Chemosensory Evaluation of Training and Oxidative Stress in Long Distance Runners

Christan Y. Whysong

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
In
Biological Systems Engineering

Parameswarakumar Mallikarjunan, Chair
Per Gunnar Brolinson
Jenny Lo
Robert Grisso

October 28, 2009
Blacksburg, VA

Keywords: electronic nose, overtraining, long distance runners, oxidative stress, noninvasive testing

Chemosensory Evaluation of Training and Oxidative Stress in Long Distance Runners

Christan Y. Whysong

ABSTRACT

Athletic performance is improved by increasing training loads but it is difficult to determine an athlete's response to a training load and the amount of stress incurred. This makes athletes susceptible to overtraining, leading to decreased performance levels, due to physical exhaustion and oxidative stress. Past studies have observed a myriad of biomarkers without conclusively identifying a clinically specific marker for overtraining due to oxidative stress. These methods require invasive testing and lengthy result times, making real-time adjustments of training programs to prevent overtraining difficult. The use of an electronic nose (enose) as a non-invasive evaluation tool will provide immediate feedback on training stress, allowing for real-time training adjustments for performance optimization.

Two long distance runners (one male and one female) completed a pilot study. Both performed a short run and the female performed a long run. Blood samples were collected from each athlete before and after each run and analyzed for catalase and GPx activity. Breath samples were also collected before and after each run and analyzed by an enose. Multivariate analyses of combined blood data yielded better results than individual analyses. Although data was limited for this pilot study, canonical discriminant analyses (CDA) showed separation between before and after run and between short and long run breath samples. Cross validations also found up to a 77.8 percent prediction accuracy for the enose. Results indicate an enose is feasible for detecting changes in the breath occurring after physically demanding exercise perhaps due to oxidative stress incurred during the exercise.

DEDICATION

"Upon the subject of education, not presuming to dictate any plan or system respecting it, I can only say that I view it as the most important subject which we as people may be engaged in."
-Abraham Lincoln

"The important thing is not to stop questioning."
-Albert Einstein

"The beautiful thing about learning is that no one can take it away from you."
-B.B. King

"Education is a better safeguard of liberty than a standing arm."
-Edward Everett

"Aerodynamically, the bumble bee shouldn't be able to fly, but the bumble bee doesn't know it so it goes on flying anyway."
-Mary Kay Ash

For my family who recognized how important an education is and gave up so much and worked so hard for me to pursue mine. You have understood when I couldn't be there or when you couldn't be here, yet been there for me when I needed you the most. Your endless love, support, and dedication to me have always made me feel as though I could fly. I have, and always will, dedicate my work to you. I will never be able to thank you enough, and can only hope that one day I will be able to give as much to you as you have given to me.

ACKNOWLEDGEMENTS

I would like to first thank Dr. Parameswarakumar Mallikarjunan who took me on as an undergraduate researcher and then graciously agreed to provide me with the opportunity to pursue my graduate studies. Thank you for always being supportive of me and encouraging me to pursue many different opportunities, including teaching, proposal writing, and professional presentations. I am especially appreciative of you working with me to find a research area that is most important to me and my family.

I would also like to thank Dr. Per Gunnar Brolinson, Dr. Jenny Lo, and Dr. Robert Grisso for agreeing to sit on my committee. I appreciate the time you took out of your overbooked schedules to review my work and provide imperative questions and suggestions for improving it.

This project would not have been possible without the support of the entire research team. Dr. Mallikarjunan, Dr. Brolinson, and Dr. Hara Misra showed important leadership and have provided invaluable suggestions from their respective disciplines. They also allowed me to take important leads in the grant writing and IRB approval processes. A special thanks to Dr. Zhenquan Jia who provided copious amounts of patience in teaching me laboratory techniques for blood analyses. Dr. Mark Rogers and Dr. Greg Beato provided clinical assistance in collecting samples. Many thanks to all of you! Additionally funding was received from the Institute for Critical Technology and Applied Science and the Edward Via Virginia College of Osteopathic Medicine's Harvey Peters Foundation, making this project possible.

For several years now BSE has been a home away from home for me. I always look forward to walking through the front door to see many smiling faces greet me throughout the building. I would like to thank all of the students, faculty, and staff in BSE for being so welcoming and helpful to me. I would especially like to thank Dr. Saied Mostaghimi who has been nothing but supportive of me and my various ventures around the university. Additionally I would like to give a special thank you to Dr. Mary Leigh Wolfe whose door has always been open to me. I have appreciated our conversations and your guidance.

I would be remiss without thanking the members of the Department Level Reform Project, along with the Department of Engineering Education's faculty and staff, particularly Dr. Vinod Lohani, Dr. Lo, and Dr. Hayden Griffin. Along with providing financial assistance, without these opportunities, I may never have discovered my passion for teaching and learning different pedagogical practices. Dr. Lo, from Hawaii to Blacksburg, I have enjoyed our many lunches and adventures. Thank you for always taking the time to check in on me and for your invaluable insights.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 2

Chapter 3

Chapter 4

Appendix D

LIST OF FIGURES

Chapter 3

Chapter 4

Appendix D

LIST OF ABBREVIATIONS

8-OHdG: 8-hydroxydeoxyguanosine

All:Both: Both participants performing both runs

ALRClinic: After long run in clinic

ALRField: After long run in field

ASRClinic: After short run in clinic

ASRField: After short run in field

BLR: Before long run

BSR: Before short run

C320: Cyranose 320

GCMS: Gas chromatography mass spectroscopy

CDA: Canonical discriminant analysis

CP: Conducting polymer

CSF: Cerebrospinal fluid

EDTA: Ethylenediaminetetraacetic acid

enose: Electronic nose

Female:Both: Female participant performing both runs

Female:Long: Female participant performing the long run

Female:Short: Female participant performing the short run

GPx: Glutathione peroxidase

GSH: Reduced glutathione

GSSG: Oxidized glutathione

HSP: Heat shock proteins

IgA: Immunoglobulin A

IPA: Isopropyl alcohol

LPS: Lipopolysaccharide

M-Distances: Mahalanobis distances

Male:Short: Male participant performing the short run

MDA: Malondialdehyde

MHPG-S: Methoxy-4-hydroxyphenylglycol-Sulphate

MLSS: maximal lactate steady state

MOS: Metal oxide sensors

MOSFETS: Metal oxide silicon field effect transistors

NOAA: National Oceanic and Atmospheric Administration

PBS: Phosphate buffer solution

PCA: Principal component analysis

POMS: Profile of Mood States

PQC: Piezoelectric Quartz Crystals

QMB: Quartz microbalance

R_0: Sensor resistance during baseline gas flow

R_{max}: Resistance for the sample headspace after reaching steady state

RBC: Red blood cells

ROS: Reactive oxygen species

SAW: Surface acoustic wave

SOD: Superoxide dismutase

TB: Tuberculosis

VCOM: Edward Via Virginia College of Osteopathic Medicine

VOC: Volatile organic compounds

Chapter 1: Introduction

1.1 BACKGROUND

Athletic performance improves with increased training length and difficulty. Unfortunately, performance levels can drop dramatically due to physical exhaustion brought on by overtraining which has been attributed to oxidative stresses (Cooper et al., 2002). Overtraining may lead to increased incidents of illness (Lakier Smith, 2003), lingering fatigue (Booth et al., 2006), mood disturbances (Anglem et al., 2008), tissue inflammation (Margonis et al., 2007), increased injuries (Finaud et al., 2006), and decreased overall athletic performance (Armstrong and VanHeest, 2002). Approximately 10 percent of rigorously training competitive athletes, such as swimmers and runners, may be affected by overtraining (Raglin, 2001). Furthermore intensive training and conditioning is becoming more common in many sports, leading to increased risks for overtraining syndrome to occur.

Proposed methods for monitoring training stresses include the detection of biomarkers related to oxidative stress. One such biomarker is pentane in breath (Cooper et al., 2002). Oxidative stress may also be detected by observing haemorheological properties such as serum viscosity and haematocrit content (El-Sayed et al., 2005). Other detection methods include analyzing blood samples for antioxidant enzymes (Margonis et al., 2007), cytokines (Shephard, 2001), lactate (Bosquet et al., 2001), and uric acid (Teeple et al., 2006). Urine (Filaire et al., 2004) and muscle sample (Halson and Jeukendrup, 2004) analyses have also been suggested as indicators of oxidative stress.

There have been a myriad of biomarkers proposed as possible indicators of overtraining. However, it appears as though the only thing previous investigators agree on is that no one clinically specific marker has been identified for the diagnosis of overtraining (Urhausen and Kindermann, 2002), and such an extensive list of possible biomarkers makes overtraining diagnosis challenging. Additionally, many of the proposed biomarkers require physical samples be collected invasively often causing discomfort to the patient. These invasive and time consuming procedures are not practical in a field setting commonly used for training athletes.

The use of smell in disease diagnosis has been a time-honored tradition (Pavlou and Turner, 2000) but one that has recently fallen out of favor as other medical diagnostic technologies have advanced. Electronic nose (enose) devices consist of a varying number of sensors designed to mimic the human nose (Munoz et al., 1999). The development of this chemosensory technology is helping to bring the use of smell back into the disease diagnosis and monitoring processes. Previous studies have been performed utilizing enoses to detect diabetes (Wang et al., 1997), pneumonia (Hockstein et al., 2004), lung cancer (Chen et al., 2005), and uremia (Lin et al., 2001) in the breath of patients.

1.2 SIGNIFICANCE AND IMPACT

Prior studies have proposed many biomarkers as indicators of overtraining, yet most of them require invasive testing and lengthy result turnaround times which do not allow athletic medical staffs to make real time adjustments to training regimens. The use of an enose to noninvasively detect oxidative stress, and thus overtraining, in the breath of athletes would allow for immediate feedback on training stresses, leading to optimization of individual athletic performances and potentially reducing injuries. Therefore this research focused on evaluating the feasibility of a conducting polymer-based electronic nose system to detect oxidative stress in the breath of two long distance runners.

If successful in detecting oxidative stress in athletes, enose technologies may also be implemented within the broader population. Other possible enose applications may be detecting oxidative stress in the breath of soldiers or construction workers who perform in physically exhausting settings, frequently in heavy and hot uniforms. Additionally an electronic nose may be able to detect diseases, such as strep throat, pneumonia, or diabetes, in situations where diagnostic equipment is hard to find, such as third world countries. This cheaper, more convenient, and less invasive method has the potential to enhance the quality of life of many individuals, near and far.

1.3 HYPOTHESIS

A conducting polymer-based electronic nose will be able to discriminate between different levels of training stress in long distance runners, and therefore may be utilized as an inexpensive, noninvasive tool in the detection and prevention of overtraining associated with oxidative stress.

1.4 OBJECTIVES

The objective of this study is to determine the feasibility of an electronic nose to detect oxidative stress leading to overtraining syndrome in the breath of long distance runners. Enose results will also be compared to those of blood analyzed for antioxidant enzymes proposed as indicators of oxidative stress.

1.5 THESIS OUTLINE

This thesis is comprised of five chapters. Chapter one provides an introduction while chapter two sets the stage for the study by reviewing previous work performed in related areas. The chapter reviews athletic training methods along with causes of and potential detection methods for overtraining. Additionally the chapter reviews previous work performed with enose systems in the medical field. Chapter two ends with the proposal of an electronic nose in the detection of overtraining in athletes. Chapter three presents the methodology utilized for this study while chapter four presents and discusses the results. Finally, study conclusions are presented in chapter five and suggestions for future work in chapter 6.

References

Aathithan, S., J.C. Plant, A.N. Chaudry, and G.L. French. 2001. Diagnosis of bacteriuria by detection of volatile organic compounds in urine using an automated headspace analyzer with multiple conducting polymer sensors. J Clin Microbiol 39:2590-3.

Anglem, N., S.J. Lucas, E.A. Rose, and J.D. Cotter. 2008. Mood, illness and injury responses and recovery with adventure racing. Wilderness Environ Med 19:30-8.

Armstrong, L.E., and J.L. VanHeest. 2002. The unknown mechanism of the overtraining syndrome: clues from depression and psychoneuroimmunology. Sports Med 32:185-209.

Aronzon, A., C.W. Hanson, and E.R. Thaler. 2005. Differentiation between cerebrospinal fluid and serum with electronic nose. Otolaryngol Head Neck Surg 133:16-9.

Booth, C.K., B. Probert, C. Forbes-Ewan, and R.A. Coad. 2006. Australian army recruits in training display symptoms of overtraining. Mil Med 171:1059-64.

Bosquet, L., L. Leger, and P. Legros. 2001. Blood lactate response to overtraining in male endurance athletes. Eur J Appl Physiol 84:107-14.

Bruno, E., M. Alessandrini, F. Ottaviani, A. Delfini, D. Di Pierro, A. Camillo, and A. De Lorenzo. 2008. Can the electronic nose diagnose chronic rhinosinusitis? A new experimental study. Eur Arch Otorhinolaryngol 265:425-8.

Chandiok, S., B.A. Crawley, B.A. Oppenheim, P.R. Chadwick, S. Higgins, and K.C. Persaud. 1997. Screening for bacterial vaginosis: a novel application of artificial nose technology. J Clin Pathol 50:790-1.

Chen, X., M. Cao, Y. Hao, Y. Li, P. Wang, K. Ying, and H. Pan. 2005. A Non-invasive detection of lung cancer combined virtual gas sensors array with imaging recognition technique. Conf Proc IEEE Eng Med Biol Soc 6:5873-6.

Cooper, C.E., N.B. Vollaard, T. Choueiri, and M.T. Wilson. 2002. Exercise, free radicals and oxidative stress. Biochem Soc Trans 30:280-5.

D'Amico, A., R. Bono, G. Pennazza, M. Santonico, G. Mantini, M. Bernabei, M. Zarlenga, C. Roscioni, E. Martinelli, R. Paolesse, and C. Di Natale. 2008. Identification of melanoma with a gas sensor array. Skin Res Technol 14:226-36.

Di Natale, C., A. Macagnano, R. Paolesse, E. Tarizzo, A. Mantini, and A. D'Amico. 2000. Human skin odor analysis by means of an electronic nose. Sensors and Actuators B 65:4.

Di Natale, C., A. Macagnano, E. Martinelli, R. Paolesse, G. D'Arcangelo, C. Roscioni, A. Finazzi-Agro, and A. D'Amico. 2003. Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors. Biosens Bioelectron 18:1209-18.

Dragonieri, S., J.T. Annema, R. Schot, M.P. van der Schee, A. Spanevello, P. Carratu, O. Resta, K.F. Rabe, and P.J. Sterk. 2008. An electronic nose in the discrimination of patients with non-small cell lung cancer and COPD. Lung Cancer.

Dragonieri, S., R. Schot, B.J. Mertens, S. Le Cessie, S.A. Gauw, A. Spanevello, O. Resta, N.P. Willard, T.J. Vink, K.F. Rabe, E.H. Bel, and P.J. Sterk. 2007. An electronic nose in the discrimination of patients with asthma and controls. J Allergy Clin Immunol 120:856-62.

Dutta, R., J.W. Gardner, and E.L. Hines. 2004. Classification of Ear, Nose, and Throat Bacteria Using a Neural-Network-Based Electronic Nose. MRS Bulletin:5.

Dutta, R., E.L. Hines, J.W. Gardner, and P. Boilot. 2002. Bacteria classification using Cyranose 320 electronic nose. Biomed Eng Online 1:4.

El-Sayed, M.S., N. Ali, and Z. El-Sayed Ali. 2005. Haemorheology in exercise and training. Sports Med 35:649-70.

Fend, R., C. Bessant, A.J. Williams, and A.C. Woodman. 2004. Monitoring haemodialysis using electronic nose and chemometrics. Biosens Bioelectron 19:1581-90.
Fend, R., A.H. Kolk, C. Bessant, P. Buijtels, P.R. Klatser, and A.C. Woodman. 2006. Prospects for clinical application of electronic-nose technology to early detection of Mycobacterium tuberculosis in culture and sputum. J Clin Microbiol 44:2039-45.
Fend, R., R. Geddes, S. Lesellier, H.M. Vordermeier, L.A. Corner, E. Gormley, E. Costello, R.G. Hewinson, D.J. Marlin, A.C. Woodman, and M.A. Chambers. 2005. Use of an electronic nose to diagnose Mycobacterium bovis infection in badgers and cattle. J Clin Microbiol 43:1745-51.
Filaire, E., B. Legrand, G. Lac, and J.M. Pequignot. 2004. Training of elite cyclists: effects on mood state and selected hormonal responses. J Sports Sci 22:1025-33.
Finaud, J., G. Lac, and E. Filaire. 2006. Oxidative stress : relationship with exercise and training. Sports Med 36:327-58.
Gendron, K.B., N.G. Hockstein, E.R. Thaler, A. Vachani, and C.W. Hanson. 2007. In vitro discrimination of tumor cell lines with an electronic nose. Otolaryngol Head Neck Surg 137:269-73.
Halson, S.L., and A.E. Jeukendrup. 2004. Does overtraining exist? An analysis of overreaching and overtraining research. Sports Med 34:967-81.
Hanson, C.W., 3rd, and E.R. Thaler. 2005. Electronic nose prediction of a clinical pneumonia score: biosensors and microbes. Anesthesiology 102:63-8.
Hockstein, N.G., E.R. Thaler, Y. Lin, D.D. Lee, and C.W. Hanson. 2005. Correlation of pneumonia score with electronic nose signature: A prospective study. Ann Otol Rhinol Laryngol 114:504-8.
Hockstein, N.G., E.R. Thaler, D. Torigian, W.T. Miller, Jr., O. Deffenderfer, and C.W. Hanson. 2004. Diagnosis of pneumonia with an electronic nose: correlation of vapor signature with chest computed tomography scan findings. Laryngoscope 114:1701-5.
Kodogiannis, V.S., J.N. Lygouras, A. Tarczynski, and H.S. Chowdrey. 2008. Artificial odor discrimination system using electronic nose and neural networks for the identification of urinary tract infection. IEEE Trans Inf Technol Biomed 12:707-13.
Lai, S.Y., O.F. Deffenderfer, W. Hanson, M.P. Phillips, and E.R. Thaler. 2002. Identification of upper respiratory bacterial pathogens with the electronic nose. Laryngoscope 112:975-9.
Lakier Smith, L. 2003. Overtraining, Excessive Exercise, and Altered Immunity: Is This a T Helper-1 Versus T Helper-2 Lymphocyte Response? Sports Med 33:347-364.
Lin, Y., H. Guo, Y. Chang, M. Kao, H. Wang, and R. Hong. 2001. Application of the electronic nose for uremia diagnosis. Sensors and Actuators B 76:4.
Machado, R.F., D. Laskowski, O. Deffenderfer, T. Burch, S. Zheng, P.J. Mazzone, T. Mekhail, C. Jennings, J.K. Stoller, J. Pyle, J. Duncan, R.A. Dweik, and S.C. Erzurum. 2005. Detection of lung cancer by sensor array analyses of exhaled breath. Am J Respir Crit Care Med 171:1286-91.
Margonis, K., I.G. Fatouros, A.Z. Jamurtas, M.G. Nikolaidis, I. Douroudos, A. Chatzinikolaou, A. Mitrakou, G. Mastorakos, I. Papassotiriou, K. Taxildaris, and D. Kouretas. 2007. Oxidative stress biomarkers responses to physical overtraining: implications for diagnosis. Free Radic Biol Med 43:901-10.
Moens, M., A. Smet, B. Naudts, J. Verhoeven, M. Ieven, P. Jorens, H.J. Geise, and F. Blockhuys. 2006. Fast identification of ten clinically important micro-organisms using an electronic nose. Lett Appl Microbiol 42:121-6.

Munoz, B.C., G. Steinthal, and S. Sunshine. 1999. Conductive polymer-carbon black composites-based sensor arrays for use in an electronic nose. Sensor Review 19:300-305.
Nonaka, A., M. Tanaka, H. Anguri, H. Nagata, J. Kita, and S. Shizukuishi. 2005. Clinical assessment of oral malodor intensity expressed as absolute value using an electronic nose. Oral Dis 11 Suppl 1:35-6.
Pavlou, A.K., and A.P. Turner. 2000. Sniffing out the truth: clinical diagnosis using the electronic nose. Clin Chem Lab Med 38:99-112.
Pavlou, A.K., N. Magan, J.M. Jones, J. Brown, P. Klatser, and A.P. Turner. 2004. Detection of Mycobacterium tuberculosis (TB) in vitro and in situ using an electronic nose in combination with a neural network system. Biosens Bioelectron 20:538-44.
Raglin, J.S. 2001. Psychological factors in sport performance: the Mental Health Model revisited. Sports Med 31:875-90.
Saraoglu, H.M., and B. Edin. 2007. E-Nose system for anesthetic dose level detection using artificial neural network. J Med Syst 31:475-82.
Shephard, R.J. 2001. Chronic fatigue syndrome: an update. Sports Med 31:167-94.
Tanaka, M., H. Anguri, A. Nonaka, K. Kataoka, H. Nagata, J. Kita, and S. Shizukuishi. 2004. Clinical assessment of oral malodor by the electronic nose system. J Dent Res 83:317-21.
Teeple, E., R.M. Shalvoy, and E.R. Feller. 2006. Overtraining in young athletes. Med Health R I 89:236-8.
Urhausen, A., and W. Kindermann. 2002. Diagnosis of overtraining: what tools do we have? Sports Med 32:95-102.
Voss, A., V. Baier, R. Reisch, K. von Roda, P. Elsner, H. Ahlers, and G. Stein. 2005. Smelling renal dysfunction via electronic nose. Ann Biomed Eng 33:656-60.
Wang, P., Y. Tan, H. Xie, and F. Shen. 1997. A novel method for diabetes diagnosis based on electronic nose. Biosens Bioelectron 12:1031-6.
Yamada, Y., Y. Takahashi, K. Konishi, and I. Katsuumi. 2007. Association of odor from infected root canal analyzed by an electronic nose with isolated bacteria. J Endod 33:1106-9.
Yates, J.W., M.J. Chappell, J.W. Gardner, C.S. Dow, C. Dowson, A. Hamood, F. Bolt, and L. Beeby. 2005. Data reduction in headspace analysis of blood and urine samples for robust bacterial identification. Comput Methods Programs Biomed 79:259-71.

Chapter 2: Literature Review

Athletes push themselves to extremes to increase their competiveness, but there is currently no consensus on how to detect if an athlete is optimizing his fitness level through his training program. Consequently athletes are susceptible to developing overtraining syndrome which significantly decreases training abilities along with causing other unwanted symptoms. This chapter reviews literature on previous studies that have investigated training optimization, causes and symptoms of overtraining, and potential overtraining detection methods. Finally, utilization of an electronic nose to detect overtraining through the breath of athletes is proposed.

2.1 TRAINING GOALS

One only has to look as far as the Olympic Games to realize how important competitive sports have become throughout the world. The 2008 games, held in China, showcased this reality as the whole world tuned in to watch a 23 year old Michael Phelps swim his way to eight gold medals (Swimming, 2004). The elite swimmer, who began training for future Olympic appearances at the age of 11 (Park, 2008), also broke seven world records (Swimming, 2004) during his exciting nine days of competition. One of the best athletes to date, Phelps' actions will unarguably reinforce the goals of many competitive athletes who push themselves to extremes to become the next Michael Phelps, or respective hero in their own sports.

The increasing number of people interested in pursuing such athletic accomplishments becomes apparent when considering the 30 to 35 million children who participate in organized sports in the United States alone (Soprano and Fuchs, 2007). Unfortunately once a particular talent has been identified, there is a tendency for the athlete to become exploited in that area. He is pushed into an over-ambitious competitive calendar, focusing on winning rather than efficient training toward his optimum athletic ability (Smith, 2003). However it is becoming increasingly clear there is no one training program that will fit all athletes, not even for a particular field. Factors such as sport, position, gender, age, previous training experience, and genetics (Smith, 2003) may play a role in a particular athlete's performance due to a training program.

Alternatively each athlete requires a unique set of training loads, consisting of specific training conditions, which when utilized lead to an individualized optimum performance (Peterson et al., 2004). This set of training loads has been found to follow a dose-response model (Morton, 1997; Peterson et al., 2004) in which the variables may consist of intensity, duration, and frequency (Smith, 2003). Immediately after an athlete has performed a training load, he will experience a slight temporary decrease in capability but after a short rest period an increase in ability will be observed (Morton, 1997). However if an athlete experiences too many training loads and training periods along with too infrequent rest periods, he may become burned out or develop overtraining. Therefore the overall training goal should be to induce optimum training doses and allow for adequate rest periods in each athlete so he can experience maximized performance potential at a given time, such as the fastest run time at a track meet, with minimal risk of fatigue (Morton, 1997; Peterson et al., 2004; Smith, 2003).

2.2 OVERTRAINING

2.2.1 TERMINOLOGY

Several investigators have researched how training loads affect athletes and at which point they become too exhausted to continue optimizing performance levels. However, literature comparisons are difficult because many of these investigators have used differing methodologies, not to mention differing terminology. Some terms that have been used to describe a state of exhaustion in athletes include, but are not limited to: staleness (Budgett et al., 2000; Kellmann and Gunther, 2000; Morgan et al., 1987; Nederhof et al., 2006; Peluso and Guerra de Andrade, 2005; Smith, 2004; Varlet-Marie et al., 2004), burnout (Kellmann and Gunther, 2000; Nicholls et al., 2009; Peluso and Guerra de Andrade, 2005; Smith, 2004), chronic fatigue in athletes (Budgett et al., 2000; Peluso and Guerra de Andrade, 2005), sports fatigue syndrome (Budgett et al., 2000; Peluso and Guerra de Andrade, 2005), underperformance syndrome (Budgett et al., 2000), overfatigue (Morgan et al., 1987; Peluso and Guerra de Andrade, 2005), overstrain (Morgan et al., 1987; Peluso and Guerra de Andrade, 2005), failure adaptation (Nederhof et al., 2006), underrecovery (Nederhof et al., 2006), training stress syndrome (Nederhof et al., 2006), unexplained underperformance syndrome (Nederhof et al., 2006; Peluso and Guerra de Andrade,

2005), overstress (Peluso and Guerra de Andrade, 2005), overuse (Peluso and Guerra de Andrade, 2005), and overwork (Peluso and Guerra de Andrade, 2005).

Two terms closely associated with each other are overtraining and overreaching which has been described as an inability to perform at expected optimums (Bosquet et al., 2001; Halson et al., 2003). However, whereas recovery from overtraining has been found to last anywhere from months (Cosca and Navazio, 2007; Halson and Jeukendrup, 2004; Smith, 2004; Teeple et al., 2006) to years (Halson and Jeukendrup, 2004) and in some instances even proves to be chronic (Smith, 2004), recovery from overreaching has been found to be much shorter (Bosquet et al., 2001; Moore and Fry, 2007), ranging from a few days to a few weeks (Armstrong and VanHeest, 2002; Budgett et al., 2000; Nicholls et al., 2009). With symptoms less severe (Halson and Jeukendrup, 2004; Moore and Fry, 2007) than overtraining, it has been suggested overreaching is the precursor to overtraining and once diagnosed actions should be taken to prevent an athlete from reaching the more extensive overtraining status (Anglem et al., 2008; Halson et al., 2003; Hynynen et al., 2006). Overreaching has also been said to occur more in team sports whereas overtraining has been found more in individual endurance sports (Halson and Jeukendrup, 2004).

For this study the term overtraining will be utilized to describe an athlete-specific syndrome characterized as a long term persistent inability to perform at expected optimums even though intense training is still being incurred (Armstrong and VanHeest, 2002; Budgett et al., 2000; El-Sayed et al., 2005; Jeukendrup and Hesselink, 1994; Lakier Smith, 2003; Margonis et al., 2007; Moore and Fry, 2007; Morgan et al., 1987; Nederhof et al., 2006; Nicholls et al., 2009; Shephard, 2001; Smith, 2004; Teeple et al., 2006; Urhausen and Kindermann, 2002; Varlet-Marie et al., 2004).

2.2.2 NUMBER AFFECTED

Overtraining will affect a large percentage of athletes at least once during their athletic careers (Armstrong and VanHeest, 2002). Although most athletes are able to manage the necessary training their sport requires, approximately 10 percent of all athletes will display excessive disturbances in mood state indicating a presence of overtraining (Raglin, 2001). In swimmers it

is not uncommon for five to 10 percent of athletes to be diagnosed as overtrained (Morgan et al., 1987) while in elite distance runners as many as 64 percent of the female and 66 percent of the male athletes will experience overtraining throughout their competitive careers (Morgan et al., 1987).

2.2.3 CAUSES OF OVERTRAINING

Previous investigators have not provided a consensus on the underlying mechanisms of overtraining (Armstrong and VanHeest, 2002; Lakier Smith, 2003; Smith, 2004). However one common thread mentioned is the delicate balance between training loads and recovery times utilized when training athletes to reach their optimum performance (Jeukendrup and Hesselink, 1994; Maso et al., 2004). In the attempt to obtain maximum athletic ability over the least amount of time (Lieberman et al., 2008), athletes may sometimes partake in an overzealous escalation of training loads (Cosca and Navazio, 2007; Lowery and Forsythe, 2006; Umeda et al., 2008) and decline in workout recovery periods leading to overtraining (Petibois et al., 2002; Teeple et al., 2006). Some athletes may also possess varying degrees of overtraining susceptibility (Morgan et al., 1987), which depend on factors such as how long an athlete has been training (Cooper et al., 2002; Moore and Fry, 2007) in a particular sport, age (Cooper et al., 2002), gender (Cooper et al., 2002; Umeda et al., 2008), and their proneness to mood disturbances (Anglem et al., 2008; Raglin, 2001). Stress has also been shown to be a factor in an athlete's performance (Nederhof et al., 2006). Specifically, stress due to training (El-Sayed et al., 2005), events occurring outside of training, and physical (Booth et al., 2006) and psychological factors (Booth et al., 2006) may play a role in increasing an athlete's susceptibility to developing overtraining syndrome. It has been suggested those athletes with strong coping skills and a good social network recover from injuries quicker than those without (Raglin, 2001). Other factors which may cause overtraining include tissue trauma (Smith, 2004), cumulative metabolism alterations (Petibois et al., 2003), and oxidative stress (Cooper et al., 2002; Lowery and Forsythe, 2006).

2.2.4 STRESS

Two athletes who have the exact same profile and training regimen should exhibit the same athletic achievements. However if one of the athletes is experiencing some kind of stress, she is at an increased risk for decreased athletic achievement and becoming overtrained (Nederhof et al., 2006). This stress could originate from training (El-Sayed et al., 2005; Nederhof et al., 2006; Raglin, 2001) or from outside of training (Nicholls et al., 2009) and could be physical (Booth et al., 2006) or psychological (Booth et al., 2006). Sport-related stressors may include injury and physical or mental errors occurring during training or competitions (Nicholls et al., 2009) while an example of a life stressor could be the death of a loved one (Nederhof et al., 2006). Additionally multiple stressors interact and compound their effect on the athlete (Nicholls et al., 2009). Stress is often associated with emotional symptoms such as anxiety and anger, elevated activation in the nervous system, humoral responses, changes in immune function, and behavioral changes (Kellmann and Gunther, 2000), all symptoms which might be displayed in an overtrained athlete. Previous investigators found athletes experience more stress on days when training occurs (Nicholls et al., 2009). In contrast stress was rated significantly better than normal on days of rest when athletes had more time to interact with their friends and participate in recreational activities (Nicholls et al., 2009). Monitoring stress in athletes is challenging; however early detection of stress, especially elevated levels, may help prevent the onset of overtraining symptoms and improve chances for obtaining optimal performance (Morgan et al., 1987; Nicholls et al., 2009).

2.2.5 SYMPTOMS

Symptoms of overtraining typically fall into four categories (Smith, 2004): physiological (Finaud et al., 2006; Nederhof et al., 2006; Smith, 2004), immunological (Booth et al., 2006; Jeukendrup and Hesselink, 1994; Lakier Smith, 2003; Petibois et al., 2002; Radak et al., 2008; Shephard, 2001; Smith, 2004), biochemical (Smith, 2004), and psychological (Kellmann and Gunther, 2000; Nederhof et al., 2006; Smith, 2004).

Physiological Symptoms

The most prominent physiological complaint is a persistent decline in performance (Booth et al., 2006; Cosca and Navazio, 2007; Finaud et al., 2006; Halson and Jeukendrup, 2004; Jeukendrup and Hesselink, 1994; Margonis et al., 2007; Moore and Fry, 2007; Morgan et al., 1987; Nederhof et al., 2006; Peluso and Guerra de Andrade, 2005; Petibois et al., 2003; Radak et al., 2008; Smith, 2004; Varlet-Marie et al., 2004) even when a period of rest is incurred (Cosca and Navazio, 2007; Peluso and Guerra de Andrade, 2005; Smith, 2004). Chronic fatigue (Armstrong and VanHeest, 2002; Booth et al., 2006; Brenner, 2007; Cosca and Navazio, 2007; Finaud et al., 2006; Halson and Jeukendrup, 2004; Kellmann and Gunther, 2000; Margonis et al., 2007; Maso et al., 2004; Nederhof et al., 2006; Nicholls et al., 2009; Peluso and Guerra de Andrade, 2005; Petibois et al., 2003; Varlet-Marie et al., 2004) is also commonly associated with overtraining as is an increase in muscular soreness (Booth et al., 2006; Urhausen and Kindermann, 2002), complaints of heavy legs (Cosca and Navazio, 2007; Peluso and Guerra de Andrade, 2005; Petibois et al., 2003; Urhausen and Kindermann, 2002; Varlet-Marie et al., 2004), and injuries (Booth et al., 2006; Finaud et al., 2006; Maso et al., 2004; Shephard, 2001), especially overuse injuries (Brenner, 2007; Cosca and Navazio, 2007). Loss of body weight (Booth et al., 2006; Moore and Fry, 2007), increased inflammation (Booth et al., 2006; Margonis et al., 2007), decreased resting heart rate (Booth et al., 2006), increased resting blood pressure (Booth et al., 2006), and changes in endocrinological function (Jeukendrup and Hesselink, 1994; Nederhof et al., 2006) and nervous system function (Jeukendrup and Hesselink, 1994; Nederhof et al., 2006) are also physiological symptoms that have been associated with overtraining.

Immunological Symptoms

Very heavy exercise may lead to a pro-inflammatory cytokine response similar to that seen in inflammation and sepsis (Shephard, 2001). Excessive training has been shown to induce chronic injuries which may increase regulation of the humoral arm of the immune system and suppress the cell-mediated arm of the adaptive immune system (Smith, 2004). This explains why overtrained athletes exhibit signs of increased illness (Booth et al., 2006; Finaud et al., 2006; Lakier Smith, 2003; Maso et al., 2004; Radak et al., 2008; Shephard, 2001; Smith, 2004),

especially those in the upper respiratory tract (Lakier Smith, 2003; Peluso and Guerra de Andrade, 2005).

Biochemical Symptoms

Previous literature has not agreed on one biochemical symptom present in overtrained athletes. However biological samples, such as blood (Aguilo et al., 2005; El-Sayed et al., 2005; Finaud et al., 2006; Lakier Smith, 2003; Margonis et al., 2007; Shephard, 2001; Teeple et al., 2006; Varlet-Marie et al., 2004), urine (Filaire et al., 2004; Margonis et al., 2007), saliva (Halson and Jeukendrup, 2004), and muscle (Halson and Jeukendrup, 2004; Lakier Smith, 2003; Petibois et al., 2002; Smith, 2004), have been collected and analyzed to detect changes in biochemical parameters such as catalase (Aguilo et al., 2005; Finaud et al., 2006; Margonis et al., 2007), creatine kinase (Lakier Smith, 2003; Smith, 2004; Umeda et al., 2008), glutathione peroxidase (GPx) (Finaud et al., 2006; Margonis et al., 2007), IgA (Halson and Jeukendrup, 2004), and testosterone to cortisol ratio (Booth et al., 2006; Filaire et al., 2004; Halson and Jeukendrup, 2004; Maso et al., 2004; Petibois et al., 2002; Teeple et al., 2006; Urhausen and Kindermann, 2002). Unfortunately most of these tests require invasive sample collection procedures followed by lengthy analysis times.

