

37 - DIFFERENTIATION OF ADAPTATIONS OF ATHLETES IN ERGOESPIROMETRIC EXERCISE TEST BY INFRARED SPECTROSCOPY

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INTRODUCTION

FT-IR spectroscopy is technique used for organic compounds and it is known as a vibrational spectroscopy since the spectrum originates from transitions between vibrational energy levels of a covalent bonding of a molecule. Infrared spectra located between visible light and microwave spectra (1 e 1.000 μm). The spectral region more useful to qualitative analysis is located between 4.000 to 400 cm⁻¹ and it is known as mid infrared (MIR) ((Wang. Mizaikoff. 2008). Based on this principles, specific spectral ranges associated with chemical functions in molecules (fatty acids, proteins, aminoacids, carbohydrates) may be used to compare structural informations or to determine molecular concentrations in biological samples (Longas et al. 2011).

As a global evaluation method, FT-IR associated to multivariate analysis have been applied to discrimination of populations by techniques of pattern recognition (Werner et al. 1998; Petibois, Délérís Cazorla. 2001).

In physiological exercise, FT-IR have been applied in global evaluation of metabolic adaptations during endurance activities (Petibois, Délérís, Cazorla, 2000; Petibois et al., 2002; Petibois, Délérís, 2003a; Petibois, Délérís, 2003b) and oxidative stress in erythrocytes (Petibois, Délérís, 2004; Petibois, Délérís, 2005).

In this context, the aim of this work was to verify the application of whole blood FT-IR to differentiate metabolic adaptations of athletes in ergospirometric exercise test regarding to the sexes.

MATERIALS AND METHODS

The methodology consisted of analytical cross-sectional descriptive study with a quantitative methodological design. The sample was composed of a group of 12 athletes of the Athletics team of UNISC, of different modalities (sprinters, middle-distance runners and runners), being six of the male gender. These athletes have voluntarily joined the study upon the signature of term of informed consent. This study is part of the project "Correlation between the blood biochemical profile and performance of running athletes in the ergospirometry test of Bruce and in specific tests using infrared spectroscopy" proposed and approved by the Ethics Committee in the protocol 2146/08.

It was recommended to the subjects a light diet and rest in the 24h prior to the test, being initially submitted to an anthropometric assessment, blood pressure and resting heart rate. In the sequence the athlete was positioned on the treadmill (ergometer), being connected to the spirometry system (respiratory gas analysis). After the beginning of the test on the treadmill, blood from fingertip was collected every three minutes until the end of test on a way similar to the one done in resting. Also during the test the heart rate was verified every 20 seconds with a digital frequencymeter and blood pressure checked every 3 minutes with a mercury sphygmomanometer.

The exercise test was carried out until to exhaustion or end of protocol after 10 min of recovery (posttest). Blood samples (5mL) were collected from cubital fossa with vacutainer without additives at pre- and posttest and from fingertip with lancet and micropipette in the transition of two states. Blood triplicates of 5 μL were introduced in eppendorfs with 150 mg of KBr (VETEC, spectroscopic grade) at pretest and posttest stages and blood simplicates were purchased at transition between two stages. Samples were lyophilized (2h15min. 1x10⁻⁴ torr) and introduced in a diffuse reflectance device with a monochromatic light source (PIKE Technologies, Madison, USA) connected to a Nicolet Magna 550 FTIR spectrophotometer (Thermo Nicolet Corporation, Madison, USA). Spectra were recorded from 4000 to 400 cm⁻¹ using a spectral resolution of 4 cm⁻¹ and 32 scans.

FT-IR spectra were normalized (0 and 1) and the mean of 18 spectral band areas of each athlete for pre- and posttest states were calculated using Nicolet's OMNIC E.S.P. 7.0 software and correlated with VO_{2max} and VCO_{2max} using Excel 97-2003 software by Pearson's correlation coefficient. Data were statistically analyzed by a 2-tailed t-test at 95% confidence level comparing male and female athletes at pre- and posttest stages.

DISCUSSION AND RESULTS

On Table 1 it can be observed the anthropometric parameters of characterization of sample investigated. Athletes, with mean age of 20.83 and 17.50 years presented results of BMI of 22.68 ± 2.26 kg.m⁻² and 20.38 ± 3.16 kg.m⁻². Concerning to the sexes, mean age were 22.7 ± 2.3 e 20.7 ± 3.9, for the males and females, respectively. These results are similar to those found in other reports. Siqueira et al. (2009) carried out a study with twenty male marathon runners with 35.5 ± 10 years and found BMI values at 21 ± 1.2 kg.m⁻². Concerning to female athletes, Nunes et al. (2009), in studies done with 112 indoor soccer players with age of 22.1 ± 5.4 years, found a BMI of 22.3 ± 1.9 kg.m⁻² and fat percentage of 23.2 ± 5.1 %.

