Cardiovascular and Ventilatory Limitations in the Oxygen Transport Pathway

by

Danielle Jessica Padilla

B.S., New Mexico State University
M.S., New Mexico State University

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Anatomy and Physiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2005

ABSTRACT

The components of the O_2 transport pathway can be divided into (along with their respective circulations) the pulmonary, cardiovascular, and skeletal muscle systems. They must operate in tight conjunction with one another, especially during dynamic exercise, to sustain ATP production within muscle mitochondria. Any limitation placed on the O_2 transport pathway will result in decreased performance. The purpose of this dissertation is to present four novel studies which examine specific limitations on (1) the pulmonary system (i.e. lungs and circulation) within the highly athletic Thoroughbred horse (Studies A & B), and (2) within the peripheral circulation (i.e. microcirculation) within a disease model of Type II diabetes, the Goto-Kakizaki (GK) rat (Studies C & D). Study A demonstrates that locomotory respiratory coupling (LRC) is not requisite for the horse to achieve maximal minute ventilation ($\dot{V}_E$) during galloping exercise because $\dot{V}_E$ remains at the peak exercising levels over the first ~13 s of trotting recovery ($\dot{V}_E$ at end exercise: 1391±88; $\dot{V}_E$ at 13 s: 1330±112 L/sec; $P > 0.05$). The horse also experiences exercise-induced pulmonary hemorrhage (EIPH) which has been linked mechanistically to increased pulmonary artery pressure (Ppa) during high intensity exercise. Therefore, in Study B, we hypothesized that endothelin-1 (ET-1), a powerful vasoconstricting hormone, would play a role in the augmented Ppa and therefore, EIPH. However, contrary to our hypothesis, an ET-1 receptor antagonist did not decrease Ppa nor prevent or reduce EIPH. Studies C and D examine potential mechanisms behind the exercise intolerance observed in humans with Type II diabetes. Utilizing phosphorescence quenching techniques (Study C) within the GK spinotrapezius muscle, we found lowered microvascular PO_2 (PO_2mv; Control: 28.8±2.0; GK: 18.4±1.8 mmHg; $P<0.05$) at rest and a PO_2mv "undershoot" during muscle contractions. After conducting intravital microscopy within the same muscle (Study D), we discovered the percentage of RBC-perfused capillaries was decreased (Control: 93±3; GK: 66±5 %; $P<0.05$) and all three major hemodynamic variables (i.e. RBC velocity, flux, and capillary tube hematocrit) were significantly attenuated. Both studies (C & D) indicate that there is reduced O_2 availability (via decreased O_2 delivery; i.e. $\downarrow\dot{Q}O_2/\dot{V}O_2$) within Type II diabetic muscle.

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Approved by:

Major Professor
David C. Poole

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ABSTRACT

The components of the O_2 transport pathway can be divided into (along with their respective circulations) the pulmonary, cardiovascular, and skeletal muscle systems. They must operate in tight conjunction with one another, especially during dynamic exercise, to sustain ATP production within muscle mitochondria. Any limitation placed on the O_2 transport pathway will result in decreased performance. The purpose of this dissertation is to present four novel studies which examine specific limitations on (1) the pulmonary system (i.e. lungs and circulation) within the highly athletic Thoroughbred horse (Studies A & B), and (2) within the peripheral circulation (i.e. microcirculation) within a disease model of Type II diabetes, the Goto-Kakizaki (GK) rat (Studies C & D). Study A demonstrates that locomotory respiratory coupling (LRC) is not requisite for the horse to achieve maximal minute ventilation ($\dot{V}_E$) during galloping exercise because $\dot{V}_E$ remains at the peak exercising levels over the first ~13 s of trotting recovery ($\dot{V}_E$ at end exercise: 1391±88; $\dot{V}_E$ at 13 s: 1330±112 L/sec; $P > 0.05$). The horse also experiences exercise-induced pulmonary hemorrhage (EIPH) which has been linked mechanistically to increased pulmonary artery pressure (Ppa) during high intensity exercise. Therefore, in Study B, we hypothesized that endothelin-1 (ET-1), a powerful vasoconstricting hormone, would play a role in the augmented Ppa and therefore, EIPH. However, contrary to our hypothesis, an ET-1 receptor antagonist did not decrease Ppa nor prevent or reduce EIPH. Studies C and D examine potential mechanisms behind the exercise intolerance observed in humans with Type II diabetes. Utilizing phosphorescence quenching techniques (Study C) within the GK spinotrapezius muscle, we found lowered microvascular PO_2 (PO_2mv; Control: 28.8±2.0; GK: 18.4±1.8 mmHg; $P<0.05$) at rest and a PO_2mv "undershoot" during muscle contractions. After conducting intravital microscopy within the same muscle (Study D), we discovered the percentage of RBC-perfused capillaries was decreased (Control: 93±3; GK: 66±5 %; $P<0.05$) and all three major hemodynamic variables (i.e. RBC velocity, flux, and capillary tube hematocrit) were significantly attenuated. Both studies (C & D) indicate that there is reduced O_2 availability (via decreased O_2 delivery; i.e. $\downarrow\dot{Q}O_2/\dot{V}O_2$) within Type II diabetic muscle.

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ACKNOWLEDGEMENTS

I would like to begin by thanking Dr. David Poole, my mentor and major professor, for his kindness, patience, encouragement, and opening me up to a world of opportunities in research. He made me feel like I was a part of his two families, the laboratory family and his "at home" family with Katherine (his wife), and children, Shayna, Connor, and little Kelton. I would also like to thank my committee members, Dr. Howard Erickson, Dr. Timothy Musch, Dr. Craig Harms, Dr. Michael Kenney, and Dr. Brian Niehoff for their support during my program of study. Next, I wish to thank the many people I have had the opportunity to work with throughout my 4 ½ years in Kansas. These include Dr. Bradley Behnke, Dr. Paul McDonough, Dr. Casey Kindig, Mrs. Sue Hagemen, Dr. Tammi Epp, Dr. Yutaka Kano, and Mrs. Pam Davis. I would also like to thank all of the student workers and volunteers who helped with my projects at the KSU Equine Treadmill Laboratory. However, I would not be at Kansas State University if I had not received generous support from the National Institutes of Health, the American Quarter Horse Association, and the Kansas Racing Commission. I also would like to express my appreciation to Dr. Tom Barstow and the Department of Kinesiology for welcoming me as a member of the faculty. Last, but certainly not least, I'd like to thank my family, Jesse (my father), Dorothea (my mother), Andy (my brother), and Ryan (my boyfriend) for their continuous support. They have all helped me to accomplish and complete my goals, and I hope to continue making them proud and I love them with all my heart.

CURRICULUM VITAE

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BIOGRAPHICAL DATA

Birthdate: March 12, 1977

Place of Birth: Fort Bragg, North Carolina

EDUCATION

August 2001-Present	Kansas State University	Anatomy & Physiology (Pre-Doctoral Fellow)
August 2001	New Mexico State University	Animal Science (M.S.) Minor: Toxicology
May 1999	New Mexico State University	Animal Science (B.S.) Emphasis: Horse Science
May 1993 to May 1995	Cochise Community College	Undergraduate Credit

RESEARCH EXPERIENCE

2001-present Pre-Doctoral Fellow/ Research Assistant at Dept. of Anatomy and Physiology, Kansas State University
Sponsored by Minority Pre-Doctoral Fellowship Program of National Institutes of Health.

Research and Technical Skills:
Equine treadmill exercise and testing (VO_2max) procedures involving operation of a bias open flow system and breath-by-breath analysis system for the determination of ventilation, O_2 uptake and CO_2 elimination, calibration of equipment needed for experimental use such as a mass spectrometer, thermistor catheter, and pressure transducers); performance, processing, and analysis of bronchoalveolar lavage samples from horses with exercise-induced pulmonary hemorrhage; blood gas analysis and acid-base analysis; microscopic slide preparation for white-blood cell differentials; participates in studies involving greyhound dogs and performance and processing of blood samples and bronchoalveolar lavage samples from greyhounds with exercise-induced pulmonary hemorrhage.

Measurements for determining capillary-to-fiber ratio, fiber cross-sectional area, and capillary density; performs surgical preparation for phosphorescence quenching procedures, participates in phosphorescence quenching experiments to study different aspects of O_2 transport which includes the microvascular O_2 pressure within healthy and diseased tissues for insights to the determinants of microvascular O_2 exchange; participates in experiments on the microcirculation of the rat spinotrapezius muscle; performs analysis of microcirculation which includes measuring sarcomere length, capillary diameter, capillary flow, and capillary hematocrit, flux and velocity; participates in several projects including diseased models such as chronic heart failure (rats), hypotension (rats), Type II diabetes (rats), and emphysema (hamsters); examining the physiology of muscle function in both healthy and exercise-induced muscle damage (rats); preparation of experiments (e.g.

exercise training of rats, catheterization, and equipment set-up), surgeries (e.g. exposure of spinotrapezius muscle for PO_2 measurements and microcirculation, and induction of heart failure in rats), data collection, and statistical analysis; other duties include providing assistance to advisors and other graduate students in their research projects.

1999-2001 Graduate Research Assistant at Dept. of Animal and Range Sciences, New Mexico State University

Sponsored by Minority Biomedical Research Support Assistantship Program/Research Initiative for Enhancement (MBRS-RISE) of the National Institutes of Health.

Research and Technical Skills: Designed, conducted, and analyzed research project pertaining to snakeweed (*Gutierrezia* spp.) toxicity in sheep. Emphasized on snakeweed's effects on reproduction and liver function. Surgical procedures conducted: ovariectomies, vasectomies, C-sections, ruminal cannulations (ovine and bovine). Sample collection: diet sampling (measuring orts), blood collection (jugular vena puncture, cardiac puncture in rats); dissection and decapitation of rats. Laboratory procedures: microtiter plate reader, radioimmunoassay, hexane extraction, Micro Kjeldahl, fiber analysis (NDF and ADF), spectrophotometer. Applied: handling of young and mature horses, handling of young and mature sheep, handling of rats, hot branding of cattle, paint branding of sheep, handling wool, inspection of meat animals and carcasses.

1996-2001 Research Assistant at Dept. of Animal and Range Sciences, New Mexico State University

Research and Technical Skills: Tested viability of snakeweed seeds through dissection after various herbicidal treatments, collected research on various

snakeweed studies, researched and collected information about range management practices from different countries for a professor, utilized a Global Positioning System, collected and organized data on range utilization, collected and organized rainfall data, helped in maintenance of college vehicles.

TEACHING EXPERIENCE

2004-present Full-time Instructor in Department of Kinesiology, Kansas State University. Classes consist of lecture and critique of current articles in Cardiovascular Exercise Physiology, Muscle Physiology, Topics in Biological Basis of Human Movement, Graduate Seminar, Comparative Exercise Physiology, and Topics in Heart Disease.

2001-present Conducted equine treadmill laboratory demonstrations and assisted in laboratories on lung, respiratory, and exercise physiology for the course Veterinary Physiology II.

1999-2001 Assisted in laboratories in courses offered by the department of Animal and Range Sciences and lecturing in the absence of the course instructor. Courses include: Animal Breeding, Stable Management, Introductory Horse Science, Introduction to Western Equitation, Introductory Animal Science, and Introduction to Meat Animal Production.

PUBLICATIONS

Refereed Journal Articles:

Padilla D.J., P. McDonough, B.J. Behnke, Y. Kano, K.S. Hageman, T.I. Musch, and D.C. Poole. Effects of Type II diabetes on muscle microvascular oxygen pressures. (in preparation).

Padilla, D.J., P. McDonough, B.J. Behnke, Y. Kano, K.S. Hageman, T.I. Musch, and D.C. Poole. Effects of Type II diabetes on capillary hemodynamics in skeletal muscle (in preparation).

Padilla, D.J., T.S. Epp, P. McDonough, D.J. Marlin, H.H. Erickson, and D.C. Poole. Effects of a specific endothelin-1_A antagonist on exercise-induced pulmonary hemorrhage in Thoroughbred horses (in preparation).

Behnke B.J., K. Ross, L. Abbo, **D.J. Padilla**, L. F. Ferreira, T.I. Musch, and D.C. Poole. Effects of hypovolemic hypotension on microvascular oxygen pressures in contracting skeletal muscle. (accepted upon revisions), 2005.

Poole D.C., B.J. Behnke, and **D.J. Padilla**. Dynamics of muscle microcirculatory exchange. Med. Sci. Sports. Exer. 37:1559-1566. 2005.

McDonough P., B.J. Behnke, **D.J. Padilla**, T.I. Musch, and D.C. Poole. Control of oxygen delivery and microvascular oxygen pressures in muscles comprised of different fiber types. J. Physiol. 563:903-913, 2005.

Kano Y., **D.J. Padilla**, B.J. Behnke, K. S. Hageman, T.I. Musch, and D.C. Poole. Effects of eccentric exercise on microcirculation and microvascular oxygen pressures in rat spinotrapezius muscle. J Appl Physiol. 99:1516-1522, 2005.

Kano Y., D.J. **Padilla**, K.S. Hageman, D.C. Poole, and T.I. Musch. Downhill running: a model of exercise hyperemia in the rat spinotrapezius muscle. J Appl Physiol. 97:1138-1142, 2004.

Epp, T.S., P. McDonough, D.E. Myers, **D.J. Padilla**, B.J. Behnke, D.C. Poole, and H.H. Erickson. The effectiveness of immunotherapy in treating exercise-induced pulmonary hemorrhage. (accepted).

Epp, T.S., P. McDonough, **D.J. Padilla**, J.H. Cox, D.C. Poole, and H.H. Erickson. The effect of herbal supplementation on the severity of exercise-induced pulmonary hemorrhage. Equine Comp. Exer. Physiol. (accepted upon revision).

Kano Y., **D.J., Padilla**, K.S. Hageman, D.C. Poole, and T.I Musch. Downhill running: a model of exercise hyperemia in the rat spinotrapezius muscle. J Appl Physiol. 97:1138-1142, 2004.

McDonough, P., C.A. Kindig, T.S. Hildreth, **D.J. Padilla**, B.J. Behnke, H.H. Erickson, and D.C. Poole. Effect of furosemide and the equine nasal strip on EIPH and time to fatigue in running Thoroughbred horses. Equine Comp. Exer. Physiol. 1: 177-184, 2003.

Padilla, D.J., P. McDonough, C.A. Kindig, H.H. Erickson, and D.C. Poole. Ventilatory dynamics and control of blood gases following maximal exercise in the Thoroughbred horse. J Appl Physiol. 96: 2187-2193, 2004.

Behnke, B.J., P. McDonough, **D.J. Padilla**, T.I. Musch, and D.C. Poole. Oxygen exchange profile in rat muscles of contrasting fibre types. J Physiol. 549:597-605, 2003.

Letters to the Editor-in-Chief:

Padilla, D.J., T.I. Musch, and D.C. Poole. Abusing the Fick Principle. Med. Sci. Sports. Exer. 37(4):702, 2005

Proceedings Papers:

Al-Ajimi, D.S., J.R. Strickland, T.T. Ross, **D.J. Padilla**, R.L. Ashley, and G. Parker. 2000. Effect of hexane and ether fractions of snakeweed (*Gutierrezia* spp.) on selected serum constituents of male Sprague-Dawley rats. Proc. West. Sec. Amer. Soc. Anim. Sci. 51:160-162.

Bollinger, J.L., T.T. Ross, **D.J. Padilla**, and D.M. Hallford. Body weight and body condition score changes and reproductive success in ewes fed Megalac® supplements. Proc. West. Sec. Amer. Soc. Anim. Sci. 52:406-409, 2001.

Padilla, D.J., T.T. Ross, J.R. Strickland, D.M. Hallford, M.W. Salisbury, and J.L. Bollinger. Effects of snakeweed (*Gutierrezia* spp.) ingestion on reproduction and liver function in sheep. Proc. West. Sec. Amer. Soc. Anim. Sci. 52:45-49, 2001.

Padilla, D.J., J. Smith, T.T. Ross, M.W. Salisbury, J.R. Strickland, and L.L. Melton. Effects of snakeweed toxicity and supplementation on gonadotropin-releasing hormone (GnRH), feed and weight change, and liver function. Proc. West. Sec. Amer. Soc. Anim. Sci. 51:136-138, 2000.

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Padilla, D.J., P. McDonough, B. Behnke, Y. Kano, K.S. Hageman, T. Musch, and D.C. Poole. Effects of Type II diabetes on capillary hemodynamics in skeletal muscle. *Med Sci Sports Exer* 37: S359 (2005).

Padilla, D.J., P. McDonough, B. Behnke, Y. Kano, K.S. Hageman, T. Musch, and D.C. Poole. Microvascular oxygen impairments in chronic heart failure (CHF) are exacerbated by concurrent type II diabetes. *FASEB J* (2005).

Padilla, D. J., P. McDonough, B.J. Behnke, Y. Kano, K.S. Hageman, T. Musch, and D.C. Poole. Effects of type II diabetes on muscle microvascular oxygen pressures (PO_2m). APS Intersociety Meeting, The Integrative Biology of Exercise, October 6-9, 2004, Austin, Texas.

Padilla, D.J., P. McDonough, Y. Kano, K. Sue Hageman, T.I. Musch, and D.C. Poole. Effects of Type II diabetes on capillary structure and function in skeletal muscle. *FASEB J* (2004).

McDonough, P., Y. Kano, D.J. Padilla, T. I. Musch and D.C. Poole. Effect of type-2 diabetes on O_2 exchange in rat skeletal muscle. *FASEB J* (2004).

McDonough, P., B.J. Behnke, D.J. Padilla, K.D. Ross, and D.C. Poole. On-off asymmetry of locomotory muscle microvascular PO_2 kinetics: effect of exercise intensity. *Med Sci Sports Exer* 35: S151, 2003.

Ross, K.D., L.A. Abbo, B.J. Behnke, D.J. Padilla, T.I. Musch, and D.C. Poole. Hypovolemic hypotension alters the dynamic balance between O_2 delivery and utilization in contracting skeletal muscle. *Med Sci Sports Exer* 35: S309, 2003

Padilla, D.J., B.J. Behnke, J.K. Hansen, and D.C. Poole. Different microvascular oxygen responses to contractions in slow and fast-twitch muscle are associated with capillarity. *Med Sci Sports Exer* 35: S43 2003.

Behnke, B.J., P. McDonough, D.J. Padilla, T.I. Musch, and D.C. Poole. Oxygen exchange in muscles of contrasting fiber types. *FASEB J* 17: A1273, 2003.

McDonough, P., B.J. Behnke, D.J. Padilla, K.D. Ross, and D.C. Poole. Control of O_2 exchange in rat locomotory muscle: effect of fiber type. *FASEB J* 17: A431, 2003.

Hildreth, T.S., P.J. McDonough, D.J. Padilla, B.J. Behnke, D.C. Poole, and H.H. Erickson. Is Immunotherapy effective in reducing exercise-induced pulmonary hemorrhage (EIPH)? *FASEB J* 17: A939, 2003.

Padilla D.J., P. McDonough, C.A. Kindig, H.H. Erickson, and D.C. Poole. Control of ventilation (V_E) and arterial CO_2 pressure ($PaCO_2$) following cessation of exercise in the Thoroughbred (TB) horse. *FASEB J* 17: A84, 2003.

Hildreth, T.S., P.J. McDonough, D.E. Myers, D.J. Padilla, B.J. Behnke, D.C. Poole, and H.H. Erickson. (April 4, 2003) The effectiveness of immunotherapy in treating exercise-induced pulmonary hemorrhage. Phi Zeta Research Day, College of Veterinary Medicine, Kansas State University.

Padilla D.J., P. McDonough, C.A. Kindig, H.H. Erickson, and D.C. Poole. (April 4, 2003) Control of ventilation (V_E) and arterial CO_2 pressure ($PaCO_2$) following cessation of exercise in the Thoroughbred (TB) horse. Phi Zeta Research Day, College of Veterinary Medicine, Kansas State University.

Hildreth, T.S., P.J. McDonough, D.J. Padilla. B.J. Behnke, D.C. Poole, and H.H. Erickson. (2003) The effectiveness of immunotherapy in treating exercise-induced pulmonary hemorrhage. 9th Annual Graduate Student Research Forum, Kansas State University.

Hildreth, T.S., P.J. McDonough, D.J. Padilla, B.J. Behnke, D.E. Myers, D.C. Poole, and H.H. Erickson. (2003) Is immunotherapy effective in reducing exercise-induced pulmonary hemorrhage? Central States American College of Sports Medicine, Kansas City, MO.

Abbo, L.A., K.D. Ross, B.J. Behnke, D. Padilla, T.I. Musch, and D.C. Poole. (August 8, 2002) Hypovolemic hypotension alters the dynamic balance between O_2 delivery and utilization in contracting skeletal muscle. Veterinary Research Scholars Program, Kansas State University.