Psychological Symptoms

The most prominent psychological symptom observed in overtrained athletes is a decline in mood (Anglem et al., 2008; Armstrong and VanHeest, 2002; Booth et al., 2006; Cosca and Navazio, 2007; Finaud et al., 2006; Halson and Jeukendrup, 2004; Jeukendrup and Hesselink, 1994; Kellmann and Gunther, 2000; Margonis et al., 2007; Maso et al., 2004; Nederhof et al., 2006; Peluso and Guerra de Andrade, 2005; Smith, 2004) or behavior (Smith, 2004). Loss of motivation (Brenner, 2007; Moore and Fry, 2007) for the athlete's sport and even other life events and sleep disturbances (Booth et al., 2006; Cosca and Navazio, 2007; Jeukendrup and Hesselink, 1994; Kellmann and Gunther, 2000; Nederhof et al., 2006; Peluso and Guerra de Andrade, 2005; Petibois et al., 2003) are also common psychological symptoms. Overtrained athletes may also complain of concentration problems (Nederhof et al., 2006; Petibois et al.,

2003), increased confusion (Booth et al., 2006; Kellmann and Gunther, 2000), eating disturbances (Nederhof et al., 2006; Peluso and Guerra de Andrade, 2005) and reduced libido (Peluso and Guerra de Andrade, 2005).

2.2.6 POTENTIAL BIOCHEMICAL BIOMARKERS

As Table 2.1 demonstrates, previous investigators have reviewed and studied an array of potential overtraining biomarkers, including hormone and enzyme levels. Other biomarkers may include haemorheological properties (Maso et al., 2004; Varlet-Marie et al., 2004) and physiological parameters. Haemorheology is the science that describes the flow characteristics of blood (El-Sayed et al., 2005) and can be influenced by whole blood viscosity (Maso et al., 2004), serum viscosity (El-Sayed et al., 2005; Maso et al., 2004), packed cell volume (Maso et al., 2004), ferritin content (Maso et al., 2004), whole blood volume (El-Sayed et al., 2005), serum volume (El-Sayed et al., 2005), haematocrit content (El-Sayed et al., 2005), and iron content, among others. Physiological markers may include performance deterioration, increased basal heart rate (Booth et al., 2006; Cosca and Navazio, 2007; Morgan et al., 1987), increased resting blood pressure (Booth et al., 2006; Morgan et al., 1987), decreased aerobic capacity (Booth et al., 2006), decreased anaerobic power (Booth et al., 2006), weight loss (Booth et al., 2006), and muscle soreness (Booth et al., 2006). Unfortunately no clinically specific overtraining biomarker has been conclusively identified. Additionally there are often conflicting results for those biomarkers observed in multiple studies. For example, a decrease in GPx activity was observed in cyclists after performing exercise in one study (Tauler et al., 2002) while stagnant GPx activity levels were found in cyclists in another study (Sureda et al., 2005). Still other studies have noted increases in GPx activity levels (Aguilo et al., 2005; Sinha et al., 2009).

Table 2.1. Potential biomarkers for identifying overtraining in athletes.

Marker	Source	Description	Reference(s)
Aluminum		Depletion	(Lakier Smith, 2003; Teeple et al., 2006)
Ammonia	Serum	Decreased concentrations at rest	(Urhausen and Kindermann, 2002)
Anti-Lipopolysaccharide immunoglobulin G	Blood	Decreased concentrations	(Bosenberg et al., 1988)
Aspartate aminotransferase	Blood	Increased levels	(Umeda et al., 2008)
Catalase	Serum	Increased levels	(Aguilo et al., 2005; Finaud et al., 2006; Margonis et al., 2007)
Cobalt		Depletion	(Lakier Smith, 2003; Teeple et al., 2006)
Copper		Depletion	(Lakier Smith, 2003; Teeple et al., 2006)
Cortisol/Cortisone ratio		Increased levels	(Teeple et al., 2006)
Creatine kinase	Blood	Increased levels indicate muscle damage	(Lakier Smith, 2003; Smith, 2004; Umeda et al., 2008)
Cytokines	Blood	Increased levels indicate inflammation	(Lakier Smith, 2003; Shephard, 2001; Smith, 2004; Teeple et al., 2006)
Ferritin	Serum	Decreased levels	(Teeple et al., 2006; Varlet-Marie et al., 2004)
Glucose	Blood	Decreased levels	(Umeda et al., 2008)
Glutamine	Serum	Decreased levels result in reductions in immune function	(Halson and Jeukendrup, 2004; Lakier Smith, 2003; Shephard, 2001; Teeple et al., 2006)
Glutamine/Glutamate ratio	Serum	Ratio below 3.58 indicates overreaching	(Halson et al., 2003)
(Oxidized) Glutathione (GSSG)	Blood	Increased levels	(Aguilo et al., 2005; Gohil et al., 1988; Margonis et al., 2007)
(Reduced) Glutathione (GSH)	Blood	Decreased levels	(Cooper et al., 2002; Gohil et al., 1988; Margonis et al., 2007; Witt et al., 1992)
Glutathione Peroxidase	Serum	Increased levels	(Finaud et al., 2006; Margonis et al., 2007)
Glycogen	Muscle	Reduced levels results in fatigue, muscle soreness and heavy legs	(Halson and Jeukendrup, 2004; Lakier Smith, 2003; Petibois et al., 2002; Smith, 2004)

Marker	Source	Description	Reference(s)
Heat Shock Proteins (HSP)		Increasing levels with exercise, particularly with body temperature	(Finaud et al., 2006)
Hematocrit	Blood	Increase levels	(El-Sayed et al., 2005; Varlet-Marie et al., 2004)
Hemoglobin	Blood	Decreased levels	(Teeple et al., 2006; Umeda et al., 2008)
IgA	Saliva	Decreased concentrations result in reductions in immune function	(Halson and Jeukendrup, 2004)
Iron	Blood	Decreased levels	(Booth et al., 2006; Teeple et al., 2006; Varlet-Marie et al., 2004)
Isoprostanes	Urine	Increased levels	(Margonis et al., 2007)
Lactate	Blood	Reduced concentrations or shift right in lactate curve	(Bosquet et al., 2001; Halson and Jeukendrup, 2004; Jeukendrup and Hesselink, 1994; Urhausen and Kindermann, 2002)
Lipopolysaccharide (LPS) endotoxin	Blood	Increased concentrations	(Bosenberg et al., 1988)
Manganese		Depletion	(Teeple et al., 2006)
Metanephrine	Urine	Increased levels	(Filaire et al., 2004)
Methaemoglobin		Increased levels	(Cooper et al., 2002)
Methoxy-4-hydroxyphenylglycol-Sulphate (MHPG-S)	Urine	Decreased levels	(Filaire et al., 2004)
Nitrogen		Negative balance	(Lakier Smith, 2003; Teeple et al., 2006)
Norepinephrine	Serum	Increased concentrations	(Urhausen and Kindermann, 2002)
Normetanephrine	Urine	Increased levels	(Filaire et al., 2004)
Pentane	Breath	Indicates increase in lipid peroxidation	(Cooper et al., 2002)
Protein Carbonyls	Serum Muscle	Increased levels	(Margonis et al., 2007; Witt et al., 1992)
Selenium		Depletion	(Lakier Smith, 2003; Teeple et al., 2006)
Superoxide Dismutase		Increased levels	(Finaud et al., 2006)

Marker	Source	Description	Reference(s)
Testosterone/Cortisol ratio	Blood Saliva	Decreased ratio indicates anabolic-catabolic balance	(Booth et al., 2006; Filaire et al., 2004; Halson and Jeukendrup, 2004; Maso et al., 2004; Petibois et al., 2002; Teeple et al., 2006; Urhausen and Kindermann, 2002)
Total Protein	Serum	Increased concentration	(El-Sayed et al., 2005)
Urea	Serum Blood	Increased concentrations indicate protein catabolism	(Lakier Smith, 2003; Teeple et al., 2006; Urhausen and Kindermann, 2002)
Uric Acid	Blood	Increased levels	(Aguilo et al., 2005; Teeple et al., 2006)
Vitamin C	Serum	Increased levels	(Cooper et al., 2002)
Vitamin E	Serum	Increased levels	(Cooper et al., 2002)
White Blood Cells	Blood	Increased levels (sustained leukocytosis)	(Margonis et al., 2007)
Zinc		Depletion	(Lakier Smith, 2003; Teeple et al., 2006)

2.2.7 EXERCISE AND OXIDATIVE STRESS

Free radicals are chemical molecules that are naturally produced in all living cells (Cooper et al., 2002; Finaud et al., 2006). These reactive molecules, or molecule fragments contain one or more unpaired electrons (Cooper et al., 2002) in their valence shells and are extremely unstable because they tend to oxidize with other molecules by capturing an electron from them (Finaud et al., 2006). Some free radicals are important in helping to protect the body against disease in the immune system but many free radicals also work against the body by oxidizing lipids, proteins, and DNA (Finaud et al., 2006). Free radicals have a short life time, ranging from nanoseconds to milliseconds (Finaud et al., 2006).

Many free radicals occurring in living organisms are reactive oxygen species (ROS) (Cooper et al., 2002). ROS are derived from oxygen and are continuously being produced externally by radiation exposure, air pollutants, and intoxication by oxygen, smoke, and alcohol and internally by oxygen metabolism (Finaud et al., 2006). ROS can damage almost all cell components including proteins, nucleic acids, and lipids (Witt et al., 1992). ROS are involved in important physiological processes, such as immunity, but also have negative consequences such as muscle

fatigue, healthy cell apoptosis, inflammation, and altered cellular functions (Finaud et al., 2006). When lipid peroxidation occurs, cell membrane fluidity is altered, reducing the cell's ability to maintain required concentration gradients, increasing membrane permeability, and increasing inflammation (Finaud et al., 2006). When blood and structural proteins are oxidized, the proteolytic system becomes inhibited, leading to changes in structural proteins and enzyme functions (Finaud et al., 2006). Every part of DNA is susceptible to ROS attack which causes strand breakage and base repair damage (Finaud et al., 2006).

To limit the harmful effects of free radicals, particularly ROS, the body has an elaborate defense system consisting of antioxidants which work by forming less active radicals (Finaud et al., 2006). Antioxidants can be enzymatic, such as catalase (Aguilo et al., 2005; Cooper et al., 2002; Finaud et al., 2006), superoxide dismutase (Aguilo et al., 2005; Cooper et al., 2002; Finaud et al., 2006), and glutathione peroxidase, (Aguilo et al., 2005; Cooper et al., 2002; Finaud et al., 2006) or non-enzymatic, such as tocopherols (Aguilo et al., 2005), ascorbate (Aguilo et al., 2005), urate (Aguilo et al., 2005), glutathione (Aguilo et al., 2005; Cooper et al., 2002; Finaud et al., 2006), Vitamin E (Cooper et al., 2002; Finaud et al., 2006; Witt et al., 1992), Vitamin A (Finaud et al., 2006), Vitamin C (Cooper et al., 2002; Finaud et al., 2006; Witt et al., 1992), flavonoids (Finaud et al., 2006), uric acid (Finaud et al., 2006), and Q_{10} (Witt et al., 1992). Micronutrients acting as enzymatic cofactors consist of iron (Finaud et al., 2006), copper (Finaud et al., 2006), zinc (Finaud et al., 2006), selenium (Finaud et al., 2006; Witt et al., 1992), and manganese (Finaud et al., 2006). The body's antioxidant defense system is usually capable of preventing substantial oxidative damage (Cooper et al., 2002). However, when the product of oxygen radicals exceeds the detoxification capacity of the antioxidants, an oxidative stress state occurs (Cooper et al., 2002; Finaud et al., 2006; Witt et al., 1992).

Previous investigators have said exhaustive exercise is affiliated with an acceleration of unbalance between ROS generation and antioxidant defenses (El-Sayed et al., 2005), resulting in oxidative stress (Aguilo et al., 2005; Cooper et al., 2002; Finaud et al., 2006; Witt et al., 1992). Among other things, this oxidative stress is associated with muscle fatigue and may lead to overtraining (Finaud et al., 2006). Therefore blood and urine indicators of oxidative stress may

prove useful in monitoring and diagnosing overtraining in athletes (Finaud et al., 2006; Margonis et al., 2007).

Catalase and Glutathione Peroxidase

Catalase is an enzymatic antioxidant present in every cell, especially in peroxysomes (Finaud et al., 2006). Catalase converts hydrogen peroxide into water and oxygen and can also use hydrogen peroxide to detoxify some toxic substances using a peroxidase reaction (Finaud et al., 2006). Glutathione peroxidase (GPx) is an enzymatic antioxidant present in cell cytosol and mitochondria and is able to transform hydrogen peroxide into water (Finaud et al., 2006). Exercise increases hydrogen peroxide production and catalase and GPx have both been shown to increase after periods of exercise (Margonis et al., 2007). GPx has been found to scavenge hydrogen peroxide more efficiently at lower concentrations, therefore at higher training volumes, hydrogen peroxide production may exceed GPx capabilities (Margonis et al., 2007). Catalase production would then be expected to increase in response to training load to compensate for the GPx inability to scavenge hydrogen peroxide during peak oxidative stress and overtraining (Margonis et al., 2007). GPx levels have been found to return to baseline levels after excessive training ends (Margonis et al., 2007). Measuring catalase and GPX activity can help evaluate the status of the antioxidant defense system (Finaud et al., 2006) as an indicator of oxidative stress present in athletes being monitored for overtraining.

2.2.8 PSYCHOLOGICAL MARKERS

Overtrained athletes, particularly females (Umeda et al., 2008), frequently exhibit negative psychological symptoms (Booth et al., 2006; Peluso and Guerra de Andrade, 2005) with the clinical diagnosis of depression demonstrated in some of the more extreme cases (Morgan et al., 1987; Peluso and Guerra de Andrade, 2005). These athletes tend to have trouble sleeping (Booth et al., 2006; Cosca and Navazio, 2007; Peluso and Guerra de Andrade, 2005) and experience mental fatigue (Booth et al., 2006; Umeda et al., 2008), high levels of confusion (Booth et al., 2006), and reduced libido (Peluso and Guerra de Andrade, 2005). Psychomotor speed, a

measure of the amount of time a person requires to perform a requested psychological task or test, has been suggested to decrease in overtrained athletes (Nederhof et al., 2006).

Although not widely accepted as a marker for overtraining, mood disturbances (Anglem et al., 2008; Cosca and Navazio, 2007; Filaire et al., 2004; Halson and Jeukendrup, 2004; Lieberman et al., 2008; Maso et al., 2004; Morgan et al., 1987; Nicholls et al., 2009; Peluso and Guerra de Andrade, 2005; Urhausen and Kindermann, 2002) have been repeatedly observed in athletes considered overtrained. A dose-response relationship has been proven between mood state and exercise, with mood improving with each dose of exercise (Filaire et al., 2004; Kellmann and Gunther, 2000; Morgan et al., 1987; Raglin, 2001). However, significantly decreased mood states have been commonly associated in people who participate in excessive exercise or overtrained athletes. Furthermore, athletes who exhibit good mood states have been shown to perform better than those with poorer mood states (Raglin, 2001). This finding has lead to a possible new, controversial, method of selecting athletes for sports teams.

Profile of Mood States

The Profile of Mood States (POMS) survey is a self-reporting tool used to measure mood states such as tension, depression, anger, fatigue, confusion, and vigor (Filaire et al., 2004) which are often combined to provide an overall mood state (Armstrong and VanHeest, 2002; Filaire et al., 2001; Lieberman et al., 2008; Morgan et al., 1987; Raglin, 2001). Subjects are provided with a list of 65 adjectives (Morgan et al., 1987) and asked to use a Likert scale to rate how each one relates to how they feel (Shacham, 1983). The overall mood state score is then calculated by adding the five negative mood states (tension, depression, anger, fatigue, and confusion) and subtracting the one positive mood state (vigor) (Filaire et al., 2004; Morgan et al., 1987). Hence a large overall mood score indicates a bad mood (Filaire et al., 2004).

One major advantage of the POMS is its ease of administration (Shacham, 1983). Investigators have even found a shortened version of the questionnaire, consisting of 37 instead of 65 adjectives (Fillion and Gagnon, 1999), works in instances when there are time constraints and still remains consistent (Shacham, 1983). However since it usually only takes healthy

individuals three to seven minutes to complete the full POMS (Shacham, 1983), it is suggested using the full 65 adjectives to obtain more information.

Athletes have been shown to demonstrate a mood state profile characterized by scoring below general population average on tension, depression, anger, fatigue, and confusion and approximately one standard deviation above the general population's average of vigor (Morgan et al., 1987). This profile is known as the iceberg profile and has been consistently seen in successful athletes (Kellmann and Gunther, 2000) such as swimmers, rowers, and wrestlers (Morgan et al., 1987).

Measuring the mood state over time, or the transient mood state (Fillion and Gagnon, 1999; Shacham, 1983), in athletes may provide a method for detecting initial symptoms of overtraining before a full blown case develops, allowing for quick action to reverse the symptoms (Raglin, 2001). To use it as an overtraining monitor, athletes should complete the POMS questionnaire before undertaking any training or during periods of rest to be used as a baseline for future mood state comparisons (Raglin, 2001). Previous investigators have had athletes complete the POMS questionnaire asking them to reflect on their mood over a specific amount of time, such as a week (Filaire et al., 2001). However to more accurately monitor how increases in training loads are affecting athletes, the POMS should be administered asking athletes to rate how they are feeling at the moment the questionnaire is being completed (Lieberman et al., 2008; Raglin, 2001).

2.2.9 OVERTRAINING DIAGNOSIS

Overtrained athletes typically exhibit symptoms in one or more of the four categories (Smith, 2004): physiological (Finaud et al., 2006; Nederhof et al., 2006; Smith, 2004), immunological (Booth et al., 2006; Jeukendrup and Hesselink, 1994; Lakier Smith, 2003; Petibois et al., 2002; Radak et al., 2008; Shephard, 2001; Smith, 2004), biochemical (Smith, 2004), and psychological (Kellmann and Gunther, 2000; Nederhof et al., 2006; Smith, 2004). For each category there have been numerous markers proposed as possible methods for diagnosing overtraining. However, as previously mentioned, the one thing previous investigators do agree on is that no

one clinically specific marker has been identified for the detection of overtraining (Armstrong and VanHeest, 2002; Finaud et al., 2006; Jeukendrup and Hesselink, 1994; Margonis et al., 2007; Petibois et al., 2003; Smith, 2003; Urhausen and Kindermann, 2002), but such an extensive list of possible biomarkers makes overtraining diagnosis difficult.

In an ideal situation all biomarkers linked to overtraining would be analyzed to ensure the most accurate diagnosis. Additionally, testing would be required at rest, after a sport-specific training load is performed, and at a set time after completing the training load (Petibois et al., 2002). Unfortunately this extensive sample collection and testing suggested are not ethically, financially, or technically feasible (Petibois et al., 2003).

As a result the only reliable diagnostic marker for overtraining remains to be declined performance (Margonis et al., 2007; Petibois et al., 2003; Urhausen and Kindermann, 2002). Unfortunately even the most experienced coaches have been forced to admit they can not predict which athletes will become overtrained (Armstrong and VanHeest, 2002). Athletic staffs (athletes, coaches, trainers, and physicians) would benefit greatly from a specific, sensitive, simple diagnostic test for overtraining (Armstrong and VanHeest, 2002). Diagnostic tools that are noninvasive, inexpensive, and may be utilized at rest or in a manner that does not disturb the training process are preferred (Urhausen and Kindermann, 2002).

2.2.10 TREATMENT

As soon as overtraining has been diagnosed immediate action must be taken to reverse its effects (Morgan et al., 1987). Unfortunately for the affected athlete, treatment usually consists of complete rest from his sport until fully recovered (Jeukendrup and Hesselink, 1994; Morgan et al., 1987). This rest period may take anywhere from months (Cosca and Navazio, 2007; Halson and Jeukendrup, 2004; Smith, 2004; Teeple et al., 2006) to years (Halson and Jeukendrup, 2004) and in some instances even proves to be chronic (Smith, 2004). At the end of this rest period and after the athlete is no longer showing symptoms of overtraining, he should gradually resume his training regimen under carful monitoring (Cosca and Navazio, 2007).

However it has been shown decreasing training will not only decrease fatigue but will also decrease performance (Morton, 1997), therefore an alternative to complete rest may be for the overtrained athlete to participate in a significantly decreased training regimen (Jeukendrup and Hesselink, 1994). This active recovery period would involve a carefully monitored, drastically reduced training program along with increased workout recovery times similar to that which would occur before a competition (Budgett et al., 2000). This progressive recovery process will not only allow for overtraining recovery but may also provide a sense of power (Kellmann and Gunther, 2000) and encouragement to the athlete who will not lose his sense of accomplishment all at one time.

2.2.11 RESULTS

Excessive training loads can be physically and emotionally draining on the athletes executing them (Teeple et al., 2006). Once athletes have surpassed their training threshold they may become overtrained which is associated with significantly decreased performance. Recovery time depends on the degree of overtraining developed. Months to years of complete sport-related rest will lead to decreased athletic ability and may lead to frustration in the athlete (Peluso and Guerra de Andrade, 2005). This frustration, accompanied by the decrease in athletic ability may result in the loss of sponsorship and forced retirement from the sport (Peluso and Guerra de Andrade, 2005). Beyond performance-related consequences, 80 percent of overtrained athletes have also been clinically diagnosed with depression (Morgan et al., 1987). Other results of overtraining appear to be hormonal (Radak et al., 2008; Shephard, 2001), nutritional (Shephard, 2001), and motivational depletion (Peluso and Guerra de Andrade, 2005). Overtrained athletes may also experience increased illnesses (Lakier Smith, 2003; Smith, 2004) due to immune suppression (Booth et al., 2006; Halson et al., 2003; Radak et al., 2008; Shephard, 2001) and increased injuries due to muscle tissue damages (Shephard, 2001).

2.2.12 PREVENTION

Once a state of overtraining occurs, an athlete suffers performance decreases and often other physiological and mental detriments. Recovery consists of significantly decreasing, if not

halting, training until symptoms subside. As they are constantly preparing for the next competition, athletes do not have the luxury of time on their side (Morgan et al., 1987) and being forced into overtraining recovery may result in the loss of several months, if not more, (Petibois et al., 2002; Smith, 2004) of training. This has potentially severe consequences including loss of sponsorship and even retirement (Peluso and Guerra de Andrade, 2005). It is, therefore, far better to try and prevent overtraining from ever occurring in the first place (Morgan et al., 1987; Peluso and Guerra de Andrade, 2005).

Most importantly athletic staffs should be educated on and practice safe training (Teeple et al., 2006). Athletes, in particular, should be taught to pay special attention to their bodies and how they are reacting to training (Smith, 2004). Some major items to look for include performance decreases, changes in psychological health, and an increase in illness or injuries (Filaire et al., 2001). Some physical performance measurements that could be used as monitors include maximal strength, anaerobic capacity, jumping ability, and stand and reach tests (Margonis et al., 2007). Recovery from a strenuous workout should take from 24 to 48 hours but if an athlete is taking longer, a note should be made (Smith, 2004). Once the athletic team notices signs of exhaustion or overtraining beginning, they should work closely with the athlete to decrease or temporarily pause training and monitor her until she makes a full recovery (Morgan et al., 1987). Only then should the athlete be allowed to return to the scheduled training program.

Some other suggestions to consider in the prevention of overtraining include taking at least one day off per week from any organized physical activity and taking from two to three months off per year from an athlete's specific sport to let injuries heal, the mind refresh, and allow for strength and conditioning (Brenner, 2007). Furthermore since not all athletes are created equal, there should be some focus on individualized training regimens (Smith, 2004).

Previous investigators have looked at a variety of biomarkers for monitoring overtraining without conclusively identifying a clinically specific marker. The majority of these tests require invasive testing and hours to days before results are known. This does not allow athletic medical staffs to make real time adjustments to training programs. Further work should be done to

identify a biomarker or method to allow for noninvasive, rapid monitoring of and altering training load to optimize athletic performance and prevent overtraining.

2.3 SMELL IN MEDICINE

The use of smell has been a time-honored tradition since 2,000 BC when both the Chinese and Greeks used it to diagnose diseases (Pavlou and Turner, 2000). These ancient medicine practitioners learned they could use the smell of body secretions such as sweat, urine, sputum, and vaginal fluid, to form conclusions as to how a person should be treated for a given illness (Pavlou and Turner, 2000). Table 2.2 presents some odors which may be used to form a diagnosis and what that diagnosis might be.

Now recall your last visit to the doctor's office: the physician uses her senses to examine you and provide a diagnosis. She may use sight to observe your complexion: are you flushed? She may use hearing to listen for congestion in your chest. She may use touch to feel for swelling or abnormalities in your abdomen. One sense you typically do not observe the physician utilizing is the sense of smell. So what happened?

Medical diagnosis changed with the coming of the industrial and technological revolution of the 18^{th} and 19^{th} centuries (Pavlou and Turner, 2000). Additionally the use of smell for medical diagnostics continued to fall out of favor along with the advancement of new molecular tests such as polymerase chain reaction (Pavlou and Turner, 2000). However, this does not mean bodily odors are not still helpful in the detection patients' illnesses.

Table 2.2. Bodily odors and their potential medical diagnosis.

Odor	Sample Source	Diagnosis	Reference
Acetone	Breath	Diabetic acidosis	(McVan, 1977)
Acetone	Breath	Ketosis	(Hanson and Thaler, 2005)
Ammonia	Breath	Uremia	(Hanson and Thaler, 2005)
(Persistent) Bad breath	Breath	Gingival inflammation	(McVan, 1977)
Bitter almond	Breath	Cyanide poisoning	(Hanson and Thaler, 2005; McVan, 1977)
Burnt rope		Marijuana use	(McVan, 1977)
Decaying apples (acetone)	Breath	Diabetes	(Rock et al., 2008; Wang et al., 1997)
Fish (Amine)	Vaginal swab	Bacterial vaginosis	(Chandiok et al., 1997)
Freshly baked brown bread	Skin	Typhoid	(McVan, 1977; Rock et al., 2008)
Freshly plucked feathers	Sweat	Rubella	(McVan, 1977; Rock et al., 2008)
Garlic		Arsenic poisoning	(McVan, 1977)
Menagerie		Variola	(McVan, 1977)
Must or horse	Infant skin	Phenylketonuria	(McVan, 1977)
Musty fish/raw liver		Hepatic failure	(Rock et al., 2008)
New-mown clover	Breath	Liver failure	(McVan, 1977)
Overripe cheese or sweaty feat	Skin or sweat	Isovaleric academia	(McVan, 1977)
Paraldehyde		Acute poisoning	(McVan, 1977)
Stale urine		Uremic acidosis	(McVan, 1977)
Sweet	Sweat	Diphtheria	(Rock et al., 2008)

2.4 ELECTRONIC NOSE

Panels of trained human noses are still considered the gold standard in measuring odors (Nagle et al., 1998; Pavlou and Turner, 2000). However this sensitive sniffing expertise comes at a price as training each panelist is expensive and requires a long time (Nagle et al., 1998; Pavlou and Turner, 2000). Additionally these panels typically do not operate in the field (Nagle et al., 1998).

Previous investigators attempted to imitate the human nose with an instrument consisting of a varying number of sensors and called the electronic nose, or enose (Munoz et al., 1999). There are several types of enoses but each consists of the same basic procedure, developed with the human nose in mind (Munoz et al., 1999; Nagle et al., 1998; Rock et al., 2008): (1) air consisting of volatiles from a sample, or the sample's headspace, is pulled into the unit by a vacuum, (2) the headspace passes over a sensor array, (3) each sensor has a specific reaction to the gas, (4) each sensor's response is recorded, and (5) the sensors are purged of the headspace gas (Nagle et al., 1998).

The main component of an electronic nose is an array of sensors each having a different specificity to a wide range of odorous molecules (Ballabio et al., 2006; Nagle et al., 1998; Rock et al., 2008). When exposed to a sample's headspace, each sensor binds differently with the volatiles present creating a pattern of individual sensor responses (Ballabio et al., 2006; Munoz et al., 1999; Rock et al., 2008). This pattern is also known as a sample's digital fingerprint or smellprint (Ballabio et al., 2006). Unlike other analytical methods, such as gas chromatography, the electronic nose does not identify specific components of the sample, however each sample can be identified by its individual smellprint once the enose has been sufficiently trained (Ballabio et al., 2006; Nagle et al., 1998).

Some common types of electronic noses are named for their sensors' components: metal oxide sensors (MOS), metal oxide silicon field effect transistors (MOSFETS), quartz microbalance sensors (QMB) surface acoustic wave devices (SAW), and conducting polymers (CP) (Nagle et al., 1998; Pavlou and Turner, 2000).

2.4.1 METAL OXIDE SENSORS

Metal oxide sensors (MOS) are a type of conductivity sensor (Nagle et al., 1998) that consist of chemically adsorbed oxygen species which interact with volatiles (Turner and Magan, 2004). A semiconducting material is deposited between two metal contacts over a resistive heating element (Nagle et al., 1998). As the volatiles pass over the material, the resistance between the two metal contacts changes (Nagle et al., 1998; Pavlou and Turner, 2000). MOS are very

sensitive to volatiles (Gouma and Sberveglieri, 2004; Pavlou and Turner, 2000; Turner and Magan, 2004), quick (Gouma and Sberveglieri, 2004), relatively inexpensive (Gouma and Sberveglieri, 2004), and are stable over long time periods (Gouma and Sberveglieri, 2004; Turner and Magan, 2004); however they run at high temperatures (Pavlou and Turner, 2000) and can be sensitive to long term drift (Pavlou and Turner, 2000; Turner and Magan, 2004).

2.4.2 METAL OXIDE SILICON FIELD EFFECT TRANSISTORS

Metal oxide silicon field effect transistors (MOSFETS) are similar to MOS; however the output is determined from a change in potential as volatiles react with the device (Turner and Magan, 2004). MOSFET sensors experience similar base-line drifts of that seen in MOS (Nagle et al., 1998).

2.4.3 QUARTZ MICROBALANCE SENSORS

Quartz microbalance (QMB) sensors are a type of piezoelectric sensors (Nagle et al., 1998; Pavlou and Turner, 2000). QMB enoses consist of piezoelectric crystals which use radio frequencies of quartz materials coated with acetyl cellulose or lecithin polymer to detect mass (Turner and Magan, 2004). When volatiles are adsorbed at the surface of the polymer, the mass is increased, decreasing the resonance frequency (Nagle et al., 1998). This deduction is inversely proportional to the mass adsorbed by the polymer (Nagle et al., 1998). One of the major disadvantages for QMB is that the sensors require complicated electronics (Nagle et al., 1998). Additionally as the polymer membrane ages, the resonant frequencies can drift (Nagle et al., 1998).

2.4.4 SURFACE ACOUSTIC WAVE DEVICES

Surface acoustic wave (SAW) devices are similar to QMB but they operate at higher frequencies (Pavlou and Turner, 2000). They are based on waves that are given off along the surface of the crystals by electric fields of surface-deposited aluminum electrodes (Turner and Magan, 2004).

SAW exhibit poorer signal-to-noise ratios, making them less sensitive than QMC in some instances (Nagle et al., 1998).

2.4.5 CONDUCTING POLYMER SENSORS

Conducting polymer (CP) sensors make up the most common kind of electronic nose used (Gouma and Sberveglieri, 2004). Like MOS, they are a type of conductivity sensor (Nagle et al., 1998). Their sensor arrays are made of unique polymers, such as polypyrol or polythio-phene (Thaler, 2002), with reversible physico-chemical properties and sensitivity to groups of volatiles (Turner and Magan, 2004). When exposed to headspace samples, volatile compounds interact and bind with these polymer backbones making them swell and altering their conductance capability or resistance (Gouma and Sberveglieri, 2004; Thaler, 2002). The response of the sensors may depend on the polymer's molecular volume, branching of the polymer chain, and hydrogen bonding (Gouma and Sberveglieri, 2004).

An alternative sensing backbone is made by mixing carbon black and a polymer in a solvent (Munoz et al., 1999). The mixture is then placed on a substrate between two metal electrodes where the solvent evaporates leaving behind a composite film (Munoz et al., 1999). In carbon black arrays, the carbon black provides conductivity while the polymer allows for selectivity (Munoz et al., 1999). These arrays undergo a change in resistance when reversibly swelling after exposure to headspace volatiles (Munoz et al., 1999). Carbon black sensors can discriminate even when high humidity may be a problem for other electronic noses (Munoz et al., 1999).

2.5 APPLICATIONS OF THE ENOSE IN THE MEDICAL FIELD

As Table 2.2 shows many diseases and disorders are commonly associated with certain smells. However due to lack of precision and subjectivity, a physician's sense of smell is not commonly used to diagnose these diseases (Thaler, 2002). Instead a patient usually must undergo testing which take a long time and in some instances is invasive and painful. Researchers have realized this and have investigated the use of electronic nose devices in disease diagnosis. Using standard cultural methods, bacterial infection diagnosis takes at least 24 to 48 hours (Turner and Magan,

2004), but an electronic nose could provide the same diagnosis within minutes (Thaler, 2002; Turner and Magan, 2004), allowing for immediate treatment of the infection. Table 2.3 showcases some areas in which an electronic nose has been utilized in the diagnosis of a given disorder or disease.

Table 2.3. Applications of electronic nose devices in the medical field.