The ergospirometry assessment observed in Table 2 attempts to differ the consumption of oxygen according to the training, particularly between the sexes, with higher oxygen uptake values (VO₂) in male athletes compared with female athletes with means of VO_{2max} of 64.69 ± 16.97 e 49.99 ± 6.71 mL.kg⁻¹.min⁻¹, respectively.

Table 1: Characteristics of athletes selected to the ergospirometric test in 2008.

Nº	Modalidade	Age (Years)	BMI (kg.m ⁻²)	VO _{2max} mL.kg ⁻¹ .min ⁻¹	VCO _{2max} mL.kg ⁻¹ .min ⁻¹
Male athletes					
01	Runner	27	21.40	93.82	108.60
02	Runner	17	25.14	52.59	52.84
03	Sprinter (200 e 400 m)	21	25.02	55.73	61.16
04	Runner	15	19.25	55.98	53.59
10	Sprinter (100 e 200m)	17	22.03	*	*
12	Runner	28	23.22	65.32	66.19
X(DP)		20.8(5.5)	22.7(2.3)	64.69(16.97)	68.48(23.10)
Female athletes					
05	Runner	19	20.00	57.52	56.86
06	Sprinter 400 m	18	20.62	*	*
07	Long jump	18	19.77	*	*
08	Middle-distance runner	17	17.07	53.16	56.84
09	Runner	15	18.52	46.99	49.00
11	Bounce off weight	28	28.30	42.28	48.51
X(DP)		19.2(4.5)	20.7(3.9)	49.99(6.71)	52.80(4.68)

BMI: body mass index; M = male; F = female. X = mean; SD = standard deviation *undetermined.

Mean spectra of pré- and posttest of male and female athletes are presented in Figure 1 and in Table 2 are the means of spectral band areas.

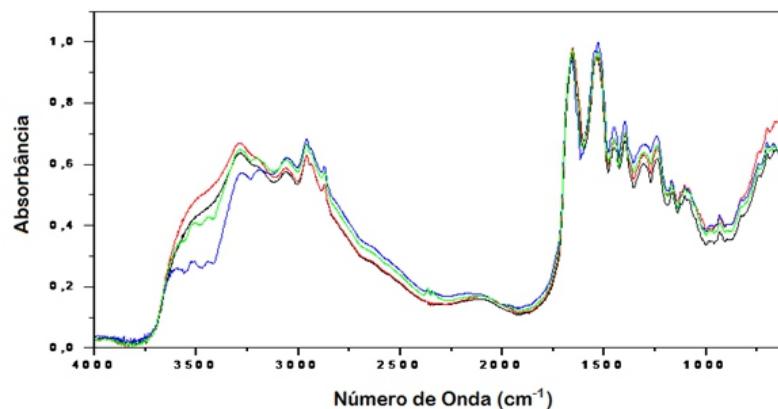


Figure 1: Mean normalized whole blood FT-IR spectra of six male and six female athletes at ergoespirometric exercise test at pre- and posttest. (—): male, pretest; (—): male, posttest; (—): female, pretest; (—): female, posttest.

Tabela 2 – Comparison of means of liophylized-whole blood FT-IR spectral band areas of athletes of Athletism team-UNISC regarding to sexes at pre- and pos- ergoespirometric exercise test evaluated in 2008.