Ross, K.D., L.A. Abbo, B.J. Behnke, D. Padilla, T.I. Musch, and D.C. Poole. (2002) Hypovolemic hypotension alters the dynamic balance between O_2 delivery and utilization in contracting skeletal muscle. Central States American College of Sports Medicine, Kansas City, Missouri

McDonough, P.J., B.J. Behnke, D.J. Padilla, K.D. Ross, and D.C. Poole, FACSM. (2002) Locomotory muscle microvascular PO_2 kinetics: influence of oxidative capacity and workload. Central States American College of Sports Medicine, Kansas City, Missouri

Hildreth, T.S., P.J. McDonough, D.J. Padilla, B.J. Behnke, D.E. Myers, D.C. Poole, and H.E. Erickson. (2002) Is Immunotherapy effective in reducing exercise-induced pulmonary

hemorrhage? Central States American College of Sports Medicine, Kansas City, Missouri

Al-Ajimi, D.S., J.R. Strickland, T.T. Ross, D.J. Padilla, R.L. Ashley, and G. Parker. (2000) Effect of hexane and ether fractions of snakeweed (*Gutierrezia* spp.) on selected serum constituents of male Sprague-Dawley rats. J. Anim. Sci. 78 (Suppl. 2): 121 (Abstr.).

Bollinger, J.L., T.T. Ross, D.J. Padilla, and D.M. Hallford. (2001) Body weight and body condition score changes and reproductive success in ewes fed Megalac® supplements. J. Anim. Sci. 79 (Suppl. 2).

Padilla, D.J., T.T. Ross, J.R. Strickland, D.M. Hallford, M.W. Salisbury, and J.L. Bollinger. (2001) Effects of snakeweed (*Gutierrezia* spp.) ingestion on reproduction and liver function in sheep. J. Anim. Sci. 79 (Suppl. 2).

Padilla, D.J., J. Smith, T.T. Ross, M.W. Salisbury, J.R. Strickland, and L.L. Melton. (2000) Effects of snakeweed toxicity and supplementation on gonadotropin-releasing hormone (GnRH), feed and weight change, and liver function. J. Anim. Sci. 78 (Suppl. 2):122 (Abstr.).

Research Briefs:

Bollinger, J.L., T.T. Ross, D.J. Padilla, and D.M. Hallford. (2001) Body weight and body condition score changes and reproductive success in ewes fed Megalac® supplements. Livestock Research Briefs and Cattle Growers' Short Course. P. 69.

Padilla, D.J., T.T. Ross, J.R. Strickland, D.M. Hallford, M.W. Salisbury, and J.L. Bollinger. (2001) Effects of snakeweed (*Gutierrezia* spp.) ingestion on reproduction and liver function in sheep. Livestock Research Briefs and Cattle Growers' Short Course. P. 69.

Padilla, D.J., J. Smith, T.T. Ross, M.W. Salisbury, J.R. Strickland, and L.L. Melton. (2000) Effects of snakeweed toxicity and supplementation on gonadotropin-releasing hormone (GnRH), feed and weight change, and liver function. Livestock Research Briefs and Cattle Growers' Short Course. P. 72.

GRANTS

Padilla, D.J. and D.C. Poole, D.C. U.S. Department of Health and Human Services; National Institutes of Health, National Heart and Lung, and Blood Institute, "Minority Pre-Doctoral Fellowship" under National Research Service Award (Amount $99,212; Accepted; Duration: 12/23/2001-9/30/2005)

Padilla, D.J., D.C. Poole, H.H. Erickson, P. McDonough, and T.S. Hildreth. Does the ET_A receptor antagonist, TBC11251, reduce pulmonary hypertension and EIPH in the exercising horse? American Quarter Horse Association. (Amount: $27,674; Accepted; Project duration: 10/1/2002-9/30/2003)

Hildreth, T.S., D.C. Poole, H.H. Erickson, D.J. Padilla, and P. McDonough. The role of pulmonary leukocytes in reducing EIPH with concentrated equine serum. Kansas Racing and Gaming Commission (Amount: $11,980.68; Accepted; Project Duration: 4/1/04-6/30/05).

McDonough, P., D.C. Poole, H.H. Erickson, T.S. Hildreth, D.J. Padilla. The Incidence and severity of Exercise-Induced Pulmonary Hemorrhage (EIPH) in racing greyhounds. (Amount $20,000; Accepted; Project Duration: 4/1/04-6/30/05).

AWARDS

Doctoral Student Award, Central States/American College of Sports Medicine, October, 2004.

First Place Basic Science Research, Oral Presentation Award, The Sigma Chapter of the Society of Phi Zeta, March 2004

Dr. Leo and Gloria Whitechair Fund Award, January 2004

First Place Research Poster Award, The Sigma Chapter of the Society of Phi Zeta, March 2003

Supplemental Graduate Fellowship, Kansas State University, Graduate School, 2002-2003

Individual National Research Service Award, Minority Pre-Doctoral Fellowship Program of National Institutes of Health, December 2001 to Present

Minority Biomedical Research Support Assistantship Program/Research Initiative for Enhancement (MBRS-RISE) of the National Institutes of Health, August 2000 to July 2001

Second Place Presentation Award, Graduate Research and Arts Symposium, April 2001

Gamma Sigma Delta, The Honor Society of Agriculture, Certificate of Membership, December 2000

First Place Poster Award, Graduate Research and Arts Symposium, April 2000

NMSU Assurance of Actual Training IACUC, Certification of training for the use of animals in research, November 1998, November 2000

Dean's Award of Excellence-New Mexico State University College of Agriculture and Home Economics, May 1999

American Society of Animal Science Scholarship Award, May 1999

Sam Steel Membership Award, Graduated with Honors, May 1999

Outstanding Hispanic Student in Animal and Range Sciences, April 1999

Crimson Scholar Three-Semester Pin Recipient, April 1999

Who's Who Among Students in American Universities and Colleges, Certificate of Election 1998-1999

Dean's List, Spring 1997, Fall 1997, Spring 1998

Alpha Zeta Membership Award, Spring 1997

Crimson Scholar Membership Award, Fall 1997

Golden Key Honor Society, Membership Award, Spring 1997

Paul T. & Jane Hutchcroft Scholarship, Recipient, Fall 1998 to Spring 1999

Nutritional Associates Scholarship, Recipient, Fall 1997 to Spring 1998

Alumni Out of State Scholarship, Recipient, Fall 1995

Award of Excellence in Science and Agriculture from University of Arizona, May 1995

ORGANIZATIONS

American College of Sports Medicine, August 2001 to present

Assistant to the Past-President of Central States Chapter/ American College of Sports Medicine, October 2003 to present

Central States/American College of Sports Medicine, August 2001 to present

Hispanic American Leadership Organization, January 2002 to present

Volunteer at 4-H events, January 2002 to present

Gamma Sigma Delta, December 2000 to present

American Society of Animal Science, Member, May 2000 to present

Animal and Range Sciences Graduate Student Association, Member, August 1999 to May 2001

Alpha Zeta, Honorary and Professional Agricultural Fraternity

Chancellor (President), 1998 to 1999; Chronicler (Philanthropist), 1997 to 1998

Golden Key Honor Society, Member, Spring 1997 to present

NMSU Horse Judging Team, Member, Spring 1997

Collegiate 4-H, Member 1995 to 1996

PRESENTATIONS

Oral:

Padilla, D.J. The Horse: A Natural Athlete. Kansas Thoroughbred Association. 19th Annual Convention and Stallion Season Auction. Sat. Jan. 15, 2005.

Padilla, D.J. Cardiovascular and ventilatory limitations in the oxygen transport pathway. Departmental Seminar, April 26, 2004, Department of Anatomy and Physiology, Kansas State University.

Padilla, D.J., P. McDonough, Y. Kano, K.S. Hageman, T.I. Musch, and D.C. Poole. Effects of Type II Diabetes on capillary structure and function in skeletal muscle. Annual Sigma Chapter of Phi Zeta Day, March 2, 2004.

Padilla, D.J., and D.J. Marlin. Contribution of Endothelin-1 to EIPH in the Performance Horse. In: Exercise-Induced Pulmonary Hemorrhage Workshop, Sixth International Conference on Equine Exercise Physiology, September 22-26, 2002, Lexington, Kentucky.

Padilla, D.J. Exercise, Endothelin-1, and EIPH in the Performance Horse. Departmental Seminar. October 14, 2002, Department of Anatomy and Physiology, Kansas State University.

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PANEL DISCUSSIONS

"Graduate Student Panel"; Kansas State University Graduate School Orientation for new graduate students, August 20, 2003.

"On what being a graduate student is like and other helpful information"; Summer Undergraduate Research Opportunity Program (SUROP) for undergraduate minority students; Kansas State University, June 17, 2003

CHAPTER 1

Introduction

The transfer of O_2 from the atmosphere to the mitochondria requires several structural and functional steps (Figure 1) that must work together in a precise manner for adequate ATP regeneration, especially during dynamic exercise (Wasserman et al., 1994; Hoppeler and Weibel, 1998). These steps include diffusion of O_2 across the blood-gas barrier of the lungs, O_2 transport via the blood (i.e. cardiac output and O_2 concentration), and O_2 movement by way of diffusion from the skeletal muscle capillary into the myocyte. Any limitation among the elements of the O_2 transport pathway is likely to constrain exercise performance (Wagner et al., 1997; Hoppeler and Weibel, 1998).

To investigate the consequences of limitations within the O_2 transport pathway, the choice of model as well as experimental technique must be taken into consideration. For example, it is an exciting opportunity to study a model with high aerobic capabilities such as the Thoroughbred (TB) horse, an animal that can reach a maximal oxygen consumption ($\dot{V}O_{2max}$) of ~180 - 220 ml/kg/min (~2 fold higher than most athletic humans; Rose et al., 1988; McDonough et al., 2002ab) at speeds over 45 mi/hr (65 km/hr; Evans and Rose, 1988; Rose et al., 1988). This phenomenon occurs by way of the horse's large O_2 carrying capacity (i.e. cardiac output) which is the result of an increase in blood volume via its contractile spleen, augmented heart rate (~7-fold), and extraction of 85-90% of O_2 by its skeletal muscle (rev. Poole and Erickson, 2004). However, despite these factors, the horse experiences limitations to its ventilatory capacity (Padilla et al., 2004), exercise-induced arterial hypoxemia (Wagner et al., 1989), and exercise-induced pulmonary hemorrhage (Roberts and Erickson, 1999; rev. Erickson and Poole, 2002; Figure 2) which may prevent the horse from reaching its full aerobic potential.

Besides studying a highly athletic species such as the TB horse, a disease, such as Type II diabetes, can also cause limitations within the O_2 transport pathway. For example, Type II diabetic patients exhibit exercise intolerance and sluggish pulmonary $\dot{V}O_2$ kinetics (Regensteiner et al., 1998; Figure 2), but the cause(s) of this phenomenon is unknown. The rate of $\dot{V}O_2$ kinetics is a critical measurement because it is highly representative of muscle $\dot{V}O_2$ dynamics

(Whipp and Mahler, 1980; Barstow and Mole, 1987; Barstow et al., 1990; Poole et al., 1992; Grassi et al., 1996), and slowed $\dot{V}O_2$ kinetics suggests that muscle mitochondrial function is compromised. Although technological limitations prevent *in vivo* mitochondrial measurements, other scientific advancements such as phosphorescence quenching techniques (Behnke et al., 2001; McDonough et al., 2001, 2005; Poole et al., 1995, 2004) and intravital microscopy (Kindig et al., 2002; Richardson et al., 2003; Russell et al., 2003) which measure microvascular O_2 pressures (i.e. at the site of blood-myocyte O_2 exchange) and red blood cell (RBC) hemodynamics, respectively, to quantify potential muscle O_2 availability and utilization may provide a mechanistic basis for the slow $\dot{V}O_2$ kinetics in diabetic humans.

This dissertation has been designed to first present pertinent background information that applies to the four novel studies (Studies A, B, C, & D) contained within this dissertation. This will be followed by four studies that investigate specific incidences of limitations placed on the O_2 transport pathway (i.e. the pulmonary system in the Thoroughbred horse (Studies A & B) and the microcirculation within an animal model of Type II diabetes (Studies C & D). Study A challenges one of the central dogmas in equine exercise physiology: the notion that locomotory-respiratory coupling (LRC; a synchronization of stride and breathing frequency) is the means by which a Thoroughbred (TB) horse achieves its high minute ventilation ($\dot{V}_E$; >1800 L/min) during exercise (Attenborrow, 1983; Bramble and Carrier, 1983). Study B tests the hypothesis that endothelin-1 (ET-1), a potent vasoconstricting hormone (Yanagisawa et al., 1988), contributes to the increased pulmonary artery pressure that is believed to be a primary cause of exercise-induced pulmonary hemorrhage (EIPH) in horse.

The latter two studies (Studies C & D) investigate skeletal muscle function and capillary RBC hemodynamics within an animal model of Type II diabetes, the Goto-Kakizaki rat (Goto and Kakizaki, 1981). This research will provide a mechanistic basis for the exercise intolerance and slowed oxygen uptake ($\dot{V}O_2$) kinetics evidenced by diabetic humans (Regensteiner et al., 1998). Although inferences about muscle $\dot{V}O_2$ from pulmonary $\dot{V}O_2$ can be made, they may be considered indirect. Therefore, the last two studies utilized phosphorescence quenching techniques (Study C) and intravital microscopy (Study D) to examine O_2 utilization at the site of O_2 exchange, i.e. the capillary-myocyte region. Indeed, the last two studies demonstrate that Type II diabetes is linked to a pathologically lowered O_2 driving pressure (i.e. PO_2mv) to move O_2 into the myocyte and decreased O_2 delivery (i.e. slowed RBC hemodynamics) within diabetic

skeletal muscle. Both of these phenomena will ultimately compromise O_2 availability (i.e. $\downarrow\dot{Q}O_2/\dot{V}O_2$ matching) to the mitochondria within the myocyte. Altogether, these four studies will provide novel insights crucial to understanding the mammalian O_2 transport system in health and how that system is compromised in diabetes leading to impaired exercise intolerance.

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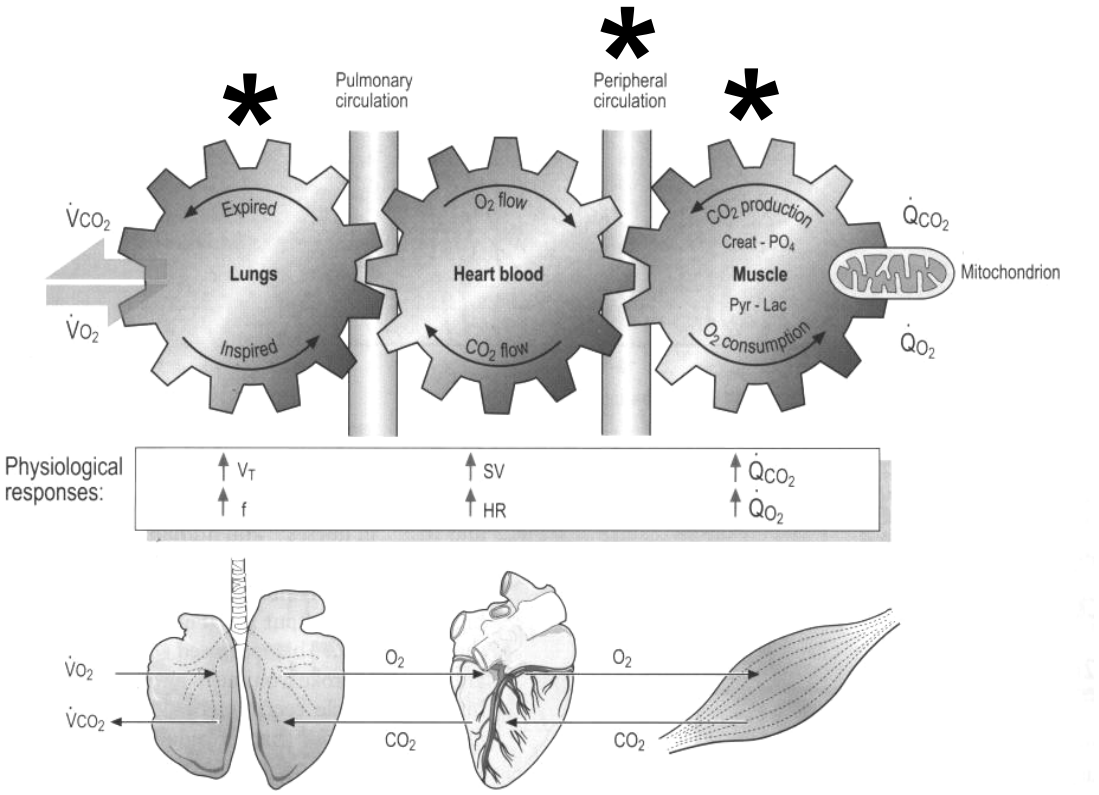

Figure 1. The O_2 transport pathway along with the organs responsible for moving O_2 from the atmosphere to the muscle mitochondria. Included are the physiological responses associated with increased O_2 delivery to the myocytes. Large asterisks implicate areas within the O_2 cascade that will be addressed by the four studies included in this dissertation (from Poole and Erickson, 2004).

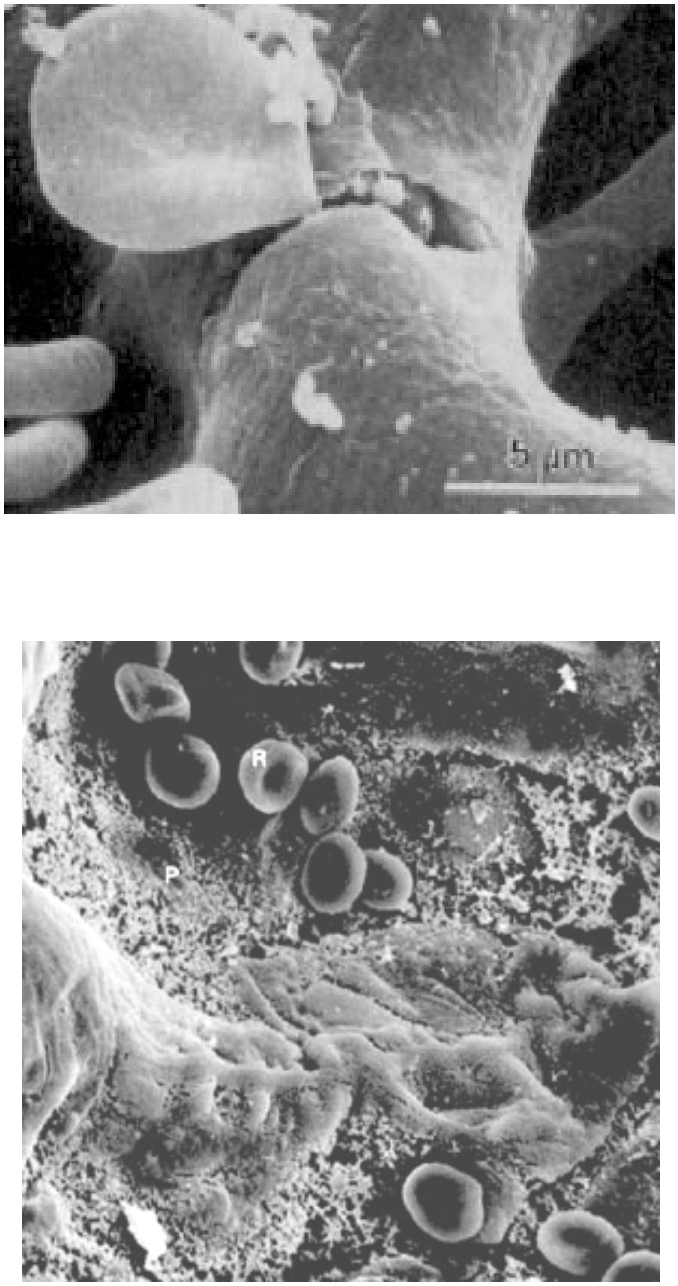

Figure 2. Elegant micrographs from Fu et al. (1992) in rabbit lungs (Top Panel) with increased propensity for blood gas barrier rupture at high lung volumes and Erickson et al. (1997) in pony lungs (Bottom Panel) after high intensity exercise clearly illustrating the breakage in the pulmonary capillary endothelium and alveolar epithelium. Red blood cells can be found along with proteinaceous fluid leaking out into the alveolar spaces.