Sample Source	Application	Sensor Technology – Instrumental System (Manufacturer)	Results	Reference
Blood	Monitor haemodialysis	CP - Bloodhound BH-114 (Bloodhound Sensors, Leeds, UK)	Discriminate between pre- and post-dialysis blood	(Fend et al., 2004)
Blood	Bacteria identification	CP - Cyranose 320 (Smith Detections, Pasadena, CA)	100% successful classification	(Yates et al., 2005)
Body Odor (leg skin)	Detect renal dysfunction	MOS - Laboratory-made	95.2% correct classification between healthy patients and those with renal failure	(Voss et al., 2005)
Breath	Detect lung cancer	SAW – Laboratory-made	Identified VOCs in breath of lung cancer patients	(Chen et al., 2005)
Breath	Lung cancer identification	QMB - Libranose, (University of Rome 'Tor Vergata' and Technobiochip, Italy)	Correctly identified 100% lung cancer and 94% reference patients	(Di Natale et al., 2003)
Breath	Asthma detection	CP - Cyranose 320 (Smith Detections, Pasadena, CA)	Up to 100% cross validation values and 5.32 M-Distance between asthmatic and control patients	(Dragonieri et al., 2007)
Breath	COPD and lung cancer discrimination	CP - Cyranose 320 (Smith Detections, Pasadena, CA)	85% cross validation values and 3.73 M-Distance	(Dragonieri et al., 2008)
Breath	Detect ventilator-associated pneumonia	CP - Cyranose 320 (Smith Detections, Pasadena, CA)	$r^2 = 0.81$ between enose and pneumonia scores	(Hanson and Thaler, 2005)
Breath	Detect pneumonia	CP - Cyranose 320 (Smith Detections, Pasadena, CA)	At least 80% prediction accuracy	(Hockstein et al., 2004)
Breath	Detect pneumonia	CP - Cyranose 320 (Smith Detections, Pasadena, CA)	At least 66% prediction accuracy	(Hockstein et al., 2005)
Breath	Diagnose uremia	PQC – (Smart Biotechnology Co., Ltd., Taipei, Taiwan)	Correctly classified 86.78% of samples	(Lin et al., 2001)
Breath	Detect lung cancer	CP - Cyranose 320 (Smith Detections, Pasadena, CA)	90.3% prediction accuracy	(Machado et al., 2005)

Sample Source	Application	Sensor Technology – Instrumental System (Manufacturer)	Results	Reference
Breath	Assess oral malodor intensity	MOS - FF-1 (Shimadzu Ltd, Kyoto, Japan)	r = 0.78 between standard test and enose score	(Nonaka et al., 2005)
Breath	Assess oral malodor	MOS - FF-1 (Shimadzu Ltd, Kyoto, Japan)	r = 0.71 between standard test and enose score	(Tanaka et al., 2004)
Breath (from nose)	Diagnose diabetes	Unknown – Laboratory-made	Discrimination between healthy and diabetic patients	(Wang et al., 1997)
CSF and serum	Distinguish CSF from serum	CP - Cyranose 320 (Smith Detections, Pasadena, CA)	M-Distances > 5.0 between groups	(Aronzon et al., 2005)
Culture	Detect bacteria that cause eye infections	CP - Cyranose 320 (Smith Detections, Pasadena, CA)	Up to 96% prediction accuracy	(Dutta et al., 2002)
Culture	Detect *M. tuberculosis*	CP - Bloodhound BH-114 (Bloodhound Sensors, Leeds, UK)	89% prediction accuracy	(Fend et al., 2006)
Culture	Detect *M. tuberculosis*	CP - Bloodhound BH-114 (Bloodhound Sensors, Leeds, UK)	Up to 100% correct identification of TB cultures	(Pavlou et al., 2004)
Culture (Microbes)	Identification of clinically important microbes	MOS - AirSense PEN2 (AirSense Analytical, Schwerin, Germany)	Up to 100% correct identification	(Moens et al., 2006)
Culture (Tumor Cell Lines)	Detect cancer cells	CP - Cyranose 320 (Smith Detections, Pasadena, CA)	M-Distances from 1.0 to 8.5	(Gendron et al., 2007)
Known Bacteria	Upper respiratory bacterial pathogen identification	CP - Cyranose 320 (Smith Detections, Pasadena, CA)	M-Distances $\geq$ 3.0	(Lai et al., 2002)
Nasal Secretion	Diagnose chronic rhino sinusitis	SAW – Libranose (Technobiochip, Italy)	6 peaks found in infected group but not control group	(Bruno et al., 2008)
Root Canal	Infection evaluation	MOS - FF-1 (Shimadzu Ltd, Kyoto, Japan)	Useful for objective evaluation of root canal odor	(Yamada et al., 2007)

Sample Source	Application	Sensor Technology – Instrumental System (Manufacturer)	Results	Reference
Serum (Badgers)	Diagnose cancer cells	CP - Bloodhound BH-114 (Bloodhound Sensors, Leeds, UK)	Diagnosis as soon as 3 weeks after infection	(Fend et al., 2005)
Serum (Cattle)	Diagnose cancer cells	CP - Bloodhound BH-114 (Bloodhound Sensors, Leeds, UK)	Diagnosis as soon as 3 weeks after infection	(Fend et al., 2005)
Sevoflurane (Anesthetic Mixture)	Detect anesthetic dose level	QMB – Laboratory-made	95% success rate	(Saraoglu and Edin, 2007)
Skin Surface	Melanoma identification	QMB – Laboratory-made	80% accuracy in melanoma prediction	(D'Amico et al., 2008)
Sputum	Detect *M. tuberculosis*	CP - Bloodhound BH-114 (Bloodhound Sensors, Leeds, UK)	Detection limit of $1x10^4$ mycobacteria per ml	(Fend et al., 2006)
Sputum	Detect *M. tuberculosis*	CP - Bloodhound BH-114 (Bloodhound Sensors, Leeds, UK)	Up to 100% correct identification of TB cultures	(Pavlou et al., 2004)
Swabs (Infection Site)	Ear, nose, and throat infection identification	CP - Cyranose 320 (Smith Detections, Pasadena, CA)	Up to 98% prediction accuracy	(Dutta et al., 2004)
Swabs (Vaginal)	Screening for bacterial vaginosis	CP – AromaScan (AromaScan plc, Crew, Cheshire, UK)	61.5% positive predictive value test	(Chandiok et al., 1997)
Sweat	Skin odor analysis	QMB – Laboratory-made	Sufficient sensitivity	(Di Natale et al., 2000)
Urine	Diagnose bacteriuria	Unknown – Osmetech Microbial Analyzer	Sensitivity of 83.5% and selectivity of 87.6%	(Aathithan et al., 2001)
Urine	Urinary tract infection identification	CP - Bloodhound BH-114 (Bloodhound Sensors, Leeds, UK)	At least 80% predictability	(Kodogiannis et al., 2008)
Urine	Bacteria identification	CP - Cyranose 320 (Smith Detections, Pasadena, CA)	80% successful classification	(Yates et al., 2005)

2.6 USE OF AN ENOSE FOR DETECTING OVERTRAINING

As Table 2.3 shows, previous researchers have used electronic noses to sniff many different kinds of biological samples for the detection of many different afflictions. Additionally exhaustive exercise has been affiliated with an accelerated induction of unbalance between ROS generation and antioxidant defenses (El-Sayed et al., 2005), resulting in oxidative stress (Aguilo et al., 2005; Cooper et al., 2002; Finaud et al., 2006; Witt et al., 1992). Therefore, it is proposed an electronic nose will be able to detect oxidative stress due to physical exercise in the breath of athletes. If proved true, an enose could be an important instrument for the detection of the onset of overtraining in athletes. In addition, an enose could be used to assist athletes in obtaining their maximum athletic potential by providing coaching and medical staffs with important information regarding current training level and thus allowing these staffs to provide more immediate changes to the training program to obtain the desired result (training optimization).

2.7 RESEARCH IMPACT

Optimal athletic performance is accomplished when athletes train at high work loads for sustained periods of time. Consequently, injuries and overtraining often occur. Previous investigators have studied a variety of biomarkers, including hormone and enzyme levels, without conclusively identifying a clinically specific marker for overtraining and oxidative stress. The above usually require invasive testing and hours to days before results are known which does not allow medical athletic staffs to make real time training load adjustments. The use of an enose is noninvasive and will allow for immediate feedback on training stress so individual athletic performance can be optimized.

Athletes and overtraining were chosen as the model for this study because of their high levels of induced oxidative stress under a controlled environment. Developing an electronic nose diagnostic method for overtraining, and oxidative stress, could lead to other medical diagnostic opportunities in the broader population. Heart disease, cancer, and other diseases due to oxidative stress may then be rapidly and noninvasively diagnosed and monitored using an electronic nose as well.

References

Aathithan, S., J.C. Plant, A.N. Chaudry, and G.L. French. 2001. Diagnosis of bacteriuria by detection of volatile organic compounds in urine using an automated headspace analyzer with multiple conducting polymer sensors. J Clin Microbiol 39:2590-3.

Aguilo, A., P. Tauler, E. Fuentespina, J.A. Tur, A. Cordova, and A. Pons. 2005. Antioxidant response to oxidative stress induced by exhaustive exercise. Physiol Behav 84:1-7.

Anglem, N., S.J. Lucas, E.A. Rose, and J.D. Cotter. 2008. Mood, illness and injury responses and recovery with adventure racing. Wilderness Environ Med 19:30-8.

Armstrong, L.E., and J.L. VanHeest. 2002. The unknown mechanism of the overtraining syndrome: clues from depression and psychoneuroimmunology. Sports Med 32:185-209.

Aronzon, A., C.W. Hanson, and E.R. Thaler. 2005. Differentiation between cerebrospinal fluid and serum with electronic nose. Otolaryngol Head Neck Surg 133:16-9.

Ballabio, D., M.S. Cosio, S. Mannino, and R. Todeschini. 2006. A chemometric approach based on a novel similarity/diversity measure for the characterisation and selection of electronic nose sensors. Anal Chim Acta 578:170-7.

Booth, C.K., B. Probert, C. Forbes-Ewan, and R.A. Coad. 2006. Australian army recruits in training display symptoms of overtraining. Mil Med 171:1059-64.

Bosenberg, A.T., J.G. Brock-Utne, S.L. Gaffin, M.T. Wells, and G.T. Blake. 1988. Strenuous exercise causes systemic endotoxemia. J Appl Physiol 65:106-8.

Bosquet, L., L. Leger, and P. Legros. 2001. Blood lactate response to overtraining in male endurance athletes. Eur J Appl Physiol 84:107-14.

Brenner, J.S. 2007. Overuse injuries, overtraining, and burnout in child and adolescent athletes. Pediatrics 119:1242-5.

Bruno, E., M. Alessandrini, F. Ottaviani, A. Delfini, D. Di Pierro, A. Camillo, and A. De Lorenzo. 2008. Can the electronic nose diagnose chronic rhinosinusitis? A new experimental study. Eur Arch Otorhinolaryngol 265:425-8.

Budgett, R., E. Newsholme, M. Lehmann, C. Sharp, D. Jones, T. Peto, D. Collins, R. Nerurkar, and P. White. 2000. Redefining the overtraining syndrome as the unexplained underperformance syndrome. Br J Sports Med 34:67-8.

Chandiok, S., B.A. Crawley, B.A. Oppenheim, P.R. Chadwick, S. Higgins, and K.C. Persaud. 1997. Screening for bacterial vaginosis: a novel application of artificial nose technology. J Clin Pathol 50:790-1.

Chen, X., M. Cao, Y. Hao, Y. Li, P. Wang, K. Ying, and H. Pan. 2005. A Non-invasive detection of lung cancer combined virtual gas sensors array with imaging recognition technique. Conf Proc IEEE Eng Med Biol Soc 6:5873-6.

Cooper, C.E., N.B. Vollaard, T. Choueiri, and M.T. Wilson. 2002. Exercise, free radicals and oxidative stress. Biochem Soc Trans 30:280-5.

Cosca, D.D., and F. Navazio. 2007. Common problems in endurance athletes. Am Fam Physician 76:237-44.

D'Amico, A., R. Bono, G. Pennazza, M. Santonico, G. Mantini, M. Bernabei, M. Zarlenga, C. Roscioni, E. Martinelli, R. Paolesse, and C. Di Natale. 2008. Identification of melanoma with a gas sensor array. Skin Res Technol 14:226-36.

Di Natale, C., A. Macagnano, R. Paolesse, E. Tarizzo, A. Mantini, and A. D'Amico. 2000. Human skin odor analysis by means of an electronic nose. Sensors and Actuators B 65:4.

Di Natale, C., A. Macagnano, E. Martinelli, R. Paolesse, G. D'Arcangelo, C. Roscioni, A. Finazzi-Agro, and A. D'Amico. 2003. Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors. Biosens Bioelectron 18:1209-18.

Dragonieri, S., J.T. Annema, R. Schot, M.P. van der Schee, A. Spanevello, P. Carratu, O. Resta, K.F. Rabe, and P.J. Sterk. 2008. An electronic nose in the discrimination of patients with non-small cell lung cancer and COPD. Lung Cancer.

Dragonieri, S., R. Schot, B.J. Mertens, S. Le Cessie, S.A. Gauw, A. Spanevello, O. Resta, N.P. Willard, T.J. Vink, K.F. Rabe, E.H. Bel, and P.J. Sterk. 2007. An electronic nose in the discrimination of patients with asthma and controls. J Allergy Clin Immunol 120:856-62.

Dutta, R., J.W. Gardner, and E.L. Hines. 2004. Classification of Ear, Nose, and Throat Bacteria Using a Neural-Network-Based Electronic Nose. MRS Bulletin:5.

Dutta, R., E.L. Hines, J.W. Gardner, and P. Boilot. 2002. Bacteria classification using Cyranose 320 electronic nose. Biomed Eng Online 1:4.

El-Sayed, M.S., N. Ali, and Z. El-Sayed Ali. 2005. Haemorheology in exercise and training. Sports Med 35:649-70.

Fend, R., C. Bessant, A.J. Williams, and A.C. Woodman. 2004. Monitoring haemodialysis using electronic nose and chemometrics. Biosens Bioelectron 19:1581-90.

Fend, R., A.H. Kolk, C. Bessant, P. Buijtels, P.R. Klatser, and A.C. Woodman. 2006. Prospects for clinical application of electronic-nose technology to early detection of Mycobacterium tuberculosis in culture and sputum. J Clin Microbiol 44:2039-45.

Fend, R., R. Geddes, S. Lesellier, H.M. Vordermeier, L.A. Corner, E. Gormley, E. Costello, R.G. Hewinson, D.J. Marlin, A.C. Woodman, and M.A. Chambers. 2005. Use of an electronic nose to diagnose Mycobacterium bovis infection in badgers and cattle. J Clin Microbiol 43:1745-51.

Filaire, E., X. Bernain, M. Sagnol, and G. Lac. 2001. Preliminary results on mood state, salivary testosterone:cortisol ratio and team performance in a professional soccer team. Eur J Appl Physiol 86:179-84.

Filaire, E., B. Legrand, G. Lac, and J.M. Pequignot. 2004. Training of elite cyclists: effects on mood state and selected hormonal responses. J Sports Sci 22:1025-33.

Fillion, L., and P. Gagnon. 1999. French adaptation of the shortened version of the Profile of Mood States. Psychol Rep 84:188-90.

Finaud, J., G. Lac, and E. Filaire. 2006. Oxidative stress : relationship with exercise and training. Sports Med 36:327-58.

Gendron, K.B., N.G. Hockstein, E.R. Thaler, A. Vachani, and C.W. Hanson. 2007. In vitro discrimination of tumor cell lines with an electronic nose. Otolaryngol Head Neck Surg 137:269-73.

Gohil, K., C. Viguie, W.C. Stanley, G.A. Brooks, and L. Packer. 1988. Blood glutathione oxidation during human exercise. J Appl Physiol 64:115-9.

Gouma, P., and G. Sberveglieri. 2004. Novel materials and applications of electronic noses and tongues. MRS Bull 29:697-702.

Halson, S.L., and A.E. Jeukendrup. 2004. Does overtraining exist? An analysis of overreaching and overtraining research. Sports Med 34:967-81.

Halson, S.L., G.I. Lancaster, A.E. Jeukendrup, and M. Gleeson. 2003. Immunological responses to overreaching in cyclists. Med Sci Sports Exerc 35:854-61.

Hanson, C.W., 3rd, and E.R. Thaler. 2005. Electronic nose prediction of a clinical pneumonia score: biosensors and microbes. Anesthesiology 102:63-8.

Hockstein, N.G., E.R. Thaler, Y. Lin, D.D. Lee, and C.W. Hanson. 2005. Correlation of pneumonia score with electronic nose signature: A prospective study. Ann Otol Rhinol Laryngol 114:504-8.

Hockstein, N.G., E.R. Thaler, D. Torigian, W.T. Miller, Jr., O. Deffenderfer, and C.W. Hanson. 2004. Diagnosis of pneumonia with an electronic nose: correlation of vapor signature with chest computed tomography scan findings. Laryngoscope 114:1701-5.

Hynynen, E., A. Uusitalo, N. Konttinen, and H. Rusko. 2006. Heart rate variability during night sleep and after awakening in overtrained athletes. Med Sci Sports Exerc 38:313-7.

Jeukendrup, A.E., and M.K. Hesselink. 1994. Overtraining--what do lactate curves tell us? Br J Sports Med 28:239-40.

Kellmann, M., and K.D. Gunther. 2000. Changes in stress and recovery in elite rowers during preparation for the Olympic Games. Med Sci Sports Exerc 32:676-83.

Kodogiannis, V.S., J.N. Lygouras, A. Tarczynski, and H.S. Chowdrey. 2008. Artificial odor discrimination system using electronic nose and neural networks for the identification of urinary tract infection. IEEE Trans Inf Technol Biomed 12:707-13.

Lai, S.Y., O.F. Deffenderfer, W. Hanson, M.P. Phillips, and E.R. Thaler. 2002. Identification of upper respiratory bacterial pathogens with the electronic nose. Laryngoscope 112:975-9.

Lakier Smith, L. 2003. Overtraining, Excessive Exercise, and Altered Immunity: Is This a T Helper-1 Versus T Helper-2 Lymphocyte Response? Sports Med 33:347-364.

Lieberman, H.R., M.D. Kellogg, and G.P. Bathalon. 2008. Female marine recruit training: mood, body composition, and biochemical changes. Med Sci Sports Exerc 40:S671-6.

Lin, Y., H. Guo, Y. Chang, M. Kao, H. Wang, and R. Hong. 2001. Application of the electronic nose for uremia diagnosis. Sensors and Actuators B 76:4.

Lowery, L., and C.E. Forsythe. 2006. Protein and overtraining: potential applications for free-living athletes. J Int Soc Sports Nutr 3:42-50.

Machado, R.F., D. Laskowski, O. Deffenderfer, T. Burch, S. Zheng, P.J. Mazzone, T. Mekhail, C. Jennings, J.K. Stoller, J. Pyle, J. Duncan, R.A. Dweik, and S.C. Erzurum. 2005. Detection of lung cancer by sensor array analyses of exhaled breath. Am J Respir Crit Care Med 171:1286-91.

Margonis, K., I.G. Fatouros, A.Z. Jamurtas, M.G. Nikolaidis, I. Douroudos, A. Chatzinikolaou, A. Mitrakou, G. Mastorakos, I. Papassotiriou, K. Taxildaris, and D. Kouretas. 2007. Oxidative stress biomarkers responses to physical overtraining: implications for diagnosis. Free Radic Biol Med 43:901-10.

Maso, F., G. Lac, E. Filaire, O. Michaux, and A. Robert. 2004. Salivary testosterone and cortisol in rugby players: correlation with psychological overtraining items. Br J Sports Med 38:260-3.

McVan, B. 1977. Odors: what the nose knows. Nursing 7:46-9.

Moens, M., A. Smet, B. Naudts, J. Verhoeven, M. Ieven, P. Jorens, H.J. Geise, and F. Blockhuys. 2006. Fast identification of ten clinically important micro-organisms using an electronic nose. Lett Appl Microbiol 42:121-6.

Moore, C.A., and A.C. Fry. 2007. Nonfunctional overreaching during off-season training for skill position players in collegiate American football. J Strength Cond Res 21:793-800.

Morgan, W.P., D.R. Brown, J.S. Raglin, P.J. O'Connor, and K.A. Ellickson. 1987. Psychological monitoring of overtraining and staleness. Br J Sports Med 21:107-14.

Morton, R.H. 1997. Modelling training and overtraining. Journal of Sports Sciences 15:335-340.

Munoz, B.C., G. Steinthal, and S. Sunshine. 1999. Conductive polymer-carbon black composites-based sensor arrays for use in an electronic nose. Sensor Review 19:300-305.
Nagle, H.T., R. Gutierrez-Osuna, and S.S. Schiffman. 1998. The How and Why of Electronic Noses. IEEE Spectrum.
Nederhof, E., K.A. Lemmink, C. Visscher, R. Meeusen, and T. Mulder. 2006. Psychomotor speed: possibly a new marker for overtraining syndrome. Sports Med 36:817-28.
Nicholls, A.R., S.H. Backhouse, R.C. Polman, and J. McKenna. 2009. Stressors and affective states among professional rugby union players. Scand J Med Sci Sports 19:121-8.
Nonaka, A., M. Tanaka, H. Anguri, H. Nagata, J. Kita, and S. Shizukuishi. 2005. Clinical assessment of oral malodor intensity expressed as absolute value using an electronic nose. Oral Dis 11 Suppl 1:35-6.
Park, A. 2008. How Phelps Made Swimming History. Time Magazine.
Pavlou, A.K., and A.P. Turner. 2000. Sniffing out the truth: clinical diagnosis using the electronic nose. Clin Chem Lab Med 38:99-112.
Pavlou, A.K., N. Magan, J.M. Jones, J. Brown, P. Klatser, and A.P. Turner. 2004. Detection of Mycobacterium tuberculosis (TB) in vitro and in situ using an electronic nose in combination with a neural network system. Biosens Bioelectron 20:538-44.
Peluso, M.A., and L.H. Guerra de Andrade. 2005. Physical activity and mental health: the association between exercise and mood. Clinics 60:61-70.
Peterson, M.D., M.R. Rhea, and B.A. Alvar. 2004. Maximizing strength development in athletes: a meta-analysis to determine the dose-response relationship. J Strength Cond Res 18:377-82.
Petibois, C., G. Cazorla, J.R. Poortmans, and G. Deleris. 2002. Biochemical aspects of overtraining in endurance sports: a review. Sports Med 32:867-78.
Petibois, C., G. Cazorla, J.R. Poortmans, and G. Deleris. 2003. Biochemical aspects of overtraining in endurance sports : the metabolism alteration process syndrome. Sports Med 33:83-94.
Radak, Z., H.Y. Chung, E. Koltai, A.W. Taylor, and S. Goto. 2008. Exercise, oxidative stress and hormesis. Ageing Res Rev 7:34-42.
Raglin, J.S. 2001. Psychological factors in sport performance: the Mental Health Model revisited. Sports Med 31:875-90.
Rock, F., N. Barsan, and U. Weimar. 2008. Electronic Nose: Current Status and Future Trends. Chemical Reviews 108:705-725.
Saraoglu, H.M., and B. Edin. 2007. E-Nose system for anesthetic dose level detection using artificial neural network. J Med Syst 31:475-82.
Shacham, S. 1983. A shortened version of the Profile of Mood States. J Pers Assess 47:305-6.
Shephard, R.J. 2001. Chronic fatigue syndrome: an update. Sports Med 31:167-94.
Smith, D.J. 2003. A framework for understanding the training process leading to elite performance. Sports Med 33:1103-26.
Smith, L.L. 2004. Tissue trauma: the underlying cause of overtraining syndrome? J Strength Cond Res 18:185-93.
Soprano, J.V., and S.M. Fuchs. 2007. Common Overuse Injuries in the Pediatric and Adolescent Athlete. Clinical Pediatric Emergency Medicine 8:7-14.
Swimming, U.S. 2004. Michael Phelps.
Tanaka, M., H. Anguri, A. Nonaka, K. Kataoka, H. Nagata, J. Kita, and S. Shizukuishi. 2004. Clinical assessment of oral malodor by the electronic nose system. J Dent Res 83:317-21.

Teeple, E., R.M. Shalvoy, and E.R. Feller. 2006. Overtraining in young athletes. Med Health R I 89:236-8.

Thaler, E.R. 2002. Candidate's thesis: the diagnostic utility of an electronic nose: rhinologic applications. Laryngoscope 112:1533-42.

Turner, A.P.F., and N. Magan. 2004. Electronic noses and disease diagnostics. Nature Reviews. Microbiology 2:161-166.

Umeda, T., K. Suzukawa, I. Takahashi, Y. Yamamoto, M. Tanabe, A. Kojima, T. Katagiri, M. Matsuzaka, M. Totsuka, S. Nakaji, and N. Sugawara. 2008. Effects of intense exercise on the physiological and mental condition of female university judoists during a training camp. J Sports Sci 26:897-904.

Urhausen, A., and W. Kindermann. 2002. Diagnosis of overtraining: what tools do we have? Sports Med 32:95-102.

Varlet-Marie, E., F. Maso, G. Lac, and J.F. Brun. 2004. Hemorheological disturbances in the overtraining syndrome. Clin Hemorheol Microcirc 30:211-8.

Voss, A., V. Baier, R. Reisch, K. von Roda, P. Elsner, H. Ahlers, and G. Stein. 2005. Smelling renal dysfunction via electronic nose. Ann Biomed Eng 33:656-60.

Wang, P., Y. Tan, H. Xie, and F. Shen. 1997. A novel method for diabetes diagnosis based on electronic nose. Biosens Bioelectron 12:1031-6.

Witt, E.H., A.Z. Reznick, C.A. Viguie, P. Starke-Reed, and L. Packer. 1992. Exercise, oxidative damage and effects of antioxidant manipulation. J Nutr 122:766-73.

Yamada, Y., Y. Takahashi, K. Konishi, and I. Katsuumi. 2007. Association of odor from infected root canal analyzed by an electronic nose with isolated bacteria. J Endod 33:1106-9.

Yates, J.W., M.J. Chappell, J.W. Gardner, C.S. Dow, C. Dowson, A. Hamood, F. Bolt, and L. Beeby. 2005. Data reduction in headspace analysis of blood and urine samples for robust bacterial identification. Comput Methods Programs Biomed 79:259-71.

Chapter 3: Methodology

Athletic performance is optimized by increasing training doses. However it is challenging to accurately monitor the degree of training stress and an individual athlete's response to a given training load. This makes real-time monitoring and adjusting of training regimens to prevent overtraining challenging. As a result many athletes experience overtraining, resulting in injuries and decreased performance. Previous investigators have studied at a variety of biomarkers but no clinically specific marker for overtraining due to excessive oxidative stress has been identified. Additionally these analyses often require invasive testing and lengthy result turnaround times which do not allow athletic and medical staffs to perform real-time training adjustments.

The aim of this study was to determine whether an electronic nose (enose) would be feasible for the detection of oxidative stresses leading to overtraining in the breath of athletes. Oxidative stresses were first observed in the blood and then compared to breath analyses performed by the enose. In future work, the enose will be trained to recognize different levels of training stress to be used in the optimization of training programs for athletes.

The flowchart (Figures 3.1a through 3.1d) below represents an overview of the steps utilized during this study. The hypothesis was: the enose will be able to differentiate levels of training stress in collegiate long distance runners. This study's objective was to determine the feasibility of using a hand-held enose utilizing conducting polymer based sensors to evaluate training loads resulting in oxidative stresses in long distance runners. Independent variables consisted of (1) weather conditions, (2) participants' diets, (3) acute training stress and (4) cumulative straining stress. Dependent variables included enose response to breath, Profile of Mood States (POMS), and blood analysis: catalase and glutathione peroxidase activities.

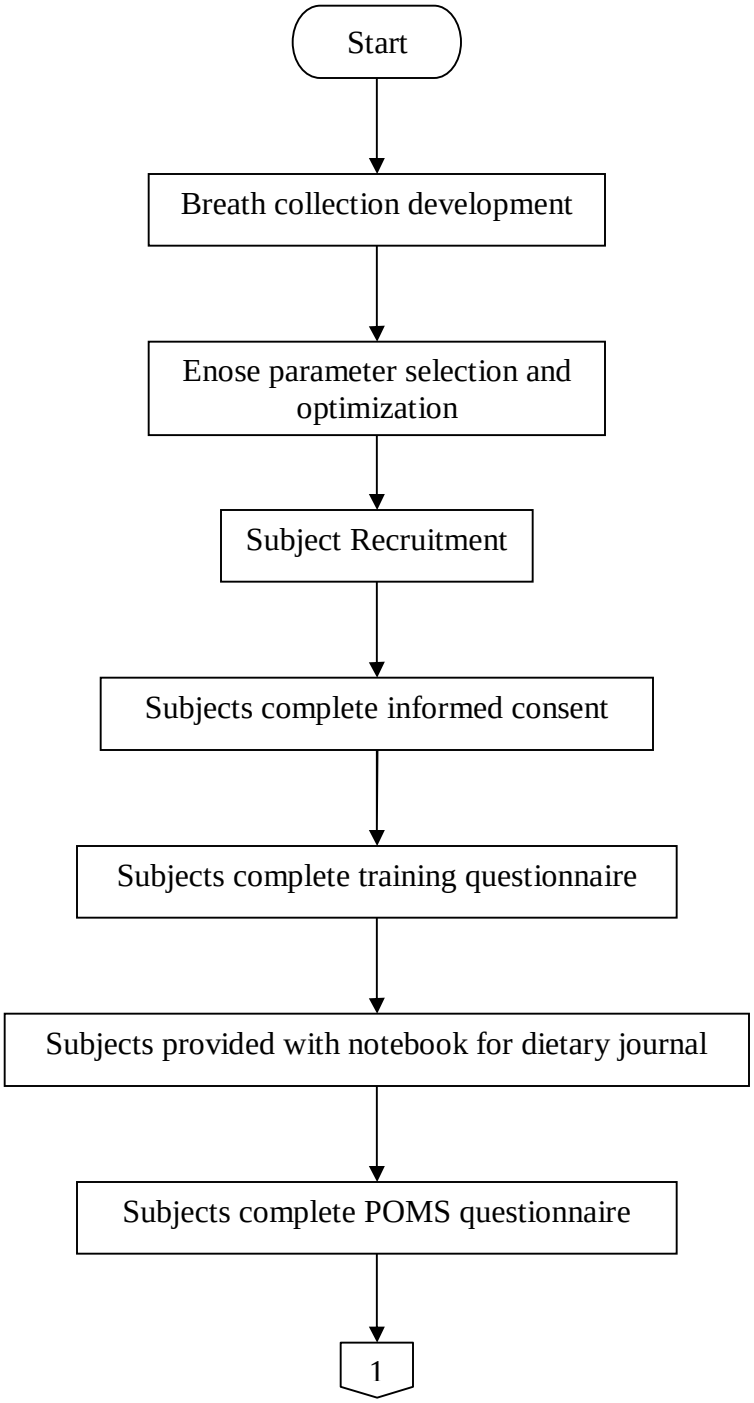

Figure 3.1(a). Flowchart indicating initial steps in the study.

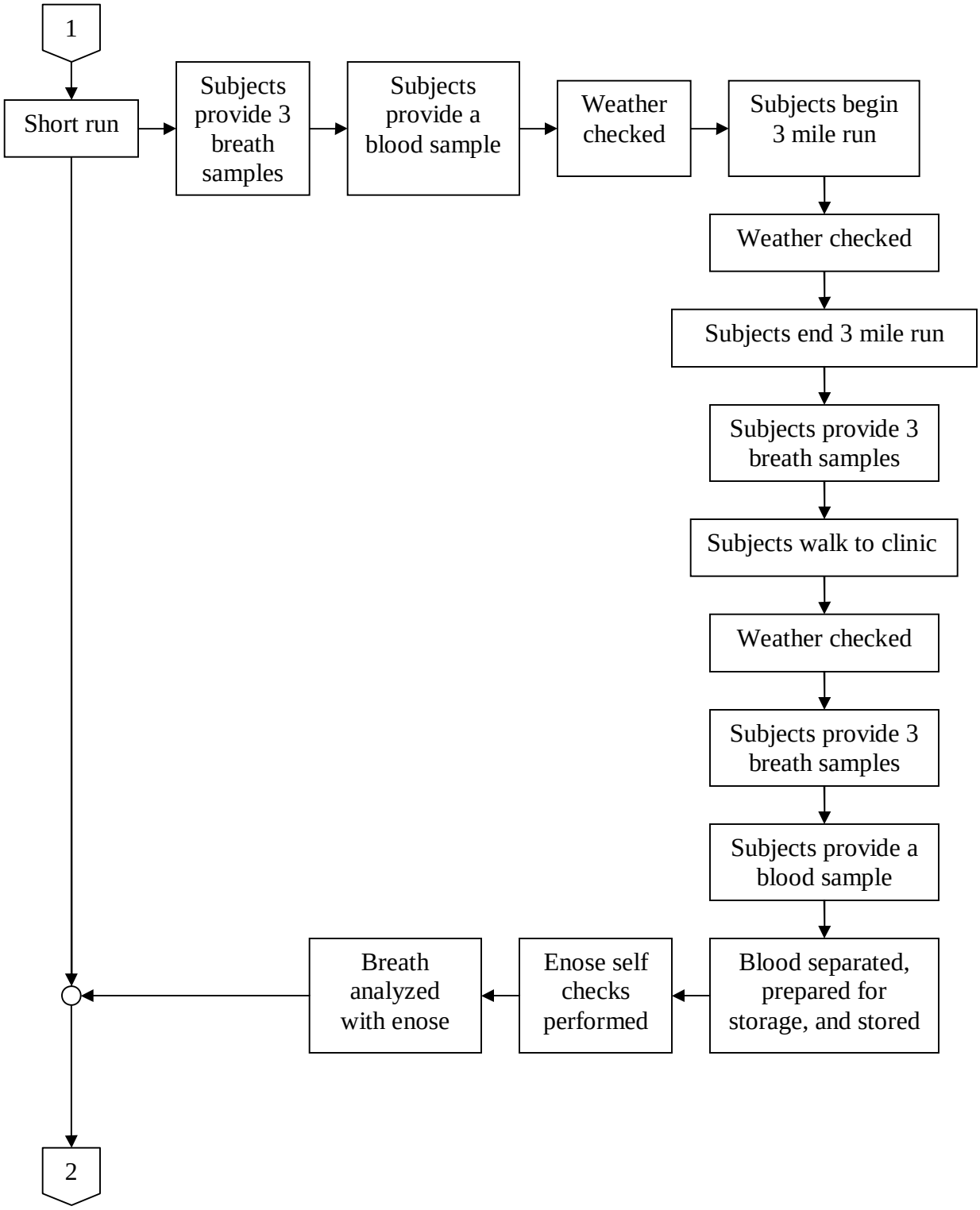

Figure 3.1(b). Flowchart indicating steps taken for the short run.

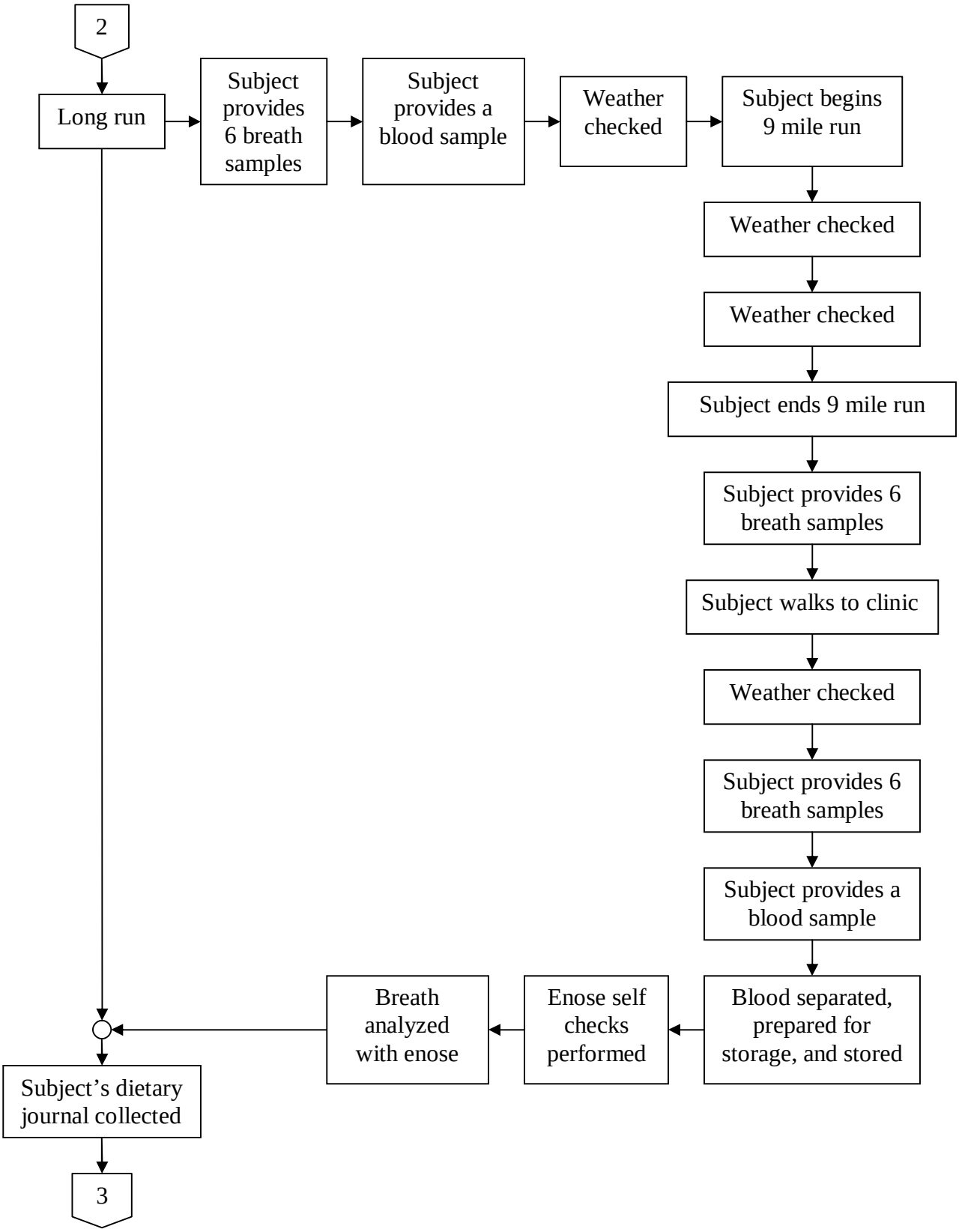

Figure 3.1(c). Flowchart indicating steps taken for the long run.

