Infrared Spectroscopy based Study of Biochemical Changes in Saliva During Maximal Progressive Test in Athletes

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Abstract
The study aims to explore biochemical changes in saliva during cardiorespiratory exercise using Attenuated-Total-Reflectance-Fourier-Transform-Infrared-Spectroscopy (ATR-FTIR). Saliva and blood samples were obtained from six athletes at rest, and after running at 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 kilometers-per-hour (km/hr) speeds on a treadmill (maximal stress test). Saliva ATR-FTIR spectra were analyzed using deconvolution and multivariate analysis. Area-under-the-curve calculations suggest differential changes in glucose, lactate, protein, lipids, carbohydrate and phosphate content in saliva during the test. Increase in glucose and lactate levels with increasing speeds were verified by simultaneous measurement of blood glucose and lactate levels using standard equipment (Roche®). Multivariate Principal-Component-Analysis (PCA) showed discrete clusters for low (rest-14 km/hr) and high (15-20 km/hr) speeds, and PCA-Linear-Discriminant-Analysis showed 100% classification of 18-20 km/hr as high speed. Overall, results suggest the possibility of using non-invasive saliva-based ATR-FTIR method for biochemical assessment during sports exercise and stress tests.
Introduction

Cardiorespiratory training is a vital component of physical activity that promotes motor development and physical fitness in youth\(^1\). Its importance in improving the quality of life and reducing the risk factors for diseases related to lifestyle is well established\(^2\). Moreover, increased aerobic endurance as a result of training is key to success of athletes in sports. However, over-training can lead to injury and serious health issues. Hence, optimizing training for maximum benefit with minimal risk is critical. Several metabolites are produced during exercise, and many of them can serve as physiological index to monitor an exercise routine, and guide effective training course. Studies show that during exercise, the oxygen consumption above which aerobic energy production supports is supplemented by anaerobic mechanisms, causing an increase in lactate and metabolic acidosis in the human body\(^3\). Its production and secretion are more sensitive to training and present higher correlation with endurance performance than other parameters\(^4\). Hence, lactate has been widely used to measure and evaluate aerobic capacity, mainly of athletes. Blood sampling for lactate concentration monitoring during exercise is done from the ear lobe or the tip of the finger using a venous catheter\(^5\). The process is invasive, requires expert technical knowledge, may cause anxiety in the athlete discouraging participation, limits adequate serial sampling, and also presents problems associated with handling and manipulating blood.

Saliva has been suggested as an alternative to blood for monitoring lactate levels during exercise. Saliva collection is easy, non-invasive, does not cause anxiety, and handling is simpler. It has been used extensively for diagnosis of hereditary, autoimmune diseases, infectious, endocrine and carcinomas\(^6\). It has also been applied to determine concentration of various substances, such as drugs and hormones\(^6\). With regard to metabolite monitoring during exercise, saliva has been used to study lactate changes in long and short distance runners and to monitor sodium, potassium and lactate levels during cycling exercises\(^5,7,9\). These studies used electrochemical or enzymatic assays for metabolite concentration determination.

Infrared spectroscopy can also be used for finding out metabolite concentrations. The technique is sensitive to vibrational modes of molecules and can provide a complete chemical profile of the sample. The advantage of the technique is that it can provide information on all chemical components in a single measurement, and this has been used for various biological and medical applications\(^10\). It has been applied in measuring blood lactate and glucose concentration during exercise\(^11\). Khaustova et al.\(^12\) used infrared spectroscopy of saliva samples obtained before and after exercise, and showed changes in immunoglobulin, amylase, and cortisol levels, which reflect response of the nervous, endocrine, and immune system to
stress. Junior et. al. showed that multivariate analysis can distinguish infrared spectra obtained from saliva before, immediately after, and two hours after a handball match\(^{13}\). However, there are no reports on changes in saliva infrared spectra/biochemical components during exercise.