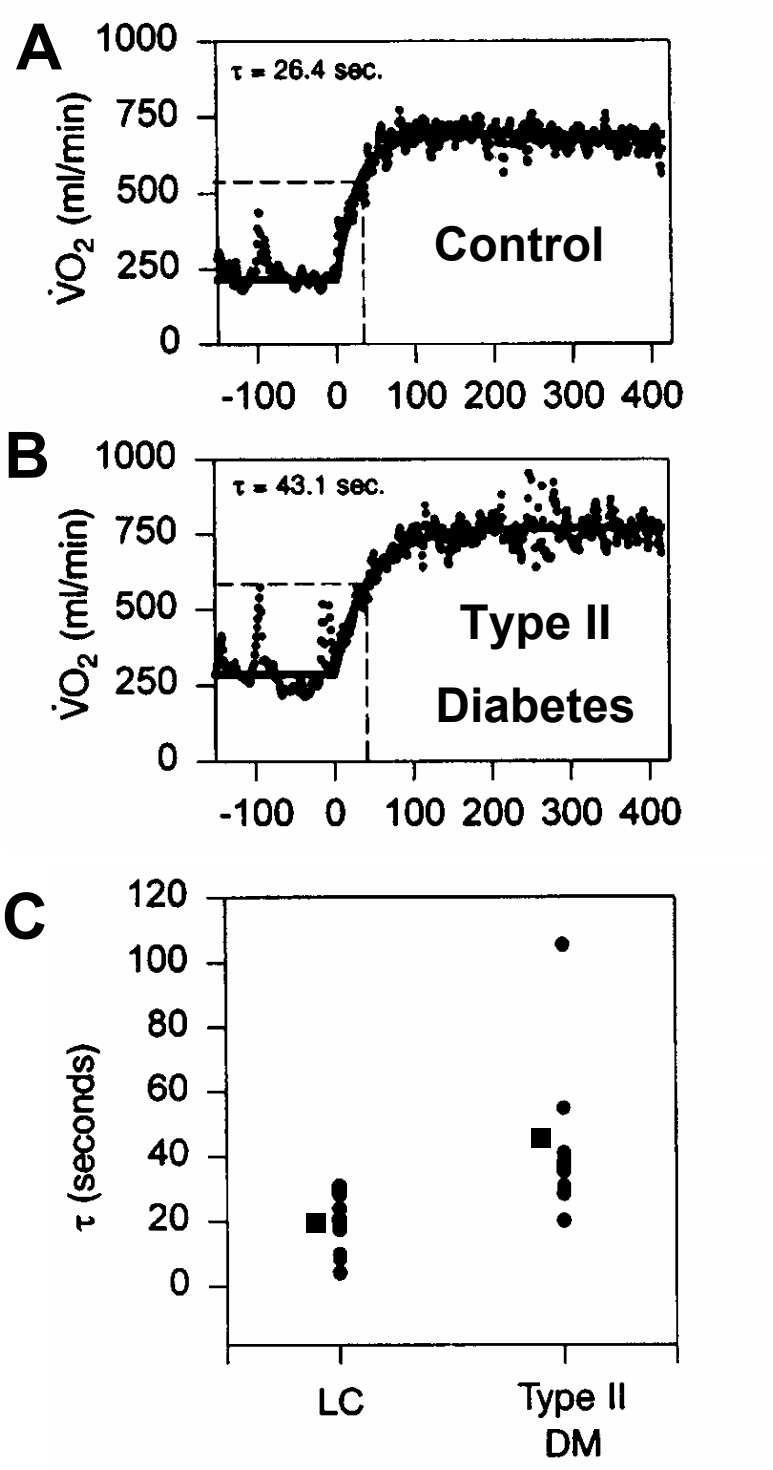

Figure 3. Panel A. Oxygen uptake ($\dot{V}O_2$) kinetics in a lean control (LC; upper panel) and a subject with Type II diabetes mellitus. Panel B. Time constants (τ) in LC and Type II diabetic women. Panel C. Squares denote mean time constant (From Regensteiner et al., 1998).

Study A: Ventilatory dynamics and control of blood gases after maximal exercise in the Thoroughbred horse

The control of minute ventilation ($\dot{V}_E$) differs between Thoroughbred (TB) horses and humans during maximal exercise. For example, the horse displays a strict 1:1 coupling of breathing to stride frequency [i.e. locomotor-respiratory coupling (LRC)] while cantering and galloping (Bramble and Carrier, 1983), whereas humans are not limited to a particular coupling ratio (Bonsignore et al., 1998). Notwithstanding this constraint, the exercising TB can achieve prodigious values for pulmonary $\dot{V}_E$ (> 1,800 l/min in extremely fit horses) and rates of gas exchange [i.e., O_2 uptake ($\dot{V}O_2$) > 70 l/min; CO_2 output ($\dot{V}CO_2$) > 80 l/min during maximal exercise; McDonough et al., 2002ab]. The relative $\dot{V}_E$ are similar in humans and horses but much less than those seen in dogs (a species of similar aerobic scope to the TB; Stray-Gunderson et al., 1986; Wagner et al., 2000), which indicates that $\dot{V}_E$ during exercise may be limited as suggested by very low ventilatory equivalents for $\dot{V}O_2$ ($\dot{V}_E/\dot{V}O_2$) and $\dot{V}CO_2$ ($\dot{V}_E/\dot{V}CO_2$) resulting in pronounced hypercapnia (McDonough et al., 2002). In this regard, and contrary to facilitating $\dot{V}_E$, it may be that LRC may actually limit $\dot{V}_E$ during brief, maximal exercise (Hornicke et al., 1983; Marlin et al., 2002).

Historically, LRC has been considered the key facilitator of $\dot{V}_E$ in TB horses, with high ventilatory volumes being achieved with supposedly little respiratory muscle contribution to the exercise hyperpnea (Bramble and Carrier, 1983). However, electromyographic studies demonstrate that the diaphragm is highly active in running horses (Ainsworth et al., 1997). Other indicators of the increased diaphragmatic activity include substantial transdiaphragmatic pressures (Slocombe et al., 1991) and high diaphragm muscle blood flows (Manohar, 1988). In addition, the horse diaphragm is highly oxidative and therefore suited to sustained high-intensity respiratory efforts (Poole et al., 2002). It has also been shown recently that rib cage expansion is very limited and out of phase with inspiration in the galloping horse which will serve to place a greater reliance on diaphragmatic vs. intercostal or accessory muscle breathing (Marlin et al., 2002).

Whereas humans usually hyperventilate during intense exercise [e.g. arterial PCO_2 ($PaCO_2$) typically falls to values < 30 Torr; Dempsey et al., 1995; Johnson et al., 1996) and defend arterial PO_2 (PaO_2) close to resting values, horses routinely become markedly

hypercapnic and hypoxemic (Bayly et al., 1989; Hodgson et al., 1990, McDonough et al., 2002ab) during brief, incremental exercise ($PaCO_2$ of ~55 - 65 Torr; PaO_2 of ~60 – 80 Torr). This hypercapnic response is a direct consequence of the extraordinarily high metabolic rate coupled with an inadequate ventilatory response in the TB (Bayly et al., 1989; McDonough et al., 2002a) and possibly the reduced red blood cell transit time in the pulmonary capillary (Wagner et al., 1989).

Horses and other quadrupeds (Bramble and Carrier, 1983) are considered to rely on LRC primarily during the canter and gallop to facilitate $\dot{V}_E$ which would act to reduce the energetic cost of $\dot{V}_E$. This would be advantageous as it would serve to minimize the redistribution of cardiac output from the exercising limbs to the respiratory muscles (Art et al., 1990; Harms et al., 1997). Indeed, in humans, reducing respiratory muscle work elevates the proportion of cardiac output available to the working limb muscles (Harms et al., 1998, 2000).

The purpose of the present investigation was to explore the notion that, if LRC is an obligatory requirement for achieving very high $\dot{V}_E$ such as those present in the galloping horse, when LRC is removed abruptly at the onset of trotting, $\dot{V}_E$ should drop immediately and precipitously. Specifically, we tested the hypothesis that at the onset of recovery from galloping (i.e. across the transition from the gallop (LRC present) to the trot at 3 m/s (no LRC)), $\dot{V}_E$ would fall precipitously and $PaCO_2$ would increase transiently above end-exercise levels. By resolving, for the first time, the breath-by-breath responses of ventilation ($\dot{V}_E$) and gas exchange ($\dot{V}O_2$ and $\dot{V}CO_2$) across the gallop-trot transition, we sought to gain novel insights into the control of breathing in the horse.

Study B: Effects of a specific endothelin-1_A antagonist on exercise-induced pulmonary hemorrhage in Thoroughbred horses

The extraordinarily high pulmonary arterial pressures (Ppa) generated by Thoroughbred horses during maximal exercise have been implicated as a principal cause of exercise-induced pulmonary hemorrhage (EIPH; Erickson *et al.* 1990; West *et al.* 1993; Birks *et al.* 1997; Meyer *et al*. 1998). While the exact cause(s) of EIPH remain controversial (see Roberts and Erickson 1999; rev. Erickson and Poole 2002), it is clear that elevations in pulmonary capillary transmural pressure are a prerequisite (due to the very high cardiac output) and mechanical stresses during

exercise do result in capillary stress failure and leakage of red blood cells (RBCs) into the alveolar spaces (West *et al.* 1993; Erickson *et al.* 1997).

Endothelin-1 (ET-1) is a potent vasoconstricting peptide produced and secreted by vascular endothelial cells (rev. Kedzierski and Yanagisawa 2001) which modulates pressures within the pulmonary circulation of the horse (Benamou *et al.* 1998, 1999, 2001, 2003), and other species (Fukuroda *et al.* 1994; MacLean *et al.* 1998; Schmect *et al.* 1999). Vasoconstriction occurs when ET-1 binds abluminally to its specific receptor subtype ET-1_A found on vascular smooth muscle. Furthermore, plasma ET-1 levels correlate with Ppa in human patients suffering from primary pulmonary hypertension (Stewart *et al.* 1991; Giaid *et al.* 1993), and the use of ET-1_A antagonists may alleviate this condition by reducing Ppa (Givertz *et al.* 2000).

The role of ET-1 in the equine lung has not been fully elucidated with some (Benamou *et al*. 1999) but not all (McKeever and Malinowski, 1999; McKeever *et al.* 2002) studies showing elevated plasma ET-1 concentrations during and after maximal exercise (McKeever et al. 2002). Studies by Benamou and colleagues (2001) have demonstrated, that in resting horses, administration of exogenous ET-1 induces a significant increase in Ppa, and these effects are completely blocked by an ET-1_A receptor antagonist. *In vitro* studies by Benamou and others (2003) have also revealed vasoconstriction of third generation equine pulmonary arteries when exposed to ET-1, and this response can be inhibited by an ET-1_A antagonist (BQ123, but not by an antagonist of the other receptor subtype, ET-1_B). Together, both studies suggest that the ET-1_A receptor subtype is present in the pulmonary arterioles in horses. Thus, we hypothesized that ET-1 would contribute to the increased Ppa and etiology of EIPH in horses during high intensity exercise. Moreover, an ET-1_A receptor antagonist, TBC3214, would reduce the augmented Ppa and ultimately the incidence and severity of EIPH.

Study C: Effects of Type II diabetes on muscle microvascular oxygen pressures

In comparison to non-diabetic individuals, Type II diabetic patients suffer from exercise intolerance and demonstrate slowed oxygen uptake ($\dot{V}O_2$) kinetics at exercise onset (Regensteiner et al., 1998). Given that pulmonary $\dot{V}O_2$ kinetics follow closely the dynamics of O_2 exchange and utilization within the muscle of a healthy individual (Grassi et al., 1996;

Bangsbo et al., 2000), impaired pulmonary $\dot{V}O_2$ kinetics in patients with Type II diabetes suggests that there may be a decrease in O_2 delivery ($\dot{Q}O_2$) to the muscle mitochondria which might ultimately compromise muscle $\dot{V}O_2$ (rev. Jones and Poole, 2005). Indeed, flow-mediated dilation in the forearm is reduced in diabetic patients at rest (Regensteiner et al., 2003), and leg $\dot{Q}$ is decreased during exercise (Kingwell et al., 2003). Furthermore, augmented plasma concentrations of the vasoconstrictor, endothelin-1 (Schneider et al., 2002), and impaired endothelium-dependent and independent dilatory responses (McVeigh et al., 1996; Williams et al., 1996; Kingwell et al., 2003) likely contribute to the reduced $\dot{Q}O_2$ responses in Type II diabetic patients. Not only may $\dot{Q}O_2$ be lowered, but patients with Type II diabetes may also evidence a reduction in fractional O_2 extraction during submaximal and maximal exercise (Baldi et al., 2003).

Slowed $\dot{V}O_2$ kinetics mandate that diabetic patients will exhibit an increased O_2 deficit, and thus greater reliance on immediate energy sources such as glycogenolysis and substrate level phosphorylation (see rev. Jones and Poole, 2005). This is substantiated by a significantly greater exercise-induced reduction of phosphocreatine (PCr; Scheuermann-Freestone et al., 2003), higher concentrations of glycolytic enzymes, decreased oxidative capacity (Kelley et al., 2002), and lower mitochondrial volume density (Mathieu-Costello et al., 2003; Ritov et al., 2005) in muscles of Type II diabetic patients. Some (Mårin et al., 1994), but not all (Andersen et al., 1993) researchers have also reported changes in muscle fiber type that include increased proportions of lower oxidative, fast-twitch glycolytic fibers in Type II diabetic subjects.

To further understand the mechanisms responsible for exercise intolerance in this patient population, examination of O_2 exchange at the level of the musculature (i.e. at the capillary-myocyte region) may prove insightful. Due to the invasiveness of these procedures, it is necessary to employ an animal model of the Type II diabetic condition. Therefore, to study O_2 exchange at the capillary-myocyte level, we chose to determine the microvascular PO_2 (PO_2mv) using phosphorescence quenching techniques within the spinotrapezius muscle of Goto-Kakizaki (GK) Type II diabetic rats (Goto and Kakizaki, 1981). The pathophysiological similarities between this model and diabetic humans are quite striking and include: insulin resistance (particularly in muscle; Steiler et al., 2003), changes in muscle fiber type (i.e. increased percentage of fast-twitch fibers; Yasuda et al., 2002), impaired endothelial and vascular function (Sandu et al., 2000; Witte et al., 2003), and elevated endothelin-1 levels (Balsiger et al., 2002).

Furthermore, the GK rat is considered a non-obese model (Goto and Kakizaki, 1981), which allows for the study of Type II diabetes without confounding factors related to obesity. Although obesity is often associated with Type II diabetes (for review, see ref. Sharma and Chetty, 2005), this does not explain the presence of insulin resistance which can be equally severe in normal-weight type II diabetic patients (DeFronzo et al., 1983). Also, endothelial dysfunction can be manifested in diabetic humans independent of obesity (Hogikyan et al., 1996).

The technique of phosphorescence quenching facilitates determination of the dynamic balance between $\dot{Q}O_2$ and $\dot{V}O_2$ (i.e. $\dot{Q}O_2/\dot{V}O_2$ ratio) as reflected in the driving pressure for O_2 (PO_2mv) within the muscle microvasculature. Any changes in the $\dot{Q}O_2/\dot{V}O_2$ ratio across time will be directly reflected by alterations in PO_2mv and its subsequent temporal response at the onset of muscle contractions (Behnke et al., 2001; McDonough et al., 2001). Therefore, if $\dot{Q}O_2$ is impaired, due to vascular dysfunction, we hypothesized that PO_2mv would be lowered at rest in the GK rat spinotrapezius muscle. Furthermore, PO_2mv dynamics during the rest-contractions transition would be altered compared to healthy Wistar rats such that PO_2mv is reduced across the non-steady state transition at the onset of contractions. Such a decreased pressure to drive O_2 into the myocyte (i.e. PO_2mv) would ultimately affect O_2 exchange at the mitochondrial level, thus contributing to the slow $\dot{V}O_2$ kinetics and the elevated O_2 deficit found at exercise onset seen in human diabetic patients.

Study D: Effects of Type II diabetes on capillary hemodynamics in skeletal muscle

Type II diabetes has been associated with increased exercise intolerance, as demonstrated by slowed oxygen uptake ($\dot{V}O_2$) kinetics at exercise onset (Regensteiner et al., 1998), lowered $\dot{V}O_{2max}$ (Regensteiner et al., 1995; Baldi et al., 2003), and a reduction in fractional O_2 extraction (Baldi et al., 2003). Diabetic patients also exhibit compromised muscle blood flow at rest (Regensteiner et al., 2003) and during exercise (Kingwell et al., 2003). The attenuated blood flow may be the result of a blunted endothelium-dependent vasodilation (McVeigh et al., 1996; Williams et al., 1996; Kingwell et al., 2003) and increased plasma concentrations of the vasoconstrictor, endothelin-1 (Schneider et al., 2002). Other changes that accompany this disease include a reduction in capillary density (Mårin et al., 1994; Mathieu-Costello et al., 2003) and decreased mitochondrial volume (Ritov et al., 2005) and function (Kelley et al., 2002). This

group of patients has also been shown to demonstrate higher amounts of Type IIb (highly glycolytic) muscle fibers by some (Mårin et al., 1994) but not all investigators (Andersen et al., 1993).

Recently, preliminary work in our laboratory (Padilla et al., 2004) has shown that O_2 exchange at the capillary-myocyte region is impaired in a muscle of the Type II diabetic Goto-Kakizaki (GK) rat, a highly representative model of the diabetic state in humans (Goto and Kakizaki, 1981). The GK rat demonstrates a decreased driving pressure for O_2 within the microvasculature (PO_2mv) of the spinotrapezius muscle, which is indicative of a slowed microvascular $\dot{Q}O_2$ relative to $\dot{V}O_2$ (Behnke et al., 2002). Direct observation of the microcirculation (via intravital microscopy; see Kindig et al., 1998, 1999; Richardson et al., 2003; Russell et al., 2003) may reveal a mechanistic basis for the decreased PO_2mv found in GK rat muscle, as thus provide insights into the exercise intolerance in diabetic humans (Regensteiner et al., 1998).

Within skeletal muscle, a functional microvascular bed is necessary for the provision of an adequate supply of O_2 and other nutrients, as well as for removal of waste products. The modeling studies of Federspiel and Popel (1986) suggest that the number of red blood cells (RBCs) adjacent to a muscle fiber (i.e. capillary tube hematocrit multiplied by the length of capillaries) is critical for O_2 exchange, and as the ratio of RBC surface area to myocyte surface area increases, muscle O_2 diffusing capacity is further augmented. Therefore, any structural or functional impairment within the capillary network of skeletal muscle caused by disease (e.g. Kindig et al, 1998; Richardson et al., 2003) will severely impact blood-myocyte O_2 and substrate exchange, consequently playing a role in fatigue and exercise intolerance. Thus, using intravital microscopy to examine the microcirculation of the spinotrapezius muscle of the GK rat, we hypothesized that the percentage of flowing capillaries would be decreased and that RBC hemodynamics would be reduced in the GK rat. These critical changes in RBC distribution will result in an attenuated O_2 diffusing capacity, and thus O_2 flux into the myocyte, which provides one potential mechanism for poor skeletal muscle performance in Type II diabetic patients.

CHAPTER 2

Study A: Methods

Animals

Five TB geldings (4 - 10 yr; 470 - 600 kg) were used in this investigation. Horses were housed in enclosed dry lots with a shaded area, fed alfalfa and grass hay, and concentrate twice daily along with water and salt available *ad libitum*. Deworming and vaccinations were administered at regular intervals. A high speed treadmill (SATO, Uppsala, Sweden) was utilized to exercise horses at least twice weekly to maintain fitness and condition. All procedures were approved by the Kansas State University Institutional Animal Care and Use Committee.

Instrumentation. Before each trial, utilizing aseptic techniques, each horse was instrumented with a 7-F introducer catheter inserted into the right jugular vein, and an 18-gauge, 2.0-in. catheter was placed in a previously elevated carotid artery or transverse facial artery (20-gauge, 1.5 in. in two TB's). Lidocaine (2%, i.e. 2 mL) was utilized subcutaneously for the insertion of the catheter in the carotid artery and jugular vein but not in the transverse facial artery (2 horses). To determine mean pulmonary artery temperature (for correction of arterial blood gases), a thermistor catheter was inserted through the 7-F introducer catheter and advanced into the pulmonary artery 8 cm past the pulmonary valve. Calibration of the thermistor catheter was conducted with the use of a Physitemp thermocouple thermometer (BAT-10, Physitemp, Clifton, NJ, USA). To withdraw arterial blood, a cannula (1.6 mm ID, 3.2 mm outer diameter) was attached to the arterial catheter.

Measurement of breath-by-breath gas exchange. To measure expired $\dot{V}_E$, an ultrasonic phase-shift flowmeter (Model FR-41eq; Flowmetrics-BDRL, Birmingham, UK) was used as described previously (Woakes et al., 1987). Briefly, horses were outfitted with a lightweight fiberglass facemask (< 1 kg). This mask is fitted internally with silicone rubber and foam gaskets to maintain an airtight seal. The flow tubes were then placed in the openings of the facemask opposite each nostril to allow measurement of airflow for each nostril. Each flow tube contained two ultrasonic transducers that quantify velocity of airflow at a resonant frequency of 40 kHz.

Each ultrasonic phase-shift flowmeter (i.e., left and right) underwent a three-point calibration at -20, 0, and +20 L/s using a rotameter (KDG Flowmeters, Burgess Hill, UK) certified by the National Board of Standards. Because the system responds linearly to changes in airflow and the design characteristics of the flow probes negate the effects of temperature and humidity (Woakes et al., 1987), the above calibration allowed for the measurement of the full extent of the exercise and recovery $\dot{V}_E$ response.

