroscopy, 57(4):396-399, 2003a.

PETIBOIS, C.; DÉLÉRIS, G. 2D-FT-IR spectrometry: a new tool for the analysis of stress-induced plasma content changes. Vibrational Spectroscopy, 32(1):117-128, 2003b.

PETIBOIS, C.; DÉLÉRIS, G. Oxidative stress effects on erythrocytes determined by FT-IR spectrometry. Analyst 129(10):912-916, 2004a.

PETIBOIS, C.; DÉLÉRIS G. Alterations of lipid profile in endurance over-trained subjects. Archives of Medical Research, 35(6):532-539, 2004b.

PETIBOIS, C.; DÉLÉRIS, G. Evidency that erythrocytes are highly susceptible to exercise oxidative stress: FT-IR spectrometric studies at the molecular level. Cell Biology International. 29(8):709-716, 2005.

SHAW, A.; MANTSCH, H. Infrared spectroscopy in clinical and diagnostic analysis. Enciclopedia of Analytical Chemistry.1-20, 2006.

WANG, L.; MIZAIKOFF, B. Application of multivariate data-analysis techniques to biomedical diagnostics based on mid-infrared spectroscopy. Analytical and Bioanalytical Chemistry, 391(5):1641-1654, 2008.

Avenida Independência. 2293 Bairro Universitário – CEP: 96.815-900
Santa Cruz do Sul – RS
valer@unisc.br

DIFFERENTIATION OF RUNNERS ATHLETES IN THE METABOLIC ADAPTATION TO THE BRUCE ERGOSPIROMETRY TEST: AN APPROACH BY DRIFTS CONSIDERING GENDER

ABSTRACT

In the present study 34 runners (12 in 2008, 14 in 2009 and 8 in 2010) from the UNISC Athletics Team were evaluated by anthropometry and ergospirometry associated with Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) of whole blood for differentiation of blood adaptation patterns with regard to gender. The athletes underwent the Bruce Protocol Treadmill Test, using gas analyzer and evaluating heart rate, maximum oxygen consumption (VO2max), maximum carbon dioxide production (CO2max) and respiratory quotient (RQ) at rest (pre-test) and every 20 seconds up to 3 minutes after the end of the test (exhaustion or termination of the protocol). The evaluation of blood pressure was performed at rest, every 3 min protocol (transition between stages) and after recovery. The blood collection by brachial venipuncture was performed in the pre-test and 10 minutes after the termination of protocol (pos-test) and blood samples were analyzed by DRIFTS through the full spectrum. It was observed significant differences between male and female athletes in the pre-test stage in the three years of collection. Significant differences were also found between the genders in the post-test stage in the three years, and in 2009 the spectral ranges that presented significant differences were larger. It is possible to conclude that the FT-IR of whole blood may be used to find differences between genders in the adaption of athletes during the Bruce Ergospirometry Test.

KEYWORDS: athletes, DRIFTS, metabolic adaption.

DIFFÉRENCIATION DE COUREURS A L'ADAPTATION METABOLIQUE DANS L'EPREUVE D'EFFORT DE BRUCE: UNE APPROCHE PAR DRS-FTIR EN FUNCTION DU SEXE

RÈSUMÉ

Dans cette étude on eté on eté avaliée 34 coureurs (12 dans l'anée 2008, 14 dans l'anée 2009 et 8 dans l'anée 2010) de l'Équipe d'Athlètisme de l'UNISC, avec anthropometrie et ergospirometrie associetée à la spectroscopie infrarouge à transformée de Fourier à réflexion diffuse (DRS-FTIR) du sang total pour la différenciation du sang modes d'adaptation en matière de genre. Lês athlètes on eté soumis en utilisant le protocol de Bruce dans un tapis roulant ergométrique couplée à l'analyseur des gaz à l'évaluation du fréquence cardiaque, de la consommation maximal d'oxygène (VO2max), de la production maximal de dioxyde de carbone (VCO2max) et de le coeficient respiratoire (QR) en vertu de repos (pré-test) et toutes le 20 second jusqu'à trois minutes aprés la fin de l'épreuve (l'epuisement ou la fin du protocole). L'évaluation de la pression artérielle a été effectuée en vertu de repos, toutes les trois minutes du protocole (transition entre lês étapes) et aprés la récupération. La collect de sang par ponction veineuse brachiale s'est tênue à l'étape de pré-test et dix minutes après la fin du protocol (pós-test) et l'es echantillons de sang ont été analysés par DRS-FTIR en utilisant le spectre complet. Des différences significatives ont été observées entre les athlètes masculins et féminins dans le pré-test dans les trois ans suivant la fin de la collecte. Des différences significatives ont également été observées entre les sexes dans le post-test pendant trois ans, et en 2009 étaient plus larges bandes spectrales qui montrent des différences significatives. Il est possible de conclure que le FT-IR de sang total peut être utilisé pour trouver des différences entre les sexes dans l'adaptation des athlètes au cours d'éprouve d'effort de Bruce.