Band	M Pre-T	F Pre-T	M Pos-T	F Pos-T	M x F Pre- T	M x F Pos- T	M Pre-T x Pos- T	F Pre-T x Pos- T
vN-H	121.12 (24.48)	72.94(32.44)	113.12 (39.97)	108.61 (8.12)	NS	NS	NS	NS
vO-H	181.69 (15.77)	152.34(32.96)	178.31 (23.96)	188.32 (8.41)	NS	NS	NS	NS
vC=CH	64.19 (7.26)	72.13(4.23)	67.95 (2.32)	75.08 (1.15)	S	S	NS	NS
v _{as} C-H(CH ₃)	28.72 (1.06)	32.22(1.50)	29.62 (1.18)	33.06 (0.53)	S	S	NS	NS
v _{as} C-H(CH ₂)	32.96 (0.98)	37.24(2.04)	34.10 (1.50)	38.47 (0.83)	S	S	NS	NS
v _s C-H(CH ₃)	15.39 (0.65)	17.83(0.93)	16.03 (0.75)	18.12 (0.35)	S	S	NS	NS
v _s C-H(CH ₂)	8.57 (0.33)	10.08(0.60)	8.98 (0.44)	10.20 (0.24)	S	S	NS	NS
vC=O	6.60 (0.35)	8.08(1.75)	7.06 (0.59)	7.39 (0.59)	S	S	NS	NS
vC=O(amide I)	107.43 (1.27)	106.09(4.74)	109.09 (2.39)	109.19 (1.29)	NS	NS	S	NS
δN-H (amide II)	82.13 (2.72)	87.98(0.55)	84.06 (3.42)	86.41 (1.79)	S	S	NS	S
vC-CH ₃	36.75 (1.37)	40.82 (1.64)	38.49 (2.26)	38.79 (2.47)	S	NS	NS	NS
v _s C=O(COO ⁻)	46.93 (1.20)	51.78 (3.34)	48.70 (1.75)	51.41 (2.74)	S	S	S	NS
Amide III	40.04 (1.88)	46.04 (2.43)	42.66 (3.68)	43.88 (2.48)	S	NS	NS	NS
v _{as} P=O	17.28 (0.62)	19.39 (1.27)	18.43 (1.44)	18.27 (1.00)	S	NS	S	NS
vC-O I	29.42 (1.23)	31.69 (1.81)	31.64 (1.79)	30.60 (2.24)	S	NS	S	NS
vC-O II	8.83 (0.60)	9.27 (0.52)	9.62 (0.57)	9.23 (0.76)	NS	NS	NS	NS
vC-O III	14.05 (0.06)	15.04 (0.93)	15.09 (0.82)	13.79 (2.51)	S	NS	S	NS
vC-O IV	37.17 (1.54)	40.67 (3.55)	41.42 (3.90)	39.15 (3.76)	S	NS	S	NS

M = male; F = female. Standard deviation in brackets. Pre-T: pretest; Pos-T: posttest; NS: non significant; S: p < 0.05; N-H: 3700-3400 cm⁻¹; O-H: 3400-3110 cm⁻¹; C=C-H: 3110-2990 cm⁻¹; asC-H(CH₃): 2990-2950 cm⁻¹; asC-H(CH₂): 2950-2890 cm⁻¹; sC-H(CH₃): 2890-2860 cm⁻¹; sC-H(CH₂): 2860-2840 cm⁻¹; C=O: 1800-1760 cm⁻¹; C=O(amide I): 1760-1590 cm⁻¹; N-H (amide II): 1590-1490 cm⁻¹; C-CH₃: 1490-1430 cm⁻¹; sC=O(COO⁻): 1430-1350 cm⁻¹; Amide III: 1310-1240 cm⁻¹; asP=O: 1240-1220 cm⁻¹; C-O I: 1200-1140 cm⁻¹; C-O II: 1140-1120 cm⁻¹; C-O III: 1120-1090 cm⁻¹; C-O IV: 1090-1000 cm⁻¹.

It is observed in the pretest that mean values of spectral band areas of male are greater than female athletes excepting bands O-H, N-H and C=O(amide I). At posttest stage these differences are located only in vibrational motions of C-H bonding of lipids, C=O and sC=O(COO⁻) of carboxylic acids and esters possibly accumulated as metabolites (lactic acid, p. ex.) and N-H(amide II) and amide III of proteins. Bands of carbohydrates and fosfodiesters were non significant between the sexes.

Considering only the group of male athletes it was observed significative differences between pretest and posttest stages at C=C-H, C=O(amide I), sC=O(COO⁻), asP=O, C-O I C-O II and C-O IV. Significant increase was observed after the test to these bands, except for the band C=O(amide I). By the other hand, the group of female athletes presented whole blood infrared spectra with little change between pre- and posttest stages. Only band N-H (amide II) presented significant differences concerning the increase of spectral band area after the test.

The spectral modifications observed in the two stages show there are differences between the sex which are larger in the pretest stage. These results show that after the ergospirometric test, there are different patterns of adaptations between the sexes, leading to blood profile in the posttest stage with greater similarity between groups, particularly concerning the contribution of metabolites of bioenergetics (compounds with fosfodiester bond and carbohydrates).

Male athletes, regardless of the modality, have higher blood modifications between the pre-and the posttest regarding to female athletes what indicates higher energy consumption and more delay to recovery homeostasis.