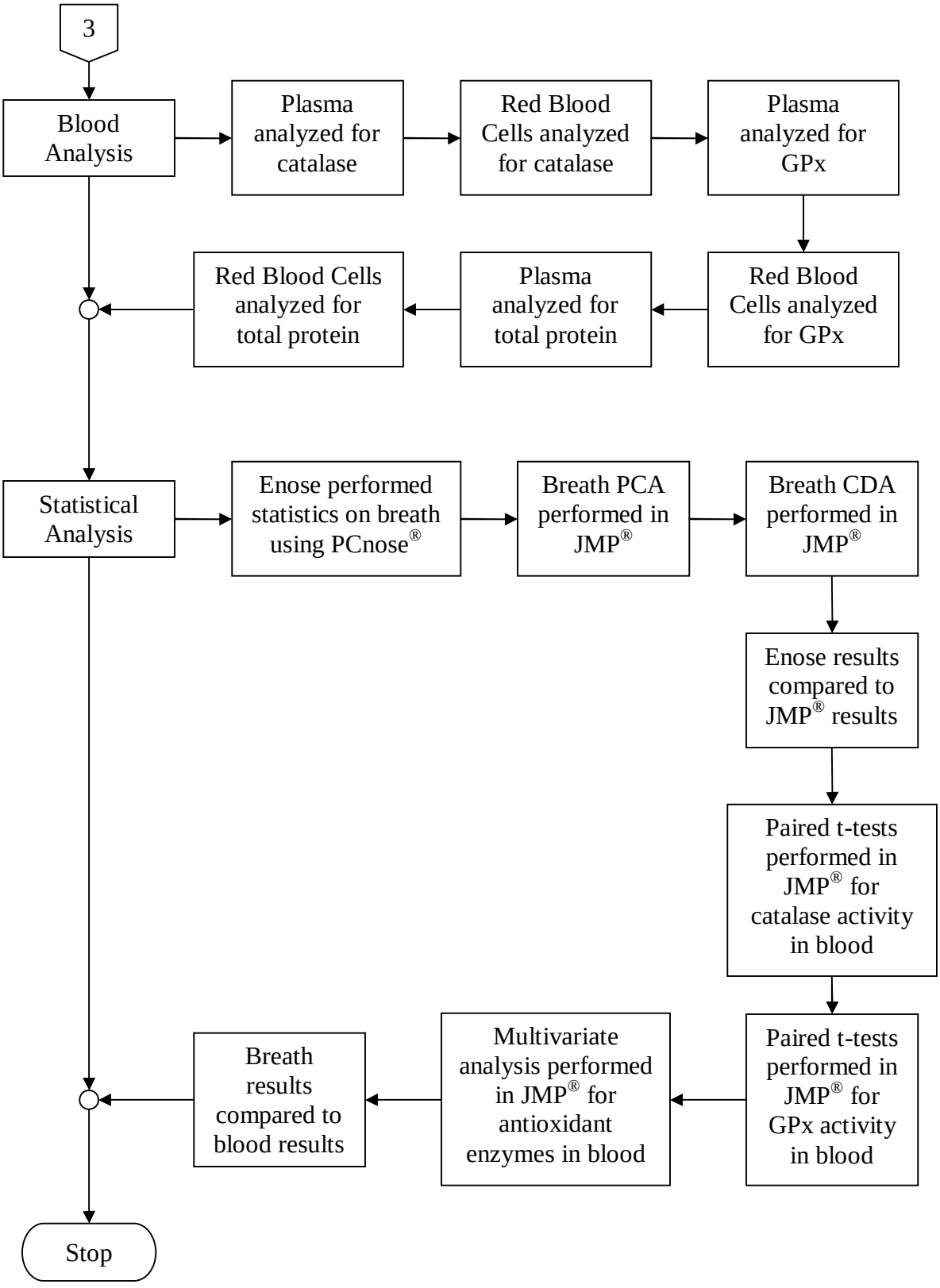

Figure 3.1(d). Flowchart indicating final steps of the study.

3.1 SUBJECTS AND EXERCISE

Two people (one male and one female) actively involved in a collegiate cross country program volunteered for this study. Each subject was informed of the purpose of this study and the possible risks involved before providing written consent to participate. The study protocol was approved by the Edward Via Virginia College of Osteopathic Medicine (VCOM) and the Virginia Tech Institutional Review Boards. Study approval documentation can be found in Appendix A.

Both participants provided three breath samples and a 30 ml blood sample before performing a short run of approximately three miles, lasting about 27 minutes. Immediately after the run, each subject provided three more breath samples. Within 15 minutes after completing the run each subject provided three final breath samples and one final 30 ml blood sample.

Twenty-two days later, the female participant provided six breath samples and a 30 ml blood sample before performing a long run of approximately nine miles, lasting about 80 minutes. Immediately after the run, the subject provided six more breath samples. Within 10 minutes after completing the run the subject provided six final breath samples and one final 30 ml blood sample. The male subject was unavailable to complete the long run.

3.2 ELECTRONIC NOSE APPARATUS

A conducting polymer based hand-held electronic nose (Model: Cyranose 320, Smiths Detection, Pasadena, CA) was used for this study. The device contains an array of 32 conducting polymer-based sensors. As the sensor array is exposed to a sample's headspace, volatiles react with each sensor causing it to reversibly swell. This dynamic response of the sensor array is adjusted by manipulating the amount of time the unit takes to perform each major task: purging and sampling. Purging is the process of bringing background air, such as nitrogen or air, across the sensor panel in order for each sensor to return to normal state. Sampling is the process of bringing sample air across the sensors in order to measure their change to the volatiles present in the sample. Each sensor responds individually and this response is recorded as a

change in resistance over time. Each sensor will respond to a volatile differently but when combined, the array's response is called a smellprint and is unique to each vapor. The sensor panel's response to different samples is compensated for by manipulating the purging and sampling times for the specified sample.

The C320 records a resistance value for each sensor which is normalized for the base resistance. The sensor resistance during baseline gas flow is represented by R_0 and the resistance for the sample headspace after reaching steady state is represented by R_{max}. Therefore the recorded resistance is calculated by $(R_{max} - R_0)/R_0$.

Unlike a gas chromatography unit, the C320 does not identify specific volatiles in a given headspace sample, but it can be trained to identify unknown samples by their unique smellprints. Using the training mode, the device is programmed to recognize samples by exposing the sensor array to the headspace of known samples. When identifying a sample, the sensor array is exposed to an unknown sample. It then compares this smellprint to the saved smellprints and attempts to identify the unknown sample.

The selected device is small and hand-held and does not require intensive training to utilize. As it is also noninvasive and provides results rapidly, it is amenable to use in the field. For this study it was used to analyze breath samples provided by long distance runners.

3.3 C320 PARAMETER OPTIMIZATION

When connected to a computer, data from the sensor array can be easily monitored and settings adjusted as necessary. PCnose® (Smiths Detection, Pasadena, CA), the C320 software package was used to optimize testing settings (Athamneh, 2006), access datasets, and to monitor the sensors' response to breath samples. Table 3.1 presents the selected times spent on each enose process during analysis of breath samples.

Table 3.1. Enose settings utilized to analyze breath.

	Setting	Time (s)
Baseline:	purge	20
Sample:	draw 1	50
	draw 2	0
Purge:	snout removal	0
	1st sample gas purge	0
	1st air intake purge	5
	2nd sample gas purge	40
	2nd air intake purge	0

3.4 BREATH

3.4.1 BREATH COLLECTION

Subjects provided breath samples by breathing into an alveolar air collection device (GaSampler System, QuinTron Instrument Company, Milwaukee, WI). As Figure 3.2 shows, the subject inhales normally and places the mouthpiece in her mouth. The subject then exhales slowly until both the discard and the collection bags are filled. A cap is then placed on the collection bag and the sample is stored until analyzed, as shown in Figure 3.3. The sample can be stored for up to 10 hours. Each sample contains 750 ml of breath, or alveolar air.

Figure 3.2. The author demonstrates the correct way to provide a breath sample.

Figure 3.3. Breath samples were collected in an alveolar air collection device and stored for up to 10 hours in the collection bag.

3.4.2 BREATH ANALYSIS

Before any breath samples were analyzed by the electronic nose, a series of checks were performed to make sure the unit was functioning properly. First a self test was performed by the unit. A result of "Test Complete-No Sensors Out of Range" was considered acceptable. Second a manual resistance check was performed. The resistance measured by the unit for each sensor was checked to make sure it was between 0.2 kΩ and 70 kΩ. Both of these tests were performed immediately prior to analyzing any breath samples.

Additionally, a test was developed to detect enose functionality when sampling was predicted a few days in advance. Two types of samples are utilized for this test: 10 mason jars with 10 ml isopropyl alcohol (IPA) and 10 mason jars sealed off with no sample (Air). Each jar has a hole cut into the lid which is covered with a piece of craft foam serving as a septum. Sample jars were made up and then left to set for at least 10 minutes. Using the settings shown in Table 3.2, the C320 was trained to each sample. Ten sample jars were then randomly chosen and presented to the C320, and the unit was asked to identify the sample in the jar. Once the enose had performed as expected, it was assumed to be working properly and sample collection and testing followed.

Table 3.2. Enose settings for analyzing air and isopropyl alcohol.

	Setting	Time (s)
Baseline:	purge	10
Sample:	draw 1	40
	draw 2	0
Purge:	snout removal	0
	1st sample gas purge	0
	1st air intake purge	5
	2nd sample gas purge	30
	2nd air intake purge	0

Sometimes the first few samples the enose analyzes are not performed correctly due to a "first sniff issue." Therefore before any samples were analyzed, the unit was purged continuously for at least five minutes. This was to ensure there were no remaining volatiles left on the sensors from previous tests. A few breath samples provided by the author were also used to train the

enose to allow for conditioning of the sensors. These data were then deleted before continuing with actual sample analysis. After completing a constant purge and the sensors were conditioned, breath samples were analyzed using the training method.

To prevent vacuum conditions within the bag, the large port cap was removed. The luer port was then quickly opened and the enose snout inserted into it (Figure 3.4). Once the C320 completed drawing the sample, the snout was removed from the bag. This was repeated for all samples in a class before moving onto the next class. Figure 3.5 shows the analysis of a breath sample and the streaming data collected using PCnose®. Breath samples were analyzed within 10 hours of collection.

Figure 3.4. The C320 snout is inserted into the luer port to analyze the breath sample.

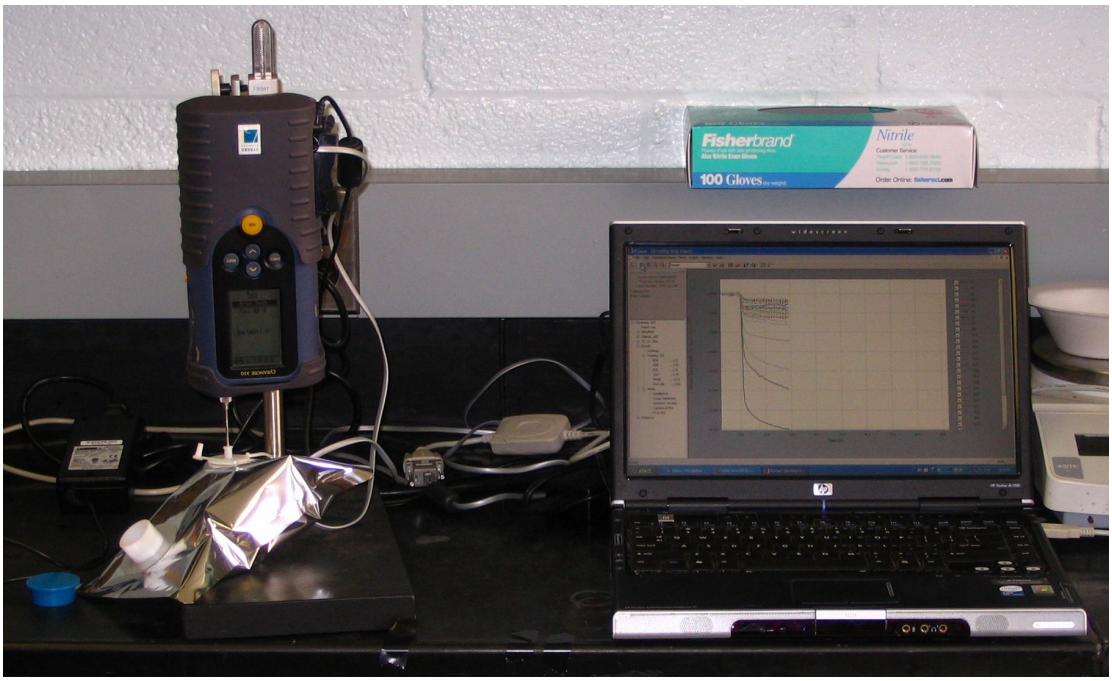

Figure 3.5. Streaming data is collected using PCnose software.

3.5 BLOOD

3.5.1 BLOOD COLLECTION

One of the sports medicine physicians venously collected blood into three 10 ml tubes (BD Vacutainer®, BD Diagnostics, Franklin Lakes, New Jersey) containing EDTA, an anticoagulant. Standard sterile procedures were utilized and any complications created by the venipuncture were immediately addressed at the time blood was drawn. The samples were immediately placed on ice and transported to the laboratory before being processed for storage.

Two tubes (or 20 ml) of blood from each sample were processed for plasma. Each tube was centrifuged (IEC Gentra GP8R) at 1000 g and 4°C for 20 minutes. The plasma from each tube was then removed and stored at -80°C until analysis.

The remaining red blood cells (RBC) were washed by resuspending them in equal amounts of sterile ice-cold phosphate buffer solution (PBS) and centrifuging at 1000 g and 4°C for 10 minutes. The supernatant was then drawn off and discarded. The red blood cells were

resuspended and centrifuged and the supernatant drawn off and discarded one more time. The red blood cells were then stored at -80°C until analysis.

The remaining one tube (or 10 ml) of blood from each sample was processed and stored as whole blood. Blood was mixed with 12 percent perchloric acid containing NEM at a one to one ratio. The mixture was then centrifuged at 10,000 g and 4°C for 15 minutes (Model: Avanti J-301, Beckman Coualter™, Fullerton, CA). The supernatant was then drawn off by pipette and stored at -80°C.

3.5.2 BLOOD ANALYSIS

Catalase Activity Analysis

Catalase is an enzyme which catalyzes the decomposition of hydrogen peroxide to water and oxygen (Equation 3.1). When in the presence of the ultraviolet (UV) range, the absorption of hydrogen peroxide increases as wavelength decreases. At a wavelength of 240 nm the decomposition of H_2O_2 by catalase can be directly observed as the absorbance decreases. These principles were utilized to analyze plasma and RBC for catalase activity (Aebi, 1983). Catalase activity was calculated using the extinction coefficient of 39.4 $M^{-1}cm^{-1}$ (Bayliak et al., 2006) and expressed as micromoles of H_2O_2 consumed per minute per milligram of sample protein.

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2 \qquad \text{Equation 3.1}$$

A 50 mM phosphate buffer was prepared by mixing 100 ml of KH_2PO_4 well with 150 ml of Na_2HPO_4. The pH was adjusted to 7.0 and the buffer was stored at room temperature until use. A 30 mM H_2O_2 solution was then prepared by mixing 34 µl of 30% hydrogen peroxide with 10 ml of the 50 mM phosphate buffer. This H_2O_2 solution was prepared right before blood analysis and kept at room temperature in between use.

Plasma and RBC samples were quickly thawed and kept on ice when not in use. The UV lamp of the spectrophotometer (Model: DU 800, Beckman Coulter, Inc., Fullerton, CA) was turned

on. A total volume of 0.6 ml was added to each of four quartz cuvettes. First 0.4 ml catalase buffer was added to each cuvette followed by 10 µl of the plasma sample or RBC dilution. All four cuvettes were placed in the spectrophotometer and the unit was calibrated. Next 0.19 ml of the H_2O_2 solution was added, and each cuvette was quickly mixed by placing Parafilm® over the top and inverting approximately five times. Each cuvette was then placed in the spectrophotometer and run at the settings seen in Table 3.3.

Table 3.3. Spectrophotometer settings utilized to analyze plasma and RBC samples for catalase activity.

	Setting	
Wavelengths:	Analytical wavelength	240 nm
	Background wavelength	400 nm
Kinetic:	Number of samples	4
	Interval time	15.0 s
	Total time	2.0 min
	Factor/units	1.0000 mg/ml
	Temperature	25.0°C
UV Lamp:	On	

A blank cuvette was created every time a new set of samples were analyzed. Blanks were created by adding 0.4 ml catalase buffer, 10 µl plasma sample or RBC dilution, and 0.19 ml of deionized water. All blanks were created using sample P14 for plasma or dilution R14 for RBC to keep all analyses consistent. Figure 3.6 shows the layout of how the cuvettes were placed in the spectrophotometer. Testing was repeated for each sample until similar results were obtained to ensure consistent methodology was being used.

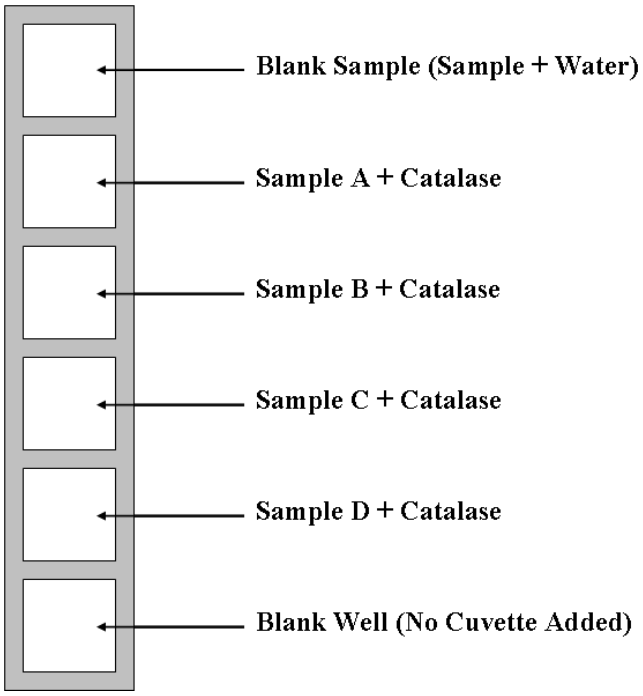

Figure 3.6. Spectrophotometer layout for catalase activity analysis in plasma and RBC samples.

Plasma samples were not diluted for catalase analysis, however RBC samples were. A 1:500 RBC dilution was obtained by first creating a 1:10 dilution by diluting 100 µl red blood cells to 1000 µl using PBS buffer. A 1:50 dilution was then performed by diluting 20 µl of the first dilution to 1000 µl using PBS buffer.

GPx Activity Analysis

Glutathione peroxidase (GPx) is an enzyme which catalyzes the oxidation of glutathione (GSH) by hydrogen peroxide, as shown in Equation 3.2. Glutathione reductase (GR) is an enzyme which catalyzes the conversion of oxidized glutathione (GSSG) by NADPH, as shown in Equation 3.3. The rate at which GSH is formed can be monitored by observing NADPH consumption, hence providing a method for measuring GPx activity (Agergaard and Jensen, 1982). GPx activity for plasma and RBC was calculated using the extinction coefficient of 6.22 mM^{-1} cm^{-1} (Zhu et al., 2008) and expressed as nanomoles of NADPH consumed per minute per milligram of sample protein.

$$2GSH + H_2O_2 \xrightarrow{GPx} GSSG + H_2O$$ Equation 3.2

$$GSSG + NADPH \xrightarrow{GR} 2GSH + NAPD$$ Equation 3.3

A 50 mM potassium phosphate buffer was created by mixing 3.4 g of KH_2PO_4 with 500 ml of deionized water. Next 4.35 g of K_2HPO_4 was mixed with 500 ml of deionized water. 300 ml of the K_2HPO_4 and 200 ml of the KH_2PO_4 were mixed well and the pH was adjusted to 7.0. A 0.05 M potassium phosphate buffer-1mM EDTA buffer was then created by mixing 186 mg of EDTA well with 500 ml of 0.05 M potassium phosphate buffer.

A 10 mM GSH solution was created by mixing 30.7 mg of GSH and 10 ml of deionized water. One ml was placed in each of ten vials. The vials were the stored at -85°C until use.

A 2.4 U/ml GSH-Reductase solution was prepared by mixing 1 µl of 1.67 U/µl GSH-Reductase with 0.7 ml of 0.05 M potassium phosphate buffer-1mM EDTA. The solution was prepared fresh before testing and kept on ice when not in use. Any solution not used was discarded after analysis was complete.

A 1.5 mM NADPH in 0.1% $NaHCO_3$ solution was then created. The 0.1% $NaHCO_3$ was created by mixing 50 mg of $NaHCO_3$ and 50 ml of deionized water. This solution was stored at room temperature. Next 10 mg NADPH and 8 ml of the 0.1% $NaHCO_3$ solution were mixed. Finally 0.5 ml was added to each of eight vials and all were stored at -85°C until use.

A 2 mM H_2O_2 solution was prepared by adding 2 µl H_2O_2 stock (30%) to 10 ml double deionized water and mixed well. This solution was prepared immediately before analysis and kept at room temperature. A GPx buffer containing 2 mM NaN_3 was also prepared immediately before experimentation by mixing 0.1 ml of the azide stock (200 mM, stored at 4°C) with 10 ml GPx buffer.

Samples, NADPH, and GSH were quickly thawed and kept on ice when not in use. A total volume of 0.6 ml was added to each of four cuvettes. First 0.350 ml of GPx buffer + NaN_3

solution was added to each cuvette followed by 10 µl of plasma sample. Next 60 µl of GSH was added, and the cuvette was vortexed briefly to mix. Next 60 µl of GR was added, all cuvettes were placed into the spectrophotometer (Model: DU 800, Beckman Coulter, Inc., Fullerton, CA), and the unit was calibrated. Next 60 µl of the NADPH was added to each cuvette, and they were incubated in the spectrophotometer at 37°C for three minutes. After the cuvettes were removed, 60 µl H_2O_2 was added to each. Each cuvette was then mixed rapidly by placing a piece of Parafilm® over each and inverting approximately five times. Each cuvette was then placed in the spectrophotometer and run at the settings seen in Table 3.4.

Table 3.4. Spectrophotometer settings utilized to analyze plasma and RBC samples for GPx activity.

	Setting	
Wavelengths:	Analytical wavelength	340 nm
	Background wavelength	500 nm
Kinetic:	Number of samples	5
	Interval time	30.0 s
	Total time	5.0 min
	Factor/units	1.0000 mg/ml
	Temperature	37.0°C
UV Lamp:	Off	

A blank cuvette was created every time samples were analyzed. Blanks were created by using the above procedure except 10 µl of deionized water was added instead of the plasma sample. Figure 3.7 shows the layout of how the cuvettes were placed in the spectrophotometer. Testing was repeated for each sample until similar results were obtained to ensure consistent methodology was being used.

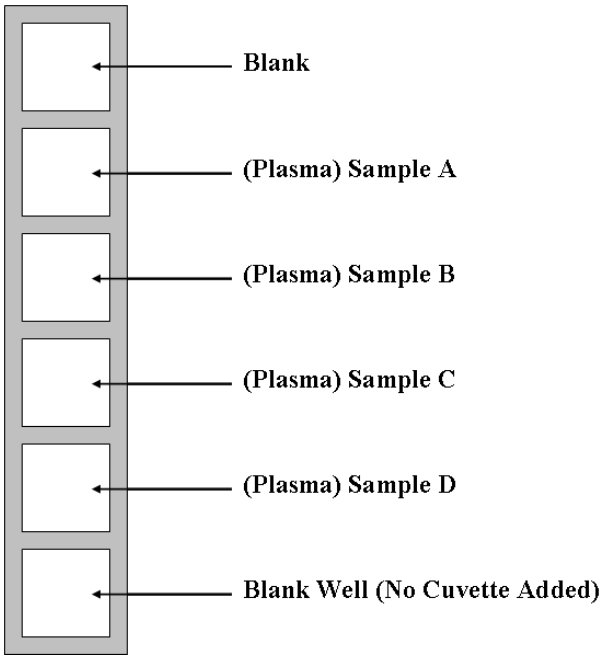

Figure 3.7. Overview of spectrophotometer layout for plasma analysis of GPx activity.

Plasma samples were not diluted for catalase analysis, however RBC samples were. A 1:10 RBC dilution was created by diluting 100 µl RBC to 1000 µl using PBS buffer. This dilution was then used during analysis for GPx activity. The above procedure utilized during plasma analysis was also used for the RBC, except 0.357 ml of the GPx buffer + NaN_3 solution and 3 µl of the RBC dilution were added to each cuvette. Similarly blanks were created by adding 3 µl of deionized water instead of the RBC dilution.

Total Protein Analysis

A Bio-Rad Protein Assay was utilized to determine total protein content in plasma and RBC samples (Laboratories, 2009). During this dye-binding assay, a color change occurs in the dye in response to protein concentration. To determine the unknown concentration of a given sample, the observed color change for that sample is compared to the color change for a known concentration of protein.

A 1:25 plasma dilution was created by diluting 20 µl plasma to 500 µl using PBS buffer. This dilution was then used for analysis for total protein content. 4 µl of the plasma dilution was

added to a clean, dry test tube followed by 796 µl deionized water. 200 µl of the dye was then added and the contents were vortexed to mix thoroughly. Tubes were incubated at room temperature for five minutes at which time the contents of each test tube were emptied into cuvettes, where the contents of one test tube went into one cuvette. The absorbance was then recorded for each cuvette using a spectrophotometer (Model: DU 800, Beckman Coulter, Inc., Fullerton, CA) at 595 nm. Testing was repeated for each sample until similar results were obtained to ensure consistent methodology was being used.

A 1:500 RBC dilution was utilized for this test. A 1:10 dilution was first performed by diluting 100 µl red blood cells to 1000 µl using PBS buffer. A 1:50 dilution was then performed by diluting 20 µl of the first dilution to 1000 µl using PBS buffer. This dilution was then used during analysis for total protein content. The above procedure was used except 20 µl of the RBC dilution and 780 µl of the deionized water were added to each test tube and then cuvette.

A concentration of 1 mg bovine serum albumin (BSA) protein per milliliter was used as the standard for this analysis. The standard was created by adding 10 µl BSA, 790 µl deionized water, and 200 µl of the dye to a test tube and then cuvette. Unless otherwise stated, all chemicals used in the preparation for storage and in analysis of blood were obtained from Sigma Chemical (St. Louis, MO).

3.6 POMS

In order to evaluate the role of mood on performance, a Profile of Mood States (POMS) was obtained from each participant before any running or sample collections were performed. As seen in Appendix B, POMS is an adjective checklist leading to the assessment of transient and distinct mood states (Shacham, 1983). Participants rated each adjective according to how they were feeling based upon a five-point scale where 1 was Not at All and 5 was Extremely. A mood state classification can then determined by adding the five negative mood states (tension, depression, anger, fatigue, and confusion) and subtracting the one positive mood state (vigor) (Filaire et al., 2004; Morgan et al., 1987).

3.7 TRAINING LOG

To better understand the training stress directly attributed to the training performed during this study, it is important to have background knowledge about training typically performed outside of the study. As Appendix C shows, a questionnaire was created to assist in the understanding of each participant's individual training program. Both subjects completed the training log before any sample collections or running took place.

3.8 DIETARY JOURNAL

Oxidative stress can overwhelm the body's antioxidant defense system. Therefore previous investigators have indicated dietary intake, such as antioxidant supplementation, specifically vitamin E and C supplements, may alter oxidative stress levels (Aghdassi et al., 2003). Diet and supplement intake information was collected through a personal dietary recall journal kept by each study participant.

3.9 WEATHER DURING RUNS

Blacksburg temperature and humidity measurements were obtained through the National Oceanic and Atmospheric Administration (NOAA) website (Service, 2009). The National Weather Service has a weather forecast station located at 1750 Forecast Drive, Blacksburg VA. Weather parameters were obtained immediately prior to the runs, during the runs, and immediately at the end of the runs in order to understand the weather conditions affecting the participants. A summary of these parameters can be seen in Table 4.1.

3.10 STATISTICAL ANALYSIS

Statistical analysis methods typically associated with electronic nose technologies have been extensively reviewed by Deventer (2001), who used an enose to detect retained printing solvent levels in food packaging, and Pathange (2003), who used an enose to detect apple maturity. Similar methods were utilized during this study in order to accomplish the primary goal of

determining the feasibility of an electronic nose to detect training stress in the breath of athletes. The results obtained from the enose unit were also compared to an independent statistical analysis to determine if this analysis could separate the identified groups further from each other. Additionally blood samples were analyzed in an attempt to identify how exercise affects catalase and GPx activity levels, hence the oxidative stress present in the body.

After all breath samples were collected for a run, an electronic nose sniffed each sample. The unit then performed a cross validation analysis and the results were examined to determine how well the training set conformed to the proposed model. A correct prediction of 100 percent indicates all sample exposures were accurately placed into one of the six named classes. For this study these classes were before short run (BSR), after short run on the field (ASRField), after short run in the clinic (ASRClinic), before long run (BLR), after long run on the field (ALRField), and after long run in the clinic (ALRClinic). The unit's software, PCnose® (Cyrano Sciences, Inc., Pasadena, CA) was then utilized to view a principal component analysis (PCA) projection plot and a canonical projection plot. These plots were visually inspected for separations between and general trends in the sample clusters. Finally the interclass Mahalanobis distances were obtained for each sample class to determine if there was enough separation between each, which was defined as five standard deviations away from all the others (Deventer, 2001).

An important part of this study was to compare the results provided by the enose to the results obtained through a separate statistical analysis performed by JMP® (JMP®, SAS Institute, Inc., Cary, NC). If the results are improved in the independent analysis, a proprietary opportunity to develop an enose, or enose statistical software, which is more sensitive to oxidative stress volatiles present in the breath may exist. A disadvantage to using the C320 and its software is that only six sample classes, at a maximum of 10 exposures each, can be analyzed at a time. Although this was not a problem at this particular time, when this study is expanded to include many more breath samples, this will be a significant constraint. Additionally, PCnose® also requires a minimum of five exposures per sample class in order to perform its statistical analyses (Cyrano Sciences, 2000). However, as this was a pilot study, it was necessary to explore how an

individual athlete may affect the enose detecting oxidative stress in the breath. As limited data was available, this was not always possible for the enose unit alone to accomplish.

Data collected from all 32 sensors were used for the independent analyses. JMP®'s Multivariate Methods function was used to analyze each of the following groups: female performing the short run, male performing the short run, both subjects performing the short run, female performing the long run, and both runners performing both runs. The female long run group is the same as both runners performing the long run as the male participant was not able to perform this run due to scheduling conflicts.

A PCA was performed for each sample group using JMP®'s Principal Components function. The number of principal components was selected by evaluating the Eigen values and cumulative variation explained for each. These components were then utilized to perform five canonical discriminant analyses (CDA) using JMP®'s Discriminant function. A table summarizing cross validation results and a plot showing the sample clusters were then obtained for each CDA analysis. Finally the Mahalanobis distance for each sample was obtained using JMP®'s Multivariate function. These distances were observed to determine whether each exposure had been placed within the correct sample class, or within five standard deviations of that group (Deventer, 2001).

Plasma and RBC samples were each analyzed for catalase and GPx activity levels. Tests were performed in at least duplicates and results presented indicate an average $\pm$ standard deviation for the sample class. JMP®'s Matched Pairs function was then utilized to perform a paired t-test for each of six groups: the female participant performing the short run, the male participant performing the short run, both participants performing the short run, the female participant performing the long run, and both runners performing both runs. The female long run situation also represents the long run being performed by both participants since the male participant was not able to complete the long run due to scheduling conflicts. The t-tests evaluated the differences between samples collected before the run and after the run for each of the given runner and run combinations. Statistical significance was first considered at an alpha level of 0.05, however because there were so few data points, the alpha level was increase to 0.10 to see

if there was an increase in significant results. P-values for both alpha levels are presented in Chapter 4.

As was mentioned in Chapter 2, a plethora of biomarkers indicating oxidative stress have been suggested but no one marker, or even a specific set of markers, has been demonstrated as a predictor of overtraining. Since data for two of these proposed markers (catalase and GPx) were collected during this study, a multivariate analysis similar to that performed with the enose data was also performed for the blood data. JMP® was utilized to perform a PCA for each sample group (female performing the short run, male performing the short run, both subjects performing the short run, female performing the long run, and both runners performing both runs), the number of principal components was chosen, and then these principal components were used to perform five CDAs. Finally a table summarizing cross validation results and a plot showing the sample clusters were obtained for each CDA analysis. These tables and plots are presented in Appendix D.

References

Aebi, H.E. 1983. Catalase, p. 273-286, *In* H. U. Bergmeyer, ed. Methods of Enzymatic Analysis, Vol. 3, 3 ed. Verlag Chemie, Deerfield Beach, Florida.

Agergaard, N., and P.T. Jensen. 1982. Procedure for blood glutathione peroxidase determination in cattle and swine. Acta Vet Scand 23:515-27.

Aghdassi, E., B.E. Wendland, A.H. Steinhart, S.L. Wolman, K. Jeejeebhoy, and J.P. Allard. 2003. Antioxidant Vitamin Supplementation in Crohn's Disease Decreases Oxidative Stress: A Randomized Controlled Trial. American Journal of Gastroenterology 98:348-353.

Athamneh, A. 2006. Electronic Nose Evaluation of Grape Maturity, Virginia Polytechnic Institute and State University, Blacksburg.

Bayliak, M., H. Semchyshyn, and V. Lushchak. 2006. Effect of hydrogen peroxide on antioxidant enzyme activities in Saccharomyces cerevisiae is strain-specific. Biochemistry (Mosc) 71:1013-20.

Cyrano Sciences, I. 2000. The Cyranose 320 Electronic Nose User's Manual. 3rd, Revision C ed. Cyrano Sciences, Pasadena, CA.

Deventer, D.V. 2001. Discrimination of retained solvent levels in printed food-packaging using electronic nose systems, Virginia Polytechnic Institute and State University, Blacksburg.

Filaire, E., B. Legrand, G. Lac, and J.M. Pequignot. 2004. Training of elite cyclists: effects on mood state and selected hormonal responses. J Sports Sci 22:1025-33.

Laboratories, B.-R. 2009. Bio-Rad Protein Assay.

Morgan, W.P., D.R. Brown, J.S. Raglin, P.J. O'Connor, and K.A. Ellickson. 1987. Psychological monitoring of overtraining and staleness. Br J Sports Med 21:107-14.

Pathange, L.P. 2003. Non-Destructive Evaluation of Apple Maturity Using an Electronic Nose System, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Service, N.W. 2009. Your National Weather Service Forecast [Online]. Available by National Oceanic and Atmospheric Administration http://forecast.weather.gov/MapClick.php?CityName=Blacksburg&state=VA&site=RNK&textField1=37.2327&textField2=-80.4284 (verified 2008).

Shacham, S. 1983. A shortened version of the Profile of Mood States. J Pers Assess 47:305-6.

Zhu, H., Z. Jia, J.S. Strobl, M. Ehrich, H.P. Misra, and Y. Li. 2008. Potent induction of total cellular and mitochondrial antioxidants and phase 2 enzymes by cruciferous sulforaphane in rat aortic smooth muscle cells: cytoprotection against oxidative and electrophilic stress. Cardiovasc Toxicol 8:115-25.

Chapter 4: Results and Discussion

Blood and breath samples were collected from two athletes before and after they performed a short run and a long run. Breath samples were then analyzed by an electronic nose (enose) followed by an independent statistical analysis performed by JMP®. If the independent analysis provides better results than the enose, a proprietary opportunity may exist for an improved enose unit or software for the unit. Blood samples were analyzed utilizing spectrophotometric methods for catalase and glutathione peroxidase (GPx), two enzymes that fight to reduce oxidative stress occurring in the body. Catalase and GPx were also compared to previous literature for similar studies with athletes. Results are presented and discussed in this chapter.

4.1 EXERCISE

Two participants (one male and one female) of a collegiate cross country team participated in this study. Both participants performed a short run of approximately three miles one morning in early August which took 27 minutes to complete. The female participant also performed a long run of approximately nine miles one morning in late August which took 80 minutes to complete. The male subject was unavailable to complete the long run due to scheduling conflicts.

4.1.1 WEATHER DURING RUNS

Blacksburg temperature and humidity measurements were obtained through the National Oceanic and Atmospheric Administration (NOAA) website (Administration, 2009). The National Weather Service has a weather forecast station located at 1750 Forecast Drive, Blacksburg VA. Weather parameters were obtained immediately prior to the runs, during the runs, and immediately at the end of the runs in order to understand the weather conditions affecting the participants. A summary of these parameters can be seen in Table 4.1. Based on the weather conditions observed, it was cooler (16°C) and more humid (96%) during the long run than the short run (25°C, 72%).

Table 4.1. Weather conditions during participant runs.