Therefore, in this study, we have investigated the biochemical changes in saliva using infrared spectroscopy during maximal stress test. Maximal stress test is widely used for assessment of cardiorespiratory fitness and training and involves running on a treadmill at increasing speeds. We collected saliva and blood from six athletes at rest and while pausing in the course of the exercise after running at speeds 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 km/hr on a treadmill. Blood glucose and lactate levels were determined using standard tests for comparison with results of spectroscopy. Attenuated Total - Reflectance Fourier Transform Infrared (ATR-FTIR) spectra acquired from saliva sample were analyzed by deconvolution and area under the curve calculations followed by multivariate analysis.

**Experimental**

**Bioethical approval**

The present research was approved by the Research Ethics Committee involving human beings of the University of Vale do Paraiba (CAEE No. 10541219.8.0000.5503), thus meeting the requirements of Resolution 196/96 of the National Health Council and in accordance with the Declaration from Helsinki. All participants were informed verbally about the research objectives, methodology, procedures, inherent risks, and the confidentiality of the information to be acquired, and signed consent obtained.

**Participants**

Six young healthy amateur male athletes (Table 1) who participate in regional 10 kilometers (kms) street race competitions were recruited. The athletes performed regular and systematic training for the sport (four to five days a week, 60 minutes per session). All participants had at least one year of competitive training and completed the 10 kms races between 30 and 40 minutes.

Exclusion criteria used was: smoking, history of heart problems, osteomioarticular lesion in the last two months, consumption of stimulant supplements, anabolic steroids, peptide hormones, drugs with effects on physical performance.
Experimental procedure

The stress tests, blood tests and salivary collections of this study were performed in the physical conditioning room of the School of Aeronautical Specialists (EEAR) and analysis of the salivary samples were carried out in the Laboratory of Infrared Spectroscopy of the University of Vale do Paraíba (UNIVAP).

Participants visited the test site on two occasions: (1) for anthropometric evaluation and familiarization with the maximal progressive stress test performed on a treadmill; (2) the following week for the actual performance of the stress test, with blood and salivary sample collection. The interval between visits was at least six and a maximum of seven days, always in the morning (9 am to 11 am), in order to avoid circadian variations.

On both occasions, subjects were also instructed to attend the test site after consuming a light meal (two hours prior to the evaluations), wearing light clothing and running shoes, refraining from vigorous physical exercise in the previous 24 hours, and do not consume caffeinated, alcoholic or any kind of stimulant supplement.

The anthropometric evaluation for sample characterization measured body mass (kg) (Filizola, ID-M 150/4, São Paulo, Brazil), height (cm) (Sanny, Standard, São Bernardo do Campo, Brazil) and skinfolds mm) of the pectoral, abdomen and thigh, using a scientific compass (Cescorf, Top Tec, Porto Alegre, Brazil). Jackson and Pollock's equation (1978) was used to calculate body density, and fat percentage was estimated by the Siri equation (SIRI, 1961). All anthropometric measures were obtained according to the recommendations of the International Society for the Advancement of Kinanthropometry, and the recording and processing of the data was performed in Avaesporte® Software (Sports Systems, MG, Brazil).

The protocol for the maximal progressive stress test was as proposed by Heck et al., where after 5 minutes of warm-up at a constant speed of 6 km/hr, the actual test was performed, with steady inclination of the treadmill at 1% and initial speed of 10 km/hr with increments of 1 km/hr every 2 minutes.

There were 30-second pauses between the stages of the test to allow the safe collection of blood and salivary samples, as well as for heart rate (bpm) and subjective perception of effort (PSE) using the Borg Scale (CR10).

The test was terminated by voluntary exhaustion or when the subject was unable to maintain the rhythm imposed by the treadmill. The criterion used to identify the truly maximum test was - maximum PSE (> 9) at the end of the last stage, blood lactate concentration greater than 8 mmol/1 in the last stage of test, and when the peak heart rate (ICF) was equal to or
greater than 90% of maximal heart rate (HRmax). Only the tests that fulfilled this criterion were included in the final analyzes.

Throughout the test, heart rate monitoring was performed by a Polar® branded heart monitor (model 610i) validated against a standard effort ECG50, allowing storage of HR behavior every 5 seconds. Afterwards, the data were transferred to the computer through an instrument compatible interface and analyzed with Polar Precision Performance SW software (Polar Electro®, Kempele, Finland).