Right and left airway flows, as well as inspired and expired O_2 and CO_2, were collected using a commercial data analysis system (DATAQ, Akron, OH) and stored for later analysis. The conversion of $\dot{V}_E$ to STPD was conducted using standard equations, while $\dot{V}O_2$ and $\dot{V}CO_2$ were calculated using the principle of mass balance (i.e. $\dot{V}O_{2\ STPD} = (\dot{V}_{I\ STPD} \times F_IO_2) - (\dot{V}_{E\ STPD} \times F_EO_{2\ STPD})$ and $\dot{V}CO_{2\ STPD} = \dot{V}_{E\ STPD} \times (F_{E\ STPD}CO_2 - F_{I\ STPD}CO_2)$. Inspired and expired gas fractions (F_I, F_E) were measured using a mass spectrometer (Perkin-Elmer, Model 1100, Pomona, CA), which was calibrated using gravimetrically-determined gas concentrations that spanned the range of O_2 and CO_2 concentrations between the inspired air and that expired by the horse. Gas was sampled continuously via a sampling port attached between the two nostril openings of the fiberglass mask (i.e., between the nares of the horse).

Experimental Protocol. An incremental exercise test was conducted with each TB on a level treadmill. The horses trotted (i.e. warmed-up) at 3 m/s for 800 m, and the speed of the treadmill was rapidly increased to 7 m/s for 1 min, and then the speed was increased in 1 m/s increments until fatigue (i.e., the horse could no longer keep up with the speed of the treadmill despite humane encouragement). The treadmill was immediately slowed to 3 m/s for 800 m, and cardiovascular, $\dot{V}_E$, and gas exchange measurements ($\dot{V}O_2$ and $\dot{V}CO_2$) were continuously recorded from the immediate off-set of maximal exercise and throughout the 4 min recovery period. Arterial blood samples were collected and pulmonary arterial temperature was recorded during the last 10 s of the final stage of exercise and at 2 and 4 min of the recovery period.

Blood Analysis. Arterial blood samples were placed immediately on ice after anaerobic withdrawal (~5 mL) into plastic, heparinized syringes. Blood gases (PaO_2 and $PaCO_2$), pH, and plasma lactate concentrations were quantified after the exercise test (within 1 – 2 h) using a blood gas analyzer (Nova Stat Profile, Waltham, MA). Blood gases and pH were then corrected

to the individual horse's pulmonary arterial temperature (Fedde, 1991). To ensure technical and internal consistency, one person conducted all blood analyses. Equipment was calibrated before and after each exercise test in accordance with manufacturers' standards.

Modeling of $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}_E$. Four-breath rolling averages for $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ were used for modeling data. Curve fitting was accomplished using KaleidaGraph software (Synergy software, Reading, PA USA) and was performed on the gallop-trot transition data using a one – component,

$$\dot{V}_{(t)} = \dot{V}_{(b)} + A_1 \cdot [1 - e^{-(t-TD)/\tau)}]$$

and a more complex two-component model,

$$\dot{V}_{(t)} = \dot{V}_{(b)} + A_1 \cdot [1 - e^{-(t-TD_1/\tau_1)} + A_2 \cdot [1 - e^{-(t-TD_2/\tau_2)}]$$

where $\dot{V}$ indicates the gas-exchange variable of interest (i.e., $\dot{V}O_2$, $\dot{V}CO_2$ or $\dot{V}_E$), t is a given time point, b is baseline (end-exercise), A_1 and A_2 are the response amplitudes, TD_1 and TD_2 are the independent time delays, and τ_1 and τ_2 are the time constants.

Goodness of model fit was determined via three criteria: 1) the coefficient of determination (i.e., r^2); 2) the sum of the squared residuals term (i.e., χ^2) and 3) visual inspection of the model. The time delay from end-exercise until the beginning of the response for $\dot{V}_E$ was determined independent of model estimates because the response varied considerably between horses (i.e., in some, $\dot{V}_E$ increased prior to falling; n = 3, while $\dot{V}_E$ remained stable in others; n = 2). Mean response times (MRT) were calculated from the model parameters using the following equations for the one-component model:

$$MRT = TD + \tau$$

and for the two-component model (see ref. MacDonald et al., 1997):

$$MRT = A_1/A_{1+2} (TD_1 + \tau_1) + A_2/A_{1+2} (TD_2 + \tau_2)$$

Statistics. A repeated measures ANOVA was used for each variable compared over time. If significance was revealed, a Student-Newman-Keuls *post hoc* test was utilized to determine the point of significance. Paired t-tests were used to determine whether the two-component model

provided a statistically better fit to the data than the one-component model. Between-variable comparisons were made by unpaired t-tests. Where a directional hypothesis was tested, a one-tailed test was utilized. Statistical significance was accepted at a p-value ≤ 0.05.

Study B: Methods

Animals. Six Thoroughbred horses (4 – 10 yr old; 470 - 600 kg) with a history of EIPH were used in this investigation. These horses were penned in dry lots, with shade, water, and salt available ad libitum. They were fed a mixture of alfalfa and grass hay, and concentrate twice daily. All horses were on a regular deworming and vaccination schedule and were conditioned, 3 d/wk on a high speed treadmill (SATO Inc., Uppsala, Sweden). Food, but not water, was withheld for two hours before experiments were conducted. Permission to conduct this investigation was granted by the Kansas State University Institutional Animal Care and Use Committee (IACUC).

Instrumentation. Before the experimental protocol, each horse was administered a local anesthetic (2% lidocaine) subcutaneously, and two 7-F introducer catheters were placed into the right jugular vein, using aseptic techniques. Another catheter (18-gauge, 2 in. Abbocath; Abbott Laboratories, North Chicago, IL) was inserted into a previously elevated carotid artery or transverse facial artery (20-gauge, 1.5 in.; without lidocaine) along with a cannula (polyethylene; 1.6 mm ID, 3.2 mm OD) for withdrawal of arterial blood (for blood gas and plasma lactate analyses) and to monitor mean arterial blood pressure (MAP; DigiMed BPA model 200, Louisville, KY).

To measure Ppa, temperature (i.e. core body), and collect mixed venous blood samples throughout the exercise protocol, a 7-F microtipped pressure transducer (Millar Instruments, model SPC-471a, Houston, TX) and thermistor catheter (Columbus Instruments, Columbus, OH) were advanced through the lumen of the introducer catheters into the pulmonary artery. Placement and calibration of the pulmonary artery transducer and thermistor catheter have been discussed previously (Meyer et al., 1998; Kindig et al., 2000, 2001ab, 2003; McDonough et al., 2004). Heart rate was determined with a Polar heart rate monitor (Mill Valley, CA).

Measurement of breath-by-breath-gas exchange. To determine oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), expired tidal volume (V_T), breathing frequency (f_B), and minute ventilation ($\dot{V}_E$), all horses were fitted with a lightweight fiberglass facemask (modified Aeromask; Trudell Medical International, London, Ontario, Canada) containing a tight-fitting rubber shroud to preventing leakage of gases. Flow-tubes, containing two ultrasonic transducers, were placed in the openings opposite each nostril to quantify airflow from each nasal passage (Model-41 eq; Flow metrics-BDRL, Birmingham, UK), as discussed previously (McDonough et al. 2002, 2004; Padilla et al. 2004).

The respiratory airflow and mass spectrometer signals were interfaced with a data analysis system (Ponemah Physiology Platform, Gould Instrument Systems, Valley View, OH) for the measurement of all ventilatory and gas exchange variables on a breath-by-breath basis (rate of digitization = 250 Hz; McDonough et al., 2002, 2004; Padilla et al., 2004). Minute ventilation ($\dot{V}_E$), $\dot{V}O_2$, and $\dot{V}CO_2$ were calculated using standard equations (see McDonough *et al.* 2004; Padilla *et al.* 2004).

Experimental Protocol. Preliminary data from our laboratory demonstrated that a dosage of 3 mg/kg of TBC3214 was capable of blocking exogenous ET-1 (0.2 μg/kg) from increasing Ppa in the resting horse (Benamou *et al.* 2001). In the current investigation, each horse served as its own control and was administered (in random order) either sterile saline (10 ml [0.9%]; CON) or the selective ET-1_A receptor antagonist, TBC3214, at 3 mg/kg (i.e. ANTAG) reconstituted in 10 ml sterile saline [0.9%]) i.v. one hour before the exercise test. Following resting measurements, the horses were warmed up on a flat (0% grade) treadmill at 3 m/s for 800 m. Subsequently, the treadmill was inclined to 6° (10%) and the speed increased in 1 m/min increments until fatigue (i.e. horse was unable to maintain treadmill speed despite humane encouragement). The treadmill was then decelerated to 3 m/s for cool down (~4 min). Exercise tests were separated by ~ 3 weeks.

Bronchoalveolar lavage (BAL) was conducted ~30 min after the exercise test to quantify EIPH, and data are presented as RBCs/ml of recovered BAL fluid values (Meyer *et al.* 1998; Kindig et al., 2003; McDonough et al., 2004). Blood analysis (PaO_2, $PaCO_2$, pH, and lactate) was conducted as in prior studies (Meyer *et al.* 1998; Kindig et al., 2003; McDonough et al., 2004). Arterial and mixed venous O_2 contents were quantified using a cooximeter (OSM 3

Hemoximeter, Copenhagen, Denmark) and used to calculate cardiac output ($\dot{Q}$) via the Fick principle (i.e. $\dot{Q} = \dot{V}O_2/(a\text{-}vO_2d)$). Measurements of time to fatigue, Ppa, $\dot{V}O_2$, $\dot{V}CO_2$, V_T, f_B, and $\dot{V}_E$ were made continuously throughout the exercise protocol with the computer based Ponemah data acquisition system, and measurements from the last 30 s of each speed and 2 and 4 min of cool-down were analyzed.

Statistical Analyses. Data are presented as mean ± SD. Specific, *a priori* hypotheses were tested using paired t-test analysis. When data were not normally distributed (i.e. EIPH), non-parametric statistics (i.e. Wilcoxin signed rank test) were conducted. Correlation analysis for non-normally distributed data was performed using Spearman Rank Order correlation test. Statistical significance was accepted at $P < 0.05$.

Study C: Methods

Experimental Animals. The Type II diabetic model chosen for this investigation was the male GK rat (Taconic Farm, Germantown, NY, USA; 6-8 mo. old; n = 7), whereas healthy male Wistar rats (n = 5) served as controls (CON). The GK model is a non-obese, hyperglycemic, hyperinsulinemic rat strain that was developed by selectively breeding glucose intolerant Wistar rats (i.e. ~5 generations; Goto and Kakizaki, 1981). Goto et al. (1975) reported that GK rats require no specially formulated diet and healthy Wistar rats may serve as ideal controls because they are of the same original strain as the GK rat.

All rats were kept in a controlled environment with a fixed 12 h light-dark cycle and with room temperature maintained at ~22ºC. Both GK and CON rats were provided standard rodent chow and water ad libitum. All experimental conditions and surgical procedures were approved by the Kansas State University Institutional Animal Care and Use Committee.

Surgical Preparation. The animals were anesthetized with sodium pentobarbital (50 mg/kg ip to effect and supplemented as necessary) and placed on a heating pad (38º C) to maintain body temperature throughout the experimental protocol. To monitor arterial blood pressure and heart rate (model 200, Digimed BPA, Louisville, KY, USA), the left carotid artery was cannulated

(polyethylene-50, Intra-Medic polyethylene tubing, Clay Adams Brands; Sparks, MD, USA). This cannula also allowed for infusion of the phosphorescent probe and blood sampling.

The spinotrapezius muscle is a postural muscle comprised of a mosaic of different fiber types (Delp and Duan, 1996) and lies in the mid-dorsal region of the rat, originating from the lower thoracic and upper lumbar region and inserts on the spine of the scapula. The right spinotrapezius muscle was exposed using a U-shaped skin incision to facilitate phosphorescence measurements of PO_2mv and electrical stimulation. After the overlying skin was reflected back and fascia removed, the muscle surface was superfused with Krebs-Henseleit solution equilibrated with 5% CO_2-95% N_2 at 38ºC and adjusted to pH 7.4. For the induction of indirect bipolar muscle contractions, stainless steel electrodes were attached to the spinotrapezius muscle in the immediate proximity of the motor point (cathode) and across the caudal end (anode) close to the spinal attachment.

Experimental Protocol. An arterial blood sample was taken during fasting conditions for analysis of blood glucose concentration (Accu-Check Advantage, Roche Diagnostics, Indianapolis, IN, USA) and for measurement of hematocrit. The phosphorescent probe (palladium *meso*-tetra(4-carboxyphenyl)porphyrin dendrimer; R2; Oxygen Enterprises, Philadelphia, PA; 15 mg/kg) was infused via the arterial cannula ~15 min before PO_2mv measurements were undertaken. Mean arterial pressure and heart rate were monitored throughout the protocol. The experiments were conducted in a darkened room to prevent contamination from ambient light. Following the 15 min stabilization period, twitch muscle contractions (1 Hz, 3-5V, 2-ms pulse duration) were elicited for 3 min using a Grass S88 stimulator (Quincy, MA). PO_2mv was determined at 2-s intervals at rest and after the rest-to-stimulation transition for 3 min. This contraction protocol has been shown to increase blood flow ~3-fold and $\dot{V}O_2$ ~ 5-fold in healthy rats without changing arterial acid-base status or elevating plasma lactate concentrations (Behnke et al., 2001, 2003). At the conclusion of each experiment, the animal was euthanized with a bolus injection of pentobarbital (>80 mg/kg i.a.).

PO_2mv Measurements. The principles behind phosphorescence quenching have been discussed previously (Rumsey et al., 1998; Behnke et al., 2001, 2002, 2003, McDonough et al., 2001). Briefly, phosphorescence quenching measures the PO_2 within the vasculature sampled by a light-

guide. The measurement is independent of the probe (R2) concentration but is volume weighted, and because capillaries comprise the greatest volume of vascular units within muscle, PO_2mv provides a measure of capillary plasma PO_2. It has been demonstrated that over the duration of the experimental paradigm used herein, measurable extravasation of the R2 probe does not occur (Poole et al., 2004).

The Stern-Volmer relationship (Rumsey et al., 1988) describes quantitatively the O_2 dependence of the phosphorescence probe (i.e. R2). R2 is a dendrimer bound to albumin at 38ºC and pH 7.4, with a quenching constant of 409 Torr/s and lifetime of decay in the absence of O_2 of 601 μs (Pawlowski and Wilson, 1992; Lo et al., 1997). PO_2mv was determined using a PMOD 1000 Frequency Domain Phosphorometer (Oxygen Enterprises, Philadelphia, PA, USA) with the common end of the bifurcated light guide placed ~2 mm above the medial region of the spinotrapezius (i.e. superficial to dorsal surface). This phosphorometer uses a sinusoidal modulation of the excitation light (524 nm) at frequencies between 100 Hz and 20 kHz, which allows for phosphorescence lifetime measurements from 10 μs to ~2.5 ms. In the single frequency mode, 10 scans (100 ms) were used to acquire the resultant lifetime of the phosphorescence (700 nm) and repeated every 2 s (for review, see Vinogradov et al., 2002). To obtain the phosphorescence lifetime, the logarithm of the intensity values was taken at each time point and by fitting the linearized decay to a straight line by the least squares method (Bevington, 1969).

Modeling of PO_2mv profiles. Curve fitting was accomplished using KaleidaGraph software (Synergy software, Reading, PA) and was performed on the PO_2mv data using a one–component,

$$PO_2mv_{(t)} = PO_2mv_{(b)} - \Delta PO_2mv_{(es)} \cdot [1 - e^{-(t-TD)/\tau}]$$

and a more complex two-component model,

$$PO_2mv_{(t)} = PO_2mv_{(b)} - \Delta PO_2mv_{(primary)} \cdot [1 - e^{-(t-TD1/\tau1)}] + \Delta PO_2mv_{(secondary)} \cdot [1 - e^{-(t-TD2/\tau2)}]$$

where "t" is a given time point, "b" is baseline (i.e. pre-contraction), and "es" is the decrease in PO_2mv from baseline to the end-stimulation values. For the two-component model, $\Delta PO_2mv_{(primary)}$ and $\Delta PO_2mv_{(secondary)}$ designate the asymptotic value to which that component of the ΔPO_2mv is projecting. TD_1 and TD_2 are the independent time delays, and τ_1 and τ_2 are the time constants.

The goodness of fit for the model was determined via three criteria: 1) the coefficient of determination (i.e. r^2); 2) the sum of the squared residuals term (i.e. χ^2) and 3) visual inspection of the model fit to the data. Mean response times (MRT) were calculated from the mono-exponential and double-exponential (i.e. primary MRT) model parameters using the following equation:

$$MRT_1 = TD_1 + \tau_1$$

Statistics. Differences between baseline (pre-contracting), nadir, and end-stimulation PO_2mv were analyzed using a repeated measures analysis of variance (ANOVA). If a significant F-value was found, a Student-Newman-Keuls post hoc test was utilized to determine the point of statistical significance. Paired t-tests were used to determine whether the two-component model provided a statistically better fit to the data than the one-component model. Between-group comparisons (i.e. cardiovascular, TD, τ, mean response time (MRT), etc.) were made using unpaired t-tests. Statistical significance was accepted at a P-value ≤ 0.05.

Study D: Methods

Experimental Animals. Male GK (Taconic Farm, Germantown, NY; 6-8 mo. old;) spontaneously diabetic rats (n = 7; body weight = 426 ± 15 g) and healthy male Wistar rats (CON; n = 5; body weight = 557 ± 19 g) were used in this investigation.

All rats were kept in a controlled environment with a fixed 12 h light-dark cycle and with a room temperature maintained at ~22ºC. Both GK and CON rats were provided a conventional rodent chow and water ad libitum. Previous investigators have reported that a conventional diet of rat chow and water is sufficient for the energy needs of the GK rat (Goto et al., 1975). All experimental conditions and surgical procedures were approved by the Kansas State University Institutional Animal Care and Use Committee.

Surgical Preparation. Before the surgical procedures, the animals were anesthetized with sodium pentobarbital (50 mg/kg ip to effect and supplemented as necessary). The rat was then placed on a heating pad (38º C) to maintain body temperature throughout the experimental

protocol. To monitor arterial blood pressure and heart rate (model 200, Digimed BPA, Louisville, KY), the left carotid artery was cannulated (polyethylene-50, Intra-Medic polyethylene tubing, Clay Adams Brands; Sparks, MD).

The spinotrapezius muscle is excellent for intravital studies of the microcirculation because: (1) it can be exteriorized without neural or substantial vascular disruption (Poole et al., 1997; Bailey et al., 2000), and (2) exteriorization permits transmission light microscopy for clear visualization of capillary structures and hemodynamics and (3) a physiological sarcomere length can be set, preventing over-stretching of the muscle and associated capillaries, thus avoiding adverse affects to the microcirculation (Poole et al., 1997). The spinotrapezius, which lies in the mid-dorsal region of the rat, originates from the lower thoracic and upper lumbar region and inserts onto the spine of the scapula. Furthermore, it is a postural muscle comprised of a mixture of fiber types (41% type I, 7% IIa, 17% IId/x, 35% IIb; ref. Delp and Duan, 1996).

For the experimental preparation, the left spinotrapezius was exteriorized and prepared in situ as described previously (Poole et al., 1997; Kindig et al., 1998, 1999; Russell et al., 2003; Richardson et al., 2003) to examine the microcirculation. Fascial removal and disturbance was minimized to avoid any associated muscle damage (Mazzoni et al., 1990). All exposed surrounding tissue as well as the dorsal surface of the spinotrapezius were superfused continuously with a Krebs-Henseleit bicarbonate-buffered solution (equilibrated with 95% N2-5% CO_2; pH 7.4; 38^{o} C) and the muscle was sutured (6.0 silk, Ethicon, Somerville, NJ) at five equidistant points around the perimeter to a thin wire horseshoe-shaped manifold. The muscle was protected with Saran Wrap (Dow Brands L.P., Indianapolis, IN) until analysis.

The rat was placed on a circulation-heated Lucite platform and the spinotrapezius was observed using an intravital microscope (Nikon, Eclipse E600-FN; X40 objective; 0.8 numerical aperture) equipped with a non-contact, illuminated lens and a high-resolution color monitor (Sony Trinitron PVM-1954Q, Ichinonya, Japan). The final magnification (X 1,184) was confirmed by initial calibration of the system with a stage micrometer (MA285, Meiji Techno). The spinotrapezius was maintained at physiological length (~2.4 μm) throughout the subsequent observation period, and any exposed tissue was continuously superfused with the Krebs-Henseleit solution. The muscle was transilluminated in a fashion that ensured clear resolution of the A-bands of the sarcomeres within 1/3 to 2/3 of the muscle fibers. The final screen magnification was X 1184 as confirmed by a stage micrometer (MA285, Meiji Techno, Japan).

This magnification is adequate for measuring all essential structural and hemodynamic variables (Poole et al., 1997).

Experimental design. Once the muscle was positioned on the platform, a microvascular viewing field (270 x 210 μm), containing ~5-8 muscle fibers and 5–10 capillaries in the midcaudal (dorsal surface) region of the muscle, was selected. Approximately 8-10 fields (~1-1.5 min ea) were recorded for each rat and images were time-referenced by frame and fields and stored on Super-VHS high resolution videocassettes (JVC S-Master XG) using a videocassette recorder (JVC BR-S822U, Elmwood Park, NJ) for subsequent offline analysis. Mean arterial pressure (MAP) was continuously monitored throughout the data-acquisition period, and the experimental protocol was no longer than 1.5–2 h in duration.