These modifications can be related with different levels of hemoconcentration during the ergospirometric exercise test moreover different habilities of utilization of carbohydrates and lipids in this test (Powers, Howley. 2000; Mcardle, Katch, Katch; 2002). FT-IR already has been used succesfully to preview metabolic modifications during fitness (Petibois, Délérés, Cazorla, 2000; Petibois et al., 2002; Petibois, Délérés, 2003a; Petibois, Délérés, 2003b) and oxidative stress in erythrocytes (Petibois, Délérés, 2004; Petibois, Délérés, 2005) and observed in endurance sports. In the work here presented there is also the potential of this method to find differences between the sexes during a cardiorespiratory fitness test.

A univariate correlation between spectral band areas of the pre-test stage with VO₂max e VCO₂max was investigated for male and female athletes. For male athletes, VO₂max e VCO₂max presented the best correlation with band area C-O II (1140-1120 cm⁻¹) with r = 0.960 and r = 0.971, respectively. For female athletes, the best correlation with VO₂max was the band

C=C-H (3110-2990 cm⁻¹) with $r = 0.893$ and with VCO₂max was the band O-H(3400-3110 cm⁻¹) with $r = 0.786$.

These results indicate a more regular and homogeneous behavior of male athletes when they were submitted at ergoespirometric exercise test as Bruce protocol. In these case, performance is strongly associated with contributions of energy components of carbohydrate metabolism. The performance of female athletes is more associated with lipid metabolism, but it was necessary more blood information trough linear combinations of spectral band areas. However, for a confirmation of this hypothesis, its necessary to rise the number of subjects aiming to elaborate a model by multiple linear regression. Linear regressions between areas of FT-IR spectral band have been reported (Petibois et al., 2002; Longas et al., 2011). Data here presented reinforce the potential of Ft-IR spectroscopy to differentiate metabolic adaptations and performance in athletes regarding to the sex.

CONCLUSION

It was possible conclude that FT-IR spectroscopy presents sensibility at 95% confidence to differentiate metabolic adaptations and performance between the sexes of athletes when submitted to ergospirometric exercise test as Bruce protocol.

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DIFFERENTIATION OF ADAPTATIONS OF ATHLETES IN ERGOESPIROMETRIC EXERCISE TEST BY INFRARED SPECTROSCOPY

ABSTRACT

Twelve athletes of Athletism team of UNISC were submitted to Bruce protocol on the treadmill (ergometer) connected to the spirometry system (respiratory gas analysis) being evaluated to the hearth rate, maximal oxygen uptake (VO₂max), maximal carbon dioxide production (CO₂max) and respiratory quotient (QR) at rest (pretest) and every 20 seconds until three minutes after the end of test (exhaustion or end of protocol). Blood pressure was verified at rest, at transition between two states and after recovery. Blood collection by brachial vein puncture was carried at pretest and 10 min after the end of protocol (posttest) and the blood samples were analysed by DRIFTS by 20 areas of bands. Significant differences ($p < 0.05$) were observed between sexes for 16 of 18 spectral bands. Differences in the posttest stage were only observed with bands C=C-H, asC-H(CH₃), asC-H(CH₂), sC-H(CH₃), sC-H(CH₂), N-H(amide II) and sC=O(CO₂-). The best univariate correlations with VO₂max were observed with band C-O(1140-1120 cm⁻¹) for male athletes ($r = 0.960$) and C=C-H(1140-1120 cm⁻¹) for female athletes ($r = 0.893$). It can be conclude that whole blood FT-IR spectroscopy is an auxiliary tool to assess differences between male and female athletes in adaptations to ergospirometric exercise test.

KEY-WORDS: athletes, FT-IR, fitness.

DIFFÉRENCIATION DES ADAPTATIONS DES ATHLÈTES DANS L' EPREUVE D'EFFORT DE BRUCE PAR LA SPECTROSCOPIE INFRAROUGE

RÉSUMÉ

Douze athlètes de l'Équipe d'Athlétisme de l'UNISC ont été évalués, dans l'année 2008, avec anthropométrie et ergospirométrie associée à la spectroscopie infrarouge à transformée de Fourier à réflexion diffuse (DRS-FTIR) du sang total pour la différenciation à l'adaptation métabolique entre les sexes. Les athlètes ont été soumis en utilisant le protocole 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 (VO_2max), de la production maximal de dioxyde de carbone (VCO_2max) et de le coefficient respiratoire (QR) à la maison (pré-test) et toutes les 20 secondes jusqu'à trois minutes après la fin de l'épreuve (l'épuisement ou la fin du protocole). L'évaluation de la pression artérielle a eu lieu à la Maison, toutes les trois minutes du protocole (transition entre les étapes) et après la récupération. La collecte de sang par ponction veineuse brachiale s'est tenue à l'étape de pré-test et dix minutes après la fin du protocole (post-test) et les échantillons de sang ont été analysés par DRS-FTIR à travers de zones de 18 bandes spectrales. Dans l'étape pré-test, aucune différence significative n'a été observée ($p < 0,05$) entre les sexes à 16 bandes spectrales. Dans l'étape post-test, les différences ont été préservées seules les bandes C=C-H, asC-H(CH3), asC-H(CH2), sC-H(CH3), sC-H(CH2), N-H(amide II) et sC=O (CO2-). Les meilleures corrélations univariées avec VO_2max ont été observées avec les bandes C-O(1140-1120 cm⁻¹) pour les hommes ($r = 0,960$) et C=C-H(1140-1120 cm⁻¹) pour les femmes ($r = 0,893$). Il peut être conclu que la spectroscopie IR-FT de sang total peut être un outil auxiliaire à trouver différences entre les sexes dans l'adaptation aux épreuves d'effort des athlètes.