**Sample collection and measurements of blood glucose and lactate**

In the hours prior to collection, the subjects performed complete oral hygiene (brushing and flossing) and thirty minutes before the test the oral cavity was previously cleaned with water to remove cell debris and other materials, discarding it in an appropriate container, according to the methods described for this procedure.

Blood sample collection was performed for each individual in the resting condition and at the end of each completed stage of the maximal progressive stress test. To collect this material, at the end of each stage, a command was activated to reduce the speed to zero. This procedure had the controlled time of 30 seconds.

The salivary samples were collected at the same time and conditions of the blood collection by the "drool method" in an Eppendorf sterilized tube (approximately 2 ml), and stored in a refrigerator (2°C). We did not centrifuge the sample before spectra acquisition.

To avoid contamination of the salivary samples with sweat, frequent cleaning of the subject's face with disposable paper towels was carried out. The salivary secretion during collection was spontaneous (not stimulated), without ingestion of any type of food or drink during the test.

Capillary blood samples (25 µl) were collected by fingertip lancet puncture, previously sterilized with 70% alcohol, for glucose and lactate analysis.

All procedures for collection of biological material and subsequent analysis, as well as the respective disposal of leftovers, strictly followed the current regulations.

**Saliva processing, ATR-FTIR spectra acquisition, and data analysis**

Spectra were acquired from passively dried saliva samples (100 µl) using a Spectrum Two FT-IR Spectrometer (Perkin-Elmer) in triplicate in the range of 4000 to 500 cm⁻¹, with 4 cm⁻¹ resolution, 32 scanning scans and a controlled temperature of 20°C. A background spectrum was obtained prior to sample spectra collection with all parameters and conditions constant,
but without the sample. The background is automatically subtracted from sample spectrum by the spectra acquisition software.

For analysis, the spectra were processed with Spectrum 5.3 software (Perkin-Elmer), performing baseline corrections and spectral smoothing with Savitzky-Golay algorithm (9 points) and for absorbance spectra was added normalization spectral considering the most intense band as basis for the calculation of relative intensity. These were used for spectral deconvolution, Gaussian curve fitting, and area under the curve calculations using OriginPro 8.5. The percent area under the curve was calculated for each band/peak.

For multivariate analysis, the following preprocessing steps were performed. First, all raw spectra were first derivatized. This corrects for the baseline. Then, spectral range to be analysed were separated from the rest of the spectrum. In this study, two spectral ranges were analysed separately, 900-1500 cm\(^{-1}\) and 2800-3300 cm\(^{-1}\). Area under these curves were then normalized. This removes the intensity related variations.

The preprocessed spectra were then subjected to Principal Component Analysis (PCA), PCA-Linear Discriminant Analysis (PC-LDA), and Leave One Out Cross Validation (LOOCV) using MATLAB.

**Serum glucose and lactate measurements**

The collected samples were immediately analyzed by specific enzymatic electromagnetic devices with a resolution of 0.1 mmol/L and the results expressed in the same unit of measurement for lactate and in mg/dL for glucose.

Before each test, the analyzer was calibrated with a standard lactate solution with known maximum and minimum concentrations according to the manufacturer's instructions (Roche®). The lactate and glucose analyzer and reagent strips model Accutrend® Plus (Roche®), validated for the intended use and disposable lancets, model Accu-Chek® SoftClix Pro (Roche®) were used.

**Results**

Figure 1 shows the mean spectrum of saliva obtained at rest and after running at different speeds during the maximal stress test, with peaks assignments detailed in table 1.