Capillary and fiber structural data analysis. Five of the fields were chosen for each rat based upon clear visualization of sarcomeres, fibers, and capillaries. Initially, each microvascular field (i.e., capillaries and myocyte boundaries) was traced directly from the television monitor. Capillaries supporting RBC flow were assessed in real time, and each capillary was placed into one of two categories (a) normal flow = 60 s of continuous or (b) impeded flow or stopped flow for > 10 out of 60 s. This was further used for determination of percentage of flowing capillaries (i.e., [no. of capillaries supporting RBC flow/ total no. of visible capillaries per area]*100). The presence and direction of RBC flow or the presence of stationary RBC's was also used to determine flowing lineal density (i.e., the number of capillaries per unit muscle width) and countercurrent flow. For all capillaries in which hemodynamics were assessed and where the capillary endothelium was clearly visible on both sides of the lumen, capillary luminal diameter (*d*c) was measured (2-4 measurements/capillary) with calipers accurate to ± 0.25 mm (± 0.17 μm at X 1,184 magnification).

Examination of the fields was conducted (30 frames/s) in real time and by frame-by-frame analysis techniques. Sarcomere length was determined from sets of 10 consecutive in-register sarcomeres (i.e. distance between 11 consecutive A bands) measured parallel to the muscle fiber longitudinal axis. This procedure was repeated 3-4 times where sarcomeres were visible to obtain a mean sarcomere length for each viewing field. For each muscle fiber in which both sarcolemmal boundaries were visible on the screen, the apparent fiber width perpendicular

to the longitudinal muscle fiber axis was measured at three locations, and a mean fiber width was determined for each fiber. Red blood cell velocity (V_{RBC}) was determined in all capillaries that were continuously RBC perfused by following the RBC path length over several frames (~5-10 capillaries/area). F_{RBC} was measured by counting the number of cells in a capillary passing an arbitrary point. For each capillary in which hemodynamic data were gathered, Hct_{cap} was calculated as:

$$Hct_{cap} = (volume_{RBC} * F_{RBC})/[\pi * (d_c/2)^2 * V_{RBC}]$$

where $volume_{RBC}$ is RBC volume, which was taken to be 61 m^3 (Altman and Dittmer, 1974), and capillaries were approximated as circular in cross section.

Statistical analysis. All data are presented as means ± SE where the group mean is that of the individual muscles rather than individual capillary measurements across muscles. Differences between CON and GK groups were tested with Student's *t*-test. Where there was clear precedence for an a priori directional hypothesis (i.e., F_{RBC} and V_{RBC}), a one-tailed test was used. Statistical significance was accepted at the $P < 0.05$ level.

CHAPTER 3

Study A: Results

$\dot{V}_E$. In the absence of overt pathology, the flow profiles for the left and right nostrils are almost superimposable. For the horses studied in the present investigation, the flow profiles from each nostril were remarkably similar throughout both exercise and recovery. Determination of inspired and expired volumes and minute $\dot{V}_E$ were made using the total flow through both the left and right nostrils.

After the gallop-trot transition, $\dot{V}_E$ remained elevated at or above end-exercise values for ~13s, after which $\dot{V}_E$ fell bi-phasically (Figure 4). Quantitatively, $\dot{V}_E$ was best characterized by a dual-exponential model with a fast and slow phase (Table 1; Figure 4) and a MRT of 85.4 ± 9.0 s (Table 1; Figure 4), which was significantly longer than the MRT of either $\dot{V}CO_2$ or $\dot{V}O_2$ (Table 1; Figures 4 & 5). The two-component nature of the response was confirmed via a significantly higher correlation coefficient (two-component: 0.96 vs. one-component: 0.93; $P < 0.05$) and a lower sum of squares residual term (two-component: 1.0×10^5 vs. one-component: 1.78×10^5; $P < 0.05$). The prolongation of the exercise $\dot{V}_E$ into recovery and the bi-phasic nature of the subsequent $\dot{V}_E$ decrease in trotting recovery were due, in part, to a radical change in breathing strategy (Table 2; Figure 6). Specifically, over the first 7-13s of recovery, tidal volume (V_T) rose and breathing frequency (f_B) fell significantly (Table 2). After this period, f_B and V_T remained at end-exercise levels for 30 s. After 30 s, V_T fell progressively with time, but f_B did not differ from end-exercise values (Figure 6; Table 2). At the end of the 4-min recovery period $\dot{V}_E$ was still 44.5 ± 18.5 % (e.g. Figures 4 & 6) above trotting baseline values.

$\dot{V}O_2$ and $\dot{V}CO_2$. $\dot{V}O_2$ and $\dot{V}CO_2$ both fell with a mono-exponential profile after the gallop-trot transition (Figures 5 & 4, respectively; Table 1) with MRTs of 39.9 ± 4.7 s and 28.9 ± 3.2 s respectively ($P<0.05$). Both $\dot{V}O_2$ and $\dot{V}CO_2$ apparently resolved to an elevated baseline (9.8 ± 36.0 and 17.5 ± 30.9 % above the pre-gallop, trotting baseline for $\dot{V}O_2$ and $\dot{V}CO_2$, respectively; e.g., Figures 5 & 4, respectively). However, compared with $\dot{V}_E$, $\dot{V}CO_2$ and $\dot{V}O_2$ were relatively closer to trotting baseline values following the 4-min recovery period (Table 1; Figures 5 & 4, respectively).

Blood gas response across the gallop-trot transition. The hypercapnia found during exercise was completely reversed within 2 min after the gallop-trot transition, and the ensuing hypocapnia was maintained through 4 min of trotting (Figure 7; $PaCO_2$ at gallop: 52.8 ± 3.2 mmHg; 2 min at trot: 25.0 ± 1.4 mmHg; 4 min at trot: 24.6 ± 1.5 mmHg; both $P < 0.05$ vs. gallop). This hyperventilation is further evidenced by the pronounced increase in $\dot{V}_E/\dot{V}CO_2$ (from ~20 at the gallop to ~60 within 1-2 min of trotting recovery; e.g. Figure 7). Plasma lactate was elevated to 25.5 ± 4.0 mM at the gallop and remained unchanged throughout the recovery period (2 min at trot: 24.9 ± 3.6; 4 min at trot: 24.5 ± 3.7 mM). Arterial pH increased from the gallop (7.217 ± 0.107) to 2 min (7.301 ± 0.093; $P < 0.05$), but did not differ between 2 min and 4 min (7.307 ± 0.098) of the recovery period.

Core temperature changes across the gallop-trot transition. Pulmonary arterial temperature decreased ($P < 0.05$) from the gallop (41.6 ± 0.9 °C) to 2 min (40.6 ± 0.7 °C), but did not change subsequently between 2 and 4 min (40.4 ± 0.7 °C) of the recovery period.

Study B: Results

Time-to-exhaustion was not different between CON and ANTAG (CON: 690 ± 40; ANTAG: 653 ± 36 s; $P > 0.05$), and neither metabolic nor gas exchange variables differed between CON and ANTAG groups either at rest or fatigue ($P > 0.05$; Tables 3 & 4). Heart rate at fatigue was also similar between groups (CON: 221 ± 5; ANTAG: 217 ± 10 beats/min; $P > 0.05$).

As shown in Figures 8 and 9 (Top Panel), respectively, resting mean values for Ppa (CON: 32 ± 8; ANTAG: 28 ± 9 mmHg) and Ppa at maximal exercise (CON: 82 ± 15; ANTAG: 92 ± 20 mmHg) did not differ between groups ($P > 0.05$). In addition, Ppa's did not differ between the two treatments throughout the last three stages of exercise or at fatigue ($P > 0.05$; Figure 8). With respect to EIPH, ANTAG tended to be higher then CON, however, differences in EIPH between the CON and ANTAG groups did not reach statistical significance (CON: 4.1 x 10^6 ± 2.9 x 10^6; ANTAG: 17.2 x 10^6 ± 19.0 x 10^6 RBCs/ml BAL fluid; Figures 9 (Bottom Panel) & 10). Moreover, there was no significant correlation between the EIPH and Ppa ($P > 0.05$; Figure 10).

Study C: Results

Animal Data. Arterial blood glucose was significantly higher and body weight was lower in GK compared to CON (Table 5) which is in agreement with previous studies (Goto and Kakizaki, 1981; Yasuda et al., 2002). Neither mean arterial blood pressure nor hematocrit differed between groups ($P > 0.05$; Table 5). However, heart rate was significantly higher in the GK rats (Table 5).

Spinotrapezius PO_2mv Profiles. As depicted in Figure 11, the PO_2mv profile at the onset of contractions was considerably different in CON vs. GK spinotrapezius muscles. Baseline PO_2mv was lower in the GK rats than the CON rats (CON: 28.8 ± 2.0 Torr; GK: 18.4 ± 1.8 Torr; $P < 0.05$; Table 6). The CON rats displayed PO_2mv profiles that consisted of a time-delay (12.3 ± 2.9 s) followed by an exponential fall ($\Delta_1 PO_2mv$: -11.2 ± 1.6 Torr) to a steady-state PO_2mv (end-stimulation PO_2mv: 17.6 ± 0.7 Torr; $P < 0.05$). This profile has been demonstrated previously for healthy rat spinotrapezius muscle (Behnke et al., 2001, 2002). In contrast, six of the seven GK rats demonstrated a close-to-exponential but transient fall after a time-delay (8.9 ± 1.5 s; not different from CON; $P > 0.05$; Table 6) that reduced PO_2mv below the end-stimulation value (e.g., See GK #4 & #5; Panels C & D in Figure 11). This behavior has been termed an "undershoot" and reduced PO_2mv (5.7 ± 3.5 Torr) below the subsequent contracting steady-state value. Subsequently, PO_2mv increased back to baseline values (resting or slightly higher) by the end of contractions (baseline PO_2mv: 18.4 ± 1.8 Torr; end stimulation PO_2mv: 18.9 ± 2.6 Torr; $P > 0.05$: Figure 11). In contrast, one GK rat displayed an unusual "overshoot" in the PO_2mv profile after a brief time-delay followed by an exponential fall in PO_2mv to the steady state (Figure 11, GK #1, Panel B).

Modeled PO_2mv responses. A simple one-component model adequately fit the PO_2mv responses of the CON rats ($r^2 = 0.97 \pm 0.01$, $\chi^2 = 56.6 \pm 12.9$; Figure 11, Panel A) and a more complex model was not chosen. For the GK rats (Figure 11, Panels B, C, & D), the double-exponential model provided a better fit for the PO_2mv profiles as evidenced by higher r-value (0.96 ± 0.01 vs

0.67 ± 0.11; $P < 0.05$) and lower χ^2 value (27.6 ± 6.3 vs 229.9 ± 114.1; double-exponential, monoexponential, respectively; $P < 0.05$).

Although, TD did not differ between the two groups (CON: 12.3 ± 2.9; GK: 9.1 ± 2.0; $P > 0.05$), τ_1 was shorter in the GK rats (CON: 15.5 ± 3.5 s; GK: 6.4 ± 1.8 s; $P < 0.05$). This resulted in an MRT (i.e. $TD_1 + \tau_1$) for the primary response that tended to be shorter in the GK vs. CON rats (CON: 27.7 ± 6.0 s; GK: 15.5 ± 2.7 s; $P = 0.08$; Table 6). However, the time necessary for the PO_2mv to fall to 63% of the difference between baseline and the nadir of the response (T_{63}; i.e. model-independent estimate of the MRT) did not differ in duration between the GK and CON rats (CON: 32.4 ± 7.6 s; GK: 31.0 ± 9.0 s; $P > 0.05$; Table 6).

Study D: Results

GK rats exhibited significantly higher fasting blood glucose levels compared to the healthy CON rats (CON: 105 ± 5 mg/dl vs. GK: 263 ± 34 mg/dl; $P < 0.05$). The GK rat is considered a non-obese model of Type II diabetes and this was reflected in the average body weights (CON: 553 ± 18 g; GK: 417 ± 14 g; $P < 0.05$). Although sustained within a normal physiological range, heart rate was higher ($P < 0.05$; Table 7) in the GK rats, but MAP did not differ between the groups ($P > 0.05$; Table 7). Systemic hematocrit was measured at the end of the experiment and did not differ between groups (CON: 47 ± 2; GK: 43 ± 1 %; $P > 0.05$).

Muscle structural data. Neither sarcomere length (CON: 2.6 ± 0.1; GK: 2.7 ± 0.1 μm) nor capillary diameter (CON: 4.7 ± 0.1; GK: 5.1 ± 0.2 μm) differed between groups (both $P > 0.05$). Although muscle fiber width was decreased in the GK rats (CON: 65.0 ± 5.2; GK: 51.5 ± 3.9 μm; $P < 0.05$), total lineal density (i.e. the total number of both perfused and non-RBC perfused capillaries per unit fiber width) did not differ ($P > 0.05$) between the GK and CON rats, but flowing lineal density was significantly attenuated in the GK rats ($P < 0.05$; Figure 13).

Hemodynamic comparisons. There was a significant decrease in the percentage of flowing capillaries in the GK rats in comparison to the CON rats (CON: 93 ± 3; GK: 66 ± 5 %), although countercurrent flow did not differ between the groups (CON: 24 ± 5; GK: 19 ± 3%; $P > 0.05$). In the RBC-perfused capillaries of the individual muscles of GK rats, dramatic decreases were also

found in Hct_{cap} (CON: 33±0.1; GK: 23±0.1 %), V_{RBC} (CON: 454 ± 20; GK: 158 ± 26 μm/s), F_{RBC} (CON: 42 ± 4; GK: 14 ± 3 RBC/s). Furthermore, V_{RBC} and F_{RBC} were significantly correlated in the individual muscles (r^2 value = 0.9275; $P < 0.05$; Figure 14). More importantly, when the product of flowing lineal density and capillary F_{RBC} was calculated (i.e. an index of O_2 delivery), the blood flow per unit of muscle was markedly lower in the GK rat spinotrapezius when compared with CON (CON: 813 ± 88; GK: 227 ± 37 RBC/s/mm; $P < 0.05$; Figure 15).

CHAPTER 4

Study A: Discussion

To the best of our knowledge, this is the first study to provide a breath-by-breath analysis of $\dot{V}_E$, gas exchange (i.e. $\dot{V}O_2$ and $\dot{V}CO_2$), and related metabolic and blood gas measurements across the gallop-trot transition at the cessation of maximal exercise in the TB horse. The finding that $\dot{V}_E$ did not decrease abruptly at the gallop-trot transition suggests that any mechanical link between locomotion and respiration (i.e. LRC) does not constitute an obligatory component of the extraordinary $\dot{V}_E$'s commensurate with those found during exercise. Specifically, an abrupt reduction in stride length and frequency, as seen at the transition from galloping to trotting (i.e., 3 m/s), and the removal of the synchrony between stride and breathing frequency (LRC) did not reduce $\dot{V}_E$ instantaneously. Furthermore, given the finite $\dot{V}CO_2$ dynamics, had a precipitous fall in $\dot{V}_E$ occurred, it would be expected to sustain or even exacerbate the exercise-induced hypercapnia (i.e. elevate $PaCO_2$ further). In contrast to this notion, our results show that the hypercapnia and hypoxemia of exercise are quickly reversed (at least within 2 min) because $\dot{V}_E$ remained at peak galloping levels for ~13 s of the post-gallop trot (due to an altered breathing strategy) before decreasing relatively slowly in the presence of more rapid reductions of $\dot{V}O_2$ and $\dot{V}CO_2$ (i.e. $\dot{V}_E/\dot{V}O_2$ and $\dot{V}_E/\dot{V}CO_2$ both increased). Thus, after the transition from gallop to trot, compensatory hyperventilation was evident. One putative interpretation of this behavior is that LRC at the gallop may have restricted the full magnitude of the exercise hyperpnea. When this constraint was removed, the $\dot{V}_E/\dot{V}CO_2$ ratio rose to a level that ensured a respiratory compensation for the metabolic acidosis ($PaCO_2 < 20$ mmHg) and restored PaO_2 to pre-exercise levels. Although the possibility remains that LRC may contribute to the exercise hyperpnea, the data from the present investigation demonstrates that the TB can attain $\dot{V}_E$ values equivalent to those during galloping in the absence of LRC during post-maximal exercise trotting recovery.

Role of LRC in the horse. Locomotory-respiratory coupling has been presented as a "general requirement for sustained aerobic activity among endothermic vertebrates" (Bramble and Carrier, 1983). Some researchers maintain that LRC functions to enhance pulmonary airflow during the gallop in the horse (Attenburrow, 1982; Hornicke et al., 1983). However, it is also

clear that racing breeds, in contrast to their more pedestrian counterparts (see ref. Dempsey and Wagner, 1999), become considerably hypoxemic and hypercapnic (Bayly et al., 1989; Butler et al., 1993; Evans et al., 1994; McDonough et al., 2002; Wagner et al., 1989) during brief, maximal exercise, suggestive of some sort of "functional" ventilatory restraint. That this restraint is not the result of an absolute limitation to ventilation, *per se*, is supported by studies using heliox inspirates (Erickson et al., 1994) and hypoxia (Pelletier and Leith, 1995). In addition, the results of the current study, where $\dot{V}_E$ remained at or rose above (3 of 5 horses) end-exercise values for the first 7-13 s of recovery also argues against any purely mechanical limitation to airflow generation.

Marlin et al. (via respiratory inductance plethysmography; Marlin et al., 2002) have shown that (during the canter and gallop) V_T is increased by abdominal elongation and expansion consistent with a major diaphragmatic contribution to inspiration, whereas Ainsworth et al. (1997) determined (via electromyogram analysis) that diaphragmatic contractions are always in phase with esophageal pressure changes and inspiratory flow generation as exercise intensity increases. These results suggest that inspiratory tidal airflow generation during running in the horse may be the sole province of the diaphragm, a contention that is supported by the extraordinary thickness, oxidative capacity and blood flow capacity of the equine diaphragm (Manohar, 1988; Poole et al., 2002).

Although LRC may limit airflow generation during running in the horse, one potential benefit of a LRC-derived constraint upon exercise ventilation can be gleaned from the work of Harms et al. (1998, 2000). These authors reported that the work of breathing can affect performance and time to fatigue in humans by redistributing flow away from the locomotory and toward the respiratory muscles. As the work of breathing increases to a much greater degree in the horse than the human (cf. ref. Aaron et al., 1992 and Art et al., 1990), this mechanism has perhaps an even greater fatigue-generation potential in the horse. This is particularly true as the TB is thought to rely primarily upon diaphragmatic breathing (Ainsworth et al., 1997; Marlin et al., 2002) during the gallop; thus any attempt to overcome the constraint resulting from LRC could well lead to both diaphragmatic and locomotory muscle fatigue.

Unlike TB horses, Standardbred horses can achieve their maximum oxygen uptakes whilst trotting or pacing when the breathing and stride frequencies are not coupled. The maximum oxygen uptake in competitive Standardbred horses (i.e., ~165 ml/kg/min; see refs. Art

and Lekeux, 1995 and Gauvreau et al., 1995) is close to that found in the TB. However, V_T is far higher (20-26 vs. 12-14 L/breath) and f_B's are lower (70-80 vs. 130 breaths/min) in the Standardbred vs. the TB horse. Therefore, for Standardbreds at the transition from the maximum speed to either resting or a lower speed, one would not expect the same response as found in the TB (i.e. increasing V_T's, lowering of f_B). Indeed, Standardbred horses increase f_B and decrease V_T across this transition. Thus, although the strategy is different, one common feature across these two breeds is that both hypoventilate during intense exercise (i.e. $\dot{V}_E/\dot{V}O_2$, $\dot{V}_E/\dot{V}CO_2$ and PaO_2 fall and $PaCO_2$ rises) and hyperventilate in recovery (Art and Lekeux, 1995; McDonough et al., 2002a).

Mechanistic basis for slow $\dot{V}_E$ recovery in the TB horse. In the TB horse, the presence of hypoventilation during maximal exercise and noticeable hyperventilation (see Figure 7) during recovery suggests strongly that, in considerable contrast to humans (Whipp and Ward, 1987), $PaCO_2$ is not a precisely controlled variable in equids (McDonough et al., 2002a; Powers et al., 1987). In addition, the observation that $\dot{V}_E$ remains at or above end-exercise values during the early recovery period (Figures 4 & 6) constitutes strong evidence that LRC may actually provide an impedance to $\dot{V}_E$ during exercise, whereas the slow, prolonged recovery profile for $\dot{V}_E$ suggests multiple sources of ventilatory control during recovery from maximal exercise.

Initial ("fast") component of $\dot{V}_E$. Throughout high intensity exercise, $PaCO_2$ is markedly elevated in the TB (McDonough et al., 2002ab). As TB horses exhibit a normal ventilatory response to CO_2 at rest (in contrast to the situation during exercise; Landgren et al., 1991), it is likely that CO_2 is providing at least some of the initial stimulus responsible for the elevation of $\dot{V}_E$ noted in the current study. Indeed, V_T increased rapidly, while f_B fell (Figure 6 and Table 2), indicative of increased alveolar ventilation and enhanced CO_2 elimination (Whipp and Ward, 1998). However, by 2 min of recovery $PaCO_2$ had fallen to hypocapnic levels (Figure 7), whereas $\dot{V}_E$ was still elevated, which strongly suggests that the ventilatory response is being maintained at this point by other stimuli.