Run	Date	Time	Temperature (°C)	Humidity (%)	Dewpoint (°C)	Heat Index (°C)
Start Short	8/ 6/2008	9:00 AM	24	74	19	-
During Short	8/ 6/2008	9:25 AM	25	69	19	26
End Short	8/ 6/2008	9:40 AM	25	74	20	26
Start Long	8/28/2008	7:55 AM	16	94	15	-
During Long	8/28/2008	8:40 AM	16	94	15	-
During Long	8/28/2008	9:09 AM	16	94	15	-
End Long	8/28/2008	9:41 AM	16	100	16	-

4.2 BREATH ANALYSIS – ELECTRONIC NOSE SYSTEM ANALYSIS

A total of 36 breath samples were collected at six different intervals and were analyzed. Half of these samples were collected for the short run: three from each participant immediately prior to performing the run (BSR), immediately after completing the run on the field (ASRField), and after completing the run and walking to the clinic (ASRClinic). The rest of the samples were collected in coordination with the long run: six from the participant immediately prior to performing the run (BLR), immediately after completing the run on the field (ALRField), and after completing the run and walking to the clinic (ALRClinic).

Once all samples were collected and sniffed by the electronic nose (Model: Cyranose 320, Smiths Detection, Pasadena, CA), the unit performed a cross validation analysis in order to examine how the training set conforms to the proposed model. This analysis is performed by removing one exposure from each sample class and constructing the model assuming the remaining exposures are the entire training set. The removed exposures are then treated as unknowns and identified as one of the trained classes. Throughout the cross validation procedure all exposures are left out once. Finally the percent correct prediction is calculated (Cyrano Sciences, 2000). The user may then review how each exposure was identified using PCnose® software (Cyrano Sciences, Inc., Pasadena, CA). For all exposures to be accurately identified into the named classes, a cross validation result of 100 percent is necessary.

As Figure 4.1 shows, 36.1 percent of the breath samples were correctly classified while 63.9 percent of the exposures were incorrectly classified. The poor classification performance by the

electronic nose could be attributed to combining both long and short runs into one group. It may also be due to an incomplete number of responses obtained from the male athlete because he did not perform the long run. Further analyses were performed to explore these variations and have been presented later in this chapter.

Breath samples were collected right at the location the run ended (Field) and then after the athletes had walked to the clinic (Clinic) which took about 10 to 15 minutes. Differences, or similarities, between the samples collected on the field and in the clinic could provide important information about how the body responds to training, specifically oxidative stresses. For example, two ASRField samples were misidentified as ASRClinic, two ASRClinic samples were misidentified as ASRField, and one ALRField sample was misidentified as ALRClinic. This may be an indication of those differences between the samples collected in the field immediately after the run and the samples collected in the clinic were insignificant. In other words, any stress occurring due to training activity detected by the electronic nose in the breath immediately at the end of a session continued until at least 15 minutes after the session has ended. It might therefore be assumed the field and clinic samples are equivalent and that only one after run collection would be necessary. Convenience would dictate this collection time be at the clinic. This would save resources in future studies and may also make real-time monitoring more feasible.

However, three ASRField samples were misclassified as BSR but no ASRClinic samples were misclassified as BSR. This may indicate stress occurring due to training is not immediately detectable by the enose in breath after a training session. Instead it might suggest volatiles due to this oxidative stress do not become detectable in the breath until at least 10 minutes after the session has indeed. If this is found to be true, then both field and clinic samples should continue to be taken during future studies. In future studies it may also be interesting to have the electronic nose detect volatiles in the breath of athletes at more intervals occurring after run completion. This would allow for the observation of how the body recovers from stress due exercising.

No BSR samples were identified as BLR and no BLR samples were classified as BSR samples. The time between runs was 22 days and it can be assumed the participants continued individualized training between the day the short run was performed and the day the long was performed. The enose unit may then be detecting a difference in oxidative stress, and therefore training level, in the athletes. Additionally, the weather conditions were different for the short and long runs and could contribute to the differences in the before run (baseline) samples.

However, one BLR sample was misclassified as ASRField, which may indicate there has been no detectable change in the oxidative stress levels from the time the short run ended to the time the long run began. One explanation for this is that the assumption the athletes had continued their individualized training was incorrect. The only time they were officially asked about their training regimen was right before the short run, so it could be possible they are at the same training level they were before. Another reason could be that the athletes did continue their individualized training but that they are not utilizing methods to fully optimize their training potential. A third explanation is that the C320 unit simply is not able to detect the volatiles due to this change in stress in the breath of the athletes. Yet another reason may be the difference in weather on the training days.

Identified As

Trained As	BSR	ASRField	ASRClinic	BLR	ALRField	ALRClinic
BSR	3	1	1	0	0	1
ASRField	3	1	2	0	0	0
ASRClinic	0	2	3	0	0	1
BLR	0	1	0	2	1	2
ALRField	2	0	0	2	1	1
ALRClinic	0	1	0	2	0	3

Correct : 36.111 %
Incorrect : 63.889 %

Figure 4.1. Chart showing cross validation results performed by the electronic nose unit.

After the C320 has performed a cross validation on the data set, the PCnose® software can be utilized to observe two statistical analyses: Principal Component Analysis (PCA) and Canonical Discriminate Analysis (CDA). These two tests are used to classify the samples, or exposures,

into groups it has been trained to recognize. In this study these classifications are BSR, ASRField, ASRClinic, BLR, ALRField, and ALRClinic. The number of principal components and the principal component scores are first retrieved from the unit and plotted using the PCnose® software, as shown in Figure 4.2. The maximum number of factors available for viewing is three (Cyrano Sciences, 2000).

The cross validation procedure also determines the optimum number of principal components to be used for a CDA. These principal component scores are then used, as opposed to the sensor response data, to calculate the canonical scores. Just like for PCA, the PCnose® software retrieves the canonical factors from the C320 and creates a canonical projection plot, as shown in Figure 4.3.

Although a clear separation between groups is the ideal situation, PCA and CDA plots (Figures 4.2 and 4.3 respectively) indicate four groups whose exposures appear to cluster together: BSR (red), ASRField (blue), ASRClinic (green), and BLR (black). Data points for the ALRField (orange) and ALRClinic (purple) groups are more dispersed. There were 22 days between runs in which it can be assumed the athletes continued their own individual training program. This should increase their athletic performance and increase the oxidative stress their bodies are undergoing which can be seen in the significant separation between BLR and BSR. The change in weather conditions, specifically the heat index present on the day of the short run, may also be responsible for this cluster separation.

Even though some overlapping occurs, there is some difference between the BSR, ASRField, and ASRClinic clusters. This is a good indication a change is occurring, perhaps due to oxidative stress, in the body during training, and that this change is detectable in the breath by an electronic nose. However the distances between the BSR and ASRField groups and between the BSR and ASRClinic groups appear to be different. This may suggest the volatiles in breath due to oxidative stress are not detectable by the C320 until at least 10 minutes after the training session has ended.

Samples collected prior to the run (BSR and BLR) are clustered within their own groups while samples taken after the run (ASRField, ASRClinic, ALRField, and ALRClinic) groups are more spread out over the plot. Furthermore samples taken after the long run are more spread out than those taken after the short run. These differences may occur because the participants' breathing is different before and after runs. Breathing was more rapid upon completing a run which would make providing a breath sample more difficult, especially since a long, steady breath is required for collection. Unfortunately samples were not collected in a fashion for which this can be proved or disproved. Any future breath samples collected should be tracked according to the order they are provided.

The reason for collecting breath samples right on the field and then after athletes walk into the clinic was to determine if they were essentially the same. One indication this is true is that some of the ASRField and ASRClinic data points are mixed together. However most of the ALRField and ALRClinic data points are not mixed together. One hypothesis for this is that volatiles due training stress are not detectable in the breath until at least 10 minutes after the training session has ended.

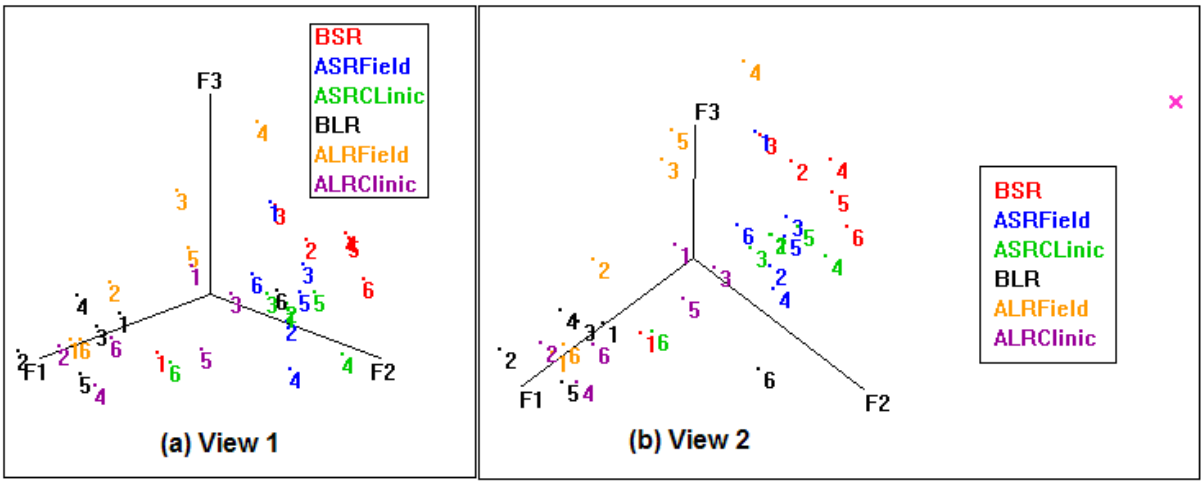

Figure 4.2. PCnose® PCA plots showing the exposures of four groups (BSR, ASRField, ASRClinic, and BLR) clustering together.

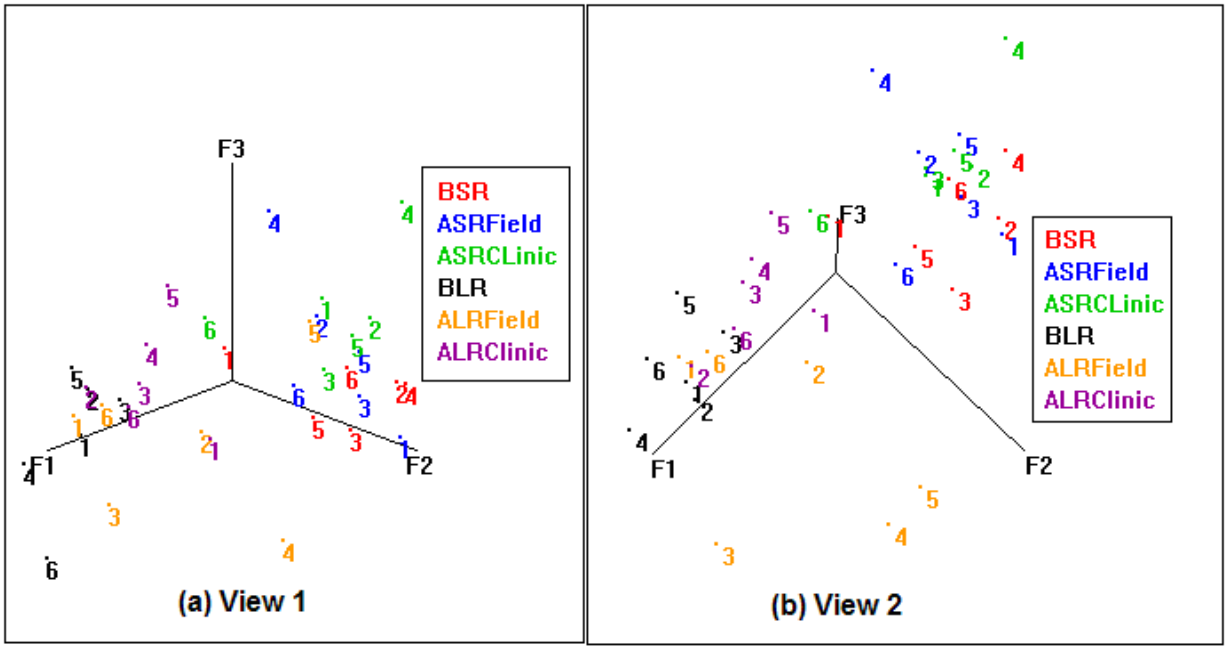

Figure 4.3. PCnose® CDA plots showing exposures from four groups (BSR, ASRField, ASRClinic, and BLR) clustering together.

Mahalanobis distance is a measure which can be used to help determine if each exposure has been placed in the correct group. It is measured in units of standard deviation from the group mean, so a value of five would represent about five standard deviations from the mean, or cover about 99 percent of the population (Deventer, 2001). Mahalanobis distances can also be used to determine how separated the data groups are from each other. Clusters separated with a Mahalanobis distance greater than five would indicate very little overlapping (Deventer, 2001). As Figure 4.4 shows, the C320 unit calculates this interclass Mahalanobis distance between the clusters. The values are then tabulated and displayed in PCnose®.

There were no interclass Mahalanobis distances greater than five for the comparisons of BSR to ASRField and ASRClinic or for the comparisons of BLR to ALRField and ALRClinic. This could be due to the electronic nose being able to detect no significant differences between the breath samples collected before the runs and the breath samples collected after the runs. There are 5.1 standard deviations between the BLR and ASRField clusters and 5.3 standard deviations between the BLR and ASRClinic clusters. This significant separation between the BLR and ASR groups may indicate a significant change in training, and therefore oxidative stress,

occurring between the times the short run ended and the long run began. Both participants are very athletic and while they were not specifically asked about their training regimen between runs, it can safely be assumed they continued their own program outside of the study to improve their athletic abilities.

Interclass M-Distances

	BSR	ASRField	ASRClinic	BLR	ALRField	ALRClinic
BSR		0.749	1.344	4.896	4.615	3.502
ASRField			0.853	5.134	4.996	3.605
ASRClinic				5.254	5.141	3.701
BLR					2.238	1.738
ALRField						2.406
ALRClinic						

Figure 4.4. Interclass Mahalanobis distances as determined by the C320 and displayed by PCnose®.

4.3 BREATH ANALYSIS – INDEPENDENT STATISTICAL ANALYSIS

4.3.1 Principal Component Analysis

A Principal Component Analysis (PCA) was performed using JMP® (SAS Institute, Inc., Cary, NC). The number of optimum principal components was determined for each of five groups by selecting those components having eigen values greater than one (Pathange, 2003) and explained cumulative variation of approximately 90 percent. These components were then saved and used to perform canonical discriminate analyses.

Although the Cyranose 320 unit automatically utilizes three principal components when performing its analyses, none of analyses performed by JMP® selected three principal components. Instead, four principal components accounted for 92.8 percent of the variability for the male runner performing the short run. Two principal components accounted for 90.3 percent of the variability for the female runner performing the short run. Two principal components accounted for 93.5 percent of the variability for the female runner performing the long run. Two

principal components accounted for 91.1 percent of the variability for both runs of the female runner. Two principal components accounted for 89.5 percent of the variability for both runs of both runners. Even though the majority of the situations analyzed selected less than three principal components, as the enose does, at least 89 percent of the total variation was still accounted for.

4.3.2 DISCRIMINANT ANALYSIS

An important part of this study is to compare the results provided by the enose to the results obtained through a separate statistical analysis. If the results are improved in the independent analysis, a proprietary opportunity to develop an electronic nose, or enose statistical software, which is more sensitive to oxidative stress volatiles present in the breath may exist. Advantages to using an external statistics program (JMP®, SAS Institute, Inc., Cary, NC) is the data are easier to manipulate and it is easier to analyze individual groups of data. The components obtained during the PCA were utilized to perform five canonical discriminate analyses (CDA), one for each of the following groups: the male participant for the short run, the female participant for the short run, the female participant for the long run, the female participant for both short and long runs, and both runners for both runs. A cross validation table and a plot showing the data clusters were then obtained for each analysis.

Male Participant – Short Run

The male participant provided nine breath samples which can be seen in Table 4.2. A cross validation correctly places 77.8 percent of the exposures and misclassifies 22.2 percent of them. One of the ASRClinic samples was misclassified as ASRField and one ASRField sample was misclassified as ASRClinic. This may suggest the volatiles present in the breath of athletes after running are the same immediately after and up to 15 minutes after completing the run. However it is important to note the analysis was able to distinguish between samples collected before the run and samples collected after the run. This indicates the electronic nose is able to detect volatiles present in the breath due to training, and therefore oxidative stress.

Figure 4.5 shows three clusters of data for the male runner performing the short run: BSR, ASRField, and ASRClinic. The individual exposures, or samples, are represented by different colored data points while the actual clusters are represented by different colored circles drawn around the data. The ASRField cluster significantly overlaps each of the BSR and ASRClinic clusters. Although there is no significant overlapping, the BSR and ASRClinic clusters are butted right up against one another. Similar to the cross validation results, Figure 4.5 shows how one ASRField point could be identified as ASRClinic and how one ASRClinic point could be classified as ASRField. Again this may indicate the volatiles present in the breath of athletes after running are not significantly different immediately after and up to 15 minutes after completing the run.

Table 4.2. Cross validation table showing 77.8 percent correct classification of samples taken from the male participant during the short run.

		Identified As		
		BSR	ASRField	ASRClinic
Actual	BSR	3	0	0
	ASRField	0	2	1
	ASRClinic	0	1	2

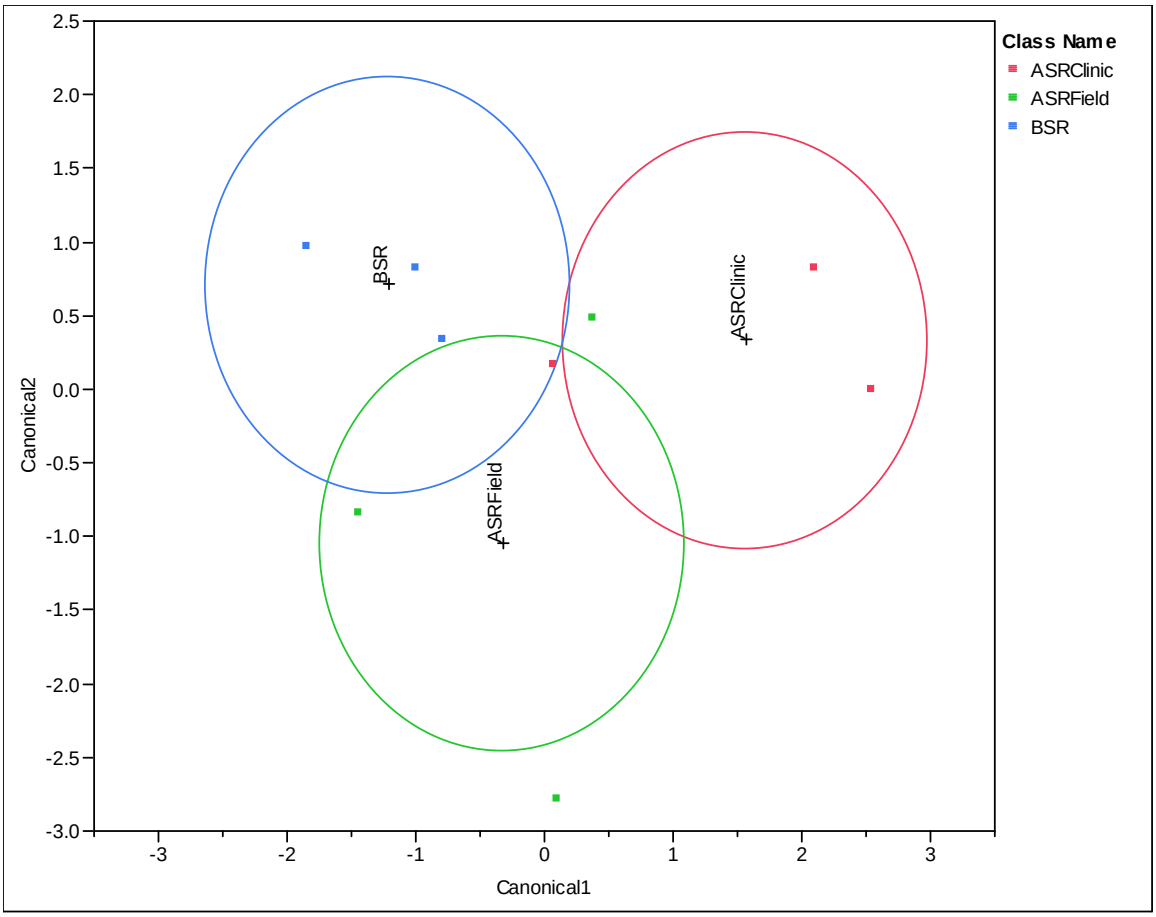

Figure 4.5. Canonical plot showing three data clusters for the male runner performing the short run.

Female Participant – Short Run

As Table 4.3 presents, the female participant provided nine breath samples during the short run. A cross validation correctly places 44.4 percent of the exposures and misidentifies 55.6 percent of them. These results demonstrate a confusion in the enose in detecting changes between the before and after run samples (one BSR was misidentified as ASRField, one BSR as ASRClinic, and one ASRField as BSR). There are two possible explanations for this confusion. First the participants are well trained long distance runners and the short run (three miles) many not have provided enough stress to be detected in the breath of the female. Secondly these changes may have been present in the breath; however the electronic nose was not able to detect them.

Additionally, One ASRField sample was misclassified as ASRClinic and one ASRClinic sample was misidentified as ASRField, indicating volatiles present in breath after running are the same immediately and up to 15 minutes after completing the run. One could then argue for only one after run sample collection period. Since the athletes are already required to visit the clinic to provide blood samples, a clinic breath sample collection may be recommended for future studies.

Figure 4.6 displays three clusters of data for the female runner performing the short run: BSR, ASRField, and ASRClinic. Just as the cross validation predicted, significant overlapping occurs between the before and after clusters. This may be due to the short run not being challenging enough to provide a detectable amount of stress in the breath of the female. Another possible reason may be that there are stresses present in the breath but the electronic nose was not able to detect them.

Both the ASRClinic and ASRField clusters significantly overlap the BSR cluster, however the ASRClinic sample overlaps more than the ASRField. Also, the ASRField cluster moves to the right and away from the BSR cluster while the ASRClinic cluster begins to move back to the left toward the BSR cluster. These movements suggest the short run induces stresses in the body which are detectable by the electronic nose immediately after the run but by the time the participant walks to the clinic and provides the after run clinic samples (10 to 15 minutes), the stress detectable in the breath is less, or more similar to that present in the baseline sample (BSR).

Table 4.3. Cross validation table showing 44.4 percent correct classification of samples taken from the female participant for the short run.

		Identified As		
		BSR	ASRField	ASRClinic
Actual	BSR	1	1	1
	ASRField	1	1	1
	ASRClinic	0	1	2

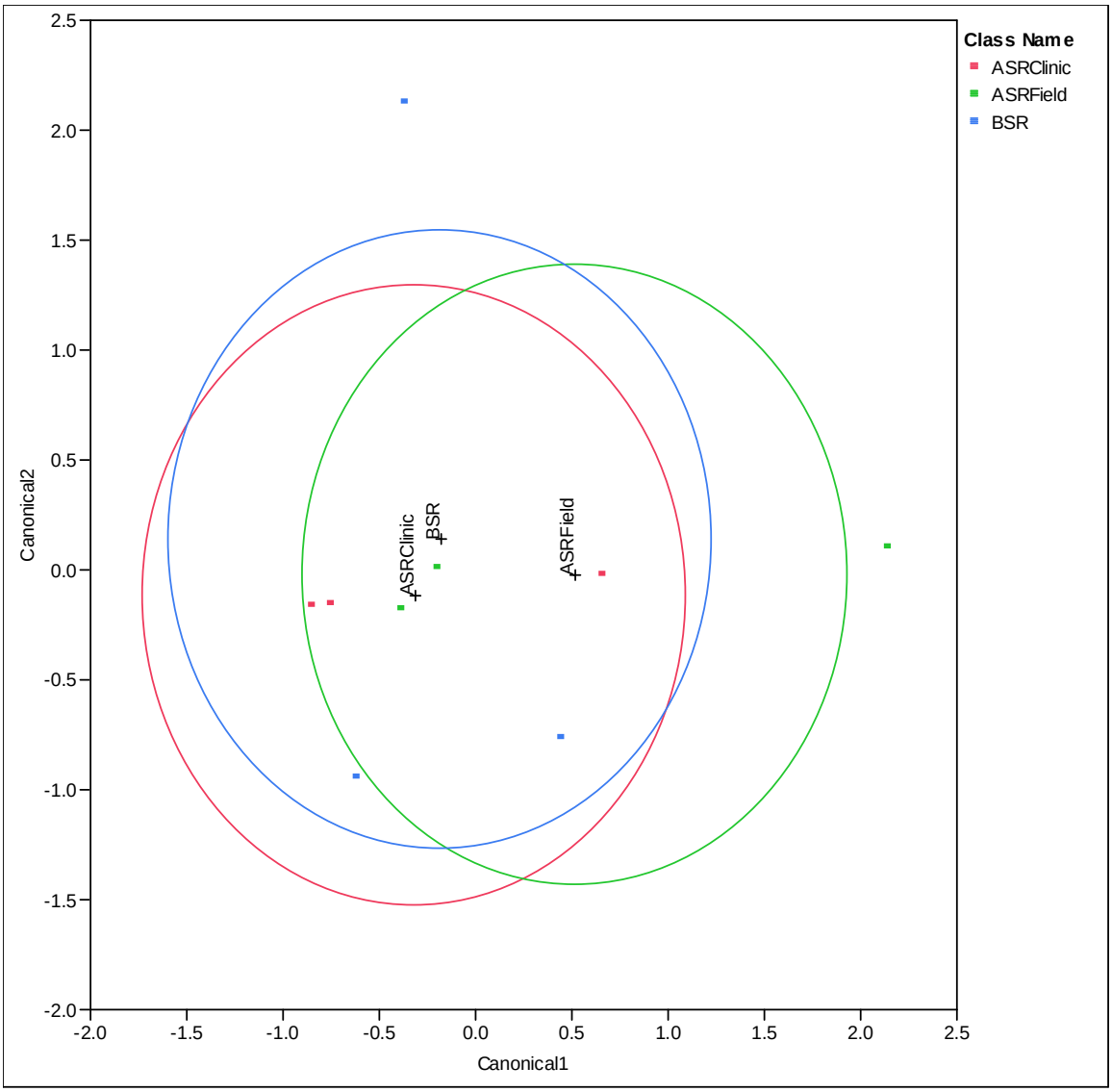

Figure 4.6. Canonical plot showing three data clusters for the female runner performing the short run.

Female Participant – Long Run

The female participant provided 18 breath samples in coordination with performing a long run as is displayed in Table 4.4 A cross validation correctly places 55.6 percent of the exposures and misclassifies 44.4 percent of them. Just as in the short run, these results indicate the electronic nose has trouble detecting changes between the before and after run samples (two BLR samples were misidentified as ALRField and two ALRClinic samples were misclassified as BLR).

Possible reasons for this include the long run (nine miles) still not being long enough to produce stress volatiles detectable in the breath and if present, the enose simply is not able to detect it.

Furthermore, three ALRField samples were misidentified as ALRClinic and one ALRClinic sample was misclassified as ALRField. These misidentifications may suggest volatiles present in breath after running are equivalent to those collected immediately after and up to 15 minutes after completing a training session. Therefore for further studies it may be necessary to collect only one after sample, preferably in the clinic due to convenience.

Figure 4.7 shows three clusters of data for the female subject performing the long run: BLR, ALRField, and ALRClinic. As the cross validation indicated, significant overlapping between the before and after run clusters occurs. A possible explanation for this is that the long run is still not challenging the athlete enough to provide a detectable amount of volatiles due to stress in the breath. A second reason may be simply that there are volatiles due to stress present in the breath but the enose unit was not able to detect them.

Just as in the short run performed by the female, both the ALRClinic and the ALRField clusters significantly overlap the BLR cluster and the ALRClinic cluster overlaps more than the ALRField. Additionally, the BLR cluster begins at the left side of the plot while the ALRField sample cluster moves to the right. However, the ALRClinic cluster has begun to move back to the left, toward the BLR cluster. Its final destination is between the BLR and ALRField clusters, overlapping them both. These movements indicate the long run induces stresses in the body which are detectable by the enose immediately after the run but less so by the time the subject walks to the clinic and provides the after run clinic samples (10 to 15 minutes). In comparison to the short run, the ALRClinic sample cluster is more in the middle and overlapping the field and before run samples than the ASRClinic sample cluster which is almost completely overlapping the before run cluster. This is a good indication the long run induces more detectable volatiles, and therefore oxidative stress, into the breath of the runner than the short run.

Table 4.4. Cross validation table showing 55.6 percent correct classification of samples taken from the female participant during the long run.

		Identified As		
		BLR	ALRField	ALRClinic
Actual	BLR	4	2	0
	ALRField	0	3	3
	ALRClinic	2	1	3

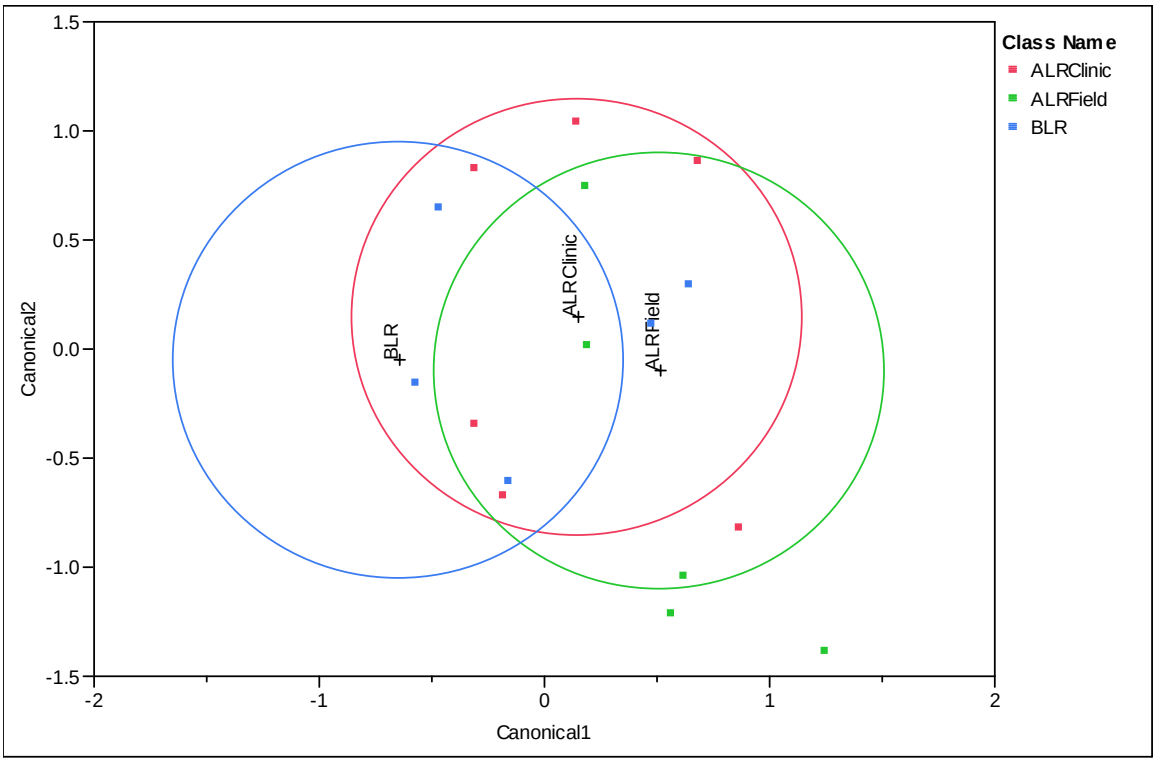

Figure 4.7. Canonical plot showing three data clusters for the female runner performing the long run.

Female Participant – Both Runs

As Table 4.5 shows the female participant provided 27 total breath samples. A cross validation correctly places 70.4 percent of the exposures and misclassifies 29.6 percent of them. One ASRField sample was misclassified as ASRClinic, two ASRClinic samples were misclassified as ASRField, two ALRField samples were misclassified as ALRClinic, and one ALRClinic sample was misclassified as ALRField. This misclassifications may indicate the volatiles present in an athlete's breath after running are the same immediately and up to 15 minutes after running.

Therefore samples may only need to be taken on the field or in the clinic, instead of both which would save resources during future studies.

It is important to note the analysis only misclassified the after run samples. It was also able to distinguish all before run samples from the after run samples and the two before run sample classes apart from each other. These results may indicate an increase in oxidative stress occurred due to increased training loads. It is safe to assume the athletes continued their training during the 22 days between performing the short and long runs. Therefore the electronic nose may be detecting this increase in oxidative stress due to increasing training loads through volatiles present in the breath.

Figure 4.8 shows six clusters of data: BSR, ASRField, ASRClinic, BLR, ALRField, and ALRClinic. Significant overlapping occurs between the ASRClinic and ASRField clusters, to the point where they almost appear to be the same cluster. There is also significant overlapping between the ALRClinic and ALRField clusters. This is evidence to support the argument that volatiles due to oxidative stress, occurring as a result of athletic training, present in breath are essentially the same immediately after and within 15 minutes of completing the run. Therefore only one set of samples may need to be collected after the run, saving time and money in the collection and analysis of breath.

A small portion of the BSR cluster is overlapped by the ASRField and ASRClinic clusters; however no overlapping occurs between the BLR and ALR samples. This suggests the electronic nose is able to detect volatiles in the breath that are due to physical training and that the longer the run, or the more oxidative stress present, the more separation will occur between the samples collected before and after the run. Samples collected from the short run are also clustered apart from the long run samples. This suggests volatiles are present in the breath at increasing levels of physical training and that the enose is able to detect the different training levels.

Table 4.5. Cross validation table showing 70.4 percent correct classification of samples taken from the female participant during both runs.

		Identified As					
		BSR	ASRField	ASRClinic	BLR	ALRField	ALRClinic
Actual	BSR	3	0	0	0	0	0
	ASRField	0	2	1	0	0	0
	ASRClinic	1	2	0	0	0	0
	BLR	0	0	0	6	0	0
	ALRField	0	0	0	0	4	2
	ALRClinic	1	0	0	0	1	4

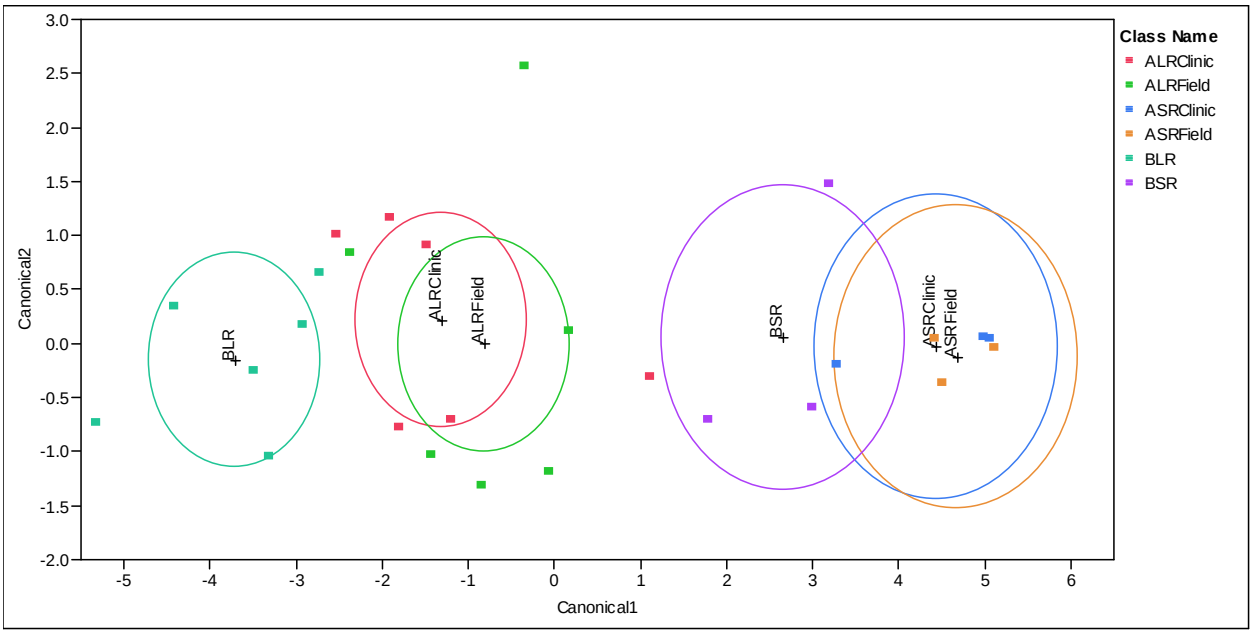

Figure 4.8. Canonical plot showing six data clusters for the female runner performing both runs.

