147 - DIFFERENTIATION OF RUNNERS ATHLETES IN THE METABOLIC ADAPTATION TO THE BRUCE ERGOSPIROMETRY TEST: AN APPROACH BY DRIFTS IN RELATION TO THE STAGE OF TEST

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INTRODUCTION

The infrared spectrum lies between the visible light spectrum and the microwaves (from 1 to 1000 mm). The region of greatest utility for qualitative analysis is situated between 4000 and 400 cm-1 and is known as mid-infrared (MIR) (Wang, Mizaikoff, 2008). Fourier transform Infrared spectroscopy (FTIR) has been increasingly enhanced by being a very useful tool for monitoring plasma or blood "in nature", because it allows the simultaneous determination of several biochemical markers in a single spectrum of plasma, as well as, a small amount of blood, equivalent to a rate extracted from the digital pulp. Also, it is possible to estimate the concentration of biomolecules that may be related to the clinical data. Therefore, this method allows a global view of the human organism (DÉLÉRIS, PETIBOIS, 2004a).

Sports figures generally move countless amounts of money in which a considerable portion is involved in improving physical conditioning and training techniques of athletes. Thus, many studies search for the improvement in technologies to improve the performance of athletes. Among these, FTIR has been shown to be sensitive in the evaluation of some diseases caused by sport such as oxidative stress (DÉLÉRIS; PETIBOIS, 2004a), and overtrainning (PETIBOIS, DÉLÉRIS, 2005), as well as quantification of biochemical parameters (LAFRANCE, LANDS , BURNS, 2004; DÉLÉRIS, PETIBOIS, 2004b; PETIBOIS et. al. 2004) and evaluation of athletes training standards. (PETIBOIS, DÉLÉRIS, CAZORLA, 2000; BENEZZEDDINE-BOUSSAIDI et. al. 2008). The FTIR connected to the sport is no longer a bet; however, there is still a lot to be researched about this technology in the sport, as it is a tool that can generate good outcomes.

Therefore the objective of this study was to evaluate the application of infrared spectroscopy of whole blood to differentiate intergeneric metabolic adaptations of runners in the Bruce ergospirometric test in relation to the stage of the test.

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 with 16 scanning pulses, respectively, in the range 4000-600 cm-1 at a spectral resolution of 4 cm-1. Then, the spectra were normalized (between 0 and 1.0) and stored in software ESP OMNIC 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 of gender:

- Male, pre-test stage versus post-test stage;
- Female, pre-test stage versus post-test stage.

Table 1 – Respective bands and vibrational motions used for describing the diffuse reflectance spectra in the infrared Fourier Transform of whole blood

N°	Spectral region (cm^{-1})	Attribution
1	3700-3400	vO-H (stO-H) = axial stretching of hydroxyl group
2	3400-3110	vN-H (stN-H) = axial stretching of amines group
3	3110-3000	vC=C-H (stC=C-H) = axial stretching C-H of vinyl group
4	2990-2950	v_{as}C-H (st_{as}C-H) = asymmetric axial stretching C-H of methyl group
5	2950-2890	v_{as}C-H (st_{as}C-H) = asymmetric axial stretching C-H of methylene group
6	2890-2860	v_sC-H (st_sC-H) = symmetric axial stretching C-H of methyl group
7	2860-2840	v_sC-H (st_sC-H) = symmetric axial stretching C-H of methylene group
8	1800-1760	vC=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$_s$C=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
	$\bar{y}$(DP)		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
	$\bar{y}$(DP)		20,47(5,89)	20,47(3,26)

LEGEND: BMI: Body mass index. M = male; F = female. $\bar{y}$ = mean; SD = standard-deviation.

The profile analysis of medium infrared spectra of the male athletes in the pre-test (rest) and post-test (recovery, Figure 1) shows the presence of significant changes alongside some similarities in certain spectral regions.

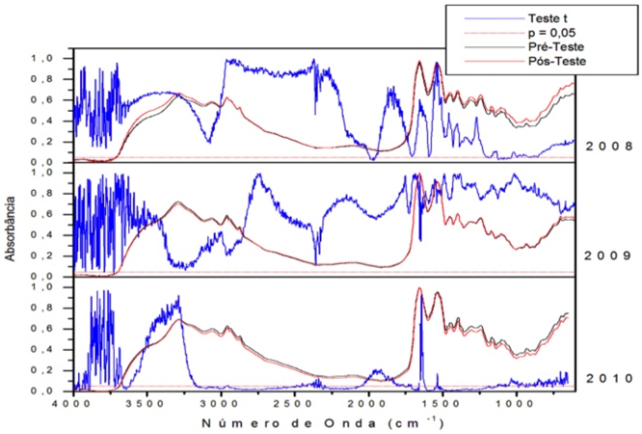

Figure 1: Medium spectra of diffuse reflectance infrared Fourier transform of whole blood from male athletes in the resting stage (pre-test) and after Bruce test (post-test) with respective p-values according to the t-test, towards the frequency for the years 2008, 2009 and 2010.