Secondary ("slow") component of $\dot{V}_E$. The secondary component of $\dot{V}_E$ during recovery started at ~90s of recovery, likely at a time point when $PaCO_2$ was already well below 40 mmHg (see

$\dot{V}_E/\dot{V}CO_2$ profile in Figure 7). Although pH at end-exercise (7.217 ± 0.107) and during recovery (2 min: 7.301 ± 0.093 and 4 min: 7.307 ± 0.098) is reduced below trotting baseline, these values are not different from those reported in humans (Stringer et al., 1992), or in the pony (Powers et al., 1987), which, unlike its more fit relative, exhibits a more rapid $\dot{V}_E$ recovery response profile. In addition, the changes in pH and $\dot{V}_E$ were not correlated.

It is also generally acknowledged that there is an extensive array of ventilatory stimuli present during and following intense exercise. Thus, the control of the exercise (and post-exercise) hyperpnea is complex and demonstrates considerable redundancy (for review, see Whipp, 1981). In early recovery following the gallop-trot transition, potential sources of ventilatory stimulation include arterial acidosis (lactic acidosis and hypercapnia), elevated catecholamines, temperature, potassium ions (K^+), osmolarlity, venous distension and/or some form of short-term potentiation (STP). This STP was originally termed "respiratory afterdischarge" by Gesell and White (1938) and has subsequently been described as an exponential ventilatory response that decays relatively slowly following removal of the stimulus. Specifically, STP decays with a time constant ranging from 36-101 s after carotid sinus nerve stimulation in cats (Eldridge, 1974; Eldridge and Gill-Kumar, 1980) and 18-39 s following hypoxic exercise in humans (Fregosi, 1991). Thus, if present in the TB, STP may have potentially played a role in the prolonged ventilatory response found during trotting recovery described herein.

The present investigation was not designed to determine which of these above mediators induced the post-galloping elevated ventilatory response. However, the temporal profile of some of these mediators decreases the likelihood that they played a major role in the extended hyperventilation seen in the trotting recovery. For example, the exercise-induced hypercapnia was resolved at least by 2 min at which time the $PaCO_2$ had been driven substantially below resting values. Whereas catecholamines were not measured in the present investigation, Snow et al. (1992) observed that blood catecholamine levels returned close to resting levels within 60 s post-exercise which is much faster that the $\dot{V}_E$ response. In addition, K^+ recovers to baseline values within ~2 min of recovery (Harris and Snow, 1988).

How are ventilation and hyperthermia linked? Respiratory heat exchange occurs in many mammalian species that primarily employ nasal breathing (Schroter and Watkins, 1989). In

large mammals such as the camel (Schroter et al., 1989) and the horse (Hodgson et al., 1993; McConaghy et al., 1995), ventilation during heat stress serves to maintain brain temperature as much as several degrees Celcius below core body temperature (McConaghy et al., 1995; Weishaupt et al., 1996). Although horses employ a robust sweating response (rates of >30 L/hr) that works in concert with the respiratory system to dissipate heat during exercise (Hodgson et al., 1993), these responses are not adequate to prevent heat storage during exercise, in part due to a relatively low surface area to body mass ratio in the racing horse. Thus, during exercise, the TB routinely achieves core body temperatures up to ~42 - 43ºC (Hodgson et al., 1993; McConaghy et al., 1995; Weishaupt et al., 1996). During trotting recovery, with the locomotory muscles operating at a much reduced metabolic rate compared with the gallop, evaporative heat loss from the respiratory tract will augment heat dissipation and provides one possible explanation for the prolonged elevation in $\dot{V}_E$ seen post-galloping. This strategy may be affected, in part, by the adoption of an altered breathing pattern during the recovery from high-intensity exercise.

Core temperature follows a slow recovery time course following incremental exercise (Marlin et al., 1996). In this investigation, core temperature was markedly elevated throughout recovery (~40.5 °C at 4-min post-gallop) and the rate of change in $\dot{V}_E$ during the secondary recovery component was highly correlated with that of core temperature (r^2=0.998; $p<0.05$). Given that hyperthermia does constitute a powerful $\dot{V}_E$ stimulus in the horse (Marlin et al., 1996) and that the temporal profiles of other potential $\dot{V}_E$ stimuli (i.e., K^+, catecholamines, $PaCO_2$) during recovery from exercise in the horse do not cohere with that of $\dot{V}_E$, the possibility must be acknowledged that the hyperthermia which attends maximal exercise and recovery in TB horses, may potentially contribute to the prolonged hyperventilatory response found during recovery.

In conclusion, rather than exhibiting the precipitous fall in $\dot{V}_E$ such as that observed in humans after intense exercise (Linnarsson, 1974; Riley and Cooper, 2002; Stringer et al., 1992), horses sustain the full magnitude of the exercise hyperpnea for several seconds into recovery followed by a prolonged biphasic decrease in $\dot{V}_E$. This sustained hyperpnea is the product of an immediate increase in V_T combined with a fall in f_B (Table 2, Figure 6). Because an immediate fall in $\dot{V}_E$ was not found during the off-transition from the gallop (LRC present) to the trot (no

LRC), we conclude that LRC does not appear requisite to achieve prodigiously high $\dot{V}_E$ equivalent to those seen during maximal exercise.

Study B: Discussion

In this current investigation, we chose to administer the ET-1_A receptor antagonist, TBC3214, into the pulmonary artery of horses to determine whether ET-1 plays an active role in the high Ppa that has been linked mechanistically to EIPH during maximal exercise. This study demonstrated that an ET-1_A antagonist does not affect exercise capacity or cardiorespiratory variables as determined by time-to-fatigue and gas exchange (i.e. $\dot{V}O_{2max}$) profiles. More importantly, the antagonist did not reduce maximal Ppa, indicating that ET-1 may not play a primary role in the rise in Ppa during intense exercise. In this investigation, only one horse demonstrated a decreased Ppa concomitant with a reduction in EIPH after ANTAG administration (Figure 9; Horse #3). In addition, there was no significant relationship between Ppa and EIPH in either the CON or ANTAG conditions. Thus, contrary to our hypothesis, EIPH was not reduced with administration of the antagonist which demonstrates that such an antagonist is not a viable treatment for the mitigation of EIPH.

Comparisons with previous literature

In agreement with Benamou and colleagues (2001), the present investigation demonstrated that an ET-1_A receptor antagonist did not result in changes in Ppa at rest, suggesting that ET-1 does not play a role in resting pulmonary vascular tone. Also, during exercise, the CON and ANTAG horses did not exhibit differences in MAP, HR, $\dot{Q}$, or plasma lactate, which concurs with previous studies in other species (Maeda *et al.* 2002; Merkus *et al.* 2003).

In the present investigation, CON values for Ppa and EIPH for CON fell within these previously reported ranges (Meyer *et al.* 1998; Kindig *et al.* 2000, 2001ab, 2003; McDonough *et al.* 2004). Also, the threshold for induction of EIPH was previously reported to be between 75 – 100 mmHg (Birks *et al.* 1997; Langsetmo *et al.* 2000), and indeed, those horses (i.e. four of the six) that showed significant bleeding in this current study met that threshold (Figure 10). However, no significant relationship was found between Ppa and EIPH, which adds to previous literature suggesting that EIPH and Ppa are not always related (Kindig *et al*. 2000, 2001b, 2003).

Exercise-induced pulmonary hypertension and EIPH

The 4 – 5 fold increase in Ppa experienced by TB horses during intense exercise has long been considered a primary cause of EIPH (Erickson *et al.* 1990; West *et al.* 1993; Birks *et al.* 1997; Meyer *et al.*, 1998; Roberts and Erickson, 1999). These elevated pressures may be the result of 1) prodigious level of $\dot{Q}$ (> 300 L/min), 2) hemoconcentration (and thus increased viscosity; Fedde and Erickson, 1998), 3) maximal recruitment and distension of the pulmonary capillaries (as suggested by the horse's high Ppa/$\dot{Q}$ ratio; Pelletier and Leith 1993), and 4) a transient increase in pulmonary venous pressure caused by an inability for the left ventricle to compensate fully for the very high $\dot{Q}$ during intense exercise (Jones *et al.* 2002). Furthermore, studies using furosemide, a diuretic, have consistently shown a decrease in Ppa and EIPH (Pascoe *et al.* 1985; Geor *et al.* 2001; Kindig *et al.* 2001a; McDonough *et al.* 2004).

More recently, the role of high Ppa's as the sole cause of EIPH has been questioned by several investigators (Poole *et al.* 2000; Kindig *et al.* 2000, 2001ab, 2003; McDonough *et al.* 2004). For example, the Flair equine nasal strip reduces EIPH by 33-50% without affecting Ppa (Poole *et al.* 2000; Geor *et al.* 2001; Kindig *et al.* 2001a; McDonough *et al.* 2004). In addition, when horses were run to fatigue while breathing nitric oxide (NO) gas, Ppa was decreased, but EIPH increased (Kindig *et al.* 2001b). Furthermore, Kindig and colleagues (2000ab) have shown that administration of an inhibitor of NO production (i.e. L-NAME) resulted in an increase in EIPH without increasing Ppa or $\dot{Q}$. Lastly, EIPH was reported to be increased with a concomitant decrease in Ppa when horses were galloped on an incline (Kindig *et al.* 2003). In agreement with these previous studies, the current investigation found no significant correlation between Ppa and EIPH, and indeed, the horse with the highest Ppa did not evidence the highest degree of EIPH (Figure 10). These results indicate that Ppa is not always a predictor of EIPH and that other factors may be involved in regulating pulmonary capillary transmural pressure and therefore determining stress failure of the blood gas barrier (see Sinha *et al.* 1996; Poole *et al.* 2000; Geor et al., 2001; Kindig *et al.* 2001ab; McDonough *et al.* 2004).

Efficacy of the Endothelin-1_A receptor antagonists

Our goal in this investigation was to administer a concentration of an ET-1_A antagonist, TBC3214, that would primarily target and be limited to the horse's lung without affecting the

systemic circulation. Indeed, one ET-1_A antagonist, Sitaxsentan (Encysive Pharmaceuticals, Houston, TX) has been shown to reduce Ppa in human patients with primary pulmonary hypertension with little or no effect on systemic vascular tone, suggesting that this ET-1_A antagonist exerts its specific action on the pulmonary circulation (Givertz *et al.* 2000).

Exact values of ET-1 concentration in equine lung tissue during exercise are currently unavailable because ET-1 operates at the autocrine/paracrine level. However, in resting horses, ET-1 venous plasma concentrations vary between 0.2 (McKeever and Malinowski, 1999; McKeever *et al.* 2002) and 5 pg/ml (Benamou *et al*. 1998), and they do not appear to differ from arterial concentrations (Benamou *et al.* 1998). Mean ET-1 concentrations in BAL fluid at rest are ~60 pg/ml (Benamou *et al.* 1998) and appear unchanged after high intensity exercise (Benamou *et al.* 1999). The ET-1 antagonist used in this study was capable of blocking the lowest concentration of exogenous ET-1 (0.2μg/kg) known to significantly increase Ppa in resting horses (Benamou *et al.* 2001) which is equivalent to plasma concentrations of 3000 pg/ml (if plasma volume is estimated to be ~65 ml/kg bwt; Benamou *et al.* 2001). Thus, the amount of antagonist administered in the present investigation should have been more than adequate to block both normal systemic (Benamou *et al*. 1998; McKeever and Malinowski, 1999; McKeever *et al.* 2002), and BAL ET-1 concentrations (Benamou et al. 1998) in the exercising TB horse.

Limitations of the Current Investigation

The functions of ET-1 are complex and highly species dependent with its receptor subtypes distributed in a heterogeneous fashion throughout the pulmonary circulation (Fukuroda *et al.* 1994; MacLean *et al.* 1998; Schmect *et al.* 1999). Although ET-1_A receptors are predominantly found in third generation equine pulmonary arterioles (Benamou *et al.* 2003), further studies must be conducted to discern which ET-1 receptors are found beyond these arterioles to further elucidate the role of ET-1 in the equine lung.

The study of ET-1 is further complicated by this hormone's ability to either act locally on receptors found on nearby smooth muscle cells to cause vasoconstriction, or in some circumstances on endothelial cells causing vasodilation (rev. Kedzierski and Yanagisawa, 2001). Although plasma and BAL ET-1 concentrations have been determined at rest, and during and after exercise in healthy horses (Benamou *et al.* 1999; McKeever and Malinowski, 1999; McKeever *et al.* 2002), these concentrations may be lower than those reported to be biologically

active at the level of the tissue (Wagner *et al.* 1992). Thus, there is the likelihood that assumptions and interpretation of data based upon changes occurring in plasma or BAL fluid may not be physiologically relevant, and therefore, in this current investigation, we can speculate that the ET-1_A antagonist, TBC3214, may not have fully blocked the effects of ET-1 at the level of the tissue.

Conclusions

To the best of our knowledge, this is the first investigation to administer an ET-1_A antagonist to a horse to determine if ET-1 plays a mechanistic role in the elevated Ppa response during exercise. Contrary to our hypothesis, we have shown ET-1 (via an ET-1_A antagonist) does not appear to contribute to the high pulmonary vascular pressures or EIPH experienced by the exercising horse. Along with previous studies, we have also demonstrated that there appears to be no significant relationship between Ppa and EIPH. Furthermore, the present investigation provides no support for the notion that treatment with the ET-1_A antagonist, TBC3214, is effective in abolishing or reducing the severity of EIPH.

Study C: Discussion

To the best of our knowledge, this is the first investigation to examine the PO_2mv response across the rest-contractions transition in a muscle from a Type II diabetic individual. The present investigation has demonstrated that (1) the PO_2mv in the spinotrapezius muscle of the GK rat is significantly lower at rest than in the CON rat indicative of a reduced O_2 driving pressure into the myocyte, and (2) at the rest-contractions transition, the kinetic profile of PO_2mv is substantially altered in the diabetic state. Specifically, in contrast to the PO_2mv response (i.e. delay followed by an exponential decline to the steady-state PO_2mv) in the CON rats, the majority (6 of 7) of GK rats elicited a time delay followed by an accelerated fall in PO_2mv that was proceeded by an either immediate or delayed return of PO_2mv to pre-contracting baseline levels. In one GK rat, the PO_2mv response profile was completely different from that seen in the other six rats: PO_2mv increased following a brief time delay at the onset of contractions before declining slightly below the baseline PO_2mv value (Figure 11, Panel B).

These PO_2mv profiles in the GK rats are indicative of a perturbed relationship between O_2 supply and O_2 utilization. The rapid fall of PO_2mv in the majority of GK rats (i.e. faster primary

'τ') followed by an "undershoot" can be interpreted as evidence for a comparatively sluggish $\dot{Q}O_2$ response at least relative to that for $\dot{V}O_2$. This would limit be expected to limit diffusive O_2 transport and thus constrain the $\dot{V}O_2$ kinetic response at the onset of contractions. Indeed, a lower arterial PO_2 (Engelen et al., 1996) and PO_2mv (McDonough et al., 2005) as well as a faster reduction in PO_2mv (Behnke et al., 2002) have all been associated with slower $\dot{V}O_2$ kinetics. The eventual return of PO_2mv in the GK rat muscle to a contracting level that was not different from CON rats indicates that in the contracting steady state, the $\dot{Q}O_2/\dot{V}O_2$ ratio did not differ between CON and GK rats. However, a lowering of PO_2mv along with perturbations in the PO_2mv response observed in the GK rat suggests a mechanistic basis for the slowed pulmonary $\dot{V}O_2$ kinetics present in human Type II diabetic patients (Regensteiner et al., 1998; Figure 12).

Interpretation of the PO_2mv responses. At any instant in time, PO_2mv will be representative of the balance between muscle $\dot{Q}O_2$ and $\dot{V}O_2$ within the microcirculation from resting conditions to the onset of contractions (Behnke et al., 2001, 2002, 2003). PO_2mv dynamics provide a measure of the driving pressure for O_2 diffusion from the blood to the contracting muscle which in turn, impacts the rate of O_2 transport into the myocyte. Fractional O_2 extraction, and thus the approximate extent to which venous and PO_2mv will fall, is determined principally by the matching between the length of the TD and τ values for $\dot{Q}O_2/\dot{V}O_2$ and their respective magnitudes within a muscle (McDonough et al., 2001; Behnke et al., 2001). Therefore assuming intracellular, and consequently mitochondrial PO_2 is close to zero (i.e. < 3 Torr; Honig et al., 1997; Richardson et al., 1999; for a contrasting opinion see Mole et al., 1999), the driving pressure for blood-muscle O_2 exchange (PO_2mv) at time any time (t) across the rest-contractions transition will be given by:

$$Pm_{O2}(t) \propto CaO_2 - CvO_2 = \frac{\dot{V}O_2\,(rest) + [\Delta\dot{V}O_2\,(1-e^{-t/\tau\dot{V}O2}]}{\dot{Q}O_2\,(rest) + [\Delta\dot{Q}O_2\,(1-e^{-t/\tau\dot{Q}O2}]}$$

Where CaO_2 and CvO_2 denote arterial and venous O_2 contents, respectively.

In healthy muscle, arteriolar vasodilation and $\dot{Q}O_2$ dynamics (i.e. $\tau\dot{Q}O_2$) are rapid in comparison to $\tau\dot{V}O_2$ (Delp et al., 1999; Bangsbo et al., 2000; Kindig et al., 2002; see Ferreira et

al., 2005 for a novel perspective on this topic). Thus, in some instances, O_2 extraction decreases (Bangsbo et al., 2000; Grassi et al., 1996) and PO_2mv (Behnke et al., 2001, 2002) may rise for the first few seconds of contractions which may be advantageous because elevating PO_2mv assures a greater O_2 gradient for driving blood-myocyte diffusion (according to Fick's law). This may also provide a higher intramyocyte PO_2 which will limit disruptions of the intracellular physicochemical milieu (i.e., mitochondrial ATP flux; Wilson et al., 1977).

In contrast to the healthy response, the GK rat exhibited a lower resting PO_2mv in the spinotrapezius. A reduced PO_2mv across the rest-exercise transition indicates that there is likely to be a reduction in O_2 exchange across the blood-myocyte interface and mitochondrial function will be compromised. Under these circumstances, this would lead to a greater reliance upon immediate energy sources (i.e. more rapid decrease in PCr, increased free ADP, and enhanced breakdown of glycogen; see rev. Jones and Poole, 2005). However, it is interesting to note that one characteristic of the diabetic individual is decreased mitochondrial function, and in turn, compromised oxidative capacity (Kelley et al., 2002). This would further serve to limit their ability to utilize more oxidative energy sources and will force diabetic individuals to utilize greater glycolytic substrates (if available) at the onset of exercise. In a patient population in whom glucose homeostasis is adversely affected with the progression of this disease, the phenomenon of decreased oxidative capacity and increased reliance upon glycolytic substrates will further contribute to the reduced exercise tolerance that is present in the diabetic condition.

Following the PO_2mv response in the GK rat muscle across the rest-exercise transition, both the CON and GK rats showed similar TD_1 (i.e. preceding their decrease in PO_2mv) of the primary responses. This does not imply that $\dot{V}O_2$ or $\dot{Q}O_2$ are stagnant, but rather, that the diabetic condition exerted an effect that was similar in proportion for $\dot{Q}O_2$ and $\dot{V}O_2$, resulting in no change in the PO_2mv. As expected in the healthy spinotrapezius of the Control rats, after the TD_1, PO_2mv fell exponentially to a steady-state during the stimulation on-transient representing an increase in mitochondrial $\dot{V}O_2$ within the myocyte (Behnke et al., 2001, 2002) relative to that of $\dot{Q}O_2$. However, the shape of the CON PO_2mv profiles was not qualitatively similar to that demonstrated for the spinotrapezius of the GK rat. The faster kinetics (expressed by the faster τ) and "undershoot" in PO_2mv in six of the seven GK rats would be consistent with a reduced blood flow response (slower $\tau\dot{Q}O_2$; Behnke et al., 2002). In addition, unlike the CON response, the GK PO_2mv profile showed a secondary TD (i.e., TD_2) that averaged ~46 s followed by the unusual

return of PO_2mv to baseline levels. Furthermore, one of the GK rats exhibited no drop in PO_2mv after the TD_1, that can only be explained by a $\dot{Q}O_2$ response that exceeded that of $\dot{V}O_2$. In summary, the PO_2mv profiles of the GK animals during contractions strongly suggests a severe mismatching of O_2 supply ($\dot{Q}O_2$) to O_2 utilization ($\dot{V}O_2$), that was likely a result of the composite of the vascular and mitochondrial impairments exhibited by Type II diabetic individuals (see below).

Mechanistic Basis for the Observed Responses. As discussed above, the primary cause of the lowered PO_2mv and unusual PO_2mv kinetic profiles in the GK rat is likely to be the lower $\dot{Q}O_2$ across the rest-contractions transition. Indeed, there is supporting evidence for decreased $\dot{Q}O_2$ in human diabetic patients such as decreased leg blood flow during exercise (Kingwell et al., 2003). The attenuation in blood flow may be explained by impaired responses to both endothelium dependent (i.e. acetylcholine) and independent (e.g. sodium nitroprusside) vasodilators in the GK rat (Sandu et al., 2000; Witte et al., 2003) as well as human diabetic patients (McVeigh et al., 1992; Williams et al., 1996). Preliminary evidence from our laboratory (Ferreira et al., unpublished data) also indicates that endothelial dysfunction (i.e. nitric oxide availability) can have profound effects on PO_2mv at rest and during muscle stimulation. The exact cause of this vascular dysfunction in diabetic individuals is unknown, but impaired glucose metabolism has been linked to decreased systemic arterial compliance (Schram et al., 2004), oxidative stress, and free radical production (Guzik et al., 2002).