Both Participants – Both Runs

In total both participants provided 36 breath samples, as is shown in Table 4.6. A cross validation correctly places 61.1 percent of the exposures and misclassifies 38.9 percent of them. Although not a perfect performance by the unit, this data suggests physical and oxidative stresses produce volatiles which are detectable in the breath by an electronic nose. However, as the discussion of these analyses suggests, there is room for improvement in this detection.

Eight field and clinic samples were misidentified: three ASRField as ASRClinic, one ASRClinic as ASRField, two ALRField as ALRClinic, and two ALRClinic as ALRField. This conveys evidence there were no significant differences between samples collected in the clinic and those collected immediately after the run. Therefore any stress attributed to physical training and detectable in the breath by an enose on the field continued for at least 15 minutes after the training session had ended. This may suggest field and clinic samples are comparable and it is not necessary to collect both. In the future only one set of samples could be collected which would decrease resources needed for this study and may make real-time monitoring of training programs more feasible. Since participants must already visit the clinic to give blood samples, a collection of breath samples in the clinic would be more convenient than those collected in the field.

An important observation to note is that the analysis was able to distinguish all but five of the before run samples from the after run samples. This is evidence to support the idea that there are volatiles present in the breath of athletes that indicate training, and therefore oxidative, stress and that an electronic nose is able to detect them. Additionally the enose is able to detect changes in these volatiles before and after a training session, or run.

The five samples misidentified were one BSR as ASRField, one BSR as ASRClinic, two ASRField as BSR, and one ASRClinic as BSR. This demonstrates stress occurring due to training may not be immediately detectable by the electronic nose in breath after a run. Instead it may take at least 15 minutes after the training session has ended for the enose to be able to detect these volatiles due to oxidative stress in the breath

Figure 4.9 presents six clusters of data for all runners performing all runs: BSR, ASRField, ASRClinic, BLR, ALRField, and ALRClinic. Significant overlapping occurs between the ASRClinic and ASRField clusters. Significant overlapping also occurs between the ALRField and ALRClinic clusters to the point where they almost appear as the same cluster. This evidence supports the argument that volatiles present in breath due to oxidative stress, occurring because of training, are not significantly altered from the time it takes to complete the run to the time required to walk to the clinic and provide samples, or a maximum of 15 minutes. This would

support the idea that it is necessary to collect only one set of samples after the run, saving time and money in the collection and analysis of breath samples.

More overlapping occurs between the samples taken before and after the short run than between the samples collected before and after the long run. One possible explanation for this is that an athlete's body undergoes less stress during the short run than during the long run and the enose is not sensitive enough to detect the amount of stress occurring during the short run but can that of the long run. This seems possible as the participants encounter an increase in distance, and perhaps stress, of 66.7 percent when performing the long run. Another possible explanation is that the male participant only performed the short run while the female participant performed both. However the data from both runners was pooled together into one group to analyze the short run.

Samples collected from the short run are clustered apart from the long run samples. There were 22 days between runs performed for this study and it can safely be assumed the participants continued their own training regimens during this time. This training would change the athletic level of the athletes and the amount of oxidative stress each body is experiencing. Therefore, the electronic nose may be detecting this difference in stress levels by sniffing volatiles present in breath. The weather conditions were also warmer and less humid on the day the short run was performed than on the one the long run was performed which may contribute to the differing results.

Table 4.6. Cross validation table showing 61.1 percent classification of samples taken from both participants for both runs.

		Identified As					
		BSR	ASRField	ASRClinic	BLR	ALRField	ALRClinic
Actual	BSR	4	1	1	0	0	0
	ASRField	2	1	3	0	0	0
	ASRClinic	1	1	4	0	0	0
	BLR	0	0	0	6	0	0
	ALRField	0	0	0	0	4	2
	ALRClinic	1	0	0	0	2	3

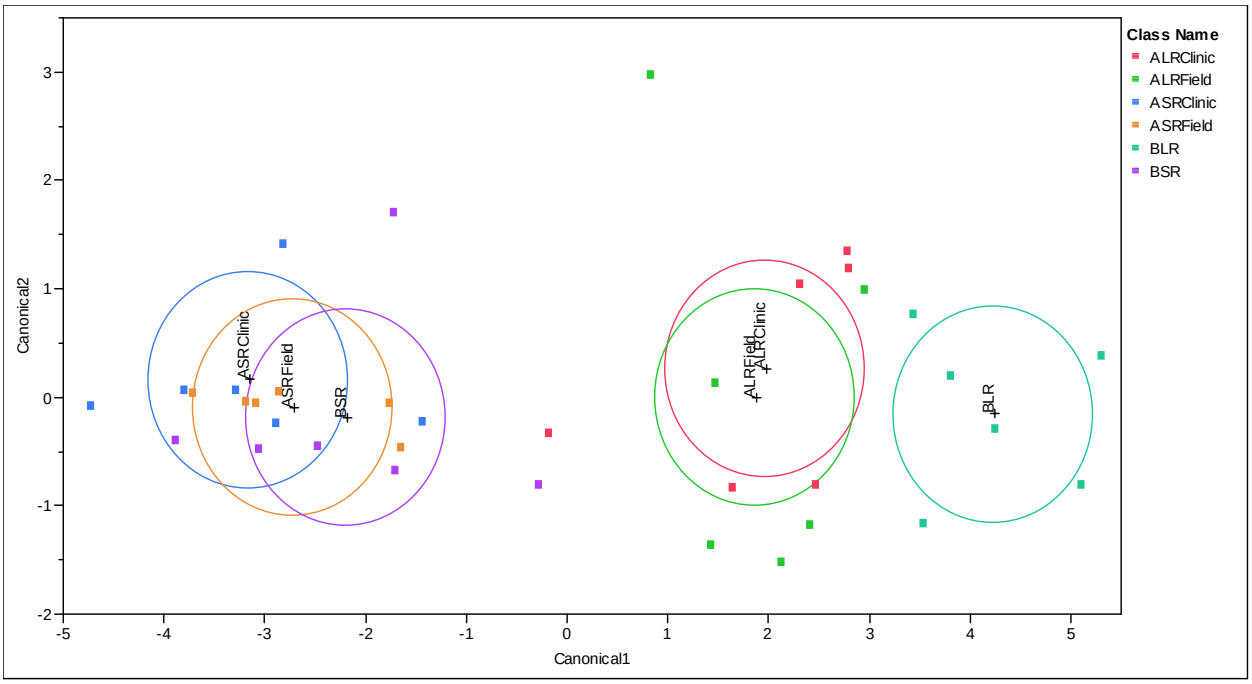

Figure 4.9. Canonical plot showing six clusters for both runners performing both runs.

4.3.3 MAHALANOBIS DISTANCES

JMP® was used to calculate the Mahalanobis distance from each sample to the class it was trained as. A value of five would indicate the point is approximately five standard deviations away from the mean of the group, or would be best fit into another cluster. According to Table 4.7, all breath samples were correctly placed in the given clusters (Mahalanobis distance of 2.60 or less) for the male runner performing the short run. As Table 4.8 shows, all breath samples were correctly placed in the clusters (Mahalanobis distance of 2.47 or less) for the female runner performing the short run. Table 4.9 also concludes all breath samples were correctly placed into the data clusters (Mahalanobis distance of 3.59 or less) for the female runner performing the long run. All breath samples provided by the female during both runs were also correctly placed into the data clusters (Table 4.10, Mahalanobis distance of 2.83 or less). Finally, Table 4.11 indicates all breath samples were correctly placed into their clusters (Mahalanobis distance of 1.89 or less) for both runners performing both runs. These results indicate there are volatiles present in the breath of athletes which are detectable by an electronic nose and that this enose, along with improved statistical analysis, would be able to correctly place samples as either before run, after run on the field, or after run in the clinic.

Table 4.7. Mahalanobis distances for the male athlete performing the short run.

Class Name	Mahalanobis Distance
BSR	1.78
BSR	0.98
BSR	2.23
ASRField	2.50
ASRField	0.78
ASRField	1.45
ASRClinic	2.43
ASRClinic	1.12
ASRClinic	2.60

Table 4.8. Mahalanobis distances for the female athlete performing the short run.

Class Name	Mahalanobis Distance
BSR	2.47
BSR	1.27
BSR	0.99
ASRField	2.25
ASRField	0.22
ASRField	0.46
ASRClinic	0.82
ASRClinic	0.93
ASRClinic	0.68

Table 4.9. Mahalanobis distances for the female athlete performing the long run.

Class Name	Mahalanobis Distance
BLR	0.57
BLR	0.67
BLR	0.45
BLR	0.66
BLR	0.82
BLR	3.59
ALRField	2.42
ALRField	0.17
ALRField	1.25
ALRField	1.39
ALRField	1.87
ALRField	0.81
ALRClinic	0.73
ALRClinic	1.10
ALRClinic	0.47
ALRClinic	1.11
ALRClinic	1.19
ALRClinic	0.92

Table 4.10. Mahalanobis distances the female athlete performing both runs.

Class Name	Mahalanobis Distance
BSR	1.90
BSR	1.15
BSR	0.96
ASRField	1.47
ASRField	1.38
ASRField	1.60
ASRClinic	1.58
ASRClinic	1.56
ASRClinic	1.05
BLR	1.14
BLR	1.44
BLR	0.94
BLR	1.87
BLR	1.12
BLR	1.56
ALRField	2.83
ALRField	0.14
ALRField	1.23
ALRField	1.32
ALRField	1.48
ALRField	1.19
ALRClinic	0.87
ALRClinic	1.41
ALRClinic	0.49
ALRClinic	1.37
ALRClinic	1.03
ALRClinic	1.10

Table 4.11. Mahalanobis distances for both runners performing both runs.

Class Name	Mahalanobis Distance
BSR	1.89
BSR	0.91
BSR	0.87
BSR	1.14
BSR	0.96
BSR	1.36
ASRField	0.74
ASRField	0.96
ASRField	1.07
ASRField	1.24
ASRField	1.03
ASRField	0.60
ASRClinic	1.27
ASRClinic	1.10
ASRClinic	0.54
ASRClinic	1.58
ASRClinic	1.00
ASRClinic	1.77
BLR	1.45
BLR	1.81
BLR	1.29
BLR	1.91
BLR	1.40
BLR	1.71
ALRField	3.17
ALRField	0.51
ALRField	1.49
ALRField	1.53
ALRField	1.77
ALRField	1.44
ALRClinic	1.19
ALRClinic	1.70
ALRClinic	0.36
ALRClinic	1.56
ALRClinic	1.05
ALRClinic	1.35

4.4 BREATH ANALYSIS – ENOSE VS. JMP® ANALYSES

Unlike gas chromatography, electronic noses do not identify specific components of a given sample. Instead these units utilize the response of each sensor in an array to create a smellprint unique to each sample category. During training, the user tells the enose unit what sample category it is sniffing. Usually several sniffs are provided for each of these categories, or classes. For this study the classes were before short run (BSR), after short run on the field (ASRField), after short run in the clinic (ASRClinic), before long run (BLR), after long run on the field (ALRField), and after long run in the clinic (ALRCLinic). Once the Cyranose 320, the enose unit used in this study, has been trained, it performs a cross validation procedure, utilizing principal component and canonical discriminant analyses to determine the robustness of the proposed model, or sample categories. At this point the user may then view the results of each analysis and determine whether to continue or not. Once the enose has been successfully trained and the model's robustness has been confirmed, the user may test the unit's ability to identify fresh samples. This study focused on the training and analysis portions of the enose in order to determine whether the model would be successful in the detection athletic training, in the form of oxidative stress, in the breath of athletes. Results obtained from the enose utilizing PCnose® were compared to similar analyses performed by JMP®.

4.4.1 CROSS VALIDATIONS

The electronic nose, along with PCnose®, was able to correctly classify 36.1 percent of the samples into the provided classes. Cross validation results for the analyses performed in JMP® ranged from 44.4 percent for the female performing the short run to 77.8 percent for the male performing the short run. Cross validation results for both runners performing both runs, which is how the enose performed the analyses, was better than that performed by the enose at 61.1 percent. It is believed the overarching explanation for this improvement in results is that data is easier to manipulate in JMP®.

The Cyranose 320 automatically uses three principal components when performing its analyses. However, during the JMP® analysis, three principal components were not selected for any of the

tested scenarios. Based on the percent cumulative variation and the eigen values for each scenario, it was decided to use two (female-short run, female-long run, female-both runs, and both runners-both runs) and four (male-short run) principal components which accounted for at least 89 percent of the total variation.

The analyses performed by the enose compared all of the runs completed by both participants while JMP® enabled analyses of five different scenarios: male performing the short run (Male:Short), female performing the short run (Female:Short), female performing the long run (Female:Long), female performing both runs (Female:Both), and both participants performing both runs (All:Both). Even though all of the data should be analyzed together, since this area of research is new, it is also necessary to observe an individual participant's response to physical training. This becomes particularly important when an athlete must drop out of the study, such as the male participant in this study who could not complete the long run because of scheduling conflicts. Many variables, such as the subjects' dietary and medicine intakes, cannot be controlled in this study, so a method for normalizing the data should be determined. Individual analyses are also important for this normalization determination process.

Results using JMP® suggests a proprietary opportunity exists. Firstly, there is a potential opportunity for improving the statistical software used with this particular unit. Additionally, all of the sensors may not be sensitive enough to detect the change in volatiles present in the breath of athletes due to exercise. The enose unit could be improved by removing those sensors which are least sensitive to oxidative stress present the breath of athletes. Similarly, sensors that are more sensitive to oxidative stress present in the breath of athletes could also be added to the sensor array.

4.4.2 CANONICAL DISCRIMINANT PLOTS

The enose, along with PCnose®, showed minimal separation between the breath classes which were presented in PCA and CDA plots. A slightly significant separation was observed between the after short run and before long run classes. This indicates the enose may only be able to detect significant differences in oxidative stress present in the breath of athletes after

compounded training loads have been incurred. For example, there were 22 days between the two runs in which it is safe to assume the participants continued training. This continuation of training would presumably increase the athletic ability of the participants, along with the oxidative stress each has experienced.

JMP® was utilized to obtain CDA plots for five scenarios: Male:Short, Female:Short, Female:Long, Female:Both, All:Both. The Female:Long situation is also the scenario for both runners performing the long run as the male participant was not able to perform the long run. No significant differences were observed between the before and after run sample clusters for the Male:Short situation, although the ASRClinic and BSR groups did not overlap each other. Significant overlapping occurred between all before and after run sample clusters for the Female:Short scenario. Overlapping was also present between the before and after run groups for the Female:Long scenario, however more separation occurred between the ALRClinic and BLR data clusters. Additionally, separation existed between the short and long run data clusters and significant separation occurred between the before and after run samples for the long run for the Female:Both and All:Both scenarios. However, some overlapping was present between the before and after run samples collected for the short run.

These results suggest the enose is able to detect volatiles present in the breath due to physical training, but the short run may not provide enough challenge to provide significant enough changes in the breath for the unit to detect. However, the long run appears to provide enough physical challenge to begin seeing separations between the sample classes collected before and after the run. This suggests the volatiles in the breath detected by the enose are due to physical training and that the longer the run, or the more oxidative stress present, the more separation will occur between the before and after run samples. This is common sense as the participants encountered an increase in distance, and perhaps stress, of 66.7 percent when performing the long run as opposed to the short run.

Additionally, short run and long run samples were clustered apart from each other. This suggests volatiles are present in the breath at increasing levels of physical training and that the enose is able to detect these different training levels. There were 22 days between the long and short runs

and it can be assumed the athletes continued individualized training between the days the runs were performed. The enose unit may then be detecting a difference in oxidative stress, and therefore training level, in the athletes.

Overall CDA plots created by JMP® showed more separation between the sample clusters than the enose did with PCnose®. The data is easier to manipulate utilizing JMP® than it is using the enose. For instance the number of principal components chosen to input into the CDA for the independent analysis was different than that chosen by the enose. Additionally analyses were performed for more individualized groups of data than the enose which just analyzed all data points together. However, the JMP® analysis for both participants performing both runs, or the circumstance analyzed by the enose, even obtained better separation between the sample classes than the enose.

Something that may have affected all enose analyses is that the male athlete was not able to complete the long run. However, the data collected from both participants were pooled together into one group for analyses including the short run. This also means double the breath samples were collected from the female athlete and analyzed for the long run such that the number of data points for the long run would be equal to that of the short run. It can be assumed the missing data from the male participant for the long run inserted some variance into the data. In future work, a larger sample size should be targeted to perform both runs.

As discussed above, improved results with JMP® suggest a proprietary opportunity may exist. The statistical software used with the Cyranose 320 could be improved. Additionally the sensor array could be improved by removing those sensors least sensitive and adding sensors more sensitive to the oxidative stress present in the breath of athletes due to physical training.

4.4.3 FIELD VS. CLINIC SAMPLES

To help understand when oxidative stress becomes detectable in breath after exercising, breath samples were collected immediately after the run was performed on the field (Field) and after the participants had walked to the clinic (Clinic) which took 10 to 15 minutes. Unfortunately the

data present conflicting results on this matter. In some instances the Field and Clinic samples appear to be equivalent, indicating the differences between the samples collected in the field immediately after the run and the samples collected in the clinic were insignificant. However in others cases, the Field and Clinic classes appear to be different indicating the differences between the samples collected immediately after the run and the samples collected a minimum of 10 minutes after completing the run were significantly different. This would imply volatiles in the breath due to physical, and perhaps oxidative, stress either are not detectable with an enose until the athlete has walked to the clinic or the volatiles become undetectable with an enose by the time the athlete has walked to the clinic. Both scenarios were presented during this study. At a minimum, future work should continue to collect both the Field and Clinic samples. It would also be interesting to collect breath samples at more intervals after run completion in order to further the understanding of how exercise affects the detection of volatiles due to exercise in the breath.

4.5 BLOOD ANALYSIS

Blood was collected from the participants at four different intervals: immediately prior to performing the short run, after completing the short run and walking to the clinic, immediately prior to starting the long run, and after completing the long run and walking to the clinic. A minimum of 10, with a maximum of 15, minutes passed between the time a run was completed and the samples were collected in the clinic. Three 10 ml tubes of blood, or 30 ml, were drawn from each participant at each collection period. Two tubes from each sample draw for each collection period were processed for plasma and red blood cells (RBC) before being stored. The plasma and red blood cells from each of these tubes were then analyzed individually for catalase and glutathione peroxidase (GPx) activities. The remaining tube from each collection period and participant were processed as whole blood and stored, however they were not used for this particular study.

4.5.1 CATALASE ACTIVITY ANALYSIS

Catalase is an enzyme that works to decompose hydrogen peroxide into water and oxygen (Kostaropoulos et al., 2006). This H_2O_2 decomposition can be observed as an absorbance decrease using a spectrophotometer at a wavelength of 240 nm under the ultraviolet light range. Catalase activity was measured in the plasma and RBC of each participant for each run and expressed as micromoles of H_2O_2 consumed per minute per milligram of sample protein. JMP® was then utilized to perform a paired t-test for each run per runner.

Catalase Activity in Plasma

Figure 4.10 presents the effects physical exercise has on catalase activity as seen in the plasma of the participants. The basal catalase activity and the activity within 15 minutes post-run are shown in the figure for four instances: the female participant performing the short run (Female:Short), the male participant performing the short run (Male:Short), the female participant performing the long run (Female:Long), and both participants performing the short run (All:Short). The Female:Long instance also represents the long run for both participants as the male participant was not able to perform this run due to scheduling conflicts.

Figure 4.10 shows an unexpected trend of catalase activity decreasing in plasma after the run is performed. In previous literature an increase of catalase activity was noted in the blood (Aguilo et al., 2005; Sinha et al., 2009), however a decrease has also been observed in neutrophils of cyclists (Tauler et al., 2002) and swimmers (Ferrer et al., 2009). As a person performs physical exercise, his body requires more oxygen which in turn accelerates the production of reactive oxygen species (ROS), such as hydrogen peroxide. Therefore the body's defense system must also increase its activity in order to break down the ROS and keep them from causing damage. However if the production of ROS exceeds the capacity of the defense system, oxidative damage occurs. The decreasing catalase activity levels seen in Figure 4.10 may be an indication the athletes' bodies are experiencing oxidative stress and damage created by this unbalanced situation.

The body's first line of defense is superoxide dismutase (SOD) which converts superoxide radicals to hydrogen peroxide which can then be converted to water by GPx. However, when hydrogen peroxide production exceeds the GPx capacity, catalase takes over this process (Kostaropoulos et al., 2006). Therefore, another possible explanation for the decrease in catalase activity may be that the participants' bodies are able to control most of the ROS being produced by using GPx. The study participants are highly trained athletes so it is not unreasonable to think their bodies are used to undertaking this amount of physical stress and are able to control the production of ROS without significantly increasing catalase activity.

Catalase activity levels for the Female:Short run were 0.299 ± 0.093 μmol H_2O_2 min^{-1} mg $protein^{-1}$ (before) and 0.248 ± 0.011 μmol H_2O_2 min^{-1} mg $protein^{-1}$ (after) while catalase levels for the for the Female:Long run were 0.680 ± 0.477 μmol H_2O_2 min^{-1} mg $protein^{-1}$ (before) and 0.472 ± 0.109 μmol H_2O_2 min^{-1} mg $protein^{-1}$ (after). Similar catalase activity levels were found in the plasma of child swimmers (Gougoura et al., 2007) and adult runners (Kostaropoulos et al., 2006). Study participants were highly trained athletes and it is safe to assume they continuously work to increase their athletic capabilities. This increase in catalase activity levels seen for the long run may indicate an increase in physical fitness and oxidative stress occurring as a result of 22 days of training performed between the two runs.

However, as Table 4.12 presents, there were only significant differences between the pre- and post-run samples when looking at the All:Short run ($p = 0.0267$) and both runs performed by both participants ($p = 0.0261$) at the 0.05 level. Additionally, increasing the alpha level to 0.1 only produces one more significant change for the Male:Short run ($p = 0.0524$). It may then be said that, although there appears to be a decreasing trend in catalase activity in plasma shown in Figure 4.10, there are no significant changes observed for each participant and run. This constant trend of catalase activity has also been seen in cyclists performing the Challenge Volta a Mallorca in 2002 (Sureda et al., 2005).

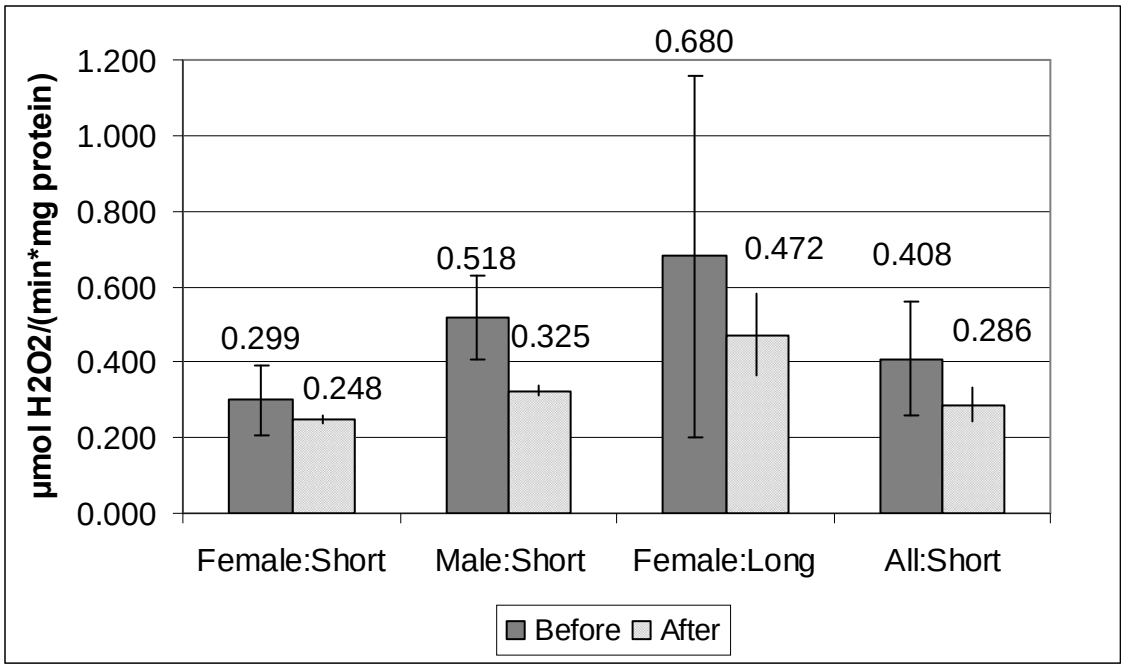

Figure 4.10. Effects of exercise on catalase activity in the plasma of runners. Values represent mean H_2O_2 present plus or minus the standard deviation.

Table 4.12. Paired t-test results for catalase activity in the plasma of runners.

Subject	Run	P-value	Significance (α = 0.05 level)	Significance (α = 0.1 level)
Male	Short	0.0524	Not Significant	Significant
Female	Short	>0.90	Not Significant	Not Significant
All	Short	0.0267	Significant	Significant
Female	Long	0.4482	Not Significant	Not Significant
All	All	0.0261	Significant	Significant

Figure 4.10 also shows a large standard deviation in catalase activity for the plasma samples collected from the female participant prior to performing the long run. Runners tend to have small veins and on this particular day it was challenging to draw blood prior to beginning the run. Three 10 ml tubes of blood were drawn at each sample draw and then plasma was collected from two of these tubes and stored in their own respective tubes. However, for one of these blood samples, only two milliliters of plasma were collected from the whole blood. Furthermore the plasma that was collected was slightly red in color. Since there were limited participants, and therefore samples, available, it was decided this plasma sample would be used in the above testing and analyses. Later, to determine whether this reddish plasma sample made a difference

in the results, it was removed and the data set was reanalyzed without it. The catalase activity using the red plasma sample was 0.68±0.477 μmol H_2O_2 min^{-1} mg $protein^{-1}$ while the catalase activity without the red plasma sample was found to be 0.170±0.242 μmol H_2O_2 min^{-1} mg $protein^{-1}$. More results are presented in Figure 4.11 and Table 4.13 and are discussed below.

As Figure 4.11 displays, removing the red-colored plasma sample alters the data to show an increase in catalase activity for the Female:Long run. Additionally a paired t-test was performed in JMP® and showed a significant change in the catalase activity for the Male:Short run (p = 0.0524) at the 0.1 level (Table 4.13). It is hypothesized the production of hydrogen peroxide in the athletes does not exceed the GPx capacity until the participants perform the long run at which point the catalase portion of the defense system must be activated, raising the catalase activity levels.

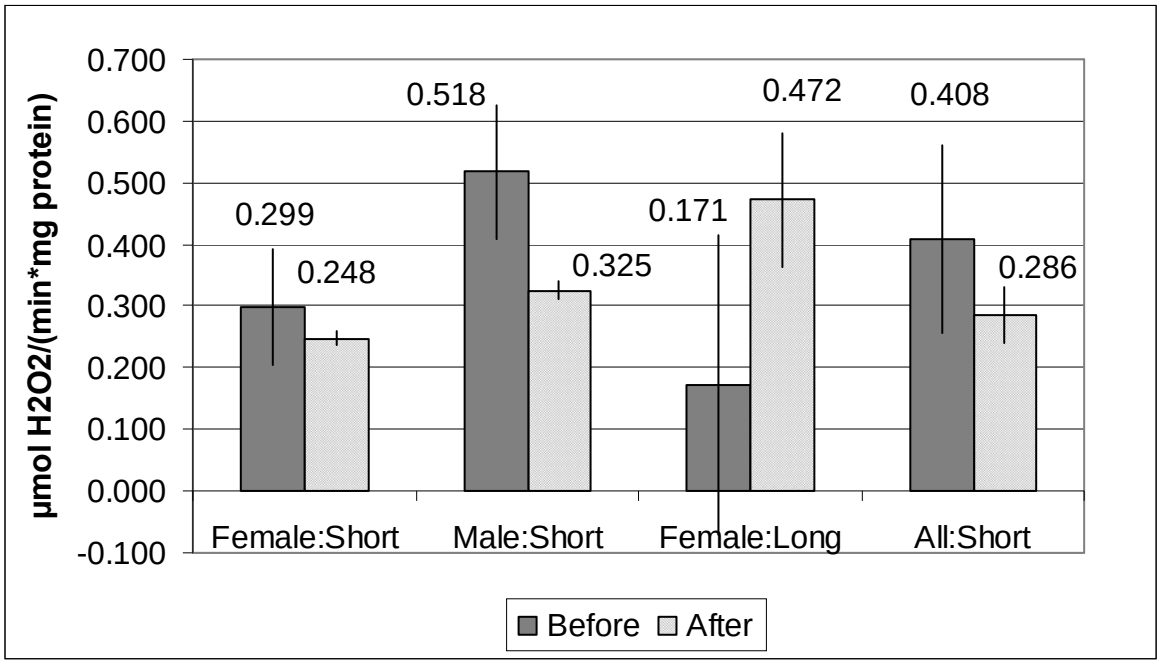

Figure 4.11. Effects of exercise on catalase activity in the plasma of runners, corrected for reddish plasma sample. Values represent mean H_2O_2 present plus or minus the standard deviation.

Table 4.13. Paired t-test results for catalase activity in the plasma of runners, corrected for reddish plasma sample.

Subject	Run	P-value	Significance (α = 0.05 level)	Significance (α = 0.1 level)
Male	Short	0.0524	Not Significant	Significant
Female	Short	>0.90	Not Significant	Not Significant
All	Short	0.0267	Significant	Significant
Female	Long	0.4482	Not Significant	Not Significant
All	All	0.0261	Significant	Significant

Catalase Activity in Red Blood Cells

Figure 4.12 presents the effects physical exercise has on catalase activity as seen in the RBC of the participants. The basal catalase activity and the activity within 15 minutes post-run are shown in the figure for four instances: Female:Short, Male:Short, Female:Long, and All:Short. Additionally the Female:Long instance also represents the long run for both participants as the male participant was unable to perform the run.

Figure 4.12 shows a small decrease in catalase activity in RBC after the Female:Short and Female:Long runs were performed. One possible explanation for this is that the body is undergoing oxidative damage due to the inability of the body's defense system to break down the increased production of ROS. Another possible explanation for these small decreases may be that the female's defense system is able to control most of the ROS production by GPx activity and so activation of the catalase portion of the defense system is not necessary.

Catalase activity for the Male:Short run appears to increase. This may indicate the participant's body is working to decrease the number of ROS by utilizing catalase, thereby increasing the catalase activity. Previous literature has also noted an increase in catalase activity in blood (Aguilo et al., 2005; Sinha et al., 2009).

Catalase activity levels for the Female:Short run were 102.9 $\pm$ 2.7 μmol H_2O_2 min^{-1} mg $protein^{-1}$ (before) and 101.4 $\pm$ 0.6 μmol H_2O_2 min^{-1} mg $protein^{-1}$ (after) while catalase levels for the Female:Long run were 100.4 $\pm$ 2.0 μmol H_2O_2 min^{-1} mg $protein^{-1}$ (before) and 96.9 $\pm$ 1.3 μmol H_2O_2 min^{-1} mg $protein^{-1}$ (after). Weather conditions during the short run were warmer (25°C)

and less humid (72%) than during the long run (16°C, 96%). It is proposed the warmer weather induced more oxidative stress and in turn increased catalase activity levels.

Table 4.14 indicates only one significant difference in the pre- and post-run samples which was when looking at the Female:Long run ($p = 0.0961$) but only at the 0.10 level. Therefore it might be said that, although there appear to be a few small changes in catalase activity for the runs, there are no major changes observed for each participant and run. This stagnant catalase activity behavior has also been seen in cyclists performing the Challenge Volta a Mallorca in 2002 (Sureda et al., 2005).

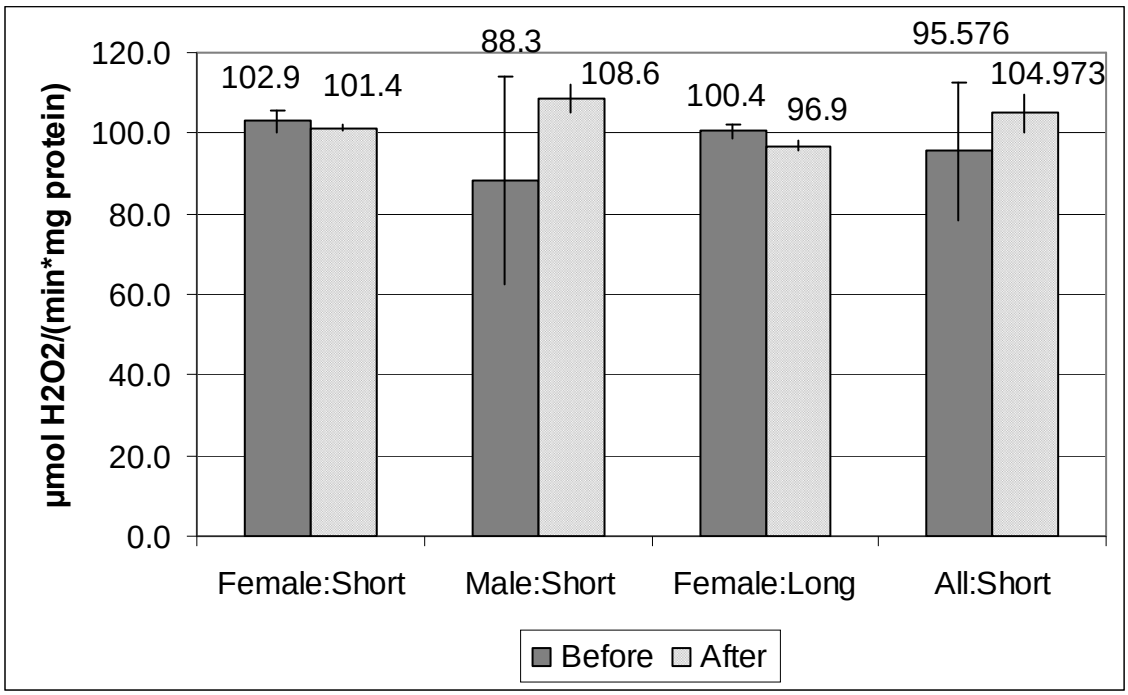

Figure 4.12. Effects of exercise on catalase activity in the RBC of runners. Values represent mean H_2O_2 present plus or minus the standard deviation.

Table 4.14. Paired t-test results for catalase activity present in RBC of runners.

Subject	Run	P-value	Significance (α = 0.05 level)	Significance (α = 0.1 level)
Male	Short	0.1094	Not Significant	Not Significant
Female	Short	0.5171	Not Significant	Not Significant
All	Short	0.8816	Not Significant	Not Significant
Female	Long	0.0961	Not Significant	Significant
All	All	0.5032	Not Significant	Not Significant

4.5.2 GPX ACTIVITY ANALYSIS

Glutathione peroxidase (GPx) is an enzyme that catalyzes the oxidation of glutathione (GSH) by hydrogen peroxide (Kostaropoulos et al., 2006). GPx activity can be measured by observing the conversion of oxidized glutathione by NADPH (Agergaard and Jensen, 1982). For this study it was measured in the plasma and RBC of each participant for each run and expressed as nanomoles of NADPH consumed per minute per milligram of sample protein.

GPx Activity in Plasma

Figure 4.13 showcases the effects physical exercise has on GPx activity in the plasma of athletes. The basal GPx activity and the activity within 15 minutes post-run are shown in the figure for four instances: Female:Short, Male:Short, Female:Long, and All:Short. The Female:Long instance also represents the long run for both participants as the male participant was not able to perform this run due to scheduling conflicts.

As a body undergoes physical exertion, such as exercise, there is an increase in demand for oxygen along with an increase in the production of ROS. When ROS production surmounts the capacity of the defense system, an imbalance occurs. GPx activity present in the plasma of a participant at the end of a run trends towards decreasing as is shown in Figure 4.13. Previous studies have also observed a decrease in GPx activity, specifically within the neutrophils (Tauler et al., 2002) and RBC (Aguilo et al., 2005) of cyclists occurring after they performed exercise. This decrease in GPx activity found in these studies and in Figure 4.13 may be indicative of an imbalance between ROS production and antioxidant defense system occurring in and causing oxidative damage to the subjects.