Of the six athletes, four athletes could sustain up to running speeds of 20 km/hr, five up to 19 km/hr, and all up to 18 km/hr., resulting in 4 saliva samples for 20 km/hr, 5 for 19 km/hr, and six for rest-18 km/hr. Since 3 spectra were acquired per saliva sample, there were 12 spectra
for 20 km/hr, 15 spectra for 19 km/hr, and 18 spectra each for speeds rest-18 km/hr. Spectral deconvolution by Gaussian curve fitting was performed on mean spectra of saliva collected from athlete at rest, and after running at speeds of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 km/hr, and percent area under the curve for each peak/band calculated. The graphs showing changes in percent area under the curves of different biomolecules for rest and different speeds are shown in Figure 2.

As can be seen, glucose increases steadily between rest and 14 km/hr, remains relatively constant between 14-17 km/hr, then rises sharply from 18-20 km/hr speeds. Phosphate/phospholipid (1066-1076 cm\(^{-1}\)) rises sharply at 10 km/hr, falls at 11 km/hr, rises again between 11-14 km/hr, falls sharply again at 15 km/hr, rises again between 15-17 km/hr, then falls between 17-19 km/hr. The peaks are in the order 17 > 14 >10 km/hr. Both carbohydrate bands show gradual but uneven decrease. Inorganic phosphate band decreases steadily and smoothly between rest and 20 km/hr speed. Lactate, on the other hand, increases gradually between rest and 20 km/hr. Lipid/protein band 1400-1404 cm\(^{-1}\) show the same trend. Phospholipids (1238-1242 cm\(^{-1}\)) and protein (1315-1318 cm\(^{-1}\)) remain relatively constant. The percent area under the curves for both these peaks is small, and that for 1445-1451 cm\(^{-1}\), 1376-1378 cm\(^{-1}\), 1205-1206 cm\(^{-1}\), 1467-1469, and 1342-1348 cm\(^{-1}\) are very low.

Change in saliva and blood lactate between rest and 20 km/hr is shown in Figure 3. Saliva lactate increases steadily after 12 km/hr, whereas blood lactate steadily increases after 15 km/hr. Saliva glucose increases between rest – 14 km/hr, drops at 15 km/hr, remains relatively unchanged between 15-17 km/hr, then rises sharply between 17-20 km/hr. Blood glucose rises between rest-10 km/hr, same as saliva glucose. However, it drops between 10-13 km/hr, unlike saliva glucose, and rises steadily between 13-17 km/hr, again unlike saliva glucose. Both blood and saliva glucose rise sharply between 17-20 km/hr.

To examine whether it is possible to distinguish the ATR-FTIR saliva spectra of different running speeds, Principal Component Analysis (PCA) was performed in the several spectral ranges. Results obtained from using 900-1500 cm\(^{-1}\) and 2800-3300 cm\(^{-1}\) spectral ranges are discussed, as they give the best separation. The scatter plot of scores of Principal Components (PC) 2 and 5 (Figure 4a) for PCA in 900-1500 cm\(^{-1}\) spectral range show that most spectra cluster together, although there is slight separation of speeds 15-17 km/hr from 18-20 km/hr. In PCA, if spectra of groups overlap, it suggests that there are no difference between the spectral signatures of the two groups. If the spectra form separate groups, that is, they
cluster separately, then the spectral signatures between the two groups differ. Thus, PCA plot in Figure 4a suggests that 15-17 and 18-20 km/hr spectra have spectral signatures different from rest-14 km/hr spectra, but there are no differences between spectra of speeds rest-14 km/hr. PC2 displays 1448 (protein), 1221 (phosphate), 1067 (ribose), and 985 cm\(^{-1}\) (polysaccharide) bands, while PC5 shows 1407 (protein), 1197 (collagen), 1079 (nucleic acids), and 974 cm\(^{-1}\) (polysaccharide) bands. PCs show spectral signatures that are responsible for clustering of groups in PCA plot. Thus, PC2 suggest that spectra of speeds 15-17 km/hr and 18-20 km/hr have difference in protein, phosphate, carbohydrate, collagen, and nucleic acid content with respect to speeds rest-14 km/hr. PCA- Linear Discriminant Analysis (PC-LDA) Leave One Out Cross Validation (LOOCV) results are shown in Table 2a confusion matrix. A confusion matrix shows how many spectra from one group is classified as itself (correct classification) and classified as other group (misclassification). For example, 56% of spectra from rest, 10, 11 km/hr group are correctly classified as rest, 10, 11 km/hr group, while 31%, 9%, and 4% are misclassified as 12,13,14 km/hr, 15,16,17 km/hr, and 18,19,20 km/hr groups, respectively. Thus, rest-11 km/hr group shares similarity with 12-14 km/hr, while the latter has some similarity to all other groups. 15-17 km/hr group spectra misclassify heavily (43%) with 12-14 km/hr, while more spectra from 18-20 km/hr groups spectra is classified as 15-17 km/hr (64%) than its own group. Correct classifications for rest-11, 12-14, 15-17, and 18-20 km/hr are 56, 57, 52, and 22 %, respectively.