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

DIFERENCIACIÓN DE ADAPTACIÓN DE LOS ATLETAS AL TESTE ERGOESPIROMETRICO DE BRUCE POR ESPECTROSCOPIA DE INFRARROJO

RESUMEN

Se evaluaron 12 atletas en el programa de atletismo de la Universidad de Santa Cruz do Sul, en el año 2008, por antropometría y ergo-espriometría relacionados con la espectroscopía de reflectancia difusa con transformada de Fourier de infrarrojo (DRIFTS) de sangre entera de los patrones diferenciales de la sangre de adaptación en relación con los sexos. Los atletas fueron presentados al protocolo de Bruce en una estera ergométrica conectado al analizador de gases siendo evaluado la frecuencia cardíaca, consumo máximo de oxígeno ($\text{VO}_2\text{máx}$), la producción máxima de CO₂ (CO₂máx) y el cociente respiratorio (RQ) en reposo (pre-test) y cada 20 segundos hasta 3 minutos después del final de la prueba (el agotamiento o la terminación del protocolo). La evaluación de la presión arterial se mantiene en reposo, cada 3 minutos (protocolo de transición entre etapas) y después de la recuperación. La colección de sangre por punción venosa del brazo se llevó a cabo en la etapa previa al juicio y 10 minutos después del final del protocolo (post-test) y las muestras de sangre fueron analizadas por DRIFTS por intermedio de las áreas de 20 bandas. En la etapa de pre-test fueron observadas diferencias significativas ($p < 0,05$) entre los sexos para las zonas media de 16 de las 18 bandas spectrales. En el estadio pos-test las diferencias se conservan sólo en las bandas C=C-H, asC-H(CH3), asC-H(CH2), sC-H(CH3), sC-H(CH2), N-H(amida II) y sC=O (CO2-). Las mejores correlaciones univariadas con VO_2max se observaron con las bandas C-O(1140-1120 cm⁻¹) para los hombres ($r = 0,960$) e C=C-H(1140-1120 cm⁻¹) para las mujeres ($r = 0,893$). Se puede concluir que la espectroscopía FT-IR de sangre entera puede ser una herramienta auxiliar para encontrar las diferencias entre los sexos en la adaptación a las pruebas de estrés de los deportistas.

PALABRAS CLAVE: atletas, FT-IR, la condición física.

DIFERENÇAÇÃO DE ADAPTAÇÃO DE ATLETAS CORREDORES AO ENSAIO ERGOESPIROMÉTRICO DE BRUCE POR ESPECTROSCOPIA NO INFRAVERMELHO

RESUMO

Foram avaliados 12 atletas do programa de atletismo da Universidade Santa Cruz do Sul, no ano de 2008, 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 frequência cardíaca, consumo máximo de oxigênio ($\text{VO}_2\text{máx}$), produção máxima de CO₂ (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 das áreas de 20 bandas. No estágio pré-teste foram observadas diferenças significativas ($p < 0,05$) entre os sexos para as médias das áreas de 16 de 18 bandas spectrais. No estágio pós-teste as diferenças foram preservadas apenas nas bandas C=C-H, asC-H(CH3), asC-H(CH2), sC-H(CH3), sC-H(CH2), N-H(amida II) e sC=O (CO2-). As melhores correlações univariadas com $\text{VO}_2\text{máx}$ foram observadas com as bandas C-O(1140-1120 cm⁻¹) para os homens ($r = 0,960$) e C=C-H(1140-1120 cm⁻¹) para as mulheres ($r = 0,893$). Pode-se concluir que a espectroscopia FT-IR de sangue total pode ser uma ferramenta auxiliar para encontrar diferenças entre os sexos na adaptação ao teste de esforço de atletas.

PALAVRAS-CHAVE: atletas, FT-IR, aptidão física.