The body has a multistep approach to combating ROS beginning with SOD converting superoxide radicals to hydrogen peroxide. GPx then works to convert H_2O_2 into water but when H_2O_2 production exceeds the GPx capacity, catalase takes over this process (Kostaropoulos et al., 2006). Hence another explanation for the decrease in GPx activity seen in Figure 4.13 may be

that the GPx capacity has been exceed and the defense system has reverted to catalase for decreasing potential damage done by H_2O_2.

Aguiló et al. (2005) studied antioxidant enzyme activities in eight male professional cyclists during the mountainous, or third, stage of the Setmana Catalana performed near Barcelona, Spain in 2000. Venous blood samples were collected from each participant at four intervals: during the morning of the cycling stage and after 12 hours of fasting conditions were observed over night, immediately after the stage was completed, three hours after subjects completed the stage, and once again on the morning of the following day and after 12 hours of fasting conditions were observed over night. The first sample collection represented the basal enzyme activity levels while the last sample collection represented the antioxidant levels present after approximately 15 hours of recovery time. The authors found GPx activity levels significantly decreased in RBC, by at least 11 percent, but returned to basal levels after the three hour recovery period (Aguilo et al., 2005). Additionally, the authors observed a significant increase in oxidized glutathione blood levels which indicated oxidative stress was present in the cyclists. Together these observations supported the conclusion that the cyclists were undergoing exhaustive exercise which induced an imbalance between ROS production and the antioxidant defense systems, leading to oxidative stress (Aguilo et al., 2005). However the three hour recovery period appears to have allowed the body's defense system to recuperate and catch up with ROS production. In future studies, it would be interesting to collect blood samples at defined intervals after completing the runs, instead of only immediately after they finish. This would allow for the observation of antioxidant enzyme behavior in the blood of runners which can later be correlated to breath analysis.

Additionally GPx activity level for the Female:Short run decreased by 0.21 nmol NADPH min^{-1} mg $protein^{-1}$ while the GPx activity level for the Male:Short run decreased by 0.18 nmol NADPH min^{-1} mg $protein^{-1}$, and the GPx activity level decreased by 0.4 nmol NADPH min^{-1} mg $protein^{-1}$ for the Female:Long run. The long run distance increased by approximately 67 percent from the short run. This increase in run distance, or training load, may explain why the largest decrease in GPx activity present in the plasma of study participants showed the largest change.

However, as Table 4.15 presents, there were only significant differences between the pre- and post-run samples for the Female:Long ($p = 0.0487$) and both runs performed by both participants ($p = 0.0045$) situations, at the 0.05 level. Additionally, increasing the alpha level to 0.1 only produces a significant change for one more situation: both runners performing the short run ($p = 0.0604$). Even though a decrease in GPx activity appears for most situations in Figure 4.13, it can be said the other situations (Female:Short and Male:Short) do not deviate from their basal levels. This steady trend of GPx activity has also been seen in the RBC of cyclists performing a mountainous stage of the Challenge Volta a Mallorca in 2002 (Sureda et al., 2005). This may indicate the production of ROS and the actions of the bodies' defense systems are in balance with one another and that the body is controlling oxidative stress efficiently.

Figure 4.13. Effects of exercise on GPx activity in the plasma of runners. Values represent mean NADPH present plus or minus the standard deviation.

Table 4.15. Paired t-test results for GPx activity present in the plasma of the participants.

Subject	Run	P-value	Significance ($\alpha = 0.05$ level)	Significance ($\alpha = 0.1$ level)
Male	Short	0.1346	Not Significant	Not Significant
Female	Short	0.2967	Not Significant	Not Significant
All	Short	0.0604	Not Significant	Significant
Female	Long	0.0487	Significant	Significant
All	All	0.0045	Significant	Significant

GPx Activity in Red Blood Cells

Figure 4.14 shows the effects physical exercise has on GPx activity present in RBC of the study participants. The basal GPx activity and the activity within 15 minutes post-run are shown in the figure for four instances: Female:Short, Male:Short, Female:Long, and All:Short. Additionally the Female:Long situation represents the long run for both participants since the male participant was unable to perform the run.

GPx activity decreases slightly for both runs performed by the female. A decrease in GPx activity has also been observed in the neutrophils (Tauler et al., 2002) and RBC (Aguilo et al., 2005) of cyclists upon completing a mountainous stage of a multiple day bike ride. A possible explanation for the decrease seen in the present study is that the female's defense system and ROS production are in a state of unbalance, or that she is experiencing oxidative damage. Another explanation may be that ROS production exceeded the capacity of the GPx and the body is now working to control the change in ROS production by using the next step in the defense system: catalase. Along with a decrease in GPx activity observed after performing exercise, Aguiló et al. (2005) also found GPx activities returned to their basal levels for the cyclists after three hours of rest. Future work may also study the GPx activity levels present in long distance runners in predetermined intervals occurring after the completion of a run to see if and when they return to basal levels.

Figure 4.14 also shows an increase in GPx activity for the Male:Short run. This increase may indicate the participant's body is working to decrease the number of ROS with GPx thereby increasing the level of GPx activity. Previous studies have also noted an increase in GPx activity (Aguilo et al., 2005; Sinha et al., 2009).

The maximum GPx activity level for the Female:Short run was 9.89 $\pm$ 0.62 nmol NADPH min^{-1} mg $protein^{-1}$ and the maximum GPx level for the Female:Long run was 10.59 $\pm$ 0.04 nmol NADPH min^{-1} mg $protein^{-1}$. Both of these values were larger than the maximum GPx level for the Male:Short run scenario which was 9.33 $\pm$ 0.55 nmol NADPH min^{-1} mg $protein^{-1}$. In a study observing antioxidant activities in healthy Chinese adults, females were also found to have higher levels of GPx in their RBC (Ho et al., 2005), although the results were not significant.

However, as Table 4.16 presents, there were no significant changes between the pre- and post-run samples collected for either participant performing either run at either the 0.05 or 0.10 alpha levels. Since the runs did not significantly change, GPx levels from the basal levels detected, the participants' antioxidant defense systems and ROS production may be under steady state conditions, with no oxidative damage occurring. A similar trend was seen in the RBC of cyclists performing a mountainous stage of the Challenge Volta a Mallorca which took place in 2002 (Sureda et al., 2005).

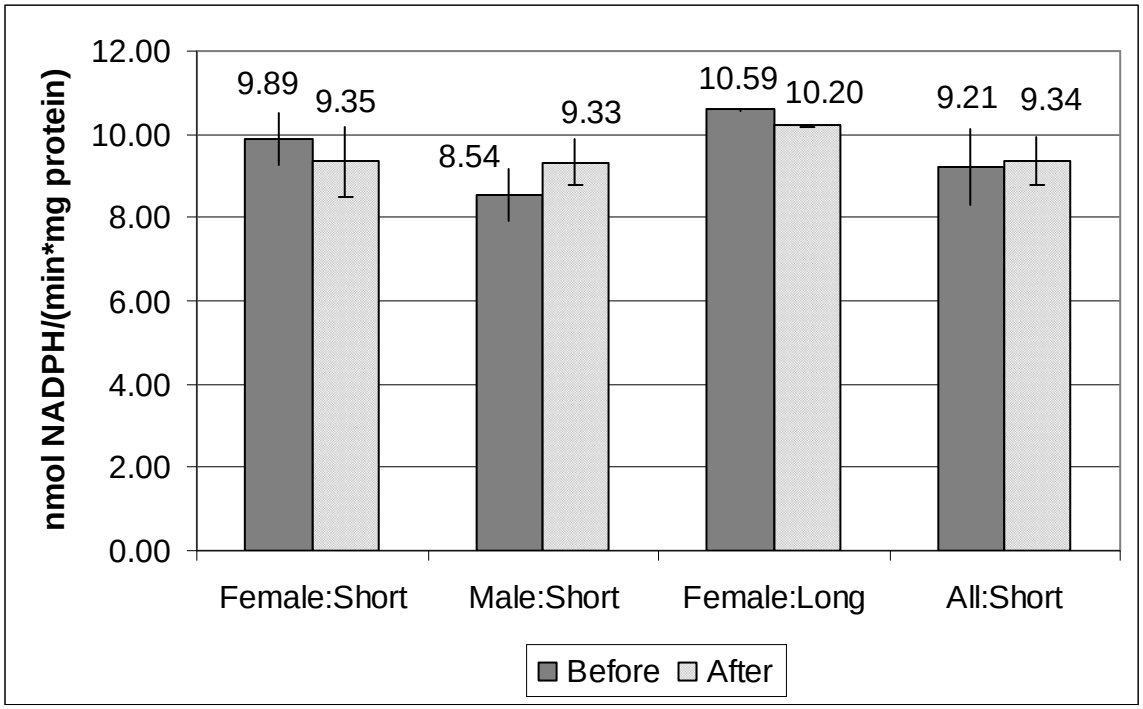

Figure 4.14. Effects of exercise on GPx activity in the RBC of runners. Values represent mean NADPH present plus or minus the standard deviation.

Table 4.16. Paired t-test results for GPx activity present in the RBC of the participants.

Subject	Run	P-value	Significance ($\alpha = 0.05$ level)	Significance ($\alpha = 0.1$ level)
Male	Short	0.2670	Not Significant	Not Significant
Female	Short	0.4344	Not Significant	Not Significant
All	Short	0.7899	Not Significant	Not Significant
Female	Long	0.4435	Not Significant	Not Significant
All	All	0.9041	Not Significant	Not Significant

4.6 BLOOD ANALYSIS VS. ENOSE

4.6.1 CATALASE ACTIVITY

Catalase activity in the plasma of the runners presented a decreasing trend in samples collected after the short run was performed. This may indicate the athlete's bodies are able to control most of the ROS production by using GPx. An increase in catalase activity in the plasma for the Female:Long scenario was also found which may indicate an uptick in catalase activity in order to combat an increase in oxidative stress. Additionally a paired t-test performed in JMP® found significant differences between the catalase activity present in the plasma of the female participant before and after performing the long run ($p = 0.0278$). It is suggested hydrogen peroxide production in plasma does not exceed GPx capacity until a long run is performed at which point the catalase portion of the defense system must be activated, increasing the catalase activity levels. A similar situation was found in the analysis of breath samples via enose. As mentioned previously, volatiles indicating oxidative stress, which might be due to physical exercise, are not detectable by an enose apparatus in the breath of athletes after completing a short run. However, these volatiles are detectable after the athletes perform a long run. A paired t-test performed for the Male:Short run scenario was also found to be significant ($p = 0.0524$) at the 0.1 alpha level while the Female:Short run scenario was found to be insignificant. A similar situation was seen as the enose sniffed the breath of the athletes before and after each performed the short run. Cross validation results were better for the Male:Short (77.8 percent) than the Female:Short (44.4 percent) scenarios. Furthermore, separations between the before and after clusters shown in the CDA plot for the Male:Short scenario were larger than those of the Female:Short circumstance.

Catalase activity present in the RBC of the male runner appears to increase which may indicate the exercise performed was enough to induce oxidative stress in the athlete, causing his catalase activity to increase. However, catalase activity present in the RBC of the female runner appears to decrease slightly. This may indicate the athlete's body is able to control most of the ROS being produced by using GPx. The only significant result ($p = 0.0961$, $\alpha = 0.1$) for a paired t-test performed by JMP® was found for the Female:Long scenario. Similar results were found during the analysis of breath samples by an enose. As previously discussed, it is suggested volatiles indicating oxidative stress, which may be due to physical exercise, are not detectable by an enose in the breath of athletes after performing a short run. However, these volatiles are detectable after a long run is performed.

4.6.2 GPX ACTIVITY

GPx activity in the plasma of the participants appears to decrease after performing a run. There may be two possible explanations for this decrease. First oxidative damage may be occurring in the athletes and secondly the GPx capacity in the athletes' bodies has been exceeded and their defense systems have reverted to catalase for decreasing potential damage done by H_2O_2. The largest decrease in GPx activity was found during the Female:Long scenario which may be explained by the 66.7 percent increase in run distance. Additionally, significant differences were observed in the GPx activity present in the plasma of the female runner after completing the long run ($p = 0.0487$). A similar situation was seen in the analysis of breath samples via enose. It is suggested volatiles indicating oxidative stress perhaps due to physical exercise are not detectable by an enose in the breath of athletes after performing a short run. However, these volatiles are detectable after the athletes perform a long run.

GPx activity found in the RBC of the female participant decreased slightly for both the short and long runs. A possible explanation for this decrease is that oxidative damage may be occurring in the athletes. A second explanation is that the GPx capacity in the athletes' bodies has been exceeded and their defense systems have reverted to catalase for decreasing potential damage done by H_2O_2. An increase in GPx activity present in the RBC of the male athlete after performing the short run increased which may indicate the participant's body is working to

decrease the production of ROS by increasing the GPx activity levels. A paired t-test indicated no significant differences occurring after any runs for either study participant.

4.7 BLOOD ANALYSIS – MULTIVARIATE STATISTICAL ANALYSIS

Previous investigators have studied many potential biomarkers for overtraining and oxidative stress but these results are not always in agreement with each other. Similarly, results for this study were found to be inconclusive for the analysis of catalase and GPx activity levels in RBC and plasma collected for study participants. However, since four data sets (catalase in plasma, catalase in RBC, GPx in plasma, and GPx in RBC) were already collected, a multivariate analysis like that performed with the breath data was performed for the blood samples. If a small set of biomarkers were identified in the prediction of oxidative stress, and thus overtraining, they could be used to assist their prevention. JMP® was utilized to perform a multivariate analysis for five different scenarios: the male participant performing the short run, the female participant performing the short run, the female participant performing the long run, the female participant performing both runs, and both participants performing both runs. Results were then compared to those obtained from the breath analysis performed by the enose. Tables and Figures referred to in this section can be found in Appendix D.

Male Participant – Short Run

As Table D.1 shows, a multivariate analysis of blood collected from the male participant yields a perfect cross validation (100 percent of samples correctly classified) indicating the short run provides enough physical exercise to stress the athlete. Figure D.1 shows two clusters of data for the male performing the short run: before short run (BSR) and after short run (ASR). Similar to the cross validation results, the BSR and ASR data clusters are completely separated indicating the run induces stress in the body. This stress can be seen in the antioxidant enzymes present in the blood which is a good indication it is due to oxidation occurring in the body. These results show similar trends seen in the enose analysis of breath samples, but the enose results show some confusion between the before and after sample clusters.

Female Participant – Short Run

As Table D.2 presents, a cross validation for the female participant performing the short run correctly places 75 percent of the blood samples and misidentifies 25 percent of them. Additionally, Figure D.2 shows some overlapping between the BSR and ASR sample clusters. Together these findings may indicate the short run induces some physical, and thus oxidative, stress in the female athlete but not as much as it does for the male participant. These results show similar trends found in the enose analysis of breath samples, but the cross validation results were better and the CDA presented better separation between the samples collected before and after the run in the blood.

Female Participant – Long Run

Table D.3 displays the results from a cross validation performed for the female participant's long run and indicates an 87.5 percent prediction accuracy. Figure D.3 presents two clusters of data for the female performing the long run: before long run (BLR) and after long run (ALR). Similar to the cross validation, separation occurs between the BLR and ALR sample clusters indicating the run induces stress in the body. Since more separation occurs between the BLR and ALR clusters than between the BSR and ASR clusters, it is proposed the long run induces more stress in the body of the athlete than the short run does. This stress can be observed by analyzing antioxidant enzymes (catalase and GPx) in the plasma and RBC collected from the athlete and thus demonstrates oxidative stress occurring in the body. In comparison to the enose analysis performed on breath samples, multivariate analysis of blood samples produces improved cross validation results (87.5 percent versus 55.6 percent for the enose) and better separation between the samples collected before the long run and those collected after for the female participant.

Female Participant – Both Runs

Table D.4 shows a cross validation for the female participant completing both runs correctly places 75 percent of the blood samples and misclassifies 25 percent of them. Of particular

interest is the one BSR sample which was misidentified as BLR. This trend was also sometimes found in the enose analysis of breath samples. A possible reason more BSR samples were not classified as BLR could be the weather which was cooler and more humid (15°C, 96%) during the long run than it was during the short run (25°C, 72%). Many uncontrollable variables, such as diet and life stressors, may occur between research subjects. Future work should address this issue by attempting to find a method for normalizing the data. A successful normalization may be seen when no differences between all samples collected before a run can be detected in the blood or breath.

Figure D.4 shows trends for the antioxidant enzymes present in blood similar to those seen in the enose analysis of breath samples. For both blood and breath, overlapping occurs between the BSR and ASR samples while separation occurs between the BLR and ALR samples. However, short run data clusters are more separated from the long run clusters for the breath while overlapping occurs between these clusters for the blood. It can be suggested changes in antioxidant enzymes can be detected in the blood at increasing levels of physical training which is to be expected.

Both Participants – Both Runs

As Table D.5 shows, a cross validation for both runners performing both runs correctly identifies 65 percent of the blood samples and misclassifies 35 percent of them. Figure D.5 presents trends for the antioxidant enzymes present in blood similar to those found in the enose analysis of breath samples. For both blood and breath analyses, overlapping occurs between BSR and ASR sample clusters. There is also some separation occurring between the short run and long runs samples for blood and breath, but some overlapping between these data clusters still occurs for the blood analysis. No overlapping occurs between the BLR and ALR sample clusters for the breath while overlapping occurs between these two clusters for the blood. However in each analysis, separation between the long run clusters is greater than that for the samples collected before and after the short run. An explanation for this is that the body undergoes more stress during the long run than the short run and the blood and breath analyses are not as sensitive to the changes in stress taking place during the short run but are to that of the long run. Intuitively,

any increase in physical activity increases the amount of stress the body is exposed to. Additionally cross validation results are only slightly improved for the blood (65 percent prediction accuracy) over the enose (61.1 percent prediction accuracy).

References

Aathithan, S., J.C. Plant, A.N. Chaudry, and G.L. French. 2001. Diagnosis of bacteriuria by detection of volatile organic compounds in urine using an automated headspace analyzer with multiple conducting polymer sensors. J Clin Microbiol 39:2590-3.

Administration, N.O.a.A. 2009. Your National Weather Service forecast.

Aebi, H.E. 1983. Catalase, p. 273-286, *In* H. U. Bergmeyer, ed. Methods of Enzymatic Analysis, Vol. 3, 3 ed. Verlag Chemie, Deerfield Beach, Florida.

Agergaard, N., and P.T. Jensen. 1982. Procedure for blood glutathione peroxidase determination in cattle and swine. Acta Vet Scand 23:515-27.

Aghdassi, E., B.E. Wendland, A.H. Steinhart, S.L. Wolman, K. Jeejeebhoy, and J.P. Allard. 2003. Antioxidant Vitamin Supplementation in Crohn's Disease Decreases Oxidative Stress: A Randomized Controlled Trial. American Journal of Gastroenterology 98:348-353.

Aguilo, A., P. Tauler, E. Fuentespina, J.A. Tur, A. Cordova, and A. Pons. 2005. Antioxidant response to oxidative stress induced by exhaustive exercise. Physiol Behav 84:1-7.

Anglem, N., S.J. Lucas, E.A. Rose, and J.D. Cotter. 2008. Mood, illness and injury responses and recovery with adventure racing. Wilderness Environ Med 19:30-8.

Armstrong, L.E., and J.L. VanHeest. 2002. The unknown mechanism of the overtraining syndrome: clues from depression and psychoneuroimmunology. Sports Med 32:185-209.

Aronzon, A., C.W. Hanson, and E.R. Thaler. 2005. Differentiation between cerebrospinal fluid and serum with electronic nose. Otolaryngol Head Neck Surg 133:16-9.

Athamneh, A. 2006. Electronic Nose Evaluation of Grape Maturity, Virginia Polytechnic Institute and State University, Blacksburg.

Ballabio, D., M.S. Cosio, S. Mannino, and R. Todeschini. 2006. A chemometric approach based on a novel similarity/diversity measure for the characterisation and selection of electronic nose sensors. Anal Chim Acta 578:170-7.

Bayliak, M., H. Semchyshyn, and V. Lushchak. 2006. Effect of hydrogen peroxide on antioxidant enzyme activities in Saccharomyces cerevisiae is strain-specific. Biochemistry (Mosc) 71:1013-20.

Booth, C.K., B. Probert, C. Forbes-Ewan, and R.A. Coad. 2006. Australian army recruits in training display symptoms of overtraining. Mil Med 171:1059-64.

Bosenberg, A.T., J.G. Brock-Utne, S.L. Gaffin, M.T. Wells, and G.T. Blake. 1988. Strenuous exercise causes systemic endotoxemia. J Appl Physiol 65:106-8.

Bosquet, L., L. Leger, and P. Legros. 2001. Blood lactate response to overtraining in male endurance athletes. Eur J Appl Physiol 84:107-14.

Brenner, J.S. 2007. Overuse injuries, overtraining, and burnout in child and adolescent athletes. Pediatrics 119:1242-5.

Bruno, E., M. Alessandrini, F. Ottaviani, A. Delfini, D. Di Pierro, A. Camillo, and A. De Lorenzo. 2008. Can the electronic nose diagnose chronic rhinosinusitis? A new experimental study. Eur Arch Otorhinolaryngol 265:425-8.

Budgett, R., E. Newsholme, M. Lehmann, C. Sharp, D. Jones, T. Peto, D. Collins, R. Nerurkar, and P. White. 2000. Redefining the overtraining syndrome as the unexplained underperformance syndrome. Br J Sports Med 34:67-8.

Chandiok, S., B.A. Crawley, B.A. Oppenheim, P.R. Chadwick, S. Higgins, and K.C. Persaud. 1997. Screening for bacterial vaginosis: a novel application of artificial nose technology. J Clin Pathol 50:790-1.

Chen, X., M. Cao, Y. Hao, Y. Li, P. Wang, K. Ying, and H. Pan. 2005. A Non-invasive detection of lung cancer combined virtual gas sensors array with imaging recognition technique. Conf Proc IEEE Eng Med Biol Soc 6:5873-6.

Cooper, C.E., N.B. Vollaard, T. Choueiri, and M.T. Wilson. 2002. Exercise, free radicals and oxidative stress. Biochem Soc Trans 30:280-5.

Cosca, D.D., and F. Navazio. 2007. Common problems in endurance athletes. Am Fam Physician 76:237-44.

Cyrano Sciences, I. 2000. The Cyranose 320 Electronic Nose User's Manual. 3rd, Revision C ed. Cyrano Sciences, Pasadena, CA.

D'Amico, A., R. Bono, G. Pennazza, M. Santonico, G. Mantini, M. Bernabei, M. Zarlenga, C. Roscioni, E. Martinelli, R. Paolesse, and C. Di Natale. 2008. Identification of melanoma with a gas sensor array. Skin Res Technol 14:226-36.

Deventer, D.V. 2001. Discrimination of retained solvent levels in printed food-packaging using electronic nose systems, Virginia Polytechnic Institute and State University, Blacksburg.

Di Natale, C., A. Macagnano, R. Paolesse, E. Tarizzo, A. Mantini, and A. D'Amico. 2000. Human skin odor analysis by means of an electronic nose. Sensors and Actuators B 65:4.

Di Natale, C., A. Macagnano, E. Martinelli, R. Paolesse, G. D'Arcangelo, C. Roscioni, A. Finazzi-Agro, and A. D'Amico. 2003. Lung cancer identification by the analysis of breath by means of an array of non-selective gas sensors. Biosens Bioelectron 18:1209-18.

Dragonieri, S., J.T. Annema, R. Schot, M.P. van der Schee, A. Spanevello, P. Carratu, O. Resta, K.F. Rabe, and P.J. Sterk. 2008. An electronic nose in the discrimination of patients with non-small cell lung cancer and COPD. Lung Cancer.

Dragonieri, S., R. Schot, B.J. Mertens, S. Le Cessie, S.A. Gauw, A. Spanevello, O. Resta, N.P. Willard, T.J. Vink, K.F. Rabe, E.H. Bel, and P.J. Sterk. 2007. An electronic nose in the discrimination of patients with asthma and controls. J Allergy Clin Immunol 120:856-62.

Dutta, R., J.W. Gardner, and E.L. Hines. 2004. Classification of Ear, Nose, and Throat Bacteria Using a Neural-Network-Based Electronic Nose. MRS Bulletin:5.

Dutta, R., E.L. Hines, J.W. Gardner, and P. Boilot. 2002. Bacteria classification using Cyranose 320 electronic nose. Biomed Eng Online 1:4.

El-Sayed, M.S., N. Ali, and Z. El-Sayed Ali. 2005. Haemorheology in exercise and training. Sports Med 35:649-70.

Fend, R., C. Bessant, A.J. Williams, and A.C. Woodman. 2004. Monitoring haemodialysis using electronic nose and chemometrics. Biosens Bioelectron 19:1581-90.

Fend, R., A.H. Kolk, C. Bessant, P. Buijtels, P.R. Klatser, and A.C. Woodman. 2006. Prospects for clinical application of electronic-nose technology to early detection of Mycobacterium tuberculosis in culture and sputum. J Clin Microbiol 44:2039-45.

Fend, R., R. Geddes, S. Lesellier, H.M. Vordermeier, L.A. Corner, E. Gormley, E. Costello, R.G. Hewinson, D.J. Marlin, A.C. Woodman, and M.A. Chambers. 2005. Use of an electronic nose to diagnose Mycobacterium bovis infection in badgers and cattle. J Clin Microbiol 43:1745-51.

Ferrer, M.D., P. Tauler, A. Sureda, J.A. Tur, and A. Pons. 2009. Antioxidant regulatory mechanisms in neutrophils and lymphocytes after intense exercise. J Sports Sci 27:49-58.

Filaire, E., X. Bernain, M. Sagnol, and G. Lac. 2001. Preliminary results on mood state, salivary testosterone:cortisol ratio and team performance in a professional soccer team. Eur J Appl Physiol 86:179-84.

Filaire, E., B. Legrand, G. Lac, and J.M. Pequignot. 2004. Training of elite cyclists: effects on mood state and selected hormonal responses. J Sports Sci 22:1025-33.

Fillion, L., and P. Gagnon. 1999. French adaptation of the shortened version of the Profile of Mood States. Psychol Rep 84:188-90.

Finaud, J., G. Lac, and E. Filaire. 2006. Oxidative stress : relationship with exercise and training. Sports Med 36:327-58.

Gendron, K.B., N.G. Hockstein, E.R. Thaler, A. Vachani, and C.W. Hanson. 2007. In vitro discrimination of tumor cell lines with an electronic nose. Otolaryngol Head Neck Surg 137:269-73.

Gohil, K., C. Viguie, W.C. Stanley, G.A. Brooks, and L. Packer. 1988. Blood glutathione oxidation during human exercise. J Appl Physiol 64:115-9.

Gougoura, S., M.G. Nikolaidis, I.A. Kostaropoulos, A.Z. Jamurtas, G. Koukoulis, and D. Kouretas. 2007. Increased oxidative stress indices in the blood of child swimmers. Eur J Appl Physiol 100:235-9.

Gouma, P., and G. Sberveglieri. 2004. Novel materials and applications of electronic noses and tongues. MRS Bull 29:697-702.

Halson, S.L., and A.E. Jeukendrup. 2004. Does overtraining exist? An analysis of overreaching and overtraining research. Sports Med 34:967-81.

Halson, S.L., G.I. Lancaster, A.E. Jeukendrup, and M. Gleeson. 2003. Immunological responses to overreaching in cyclists. Med Sci Sports Exerc 35:854-61.

Hanson, C.W., 3rd, and E.R. Thaler. 2005. Electronic nose prediction of a clinical pneumonia score: biosensors and microbes. Anesthesiology 102:63-8.

Ho, S.P., M. Chan-Yeung, K.K. Chow, M.S. Ip, and J.C. Mak. 2005. Antioxidant enzyme activities in healthy Chinese adults: influence of age, gender and smoking. Respirology 10:305-9.

Hockstein, N.G., E.R. Thaler, Y. Lin, D.D. Lee, and C.W. Hanson. 2005. Correlation of pneumonia score with electronic nose signature: A prospective study. Ann Otol Rhinol Laryngol 114:504-8.

Hockstein, N.G., E.R. Thaler, D. Torigian, W.T. Miller, Jr., O. Deffenderfer, and C.W. Hanson. 2004. Diagnosis of pneumonia with an electronic nose: correlation of vapor signature with chest computed tomography scan findings. Laryngoscope 114:1701-5.

Hynynen, E., A. Uusitalo, N. Konttinen, and H. Rusko. 2006. Heart rate variability during night sleep and after awakening in overtrained athletes. Med Sci Sports Exerc 38:313-7.

Jeukendrup, A.E., and M.K. Hesselink. 1994. Overtraining--what do lactate curves tell us? Br J Sports Med 28:239-40.

Kellmann, M., and K.D. Gunther. 2000. Changes in stress and recovery in elite rowers during preparation for the Olympic Games. Med Sci Sports Exerc 32:676-83.

Kodogiannis, V.S., J.N. Lygouras, A. Tarczynski, and H.S. Chowdrey. 2008. Artificial odor discrimination system using electronic nose and neural networks for the identification of urinary tract infection. IEEE Trans Inf Technol Biomed 12:707-13.

Kostaropoulos, I.A., M.G. Nikolaidis, A.Z. Jamurtas, G.V. Ikonomou, V. Makrygiannis, G. Papadopoulos, and D. Kouretas. 2006. Comparison of the blood redox status between long-distance and short-distance runners. Physiol Res 55:611-6.

Laboratories, B.-R. 2009. Bio-Rad Protein Assay.

Lai, S.Y., O.F. Deffenderfer, W. Hanson, M.P. Phillips, and E.R. Thaler. 2002. Identification of upper respiratory bacterial pathogens with the electronic nose. Laryngoscope 112:975-9.

Lakier Smith, L. 2003. Overtraining, Excessive Exercise, and Altered Immunity: Is This a T Helper-1 Versus T Helper-2 Lymphocyte Response? Sports Med 33:347-364.

Lieberman, H.R., M.D. Kellogg, and G.P. Bathalon. 2008. Female marine recruit training: mood, body composition, and biochemical changes. Med Sci Sports Exerc 40:S671-6.

Lin, Y., H. Guo, Y. Chang, M. Kao, H. Wang, and R. Hong. 2001. Application of the electronic nose for uremia diagnosis. Sensors and Actuators B 76:4.

Lowery, L., and C.E. Forsythe. 2006. Protein and overtraining: potential applications for free-living athletes. J Int Soc Sports Nutr 3:42-50.

Machado, R.F., D. Laskowski, O. Deffenderfer, T. Burch, S. Zheng, P.J. Mazzone, T. Mekhail, C. Jennings, J.K. Stoller, J. Pyle, J. Duncan, R.A. Dweik, and S.C. Erzurum. 2005. Detection of lung cancer by sensor array analyses of exhaled breath. Am J Respir Crit Care Med 171:1286-91.

Margonis, K., I.G. Fatouros, A.Z. Jamurtas, M.G. Nikolaidis, I. Douroudos, A. Chatzinikolaou, A. Mitrakou, G. Mastorakos, I. Papassotiriou, K. Taxildaris, and D. Kouretas. 2007. Oxidative stress biomarkers responses to physical overtraining: implications for diagnosis. Free Radic Biol Med 43:901-10.

Maso, F., G. Lac, E. Filaire, O. Michaux, and A. Robert. 2004. Salivary testosterone and cortisol in rugby players: correlation with psychological overtraining items. Br J Sports Med 38:260-3.

McVan, B. 1977. Odors: what the nose knows. Nursing 7:46-9.

Moens, M., A. Smet, B. Naudts, J. Verhoeven, M. Ieven, P. Jorens, H.J. Geise, and F. Blockhuys. 2006. Fast identification of ten clinically important micro-organisms using an electronic nose. Lett Appl Microbiol 42:121-6.

Moore, C.A., and A.C. Fry. 2007. Nonfunctional overreaching during off-season training for skill position players in collegiate American football. J Strength Cond Res 21:793-800.

Morgan, W.P., D.R. Brown, J.S. Raglin, P.J. O'Connor, and K.A. Ellickson. 1987. Psychological monitoring of overtraining and staleness. Br J Sports Med 21:107-14.

Morton, R.H. 1997. Modelling training and overtraining. Journal of Sports Sciences 15:335-340.

Munoz, B.C., G. Steinthal, and S. Sunshine. 1999. Conductive polymer-carbon black composites-based sensor arrays for use in an electronic nose. Sensor Review 19:300-305.

Nagle, H.T., R. Gutierrez-Osuna, and S.S. Schiffman. 1998. The How and Why of Electronic Noses. IEEE Spectrum.

Nederhof, E., K.A. Lemmink, C. Visscher, R. Meeusen, and T. Mulder. 2006. Psychomotor speed: possibly a new marker for overtraining syndrome. Sports Med 36:817-28.

Nicholls, A.R., S.H. Backhouse, R.C. Polman, and J. McKenna. 2009. Stressors and affective states among professional rugby union players. Scand J Med Sci Sports 19:121-8.

Nonaka, A., M. Tanaka, H. Anguri, H. Nagata, J. Kita, and S. Shizukuishi. 2005. Clinical assessment of oral malodor intensity expressed as absolute value using an electronic nose. Oral Dis 11 Suppl 1:35-6.
Park, A. 2008. How Phelps Made Swimming History. Time Magazine.
Pathange, L.P. 2003. Non-Destructive Evaluation of Apple Maturity Using an Electronic Nose System, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.
Pavlou, A.K., and A.P. Turner. 2000. Sniffing out the truth: clinical diagnosis using the electronic nose. Clin Chem Lab Med 38:99-112.
Pavlou, A.K., N. Magan, J.M. Jones, J. Brown, P. Klatser, and A.P. Turner. 2004. Detection of Mycobacterium tuberculosis (TB) in vitro and in situ using an electronic nose in combination with a neural network system. Biosens Bioelectron 20:538-44.
Peluso, M.A., and L.H. Guerra de Andrade. 2005. Physical activity and mental health: the association between exercise and mood. Clinics 60:61-70.
Peterson, M.D., M.R. Rhea, and B.A. Alvar. 2004. Maximizing strength development in athletes: a meta-analysis to determine the dose-response relationship. J Strength Cond Res 18:377-82.
Petibois, C., G. Cazorla, J.R. Poortmans, and G. Deleris. 2002. Biochemical aspects of overtraining in endurance sports: a review. Sports Med 32:867-78.
Petibois, C., G. Cazorla, J.R. Poortmans, and G. Deleris. 2003. Biochemical aspects of overtraining in endurance sports : the metabolism alteration process syndrome. Sports Med 33:83-94.
Radak, Z., H.Y. Chung, E. Koltai, A.W. Taylor, and S. Goto. 2008. Exercise, oxidative stress and hormesis. Ageing Res Rev 7:34-42.
Raglin, J.S. 2001. Psychological factors in sport performance: the Mental Health Model revisited. Sports Med 31:875-90.
Rock, F., N. Barsan, and U. Weimar. 2008. Electronic Nose: Current Status and Future Trends. Chemical Reviews 108:705-725.
Saraoglu, H.M., and B. Edin. 2007. E-Nose system for anesthetic dose level detection using artificial neural network. J Med Syst 31:475-82.
Service, N.W. 2009. Your National Weather Service Forecast [Online]. Available by National Oceanic and Atmospheric Administration http://forecast.weather.gov/MapClick.php?CityName=Blacksburg&state=VA&site=RNK&textField1=37.2327&textField2=-80.4284 (verified 2008).
Shacham, S. 1983. A shortened version of the Profile of Mood States. J Pers Assess 47:305-6.
Shephard, R.J. 2001. Chronic fatigue syndrome: an update. Sports Med 31:167-94.
Sinha, S., U.S. Ray, M. Saha, S.N. Singh, and O.S. Tomar. 2009. Antioxidant and redox status after maximal aerobic exercise at high altitude in acclimatized lowlanders and native highlanders. Eur J Appl Physiol 106:807-14.
Smith, D.J. 2003. A framework for understanding the training process leading to elite performance. Sports Med 33:1103-26.
Smith, L.L. 2004. Tissue trauma: the underlying cause of overtraining syndrome? J Strength Cond Res 18:185-93.
Soprano, J.V., and S.M. Fuchs. 2007. Common Overuse Injuries in the Pediatric and Adolescent Athlete. Clinical Pediatric Emergency Medicine 8:7-14.