We found the best classification to be in the 2800-3300 cm\(^{-1}\) range. Scatter plot of scores of PCs 4 and 5 in this range (Figure 4b) show four clusters, although there is overlap between each group. Bands responsible for classification were 2962 (\(\nu_{as} \text{ CH}_3\)), 2927 and 2886 (C-H stretching), and 2856 cm\(^{-1}\) (lipid) (PC4) and 2929 and 2849 cm\(^{-1}\) (C-H stretching) (PC5). Notably, rest-11 km/hr and 12-14 km/hr form a cluster that is well-separated from 15-17 km/hr and 18-20 km/hr, suggesting that saliva from athletes running at rest-14 km/hr speeds can be distinguished from those obtained from saliva of athletes running at speeds ranging between 15-20 km/hr. PC-LDA LOOCV confusion matrix (Table 2b) of spectra in the same range demonstrates this. 56% of rest,10,11 km/hr group classify correctly, while 28% misclassify as 12,13,14 km/hr. Thus, 80% (56%+28%) spectra get classified as belonging to groups between speeds rest-14 km/hr. Similarly, 76% of 15-17 km/hr are classified as belonging to groups between 15-20 km/hr (57% as 15,16,17 km/hr and 19% as 18,19,20 km/hr), and 100% 18-20 km/hr are classified as belonging to groups between 15-20 km/hr (31% as 15, 16, 17 km/hr and 69% as 18,19, 20 km/hr).
**Discussion**

The aim of the study was to explore biochemical changes in saliva of athletes at rest and with 1 km/hr increment in speed between 10 - 20 km/hr speeds using ATR-FTIR. Our results suggest gradual increase in glucose and lactose with increasing speeds, with an initial jump in concentration at 10 km/hr speed. Ohkuwa *et. al.* have shown that salivary lactate is higher in sprinters after 400 m run compared to long distance runners, possibly suggesting that acceleration plays a role in lactate increase, which may explain the sudden jump in saliva lactate between 10-12 km/hr speed. Santos *et. al.* collected blood and saliva at rest and after every 6 km in a run of total 30 km, and found blood lactate to increase after 6 km and stay constant, while salivary lactate increased only after 18 km compared to at-rest. In our study, with 2 minutes intervals between increasing speeds by 1 km/hr, total distance covered was ~ 3 km. The blood lactate started rising after ~ 2 km, and kept increasing, while salivary lactate started rising after ~ 1km, and kept increasing. This again suggests the role of acceleration in rise of lactate levels. Segura *et. al.* studied the correlation between salivary and blood lactate during cycling exercise with increasing loads at 3 minute intervals, and found that lactate concentration followed the same pattern of evolution during the exercise. In our study, we found that ATR-FTIR measure of salivary lactate as well as Roche instrument measured blood lactate concentration increase steadily over time, although the pattern is not exactly similar. Marliss *et. al.* have reported increase in blood glucose with intense exercise. Our study shows increase in saliva and blood glucose as the intensity of the exercise increases. Apart from the changes in lactate and glucose, we also found changes in phosphate, phospholipids, carbohydrates, lipids, and proteins in saliva during exercise and cardiorespiratory training. Among all the biochemical bands investigated, percent area under the curve for carbohydrate band 1106-1125 cm\(^{-1}\) was highest (16%) at rest, followed by glucose (13%) > 1066-1076 cm\(^{-1}\) phosphate/phospholipid (12%) = 983-988 cm\(^{-1}\) phosphate (12%) >1400-1404 cm\(^{-1}\) lipids/protein (6%) > lactate (4%). The highest contribution at any speeds are 1066-1076 cm\(^{-1}\) phosphate/phospholipid at 17 km/hr (28%) > glucose at 20 km/hr (24.9%) > 1106-1125 cm\(^{-1}\) carbohydrate band at 13 km/hr (13.8%) > 983-988 cm\(^{-1}\) phosphate at 11 km/hr (11.8%) > 1400-1404 cm\(^{-1}\) lipids/protein at 19 km/hr (10.3%) > Lactate at 19 km/hr (8%). It is clear that every biochemical component changes differently during the maximal progressive exercise test, and can be detected using saliva based FTIR, which may be useful for non-invasive in vivo delineation and study of various biochemical processes during intensive exercise. Although
PCA and PCA-LDA could not distinguish spectra of saliva from different speeds in the 900-1500 cm\(^{-1}\) range, it could distinguish high speeds (18-20 km/hr) from low speeds (rest – 14 km/hr) in the 2800-3300 cm\(^{-1}\) range with 100% efficiency.