Greater differences were observed between the two stages in the years 2008 and 2010 and the last showed numerous regions with significant differences. In 2008 these differences occurred in the spectral ranges 1995 to 1950 cm-1, 1190-1180 cm-1 and 1125-1035 cm-1. In 2010 the spectral regions were 3660-3620 cm-1, 3170-2970 cm-1, 2950-2490 cm-1, 2315-2050 cm-1, 1710-1660 cm-1, 1620-1540 cm-1, 1530-1130 cm-1. In addition to that, it can be seen that in 2008 and 2010 the region comprising 1190 to 1180 cm-1 was common to both years, while in other years there were no similarities in different areas.

Comparing the spectra of male athletes in the stages of pre-test and post-test, collected for three years, there were several significant differences. In 2010, there was a reduction in the region of absorption of lipids and carbohydrates comparing pretest to posttest, indicating occurrence of physiological adaptations combined with the consumption of energy sources. There were also changes in the region of lipids such as cholesterol, cholesterol esters, free fatty acids and triglycerides, which comprise the lipid profile of the athlete and it may be mentioned the occurrence of lipid mobilization during exercise, composing an aerobic work during testing. Another point to observe refers to the portion of the protein, region 1600-1480 cm-1 of the spectrum on which it can be inferred that probably there was a process of hemoconcentration with the test, resulting in an increase in hematocrit and consequently total protein. As in that same region of the spectrum showed the presence of increased intensity of deformation of α-helix proteins, which are associated with the presence of large amounts of hemoglobin and myoglobin. Thus, it may be concluded that due to the effort and process oxidative stress, there was rhabdomyolysis, which will release myoglobin into the bloodstream. (PETIBOIS et. al. 2001; PETIBOIS et. al. 2002; PETIBOIS; DÉLÉRIS, 2004a)

The range of saccharides, region 1200 to 1150 cm-1 of the spectrum, is in the tests of the years 2008 and 2010, but in different ways. In 2008, it is shown that in the post-test there was an increase in this region, the explanations may be associated with a possible increase of glycemia in the beginning of the activity, in which the body is still mobilizing liver glycogen and therefore glucose for muscles. In 2010, the response was the reduction of carbohydrates in the region post-test, being acceptable to say that there was a reduction in blood glucose. Still in the sample of 2010, there is a difference in the region near 1190 to 1180 cm-1, comprising lactate, and may be an indication that there was anaerobic work. A justification for this approach can be tied to the phases of pre-season training, in which the athletes were not well adapted to the training, which were in the periods of data collection in 2010, consistent with a reduced rate of VO2. Therefore, in 2008, being in more advanced training phase, an increase in blood glucose, aerobic work performance and an index of increased VO2 (PETIBOIS et. al. 2001; LAFRANCE; LANDS; BURNS, 2004; PETIBOIS; DÉLÉRIS, 2004b; PETIBOIS ; DÉLÉRIS, 2005).

For female athletes (Figure 2), within three years of collection, the spectral bands that showed significant differences were more numerous in 2010. In 2008, the significant spectral ranges were from 1550 to 1530 cm-1. And in 2010, the spectral bands with p <0.05 were the ranges 3400-3230 cm-1. Moreover, it was not observed in any of the years of collection, spectral ranges with significant differences in common.

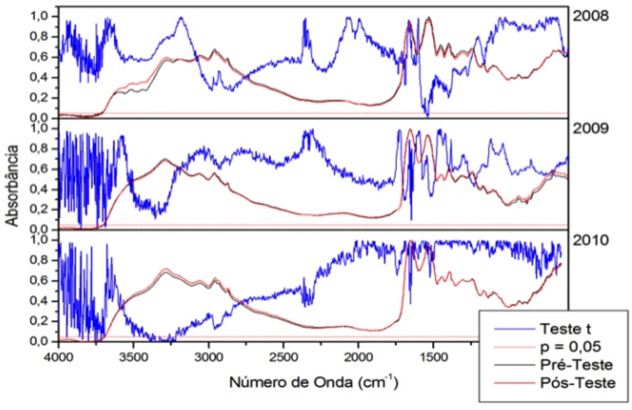

Figure 2: Medium spectra of diffuse reflectance infrared Fourier transform of whole blood from female athletes in the resting stage (pre-test) and after Bruce test (post-test) with respective p-values according to the t-test, towards the frequency for the years 2008, 2009 and 2010.