MOTS-CLÉS: athlètes, IR-FT, adaptation métabolique

DISTINCIÓN DE ATLETAS CORREDORES EN LA ADAPTACIÓN METABÓLICA AL ENSAYO ERGOESPIROMÉTRICO DE BRUCE: UN ABORDAJE POR DRIFTS CONSIDERANDO GÉNERO

RESUMEN

En este estudio fueron evaluados 34 atletas corredores (12 en 2008, 14 en 2009 y ocho en 2010) del equipo de Atletismo de UNISC, mediante antropometría y ergoespirometría asociados a la espectroscopía de reflectancia difusa en el infrarrojo con Transformada de Fourier (DRIFTS) de sangre total para diferenciación de padrones de adaptación sanguínea con relación a los sexos. Los atletas han sido sometidos al protocolo de Bruce en cinta caminadora acoplada a analizador de gases siendo evaluados frecuencia cardíaca, consumo máximo de oxígeno (VO2máx), producción máxima de dióxido de carbono (CO2máx) y cociente respiratorio (CR) en reposo (pre-prueba) y cada 20 segundos hasta tres minutos tras el fin de la prueba (agotamiento o término del protocolo). La evaluación de la tensión arterial se la hizo en el reposo, cada tres minutos del protocolo (transición entre los periodos) y tras la recuperación. La recolecta sanguínea por punción venosa braquial ha sido realizada en el periodo pre-prueba y 10 minutos después de terminado el protocolo (post-prueba) y las muestras de sangre se han analizado por DRIFTS a través del espectro total. Han sido observadas distinciones significativas entre atletas masculinos y femeninos en el periodo pre-prueba en los tres años de la realización de la recolecta. Diferencias significativas también se encontraron entre los sexos en el periodo post-prueba para los tres años, siendo que en 2009 fueron más amplias las líneas espectrales que presentaron distinciones significativas. Es posible concluir que la FT-IR de sangre total podrá ser utilizada para encontrar diferencias entre los sexos en la adaptación de atletas durante el ensayo ergoespirométrico de Bruce.

PALABRAS-CLAVE: atletas, DRIFTS, adaptación metabólica.

DIFERENCIAÇÃO DE ATLETAS CORREDORES NA ADAPTAÇÃO METABÓLICA AO ENSAIO ERGOESPIROMÉTRICO DE BRUCE: UMA ABORDAGEM POR DRIFTS EM RELAÇÃO AO GÊNERO

RESUMO

Neste estudo foram avaliados 34 atletas corredores (12 em 2008, 14 em 2009 e 8 em 2010) da equipe de Atletismo da UNISC, por antropometria e ergoespirometria associados à espectroscopia de reflectância difusa no infravermelho com Transformada de Fourier (DRIFTS) de sangue total para diferenciação de padrões de adaptação sanguínea em relação aos sexos. Os atletas foram submetidos ao protocolo de Bruce em esteira ergométrica acoplada a analisador de gases sendo avaliados freqüência cardíaca, consumo máximo de oxigênio (VO2máx), produção máxima de dióxido de carbono (CO2máx) e quociente respiratório (QR) no repouso (pré-teste) e a cada 20 segundos até 3 minutos após o final do teste (exaustão ou término do protocolo). A avaliação da pressão arterial foi realizada no repouso, a cada 3 min do protocolo (transição entre os estágios) e após a recuperação. A coleta sanguínea por punção venosa braquial foi realizada no estágio pré-teste e 10 minutos após o término do protocolo (pós-teste) e as amostras de sangue foram analisadas por DRIFTS através do espectro total. Foram observadas diferenças significativas entre atletas masculinos e femininos no estágio pré-teste nos três anos da realização da coleta. Diferenças significativas também foram encontradas entre os sexos no estágio pós-teste para os três anos, sendo que em 2009 foram mais amplas as faixas espectrais que apresentaram diferenças significativas. É possível concluir que a FT-IR de sangue total pode ser utilizada para encontrar diferenças entre os sexos na adaptação de atletas durante o ensaio ergoespirométrico de Bruce.

PALAVRAS-CHAVE: atletas, DRIFTS, adaptação metabólica.