Chemometric techniques such as PC-LDA may allow real-time measurement of glucose/lactose/other biochemical components. In this study, we have trained a PC-LDA model using spectral data from saliva of six athletes at different speeds. This model was able to differentiate low from high speeds in the 2800-3300 cm\(^{-1}\) range. Analysis of principal components suggested that lipid signatures are responsible for this distinction. By training the model with larger dataset obtained from a bigger sample size may help delineate the speeds further and give better measure of the changes in lipids using the 2800-3300 cm\(^{-1}\) range and other biochemicals using the 900-1500 cm\(^{-1}\) range. Since such analysis can be automated, the results can be calculated and observed as the spectra are measured from saliva. Using in vivo probes and PC-LDA programs, spectra can be obtained from athlete saliva without interrupting the exercise and a continuous read of biochemical changes can be obtained. Such data will help better monitor, design and control exercise routines.

Conclusions

This study shows possibility of determining glucose and lactate levels from saliva during exercise routines using ATR-FTIR, as well as that of investigating other biochemical changes. The methodology does not have pre- analytic steps (freezing, centrifugation for separation of supernatants, washing of cells and acquisition of dilution spectra), and shows good reproducibility after spontaneous drying of the salivary sample at room temperature. The technique is non-invasive in nature, which reduces the problems associated with asepsis, contamination and reluctance of some athletes associated with collection of blood. This would allow frequent physiological evaluation and a more precise prescription of the trainings for increasing the athletic performance.

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Table 1. Sample characterization in terms of anthropometric measurements, body composition and maximal oxygen consumption (\(\dot{V}\text{O}_{2\text{MAX}}\)) of adults athletes during a maximal incremental test (n = 6).

| Parameters               | Mean±SD   |
|--------------------------|-----------|
| Age (years)              | 20.6 ± 1  |
| Body weight (kg)         | 71.1 ± 6.8|
| Height (cm)              | 1.77 ± 0.03|
| BMI (kg/m\(^2\))        | 22.7 ± 2.5|
| Fat mass (%)             | 7.9 ± 3.7 |
| Lean Mass (%)            | 91.9 ± 3.7|
| Fat Mass (kg)            | 5.8 ± 2.7 |
| Lean Mass (Kg)           | 63.5 ± 8.3|
| \(\dot{V}\text{O}_{2\text{max}}\) relative \((\text{ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1})\) | 72.91 ± 5.14 |
| \(\dot{V}\text{O}_{2\text{max}}\) absolute \((\text{L} \cdot \text{min}^{-1})\) | 5.17 ± 0.33 |

Legend: n = number of subjects; BMI = body mass index; \(\dot{V}\text{O}_{2\text{max}}\) = maximum oxygen consumption.