Following our study, we analyzed the spectra of female athletes in the same stages that male athletes (pre and post-test). However, it was observed that there was not, in any period, many differences in the stages of pre-and post-test, which induced in a response that, when it comes to female athletes, physiological and biochemical changes are not apparent, making us believe that either they are little impacted by exercise, or they have a speedy recovery to the impacts caused by the same. However, in the next spectral range of 1550-1540 cm-1, which comprise proteins, it was observed significant differences (p <0.05) in 2008, while in the pre-test it showed more intensity than compared with the post-test, indicating a possible depletion of proteins. Moreover, it was shown in 2009 and 2010 an indication that a greater "n" can become an area with significant differences. A possible explanation is that this region suffered a decrease in absorbance in the post-test function for mobilization of energy via the protein amino acids, reduced the amount of blood proteins by gluconeogenesis (PETIBOIS et. al. 2001).

CONCLUSION

Fourier Transform Infrared Spectroscopy (FT-IR) was shown to be sufficiently sensitive to evaluate intergeneric adaptation of athletes during the Bruce Ergospirometry Test, enabling the characterization of particularities between genders. Thus, it is extremely timely the use of infrared equipment and consequently the FT-IR to evaluate metabolic adaptation during a workout.

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

ABSTRACT

For 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 (pretest) 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 (post test) and blood samples were analyzed by DRIFTS through the full spectrum. For the male athletes, largest spectral differences were observed between the two stages in the years 2008 and 2010 and the last one showed various regions with significant differences. For the female athletes, in the three years of collection the spectral ranges that showed significant differences were more numerous in 2010. This technique has proved to be sufficiently sensitive to the evaluation of adaptation of athletes during the Bruce Ergospirometry Test, besides being possible to observe particularities regarding the different genders of athletes.

KEYWORDS: athletes, DRIFTS, physical fitness.

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

RÉSUMÉ

Trente-quatre 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 on eté avaliée, avec anthropometrie et ergospirometrie associetée à la spectoscopie infrarouge à transformée de Fourtier à réflexion diffuse (DRS-FTIR) du sang total pour la différenciation à l'adaptation métabolique em fonction d'etape d'éprouve. 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 eu lieu 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. Pour les athlètes masculins les plus grandes différences spectrales ont été observées entre les deux étapes dans les années 2008 et 2010, et le dernier a montré de nombreuses régions avec des différences significatives. En outre, pour les athlètes féminines dans les trois ans suivant l'achèvement de la collection spectrale qui montrent des différences significatives ont été plus nombreuses en 2010. Cette technique s'est avérée être suffisamment sensible pour évaluer l'adaptation des athlètes pendant l'epreuve d'effort de bruce, en plus d'être possible d'observer des détails concernant les étapes de l'analyse.

MOTS-CLÉS: athlètes, IR-FT, aptitude physique

DISTINCIÓN DE ATLETAS CORREDORES EN LA ADAPTACIÓN METABÓLICA AL ENSAYO ERGOESPIROMÉTRICO DE BRUCE: UN ABORDAJE POR DRIFTS EN RELACIÓN A LA ETAPA DE LA PRUEBA

RESUMEN

Para este estudio fueron evaluados 34 atletas corredores (12 en 2008, 14 en 2009 y ocho en 2010) del equipo de Atletismo de UNISC, por antropometría y ergoespirometría asociados a la espectroscopía de reflectancia difusa en el infrarrojo con Transformada de Fourier (DRIFTS) de sangre total para distinción 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 en 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. Para los atletas masculinos, mayores distinciones espectrales se observaron entre los dos periodos en los años 2008 y 2010, siendo que el último presentó innumeras regiones con diferencias significativas. Ya para las atletas femeninas, en los tres años de la realización de la recolecta, las líneas espectrales que presentaron distinciones significativas fueron más numerosas en 2010. Esta técnica ha mostrado ser suficientemente sensible para evaluación de la adaptación de atletas durante la prueba ergoespirométrica de Bruce y asimismo se pudo observar particularidades de los diferentes géneros de los atletas.

PALABRAS-CLAVE: atletas, DRIFTS, aptitud física.

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

RESUMO

Para o presente 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. Para os atletas masculinos maiores diferenças espectrais foram observadas entre os dois estágios nos anos 2008 e 2010 sendo que o último apresentou inúmeras regiões com diferenças significativas. Já para as atletas femininas, nos três anos da realização da coleta as faixas espectrais que apresentaram diferenças significativas foram mais numerosas em 2010. Esta técnica mostrou ser suficientemente sensível para avaliação da adaptação de atletas durante o teste ergoespirométrico de Bruce, além de ser possível observar particularidades a cerca dos diferentes gêneros dos atletas.

PALAVRAS-CHAVE: atletas, DRIFTS, aptidão física.