Diabetic subjects also exhibit decreased mitochondrial volume density and functional changes within skeletal muscle mitochondria (Mathieu-Costello et al., 2003; Kelley et al., 2002; Ritov et al., 2005) which may also be considered as another factor that plays a role in unusual GK PO_2mv responses. In the GK rat, no changes in oxidative capacity have been found to occur in skeletal muscle (Yasuda et al., 2002), but changes in mitochondrial function have been reported in brain (Moreira et al., 2003) and heart tissue (Santos et al., 2003). Nonetheless, to what extent mitochondrial dysfunction played a role in the PO_2mv responses at rest and during exercise in the present investigations remains uncertain.

Model and Experimental Considerations. The GK rat was chosen for this investigation because it has been considered an excellent model for human Type II diabetes and may provide insight

into muscle and exercise dysfunction in this disease. However, there are some important facets of the disease that have been not been evaluated in GK rats as they have in humans. To date, extensive morphological studies of both capillarization and muscle mitochondrial organization and function have not been performed in muscles of the GK model. Changes at those levels may affect the functional capacity for transport, exchange, and utilization of O_2, and may have contributed to the substantially lowered PO_2mv as well as the blunted PO_2mv responses present during muscle contractions in the GK rat, as reported in this current study.

It must also be acknowledged that the results should be interpreted within the context and limitations of the model chosen and experimental design. For example, only one muscle was examined at the onset of muscle contractions, but fiber type and oxidative capacity differences may affect the PO_2mv responses found within other muscles (see Behnke et al., 2003; McDonough et al., 2005). Furthermore, during in vivo voluntary muscular contractions, motor unit recruitment occurs in a heterogeneous fashion (Gollnick et al., 1974). However, when a muscle is electrically stimulated, as in this investigation, activation of all fibers of the motor unit occurs which may result in differences in blood flow between both conditions. In addition, cardiac output and MAP are elevated during voluntary muscle activation (DeCort et al., 1991), which may not occur under the effects of anesthesia and electrical stimulation.

Conclusions. In comparison to healthy rat muscle, we have demonstrated both a lowered driving pressure to move O_2 (i.e. $\downarrow PO_2mv$) across the blood-myocyte interface and profound differences in PO_2mv dynamics during muscle contractions in a model of Type II diabetes (i.e. the GK rat). The attenuated PO_2mv at the transition to a higher metabolic rate provides a mechanistic basis for the slowed $\dot{V}O_2$ kinetics at exercise onset in patients with Type II diabetes (Regensteiner et al., 1998). In addition, perturbations of O_2 exchange at the level of the myocyte will have severe consequences on the regulation of intracellular mitochondrial function either by lowering intramyocyte PO_2 or elevating the O_2 deficit, or both. If treatment modalities such as exercise training do act to improve vascular control and thereby elevate PO_2mv, this may decrease the intracellular perturbation necessary to achieve a given mitochondrial ATP flux. Such a response would be important for reducing PCr depletion and conserving finite glycogen reserves thus improving exercise tolerance and the ability to perform daily activities in the diabetic patient.

Study D: Discussion

To the best of our knowledge, this is the first investigation to examine skeletal muscle capillary hemodynamics within the microcirculation of a Type II diabetic individual. The measurements for CON rats in this current investigation are within ranges reported in previous studies (Kindig et al., 1998, 1999; Russell et al., 2003; Richardson et al., 2003), but the proportion of continuously RBC-perfused capillaries in the GK spinotrapezius at rest was significantly decreased (by ~29%), and was further accompanied by a reduction in V_{RBC} (by ~65%) and F_{RBC} (by ~66%) without any discernable structural changes in the capillary network. Capillary tube hematocrit (i.e. Hct_{cap}) and $\dot{Q}O_2$ were also decreased by 30% and 72%, respectively, in the diabetic muscle which may provide one of the mechanistic bases for the altered O_2 extraction characteristic of skeletal muscle associated with this disease (Baldi et al., 2003). Another potential reason for the attenuated O_2 extraction may be changes involved with distribution of $\dot{Q}$ within and between muscles and peripheral tissues in the Type II diabetic condition, but to the best of our knowledge, this has not yet been determined. Collectively, a redistribution of $\dot{Q}$, vascular dysfunction (McVeigh et al., 1996; Williams et al., 1996; Kingwell et al., 2003), and the decrease in Hct_{tube} (present study) may provide a potential mechanistic basis for the lowered PO_2mv found in the spinotrapezius muscle of the GK rat at rest (Padilla et al., 2004). Furthermore, alteration in capillary blood flow distribution during resting conditions may have significant consequences at the onset of exercise, and this may be one factor involved in performance decrements and slowed $\dot{V}O_2$ kinetics in human patients with Type II diabetes (Regensteiner et al., 1998).

Theoretical basis for hemodynamic alterations. There are several possible underlying causes of the reduction in skeletal muscle RBC flux presented in the GK rats. First, increased muscle stretch (Welsh and Segal, 1996; Poole and Mathieu-Costello, 1997; Poole et al., 1997) and morphological changes caused by a specific disease condition such as a decrease in capillary luminal diameter concomitant with augmented capillary resistance (e.g. Type I diabetes: Kindig et al., 1998) have been previously reported to slow RBC movement through the capillary. However, particular care was taken in this current study to maintain a physiological sarcomere length (~2.7 μm; Poole et al., 1997; Kindig and Poole, 2001) within the spinotrapezius during

experimental preparation of both the CON and GK rat so that stretch-induced alterations *per se* would not occur. Secondly, the ability to increase $\dot{Q}O_2$ within the muscle may become compromised by increased glycosylation of the RBC membrane protein which would, in turn, increase plasma viscosity and increase red blood cell rigidity (i.e. reduced deformability of RBC's) associated with Type II diabetes (Macrury et al., 1992; Chung et al., 1993). This could perhaps limit the ability of RBC's to travel freely through the capillary bed. However, no correlation has been found between increased blood viscosity and exercise performance in Type II diabetic humans (Regensteiner et al., 1995). Furthermore, when examining the capillary network in the GK compared to the Con muscle, neither a decrease in capillary luminal diameter nor obvious blockages within the capillaries were observed within the spinotrapezius of the GK rat.

Since blood flow to the capillary is controlled at the arteriolar level via endothelium-dependent vasodilation (rev. Delp and Laughlin, 1998; Pohl et al., 2000), it is quite plausible that the reduced proportion of RBC-flowing capillaries and slowed capillary hemodynamics may be the result of impaired vascular function. For instance, it has been reported previously that the GK rat model demonstrates increased arteriolar tone caused by impaired endothelium-dependent vasodilation (Bitar et al., 2005), a phenomenon which occurs in human diabetic patients as well (McVeigh et al., 1992; Williams et al., 1996). Also, some (McVeigh et al., 1992; Williams et al., 1996) but not all (Avogaro et al., 1997) investigators have reported a decreased response to endothelium-independent vasodilators (e.g. sodium nitroprusside) in Type II diabetic humans. Lastly, as in humans (Schneider et al., 2002), GK rats demonstrate increased plasma concentrations of the potent vasoconstrictor, endothelin-1 (Balsiger et al., 2002). As a result of endothelial dysfunction, human diabetic patients demonstrate decreased basal forearm (Regensteiner et al., 2003) and leg blood flow at rest and during muscle contractions (Kingwell et al., 2003). Thus, from the available evidence, it is likely that vascular dysfunction in the GK rat is responsible, at least in part, for the decreased V_{RBC} and F_{RBC} (i.e. reduced capillary blood flow) evidenced by the GK rats.

Implications of decreased hemodynamics. Although diffusing capacity for O_2 (DO_2m) within the capillary bed is dependent upon the size of the capillary network (e.g. capillary volume, capillary length, capillary-to-fiber ratio, and capillary surface area per fiber surface area) as well as the

number of RBCs within the capillaries, it is fractional O_2 extraction which indicates the balance between O_2 availability and O_2 utilization. Fractional O_2 extraction is determined by the relationship between DO_2m and blood flow ($\dot{Q}$) such that $\dot{V}O_2 = \dot{Q}O_2 (1\text{-}e^{\text{-}DO2/\beta\dot{Q}})$ and therefore $\dot{V}O_2/\dot{Q}O_2 = O_2$ extraction $= 1\text{-}e^{\text{-}DO2/\beta\dot{Q}}$, where β is the slope of the O_2 dissociation curve in the physiologically relevant range (Wagner et al., 1997). As introduced previously, the effective O_2 diffusing capacity is dependent upon capillary tube hematocrit (i.e. the number of RBC's contained within the capillary) and capillary surface area (Federspiel and Popel, 1986; Groebe and Thews, 1990; Mathieu-Costello et al., 1991). In this current investigation we found that capillary tube hematocrit was significantly decreased in diabetic muscle (along with no change in lineal capillary density) demonstrating that effective DO_2 will be severely compromised (by ~30%) in the GK rat. Furthermore, the lineal density of RBC flowing capillaries, V_{RBC}, F_{RBC}, and $\dot{Q}O_2$ (see Figure 15) were also decreased in the GK diabetic rats. However, according the ratio of $DO_2m/\beta\dot{Q}$, if DO_2 is attenuated by ~30% and $\dot{Q}O_2$ is decreased by ~72%, it would appear that DO_2m is increased in the spinotrapezius muscle of the GK rats. An increase in DO_2 would contradict the lowered PO_2mv reported in the Type II diabetic GK rat during resting conditions (Padilla et al., 2004). However, a likely explanation for the decreased PO_2mv, although not yet determined, may be a redistribution of $\dot{Q}$ among and within muscles of the GK rat.

The consequences of a decreased blood flow (Regensteiner et al., 2003), lowered PO_2mv (Padilla et al., 2004), decreased $\dot{Q}O_2$ (present study), and attenuated O_2 extraction (Baldi et al., 2003) in the diabetic resting muscle may have even greater significance during exercise when capillary RBC transit time (i.e. V_{RBC} and F_{RBC}) become increased. To achieve a given $\dot{V}O_2$ while exercising in a diabetic patient, O_2 flux in the mitochondria would have to be increased either by increasing fractional O_2 extraction or by inducing a fall of intracellular PO_2 to a lower level. However, a reduced intracellular PO_2 will result in increased intracellular phosphate (i.e. increased [ADP]), which will in turn, simulate enhanced utilization of glucose, glycogen degradation, and exacerbation of intracellular acid-base disturbances. These events are even more unfavorable when pathological components of a disease such as Type II diabetes includes mitochondrial dysfunction (Kelley et al., 2002) along with impaired glucose uptake and regulation.

Methodological considerations. In the current investigation, only a small area (270 x 210 μm) of muscle tissue per screen was observed. However, analysis was conducted on five representative areas, which were not significantly different from one another which demonstrates that within a given muscle, analysis was adequate for representing the microcirculatory hemodynamics occurring in both the CON and GK spinotrapezius muscle. Furthermore, there is also the possibility that capillaries without RBCs in the lumen could not be observed by the investigator, but it has been previously demonstrated that very few capillaries (~2%) fall into this category (Damon and Duling, 1984). Moreover, total lineal density did not differ between CON and GK rats, and therefore it is unlikely that the altered flowing lineal capillary lineal density reported in GK rats arose from an inability to visualize individual capillaries.

Under certain circumstances, O_2 diffusion may occur also at the arteriolar level (Kuo and Pittman, 1988), but measurements of arteriolar hemodynamics, density, or luminal diameter were not performed in this investigation. Rather, we chose to examine the hemodynamics occurring within the capillaries of a muscle (as a representation of the whole muscle hemodynamics) because previous research efforts have shown this method to be a valid index of O_2 delivery (Kindig et al., 1999).

Mechanistic Basis for Slowed $\dot{V}O_2$ Kinetics in Diabetes. A healthy young individual demonstrates a very rapid rise (represented by a short time constant (τ ~ 30 s)) in pulmonary $\dot{V}O_2$ kinetics at the onset of submaximal exercise to reach a steady state within ~ 3 min (rev. Jones and Poole, 2005), but in patients with Type II diabetes, the rate of $\dot{V}O_2$ kinetics is significantly slowed (τ ~ 45-70 s; Regensteiner et al., 1998). Although a single rate limiting step for $\dot{V}O_2$ kinetics has not been unequivocally identified, researchers have shown that in a healthy individual, muscle $\dot{Q}O_2$ does not appear to be limiting $\dot{V}O_2$ during upright exercise. Alternatively, when $\dot{Q}O_2$ is reduced by either limiting blood flow or by disease conditions such as chronic heart failure, the rate of the $\dot{V}O_2$ response is slowed (see rev. Jones and Poole, 2005). This is further substantiated by preliminary evidence in which a lowered PO_2mv has been found in the spinotrapezius of the GK rat at the onset of muscle contractions when compared to their healthy counterparts (Padilla et al., 2004). As PO_2mv denotes the ratio of $\dot{Q}O_2$-to-$\dot{V}O_2$, and in lieu of a decreased $\dot{V}O_2$ found in type II diabetic humans at rest (Regensteiner et al., 1998), the lowered PO_2mv may most likely be accounted for by a decrease in $\dot{Q}O_2$ as well as possible $\dot{Q}$

redistribution among and within peripheral tissues and muscles. Furthermore, a sluggish $\dot{V}O_2$ kinetic response at exercise onset also signifies an increased O_2 deficit which is associated with a greater utilization of phosphocreatine stores, elevated free ADP and Pi, and increased reliance on glycolysis (rev. Jones and Poole, 2005).

It is also important to note that the pulmonary $\dot{V}O_2$ kinetic response is also highly representative of the $\dot{V}O_2$ response found at the level of the exercising muscle (Grassi et al., 1996; Bangsbo et al., 2000). With this in mind, we chose to examine a muscle of a Type II diabetic model to determine the possible mechanistic basis for the slowed $\dot{V}O_2$ response, as well as gain insight into the microcirculatory alterations, that occur with type II diabetes. In this investigation, we found that the RBC hemodynamics are altered at rest which may potentially be indicative of unfavorable effects on muscle DO_2 and ultimately O_2 exchange during exercise. Thus the present findings may indicate decrements in O_2 availability occur, both spatially and temporally, within contracting skeletal muscle in the Type II diabetic patient.

Conclusions. Our investigation in the GK model of Type II diabetes demonstrates significant attenuation in the percentage of capillaries supporting RBC perfusion and muscle capillary hemodynamics without marked capillary structural alterations. The reduced density of RBC perfused capillaries as well as impaired capillary RBC hemodynamics (V_{RBC} and F_{RBC}), and Hct_{cap} found during resting conditions in diabetic muscle are likely to compromise the matching of $\dot{Q}O_2$ and $\dot{V}O_2$ during periods of elevated metabolic demand in human patients with Type II diabetes.

Overall Summary

In brief, Studies A & B focused on the highly athletic Thoroughbred horse. Study A found that horses will sustain the full magnitude of the exercise hyperpnea (that occurred during galloping exercise) for several seconds into trotting recovery followed by a prolonged biphasic decrease in $\dot{V}_E$. These results signify that LRC does not appear requisite to achieve prodigiously high $\dot{V}_E$ equivalent to those seen during maximal exercise. Study B showed that ET-1 (via an $ET\text{-}1_A$ antagonist) does not appear to contribute to the high pulmonary vascular pressures or EIPH experienced by the exercising horse. Furthermore, we have also demonstrated that treatment with the $ET\text{-}1_A$ antagonist, TBC3214, is not effective in abolishing or reducing the

severity of EIPH. The latter studies (Studies C & B) demonstrated the consequences of Type II diabetes such that, in comparison to healthy rat muscle, there is a lowered driving pressure to move O_2 (i.e. ↓ PO_2mv) across the blood-myocyte interface and profound differences in PO_2mv dynamics occurred during muscle contractions in a model of Type II diabetes (i.e. the GK rat; Study C). Our other investigation in the GK model of Type II diabetes (Study D) demonstrates significant attenuation in the percentage of capillaries supporting RBC perfusion and muscle capillary RBC hemodynamics. The reduced PO_2mv (Study C) in conjunction with attenuated density of RBC perfused capillaries and impaired capillary RBC hemodynamics, and Hct_{cap} found during resting conditions (Study D) in diabetic muscle are likely to compromise the matching of $\dot{Q}O_2$ and $\dot{V}O_2$ during periods of elevated metabolic demand in human patients with Type II diabetes and provide a mechanistic basis for the slowed $\dot{V}O_2$ kinetics at exercise onset in patients with Type II diabetes (Regensteiner et al., 1998).

Table 1. Model parameters of $\dot{V}_E$, $\dot{V}CO_2$, and $\dot{V}O_2$ responses during the trotting recovery period from maximal exercise.

Variable	$\dot{V}_E$	$\dot{V}CO_2$	$\dot{V}O_2$
End-exercise baseline (L/min)	1411.5 ± 90.1	68.8 ± 6.2	64.2 ± 6.3
Δ_1 (L/min; Primary component Δ)	-301.0 ± 67.4	-48.6 ± 4.3	-46.0 ± 4.3
Δ_2 (L/min; Secondary component Δ)	-172.5 ± 34.8	—	—
End recovery value (L/min)	937.9 ± 125.3	20.2 ± 2.1	18.2 ± 2.1
TD_1 (Time delay 1; s)	6.3 ± 1.8	9.6 ± 1.0	11.1 ± 1.9
TD_2 (Time delay 2; s)	88.5 ± 25.3	—	—
τ_1 (time constant 1; s)	15.0 ± 5.6	30.3 ± 4.2*	17.8 ± 3.1
τ_2 (time constant 2; s)	112.2 ± 15.6	—	—
MRT (Mean response time; s)	85.4 ± 9.0	39.9 ± 4.7†	28.9 ± 3.2†‡

Values are means ± SE (n = 5). Δ_1, primary component; Δ_2, secondary component; TD, time delay; τ, time constant; MRT, mean response time; $\dot{V}_E$, minute ventilation; $\dot{V}CO_2$, CO_2 output; $\dot{V}O_2$, O_2 uptake.

$^*P < 0.05$ between $\dot{V}CO_2$ and $\dot{V}O_2$.

$^\dagger P < 0.05$ between $\dot{V}_E$ and $\dot{V}CO_2$ or $\dot{V}O_2$.

$^\ddagger P < 0.05$ between $\dot{V}O_2$ and $\dot{V}CO_2$.

Table 2. Recovery values at specified times for f_B, V_T, and $\dot{V}_E$.

Time	f_B	V_T	$\dot{V}_E$
End-exercise	113.8 ± 5.2	12.6 ± 1.2	1391 ± 88
~13 s	97.7 ± 5.9*	13.9 ± 1.6*	1330 ± 112
30 s	100.5 ± 6.1	11.9 ± 1.5†	1171 ± 104*†
2 min	108.7 ± 8.1	10.1 ± 1.5†	1061 ± 103*†‡
4 min	110.4 ± 5.6	8.9 ± 1.5*†‡£	963 ± 127*†‡

Values are means ± SE (n = 5). f_B, breathing frequency; V_T, tidal volume, $\dot{V}_E$, minute ventilation.
*$P < 0.05$ from end-exercise.
†$P < 0.05$ from 13 s.
‡$P < 0.05$ from 30 s.
£P < 0.05 from 2 min.

Table 3. Metabolic variables at rest and fatigue for horses administered saline (CON; n = 6) and the endothelin-1_A antagonist, TBC3241 (ANTAG; n = 6).

Variable	Treatment	Rest	Fatigue
Mean arterial pressure (mmHg)	CON	134 ± 28	210 ± 8
	ANTAG	131 ± 11	209 ± 17
Core Temperature (°C)	CON	36.6 ± 0.5	40.6 ± 1.3
	ANTAG	36.3 ± 0.3	40.6 ± 0.7
PaO_2 (mmHg)	CON	91.3 ± 5.0	63.4 ± 6.4
	ANTAG	90.1 ± 7.1	62.9 ± 6.4
$PaCO_2$ (mmHg)	CON	46.4 ± 1.8	63.7 ± 11.7
	ANTAG	46.6 ± 3.9	66.4 ± 11.1
pH	CON	7.486 ± 0.022	7.209 ± 0.079
	ANTAG	7.482 ± 0.020	7.174 ± 0.038
Plasma lactate (mmol/L)	CON	0.8 ± 0.2	20.6 ± 6.4
	ANTAG	0.8 ± 0.3	23.6 ± 4.8
Bicarbonate (mmol/L)	CON	35.6 ± 1.2	22.6 ± 2.3
	ANTAG	35.2 ± 2.2	21.2 ± 1.5
Hematocrit (%)	CON	41 ± 5	62 ± 2
	ANTAG	43 ± 6	63 ± 1

Data are expressed as mean ± SD.
No significant differences ($P > 0.05$) between CON and ANTAG for any variable.