Table 2. General band assignment of FT-IR spectrum of saliva.

| Peak | Wavenumber \((1/\text{cm})\) | Assignment                                                                 |
|------|-------------------------------|-----------------------------------------------------------------------------|
| 01   | 3282                          | Amide A (mainly N-H stretching of hydrogen-bonded amide groups of proteins) |
| 02   | 2965                          | CH\(_3\) asymmetric stretch: mainly lipids                                  |
| 03   | 1633                          | Amide I (C=O stretching in proteins (80%) \(\alpha\)-Helix)                  |
| 04   | 1544                          | Amide II (proteins, N-H bending (60%) and the C-N stretching (40 %))        |
| 05   | 1452                          | CH\(_2\) bending: mainly lipids                                            |
| 06   | 1403                          | COO\(^-\) symmetric stretch: fatty acids, amino acid side groups            |
| 07   | 1320                          | Amide III (protein C-N stretching (40%), N-H bending (30%) and C-C stretching (20%)) |
| 08   | 1242                          | PO\(^2-\) asymmetric stretch, fully hydrogen-bonded: mainly nucleic acids   |
| 09   | 1071                          | PO\(^2-\) symmetric stretch: nucleic acids and phospholipids C-O stretch:glycogen, polysaccharides, glycolipids |
| 10   | 994                           | C-N\(^1-\)C stretch: nucleic acids, ribose-phosphate main chain vibrations of RNA |
| 11   | 874                           | \(z\)-type DNA Vibrations in N-type sugars in nucleic acid backbone          |
Table 3. Principal Component Linear Discriminant Analysis Leave One Out Cross Validation.

| PC-LDA LOOCV (%) | 900-1500 cm⁻¹ | 2800-3300 cm⁻¹ |
|------------------|---------------|---------------|
|                  | Rest, 10, 11 km/hr | 12, 13, 14 km/hr | 15, 16, 17 km/hr | 18, 19, 20 km/hr |
| a. 900-1500 cm⁻¹ |               |               |               |               |
| Rest, 10, 11 km/hr | 56            | 31            | 9             | 4             |
| 12, 13, 14 km/hr | 13            | 57            | 13            | 17            |
| 15, 16, 17 km/hr | 6             | 43            | 52            | 0             |
| 18, 19, 20 km/hr | 4             | 9             | 64            | 22            |
| b. 2800-3300 cm⁻¹ |               |               |               |               |
| Rest, 10, 11 km/hr | 52            | 28            | 20            | 0             |
| 12, 13, 14 km/hr | 28            | 52            | 19            | 2             |
| 15, 16, 17 km/hr | 2             | 22            | 57            | 19            |
| 18, 19, 20 km/hr | 0             | 0             | 31            | 69            |
Figure Captions

Fig. 1: ATR-FTIR spectrum of Saliva, with peak assignments in Table 1

Fig. 2: Percent area under the curves for different ATR-FTIR bands plotted against speeds showing relative changes in biochemical components with changing speeds (dashed line is the plot for percent area of carbohydrate peak 1081-1118 cm\(^{-1}\) / plot for percent area of carbohydrate peak 1342-1348 cm\(^{-1}\)).

Fig. 3: Percent area under the curves for saliva lactate and glucose/carbohydrate plotted against speed (left) showing relative changes in lactate/carbohydrate with changing speeds, and amounts of the lactate/glucose measured in blood using standard tests (right) (Note: The units in the left and right panel y-axes are different, hence cannot be directly correlated. Only the trends appear to be similar over changing speeds).

Fig. 4: Principal Component Analysis (PCA) scatter plots in ranges a. 900-1500 cm\(^{-1}\), and b. 2800-3300 cm\(^{-1}\) (Legends: rest, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20 km/hr speeds)
Fig. 1
Fig. 2
Fig. 3

Saliva Lactate (FTIR, 1445-1451 cm\(^{-1}\))

Saliva Glucose (FTIR, 1028-1034 cm\(^{-1}\))

Blood Lactate (Roche\(^{e}\))

Blood Glucose (Roche\(^{e}\))

Speeds

Fig. 4

PC2

PC5

PC4

PC5