Sureda, A., P. Tauler, A. Aguilo, N. Cases, E. Fuentespina, A. Cordova, J.A. Tur, and A. Pons. 2005. Relation between oxidative stress markers and antioxidant endogenous defences during exhaustive exercise. Free Radic Res 39:1317-24.

Swimming, U.S. 2004. Michael Phelps.

Tanaka, M., H. Anguri, A. Nonaka, K. Kataoka, H. Nagata, J. Kita, and S. Shizukuishi. 2004. Clinical assessment of oral malodor by the electronic nose system. J Dent Res 83:317-21.

Tauler, P., A. Aguilo, N. Cases, A. Sureda, F. Gimenez, G. Villa, A. Cordova, and A.P. Biescas. 2002. Acute phase immune response to exercise coexists with decreased neutrophil antioxidant enzyme defences. Free Radic Res 36:1101-7.

Teeple, E., R.M. Shalvoy, and E.R. Feller. 2006. Overtraining in young athletes. Med Health R I 89:236-8.

Thaler, E.R. 2002. Candidate's thesis: the diagnostic utility of an electronic nose: rhinologic applications. Laryngoscope 112:1533-42.

Turner, A.P.F., and N. Magan. 2004. Electronic noses and disease diagnostics. Nature Reviews. Microbiology 2:161-166.

Umeda, T., K. Suzukawa, I. Takahashi, Y. Yamamoto, M. Tanabe, A. Kojima, T. Katagiri, M. Matsuzaka, M. Totsuka, S. Nakaji, and N. Sugawara. 2008. Effects of intense exercise on the physiological and mental condition of female university judoists during a training camp. J Sports Sci 26:897-904.

Urhausen, A., and W. Kindermann. 2002. Diagnosis of overtraining: what tools do we have? Sports Med 32:95-102.

Varlet-Marie, E., F. Maso, G. Lac, and J.F. Brun. 2004. Hemorheological disturbances in the overtraining syndrome. Clin Hemorheol Microcirc 30:211-8.

Voss, A., V. Baier, R. Reisch, K. von Roda, P. Elsner, H. Ahlers, and G. Stein. 2005. Smelling renal dysfunction via electronic nose. Ann Biomed Eng 33:656-60.

Wang, P., Y. Tan, H. Xie, and F. Shen. 1997. A novel method for diabetes diagnosis based on electronic nose. Biosens Bioelectron 12:1031-6.

Witt, E.H., A.Z. Reznick, C.A. Viguie, P. Starke-Reed, and L. Packer. 1992. Exercise, oxidative damage and effects of antioxidant manipulation. J Nutr 122:766-73.

Yamada, Y., Y. Takahashi, K. Konishi, and I. Katsuumi. 2007. Association of odor from infected root canal analyzed by an electronic nose with isolated bacteria. J Endod 33:1106-9.

Yates, J.W., M.J. Chappell, J.W. Gardner, C.S. Dow, C. Dowson, A. Hamood, F. Bolt, and L. Beeby. 2005. Data reduction in headspace analysis of blood and urine samples for robust bacterial identification. Comput Methods Programs Biomed 79:259-71.

Zhu, H., Z. Jia, J.S. Strobl, M. Ehrich, H.P. Misra, and Y. Li. 2008. Potent induction of total cellular and mitochondrial antioxidants and phase 2 enzymes by cruciferous sulforaphane in rat aortic smooth muscle cells: cytoprotection against oxidative and electrophilic stress. Cardiovasc Toxicol 8:115-25.

Chapter 5: Summary and Conclusions

5.1 SUMMARY

Athletic performance is improved by increasing training loads. Unfortunately it is difficult to accurately monitor the response of an athlete to a given training load and the amount of stress incurred as a result of it. This makes the prevention of overtraining through real-time adjustments of training programs difficult. The hypothesis of this study was that a conducting polymer-based electronic nose will be able to discriminate between different levels of training stress in long distance runners, and therefore may be utilized as an inexpensive, noninvasive tool in the detection and prevention of overtraining associated with oxidative stress.

Two long distance runners (one male and one female) performed a short run consisting of about three miles. Twenty-two days later, the female runner performed a long run of about nine miles. Unfortunately, the male athlete was not able to complete the long run due to scheduling conflicts.

Breath samples were collected from each individual before and after each run. An enose was then utilized to sniff and analyze each sample. An analysis performed by the enose unit was then compared to an independent statistical analysis performed by JMP®. Blood samples were also collected from each individual prior to beginning and after completing each run. Plasma and red blood cells (RBC) collected from each sample were then each analyzed for catalase and glutathione peroxidase (GPx) activity. Catalase and GPx results were compared to results of the enose analyses of the breath samples. Conclusions about the analyses performed and proposed future work are presented in this chapter.

5.2 CONCLUSIONS

The original protocol for this study called for 10 research subjects. However in an effort to provide proof-of-concept and secure data for large-scale proposals, a small pilot-scale study was performed with two highly trained long distance runners. Both participants performed a short run but unfortunately scheduling conflicts prevented the male athlete from performing the long

run. This essentially reduced the number of study participants to one which provided very limited data for analyses. Although enough data to conclusively confirm the feasibility of an enose to detect stress due to exercise in athletes was not available, enough data was collected to determine proof-of-concept and provide methodology suggestions. However, future work should utilize a larger sample size to determine the full capability of the enose as a part of the measures taken toward the prevention of the onset of overtraining.

This study found a conducting polymer based hand-held enose (Model: Cyranose 320, Smiths Detection, Pasadena, CA) is feasible for the detection of volatiles present in the breath of athletes incurred through physical exercise and likely oxidative stress. Additionally the enose unit was able to detect a greater difference between breath samples collected before and after the long run than the difference detected between the samples collected before and after the short run. The enose showed some confusion in distinguishing between samples collected immediately after the runs were completed on the field and those collected from 10 to 15 minutes after finishing the runs and walking to the clinic. Differences between the field and clinic samples were found to be either significant or insignificant depending on the type of participant-run scenario being analyzed. Therefore, it is proposed any future work should continue collecting at least both the field and clinic samples until further analyses can be performed to verify one theory or the other.

For cross validation and canonical discriminant analysis (CDA) plots performed on the breath sniffed by the enose, results obtained by JMP® were improved from those obtained by the enose and PCnose®. For example a cross validation performed by the enose was able to correctly place 36.1 percent of the breath samples within their respective clusters. However, cross validations performed by JMP® were able to correctly place up to 77.8 percent (male performing the short run) of the breath samples within their respective groups. JMP® allowed for a relatively easy analysis of individual scenarios, such as the male performing the short run or the female performing the long run, as opposed to analysis performed by the enose which analyzed the entire data set at one time. Additionally, the number of principal components, for each given scenario, were easily selected and input for the CDA. Alternatively, the enose automatically chose the number of principal components and performed the CDA without scrutiny from the user. It is believed the improvements seen by the JMP® analyses indicate a proprietary

opportunity for this particular application of the enose. The response for each of the sensors on the enose array should be reviewed to determine which ones are most sensitive to volatiles present in the breath due to physical exercise. The least sensitive ones could then be removed and, if found, more sensitive sensors could be added to the array. Additionally, the software utilized by the enose unit can be improved.

Catalase and GPx activity levels found in the blood samples indicate physical exercise induces oxidative stress in the plasma and RBC of long distance runners. Although results were generally inconclusive, the differences between the catalase and GPx activity levels in the samples collected before and after a run were more significant when the athlete performed the long run as opposed to the short run. It is believed this is an indication a longer training load, or run, induces more oxidative stress in the blood of athletes than a short run does. This conclusion was reinforced by a multivariate analysis of the blood data which analyzed each biomarker (catalase and GPx) for each sample (plasma and RBC) together. This multivariate analysis yielded more conclusive results than the individual analyses for each marker performed for each sample. However, the multivariate analysis of the blood data was only slightly better than that of the enose (65 versus 61.1 percent prediction accuracy) but both indicated similar trends in the data.

Catalase and GPx activity levels were comparable to those received from the enose analyses. Therefore one can conclude volatiles, which may be caused by oxidative stress, are present in the breath of athletes after performing physical exercise, such as running. Enose technology has shown promise in the detection of these volatiles and may be a faster, noninvasive, method for detecting the onset of oxidative stress, and therefore overtraining. Future work should be performed to determine the complete feasibility of an enose in the detection of training stress in the breath of athletes.

Chapter 6: Future Work

This pilot-scale study found a conducting polymer based hand-held enose is able to detect volatiles present in the breath of athletes incurred through physical exercise and likely oxidative stress. The next phase of this project should focus on testing and correlating more suggested biomarkers of oxidative stress and overtraining for an expanded sample size. A sample of five female and five male collegiate long distance runners will be targeted. Breath will continue to be analyzed by the Cyranose 320 but will also be analyzed by a gas chromatography mass spectroscopy (GCMS) unit for pentane. Blood will continue to be analyzed for catalase and GPx activity levels, along with malondialdehyde (MDA), nitric oxide, SOD, maximal lactate steady state, (MLSS), and total glutathione content. Additionally, urine will be collected before and after each run and then analyzed for MDA and 8-hydroxydeoxyguanosine (8-OHdG). If funding exists, and it is not a large imposition to the participants, breath, blood, and urine samples will also be collected at more intervals after run completion in an effort to study the progression of the body's response to exercise and oxidative stress. Data for each biomarker will be analyzed independently and then together using multivariate methods. A correlation between them all is sought. Beyond testing the feasibility of the enose in detecting oxidative stress due to exercise in the breath of athletes, a goal of conclusively identifying at least one clinically specific biomarker for the detection of the onset of overtraining exists.

If possible, data should be collected for an athlete who is overtrained. Results from this study indicate the chosen run lengths induced different levels of training, and thus oxidative, stress but neither participant showed any symptoms for overtraining. Intentionally submitting any person to conditions which would induce overtraining is harmful to that person and unethical. However, long distance runners who are already members of the cross country team readily submit themselves to extreme training conditions as part of their participation on the team. Therefore the chance of having an athlete overtrain at some point during the season exists. The study protocol states any athlete participating in the study and who has been clinically diagnosed as overtrained will be asked to provide biological samples for testing.

Although the main focus of this study is the detection of the onset of overtraining in the breath of athletes, if the electronic nose is proven to be capable of detecting oxidative stress in the human breath on a large scale, there are many other exciting opportunities available outside this area. Broader clinical applicability may be seen in the diagnosis and management of infections and metabolic diseases due to oxidative stress such as diabetes, cancer and pneumonia. Other applications may include the detection of exhaustion in the breath of people who are using exercise as a means to lose weight or in construction workers and service people who continuously perform in physically exhausting settings. Additionally, the enose may be used in situations where diagnostic equipment is hard to find, such as third world countries, and closer to home: in Appalachia. This cheaper, more convenient, and less invasive method has the potential to enhance the quality of life of many individuals, near and far.

Appendix A.1: VCOM IRB Approval

Edward Via
Virginia College of Osteopathic Medicine
Physicians & Research for the 21st Century

Institutional Review Board
Dr. Hara Misra
Chairman
540.231.3693
misra@vcom.vt.edu

VCOM Institutional Review Board
Notice of Review

February 21, 2008

P. Gunnar Brolinson, DO
Sports Medicine

RE: IRB#2007/001, Chemosensory Evaluation of Training and Oxidative Stress in Runners

Dear Dr. Brolinson:

The proposed research is eligible for expedited review according to the specifications authorized by 45CFR 46.110 and 21 CFR 56.110. Your protocol has been reviewed via expedited procedure by two members of the VCOM IRB. One reviewer had some minor questions and comments which were sent to you via email on October 2, 2007. I have reviewed your response to these minor questions which was received on February 14, 2008 and have **approved your project**.

Federal guidelines dictate that IRB-approved research must be reviewed no less than once a year. Note that your continuation review will be February 20, 2009. Approximately 30 days before this date, you will receive a Progress Report Form from the IRB Coordinator. Please fill out this report and submit it to the IRB Coordinator at least two weeks prior to your review date.

Please remember that as the PI, you are responsible for promptly reporting to the IRB any proposed changes in the research activity prior to being implemented. You are also responsible for promptly reporting any injuries or adverse events or unanticipated risks to subjects.

Please be advised that the VCOM IRB will be conducting routine audits as a means of ensuring compliance with VCOM and federal policies in an effort to assure the protection of human subjects. Your project may, at any time throughout the approval period, be subject to this type of monitoring.

Thank you for your cooperation. If you have any questions or concerns, please do not hesitate to contact the IRB Coordinator, Sharon Kauffman at skauffman@vcom.vt.edu or 231-4512.

Sincerely,

Hara P. Misra

Hara P. Misra, DVM, PhD
Chairman, VCOM Institutional Review Board

2265 Kraft Drive, Blacksburg, Virginia 24060
Phone: 540.231.4000 Fax: 540.231.5252

Appendix A.2: Virginia Tech IRB Approval

VirginiaTech

Institutional Review Board
David M. Moore, Chair
2000 Kraft Drive (0497)
Blacksburg, Virginia 24060
540/231-4991 Fax: 540/231-0959
E-mail: moored@vt.edu
www.irb.vt.edu

DATE: June 5, 2008

MEMORANDUM

TO: Per Gunnar Brolinson
Kumar Malikarjunan
Hara P. Misra
Zhenquan Jia
Christan Whysong

FROM: David M. Moore

SUBJECT: Decision to Cede Authority for Review/Approval/Monitoring of Human Subjects Research, IRB #08-344

On June 5, 2008, Virginia Tech (VT) Institutional Review Board, in evaluating the protocol entitled "Chemosensory Evaluation of Training and Oxidative Stress in Runners", has ceded authority for review, approval, and continuing review activities to the Edward Via Virginia College of Osteopathic Medicine IRB with the requirement that investigators inform the VT IRB of any serious or unanticipated adverse events related to VT human subjects.

cc: File

Appendix B: Profile of Mood States (POMS) for Participants in Chemosensory Evaluation of Training and Oxidative Stress in Runners Study

Edward Via Virginia College of Osteopathic Medicine

A Profile of Mood States (POMS) is a checklist of adjectives leading to the assessment of mood states and will be used to evaluate the role mood has on athletic performance. Each adjective is rated based upon a five-point scale for analysis leading to an overall mood state classification. Please take a few minutes to circle the appropriate rating for how you are feeling at this time for each adjective.

Initials: _____ Identification Number: _____

Birth date: _____ POMS Completion Date: _____

Feeling/Adjective	Not at all	A little	Moderate	Quite a bit	Extremely
Friendly	1	2	3	4	5
Tense	1	2	3	4	5
Angry	1	2	3	4	5
Worn Out	1	2	3	4	5
Unhappy	1	2	3	4	5
Clear-headed	1	2	3	4	5
Lively	1	2	3	4	5
Confused	1	2	3	4	5
Sorry for things done	1	2	3	4	5
Shaky	1	2	3	4	5
Listless	1	2	3	4	5
Peeved	1	2	3	4	5
Considerate	1	2	3	4	5
Sad	1	2	3	4	5
Active	1	2	3	4	5
On edge	1	2	3	4	5
Grouchy	1	2	3	4	5
Blue	1	2	3	4	5
Energetic	1	2	3	4	5
Panicky	1	2	3	4	5
Hopeless	1	2	3	4	5
Relaxed	1	2	3	4	5

Unworthy	1	2	3	4	5
Spiteful	1	2	3	4	5
Sympathetic	1	2	3	4	5
Uneasy	1	2	3	4	5
Restless	1	2	3	4	5
Unable to concentrate	1	2	3	4	5
Fatigued	1	2	3	4	5
Helpful	1	2	3	4	5
Annoyed	1	2	3	4	5
Discouraged	1	2	3	4	5
Resentful	1	2	3	4	5
Nervous	1	2	3	4	5
Lonely	1	2	3	4	5
Miserable	1	2	3	4	5
Muddled	1	2	3	4	5
Cheerful	1	2	3	4	5
Bitter	1	2	3	4	5
Exhausted	1	2	3	4	5
Anxious	1	2	3	4	5
Ready to fight	1	2	3	4	5
Good-natured	1	2	3	4	5
Gloomy	1	2	3	4	5
Desperate	1	2	3	4	5
Sluggish	1	2	3	4	5
Rebellious	1	2	3	4	5
Helpless	1	2	3	4	5
Weary	1	2	3	4	5
Bewildered	1	2	3	4	5
Alert	1	2	3	4	5
Deceived	1	2	3	4	5
Furious	1	2	3	4	5
Effacious	1	2	3	4	5
Trusting	1	2	3	4	5
Full of pep	1	2	3	4	5
Bad-tempered	1	2	3	4	5
Worthless	1	2	3	4	5
Forgetful	1	2	3	4	5

Carefree	1	2	3	4	5
Terrified	1	2	3	4	5
Guilty	1	2	3	4	5
Vigorous	1	2	3	4	5
Uncertain about things	1	2	3	4	5
Bushed	1	2	3	4	5

Table B.1. A summary of the athletes' responses for the POMS survey collected pre-study. Results were similar for both athletes and did not warrant further analysis.

Athlete's Selection		**Corresponding Feelings/Adjectives**
Male	**Female**	
1	1	angry, shaky, peeved, grouchy, panicky, hopeless, unworthy, spiteful, unable to concentrate, resentful, miserable, bitter, ready to fight, gloomy, desperate, helpless, bewildered, deceived, furious, bad-tempered, worthless, terrified, guilty, and bushed
1	2	unhappy, listless, sad, on edge, blue, discouraged, lonely, muddled, anxious, and weary
1	3	tense, sorry for things done, and exhausted
1	4	worn out
1	5	not selected
2	1	uneasy, restless, annoyed, nervous, rebellious, and forgetful
2	2	confused, fatigued, sluggish, and uncertain about things
2	3	not selected
2	4	not selected
2	5	not selected
3	1	Sympathetic
3	2	not selected
3	3	lively, cheerful, effacious, full of pep, and vigorous
3	4	not selected
3	5	not selected
4	1	not selected
4	2	not selected
4	3	energetic, relaxed
4	4	friendly, clear-headed, considerate, active, helpful, good-natured, alert, trusting, and carefree
4	5	not selected
5	1	not selected
5	2	not selected
5	3	not selected
5	4	not selected
5	5	not selected

Appendix C: Training Questionnaire for Participants is Chemosensory Evaluation of Training and Oxidative Stress in Runners Study

Edward Via Virginia College of Osteopathic Medicine

The purpose of this study is to evaluate a non-invasive method for measuring training stress incurred through long distance running. To better understand the training stress each participant undergoes during the regular Virginia Tech Cross Country program, it is important to have background knowledge about typical training performed outside of the cross country program. This questionnaire was created to assist study investigators in understanding participants' training background. Please take a few minutes to complete it.

Name: _____ **Male** _____ **Female** _____
Last First MI

Date of Birth: _____ **Ethnicity:**_____
Month Day Year

Height: _____ft _____in **Weight:** _____lb **Do you smoke?** _____

How often do you drink (alcohol):

_____ Never

_____ Occasionally (3 to 5 drinks per week)

_____ Frequently (more than 5 drinks per week)

If you selected occasionally or frequently, do you drink in season, out of season, or both:

_____ In season _____ Out of season _____ Both

Please list any over-the-counter medications you are currently taking or have taken during the past 30 days:

_____

_____

_____

_____

_____

Please list any prescription medications you are currently taking or have taken during the past 30 days:

_____

_____

_____

_____

_____

Please list any nutritional supplements you are currently taking or have taken during the past 30 days:

_____

_____

_____

_____

_____

Please list any injuries you have incurred over the last 30 days:

_____

_____

_____

Please list any illnesses you have incurred over the last 30 days:

_____

_____

_____

Please describe training activities you have been involved in over the past 30 days:

Miles run per week: _____

Number of strength training sessions per week _____

Other training activities (Explain):

_____

_____

_____

Athlete's responses: Each athlete completed the training questionnaire prior to beginning the study. At this time, both athletes reported not smoking. The male athlete reported drinking occasionally out of season while the female athlete responded she never drinks. Additionally, both athletes reported they run at least 40 miles each week and participate in two strength training sessions each week. Neither athlete reported any injuries or illnesses during the 30 days prior to the study. Finally, neither athlete reported taking any nutritional supplements during the 30 days prior to the study.

Appendix D: Multivariate Statistical Analysis of Blood

Table D.1. Cross validation table showing 100 percent correct classification of blood samples taken from the male participant during the short run.

		Identified As	
		BSR	ASR
Actual	BSR	3	0
	ASR	0	2

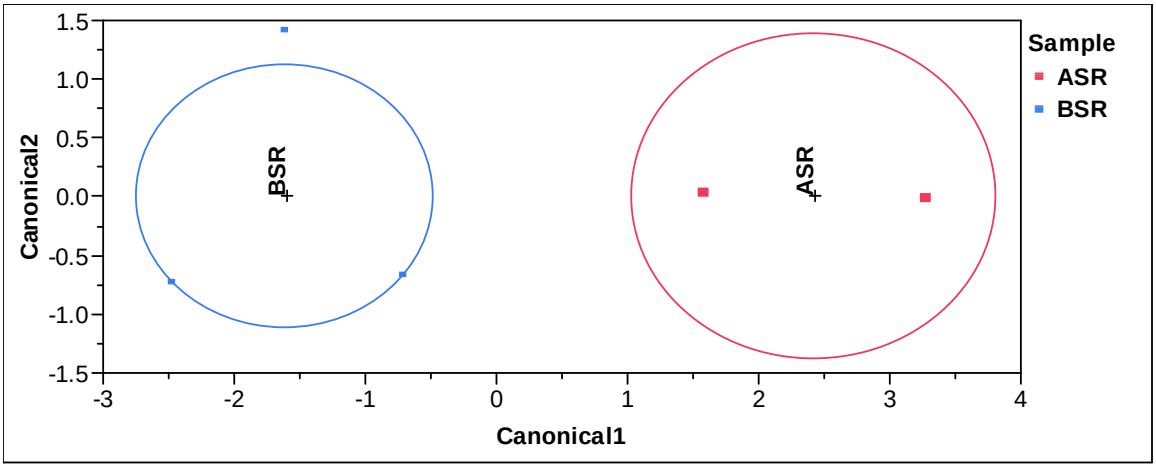

Figure D.1. Canonical plot showing two data clusters for the male runner performing the short run.

Table D.2. Cross validation table showing 75 percent correct classification of blood samples taken from the female participant during the short run.

		Identified As	
		BSR	ASR
Actual	BSR	2	2
	ASR	0	4

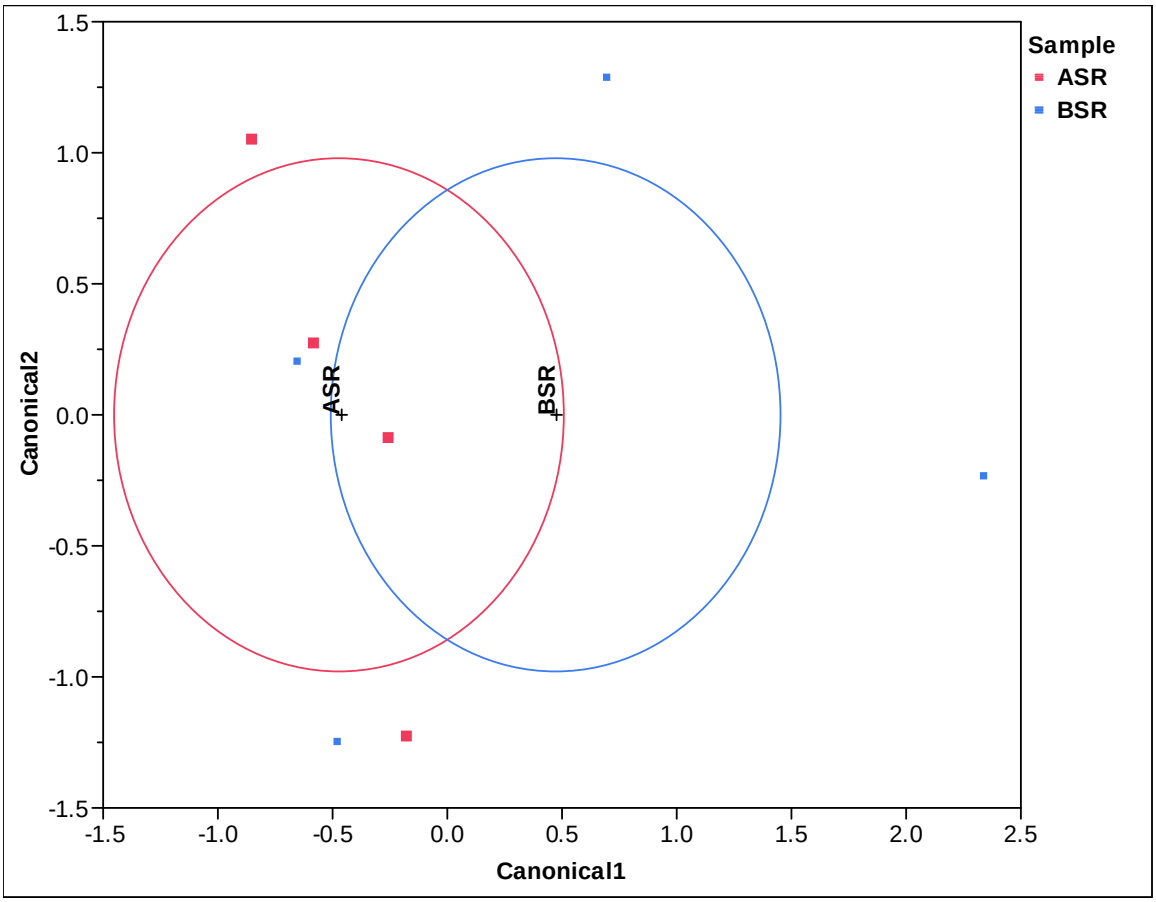

Figure D.2. Canonical plot showing two data clusters for the female runner performing the short run.

Table D.3. Cross validation table showing 87.5 percent correct classification of blood samples taken from the female participant during the long run.

		Identified As	
		BLR	ALR
Actual	BLR	3	1
	ALR	0	4

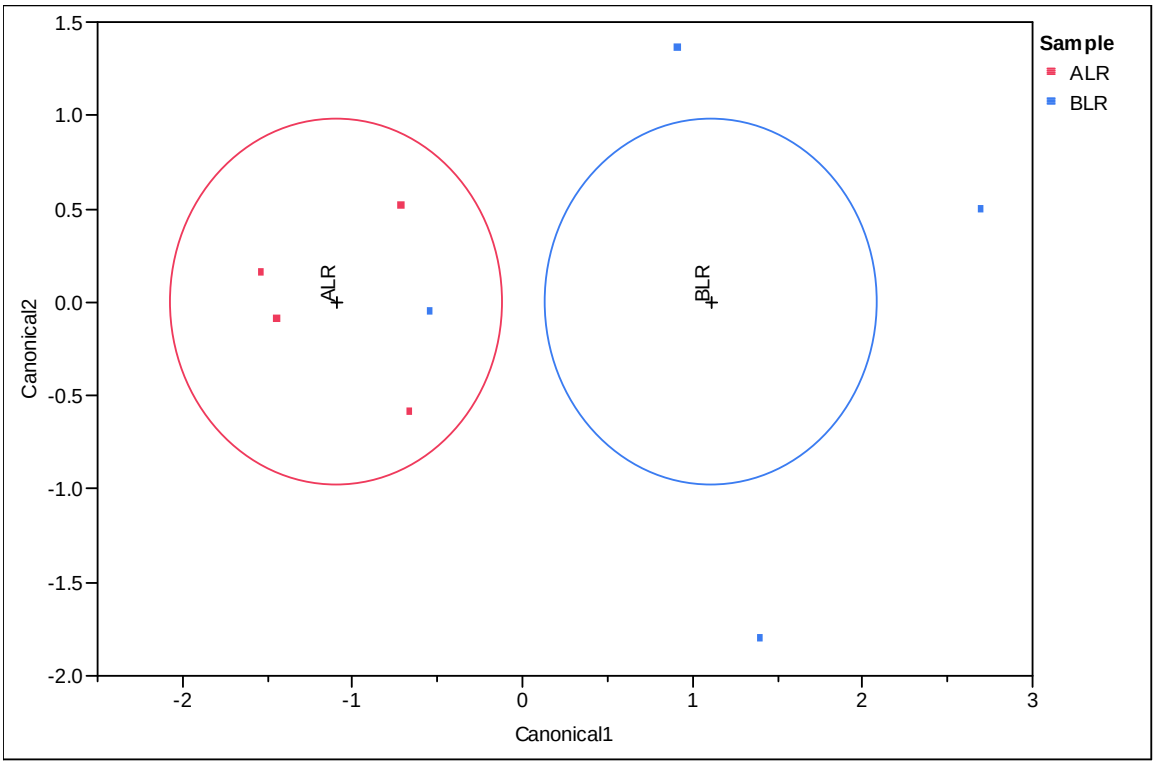

Figure D.3. Canonical plot showing two data clusters for the female runner performing the long run.

Table D.4. Cross validation table showing 75 percent correct classification of blood samples taken from the female participant during both runs.

		Identified As			
		BSR	ASR	BLR	ALR
Actual	BSR	2	1	1	0
	ASR	1	3	0	0
	BLR	0	0	3	1
	ALR	0	0	0	4

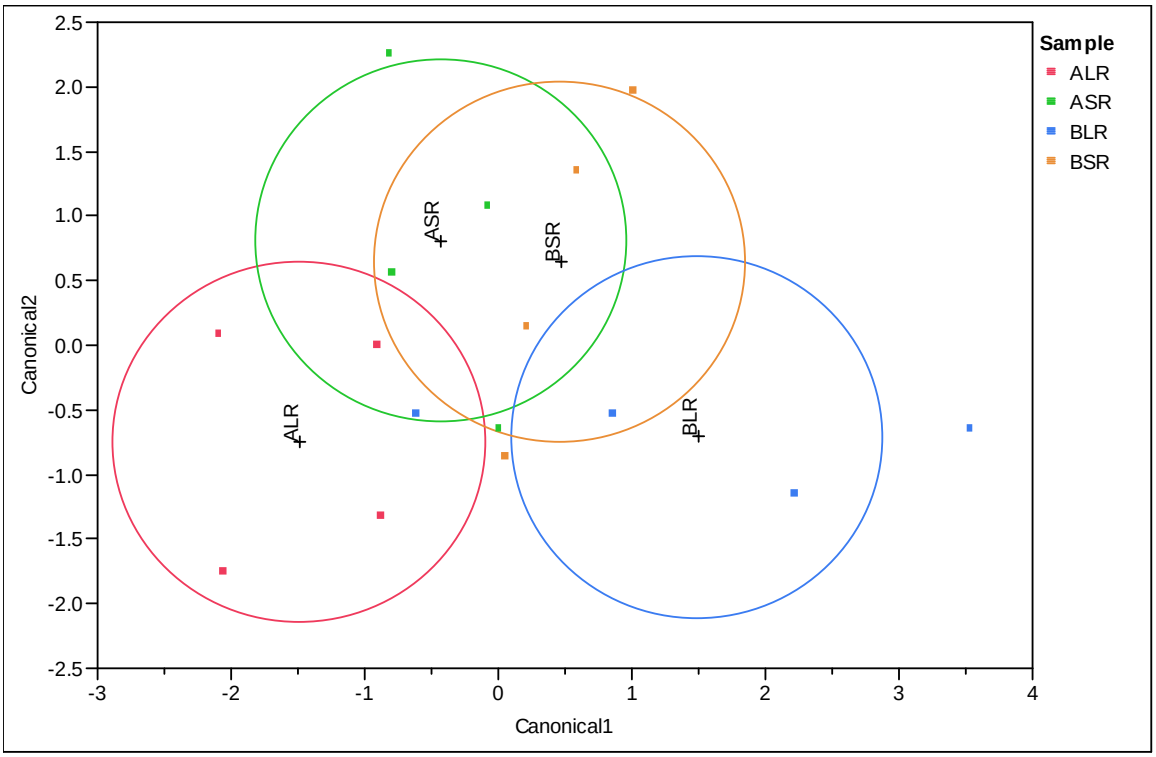

Figure D.4. Canonical plot showing four data clusters for the female runner performing both runs.

Table D.5. Cross validation table showing 65 percent correct classification of blood samples taken from the both participants during both runs.

		Identified As			
		BSR	ASR	BLR	ALR
Actual	BSR	3	2	0	1
	ASR	1	4	0	1
	BLR	0	1	2	1
	ALR	0	0	0	4

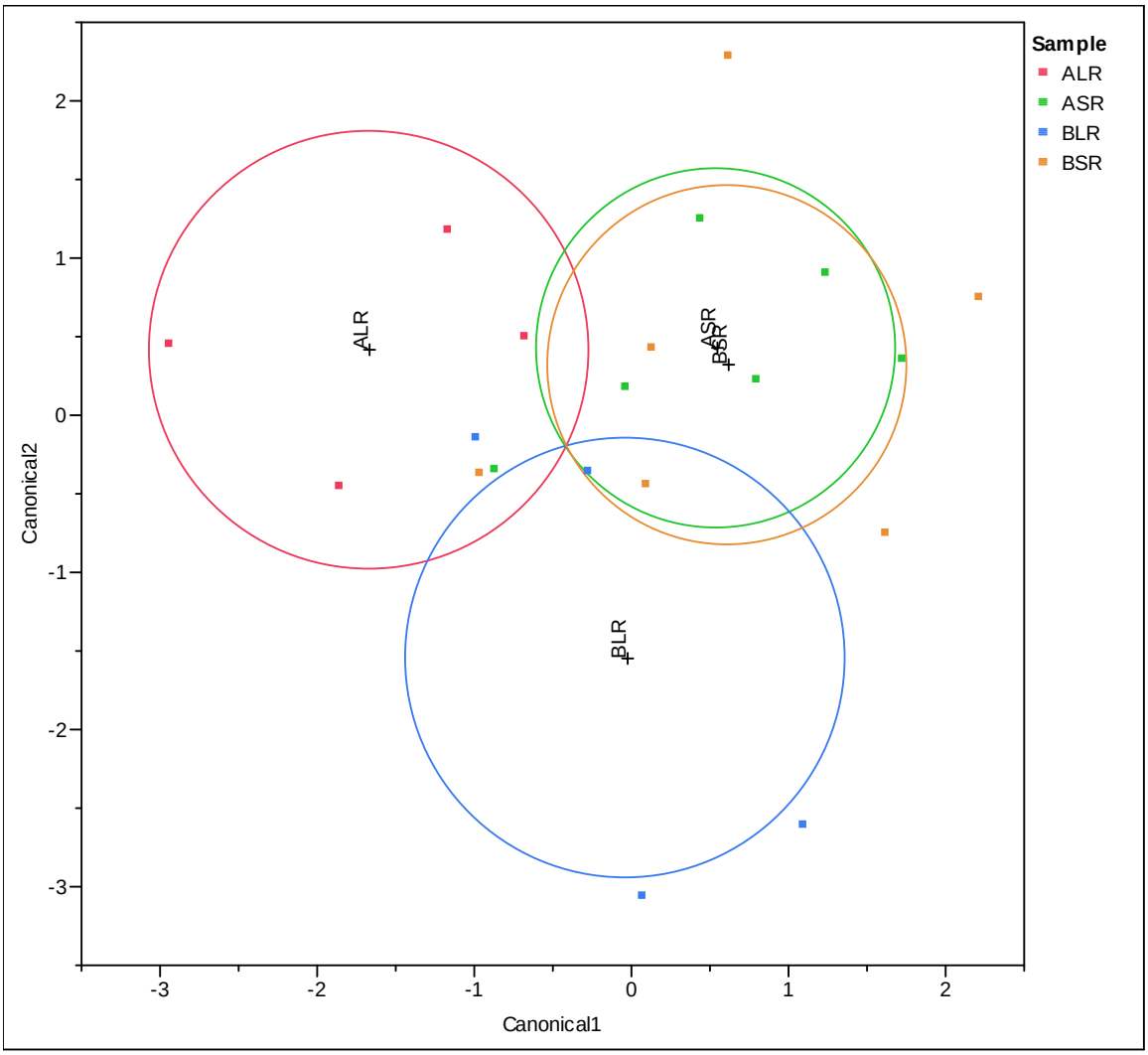

Figure D.5. Canonical plot showing four data clusters for both runners performing both runs.