Table 4. Gas exchange, ventilatory, and cardiac variables at fatigue for horses administered saline (CON; n = 6) and the endothelin-1_A antagonist, TBC3241 (ANTAG; n = 6).

Variable	Treatment	Fatigue
$\dot{V}O_{2max}$ (L/min)	CON	76.5 ± 10.4
	ANTAG	73.4 ± 14.3
$\dot{V}CO_{2max}$ (L/min)	CON	93.7 ± 11.7
	ANTAG	90.7 ± 24.2
f_B (breaths/min)	CON	113 ± 5
	ANTAG	115 ± 5
V_T (L)	CON	13.0 ± 2.1
	ANTAG	13.1 ± 2.9
$\dot{V}_E$ (L/min)	CON	1592 ± 200
	ANTAG	1552 ± 345
a-vO_2d (ml O_2/dl)	CON	23.4 ± 2.2
	ANTAG	23.3 ± 3.2
$\dot{Q}$ (L/min)	CON	327.2 ± 30.7
	ANTAG	322.8 ± 87.9

Data are expressed as mean ± SD. $\dot{V}O_{2max}$ = maximal oxygen uptake; $\dot{V}CO_{2max}$ = maximal carbon dioxide output; f_B = breathing frequency; V_T = tidal volume; $\dot{V}_E$ = minute ventilation; a-vO_2d = arteriovenous oxygen difference; $\dot{Q}$ = cardiac output; No significant differences ($P > 0.05$) between CON and ANTAG for any variable.

Table 5. Animal Data.

Variable	CON (n = 5)	GK (n = 7)
Body weight (g)	557 ± 19	417 ± 16*
Heart rate (beats/min)	368 ± 28	436 ± 11*
Mean arterial pressure (Torr)	104 ± 10	119 ± 11
Hematocrit (%)	43 ± 1	42 ± 1
Plasma glucose (mg/dL)	105 ± 5	249 ± 33*

Values are means ± SE; n = number of animals; *P<0.05 compared with CON.

Table 6. Model parameters of microvascular PO_2 (PO_2mv) response in the spinotrapezius muscle of control (CON) and the Goto-Kakizaki Type II diabetic rats (GK) during electrical stimulation (1 Hz; 3-5 V).

Parameter	CON (n = 5)	GK (n = 7)
Baseline PO_2mv (Torr)	28.8 ± 2.0	18.4 ± 1.8*
Δ_1 PO_2mv (primary component Δ, Torr)	-11.2 ± 1.6	-4.5 ± 1.4*
Δ_2 PO_2mv (secondary component Δ, Torr)	NA	5.7 ± 3.5
End-stimulation PO_2mv (Torr)	17.6 ± 0.7†	18.9 ± 2.6
Baseline-End PO_2mv Difference (Torr)	11.2 ± 1.6	-0.4 ± 2.3*
TD_1 (Time delay 1; s)	12.3 ± 2.9	9.1 ± 2.0
TD_2 (Time delay 2; s)	NA	46.1 ± 12.9
τ_1 (time constant 1; s)	15.5 ± 3.5	6.4 ± 1.8*
τ_2 (time constant 2; s)	NA	70.3 ± 32.3
MRT_1 (Mean response time; s)	27.7 ± 6.0	15.5 ± 2.7‡

Values are means ± SE; n, number of animals; MRT_1, mean response time of primary response ($TD_1 + \tau_1$); NA, not-applicable. *$P < 0.05$ compared with CON; †$P < 0.05$ compared with baseline PO_2mv; ‡ $P = 0.08$ compared with CON.

Table 7. Cardiovascular variables during the experimental protocol.

Variable	Control (n = 5)	Diabetic (n = 7)
Heart Rate (bpm)	371 ± 13	437 ± 14*
Mean Arterial Pressure (Torr)	117 ± 6	110 ± 10

Values are mean ± S.E. *P < 0.05

Table 8. Muscle and capillary structural changes in the spinotrapezius of control and diabetic rats.

Variable	Control (n = 5)	Diabetic (n = 7)
Sarcomere length (μm)	2.5 ± 0.1	2.6 ± 0.1
Fiber diameter (μm)	55 ± 3	45 ± 1*
Capillary diameter (μm)	4.7 ± 0.1	5.1 ± 0.2

Values are mean ± SE. *P < 0.05

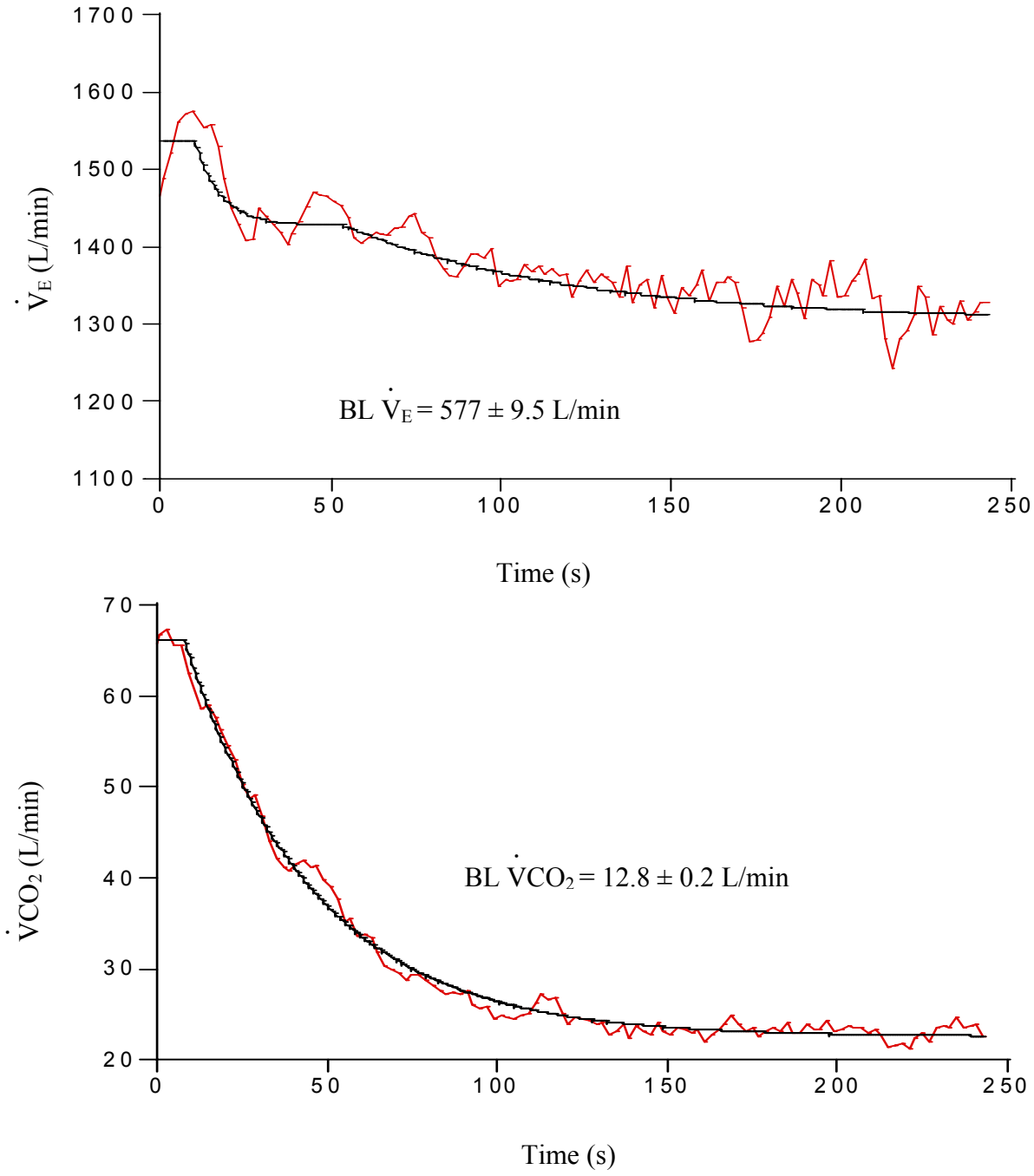

Figure 4. Minute ventilation ($\dot{V}_E$) and CO_2 output ($\dot{V}CO_2$) responses at the gallop-trot transition (~14 m/s to 3 m/s) and throughout trotting recovery (4 min) in a representative horse. Data are fit with a time delay plus 2-component exponential ($\dot{V}_E$, *top*) and 1-component exponential ($\dot{V}CO_2$, bottom) model. Trotting baseline (BL) values are shown for this horse. Note the markedly different temporal responses between $\dot{V}_E$ and $\dot{V}CO_2$.

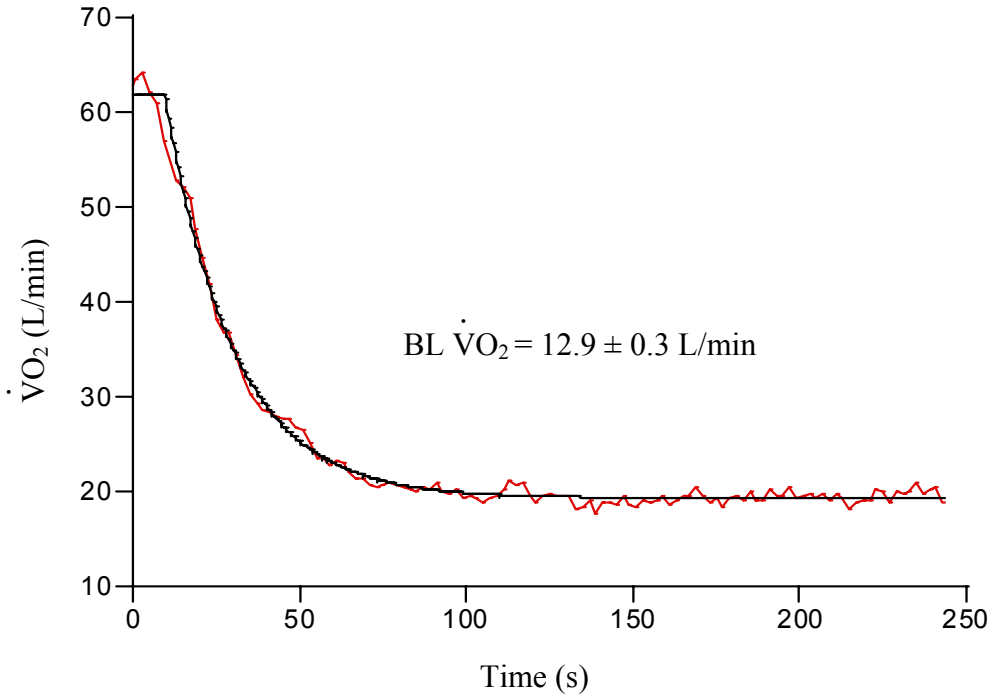

Figure 5. O_2 uptake ($\dot{V}O_2$) response at the gallop-trot transition (~14 m/s to 3 m/s) and throughout trotting recovery (4 min) for the same representative horse as in Figure 4. Data are fit with a time delay plus 1-component exponential model. Trotting baseline (BL) value is shown for this horse.

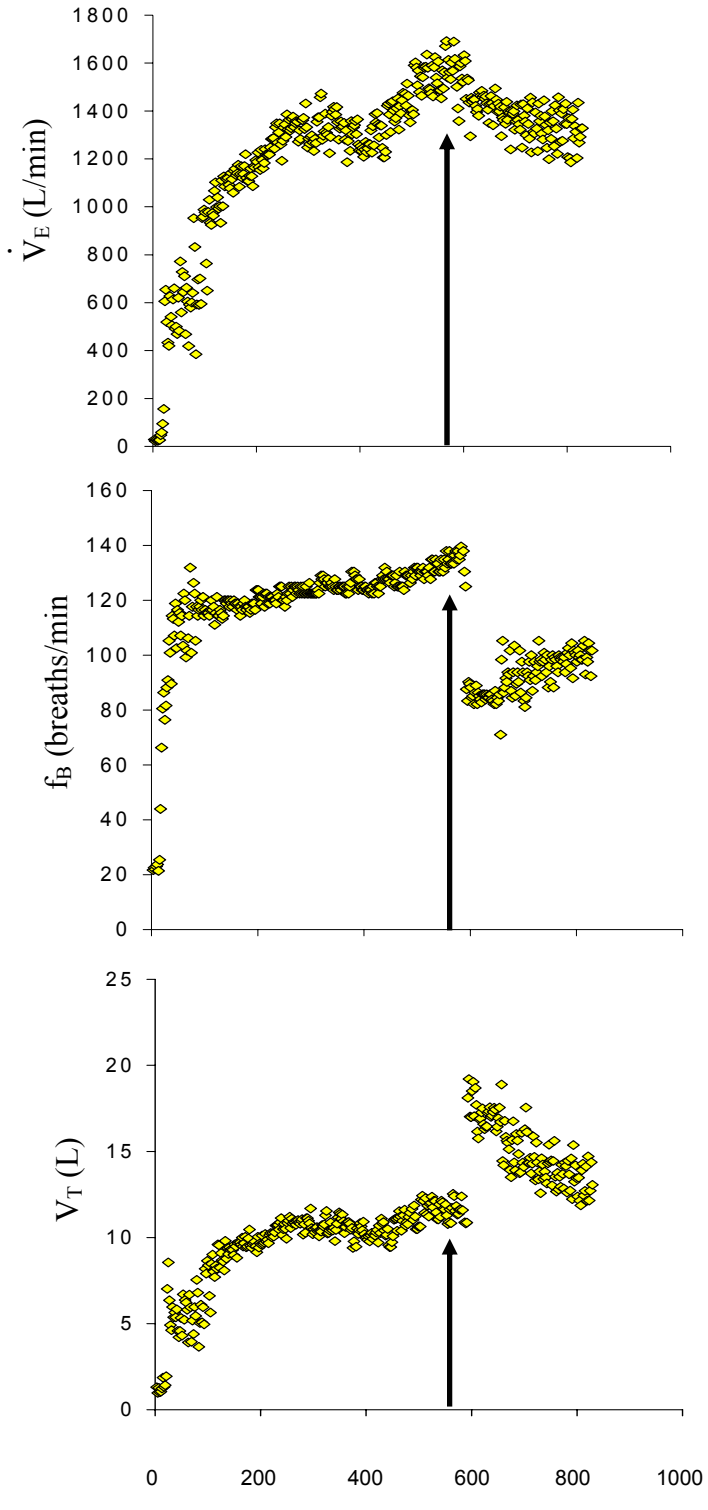

Figure 6. Ventilation ($\dot{V}_E$), breathing frequency (f_B), and tidal volume (V_T) for the same representative horse as in Figures 4 and 5 at rest and during the incremental exercise test and trotting recovery. Arrows denote beginning of trotting recovery period.

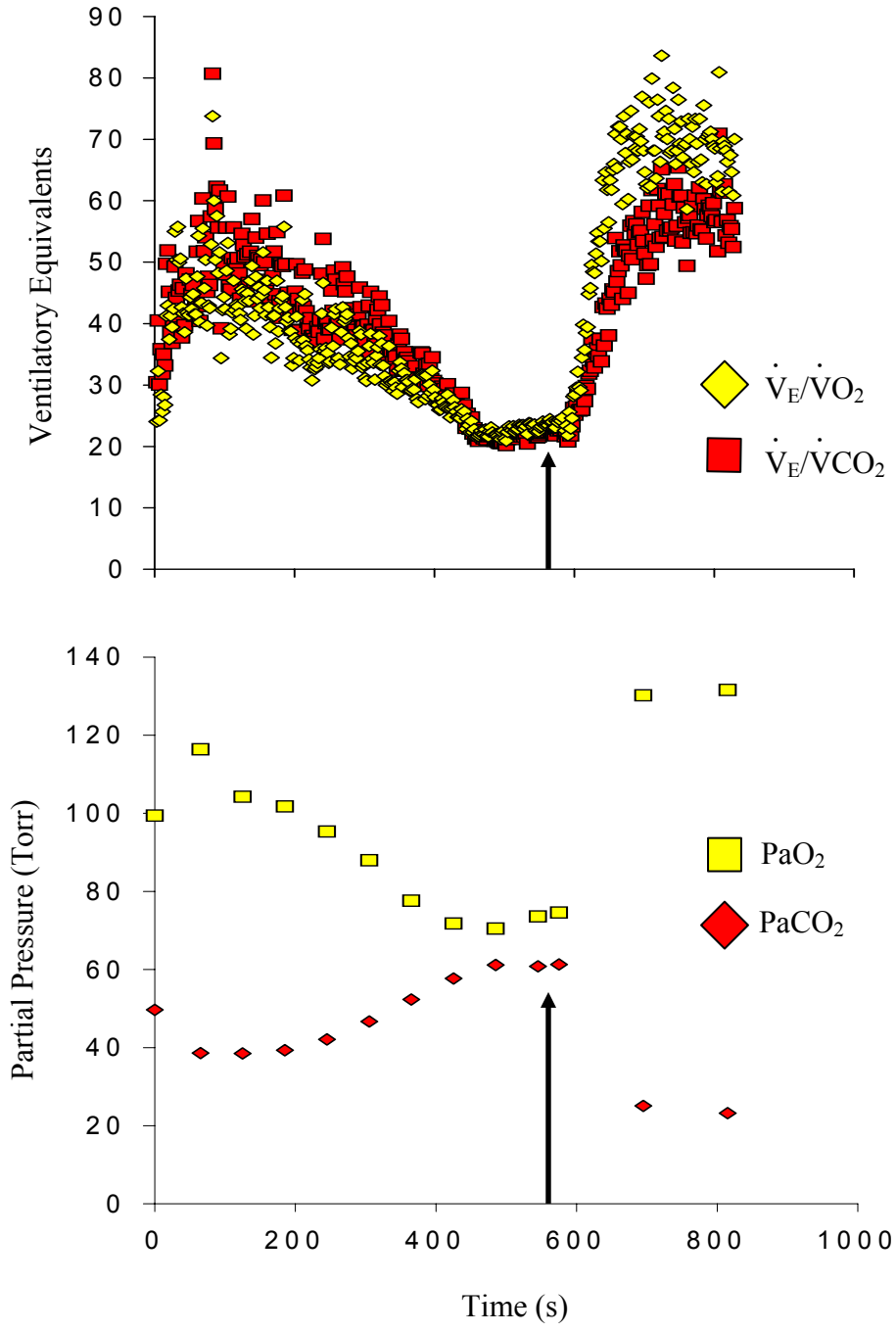

Figure 7. Ventilatory equivalents for $\dot{V}O_2$ ($\dot{V}_E/\dot{V}O_2$) and $\dot{V}CO_2$ ($\dot{V}_E/\dot{V}CO_2$) and arterial PO_2 (PaO_2) and arterial PCO_2 ($PaCO_2$) for the same representative horse as in Figures 4 and 5 at rest and during incremental exercise and trotting recovery. Arrows denote beginning of trotting recovery period.

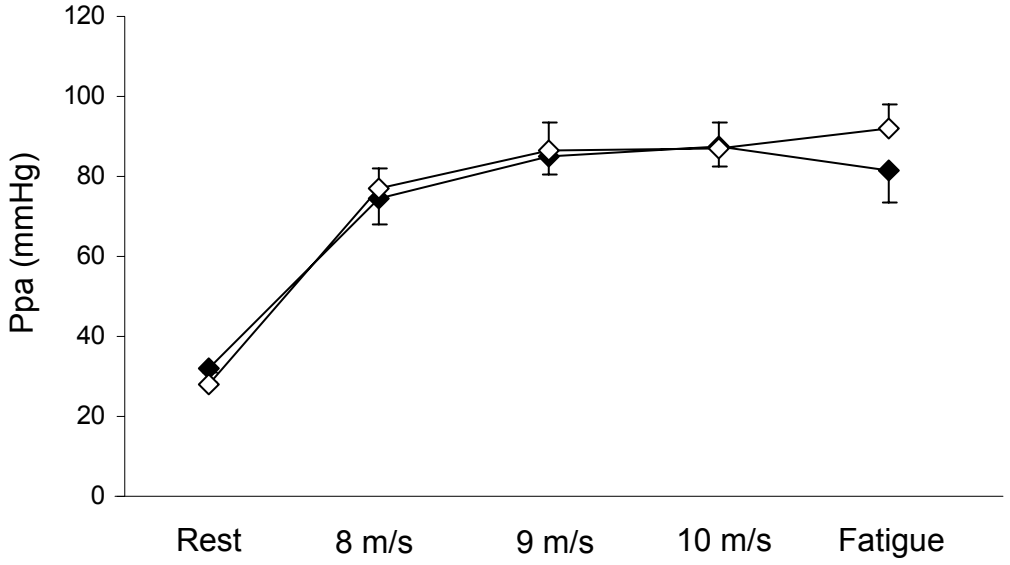

Figure 8. Mean pulmonary artery pressures (Ppa) in horses (n = 6) administered saline (i.e. Control; solid diamonds) or the ET-1_A antagonist, TBC3214 (n = 6; open diamonds), from rest, throughout the last three speeds of the incremental exercise test and at fatigue. $P > 0.05$ for Control vs. Antagonist for all points.

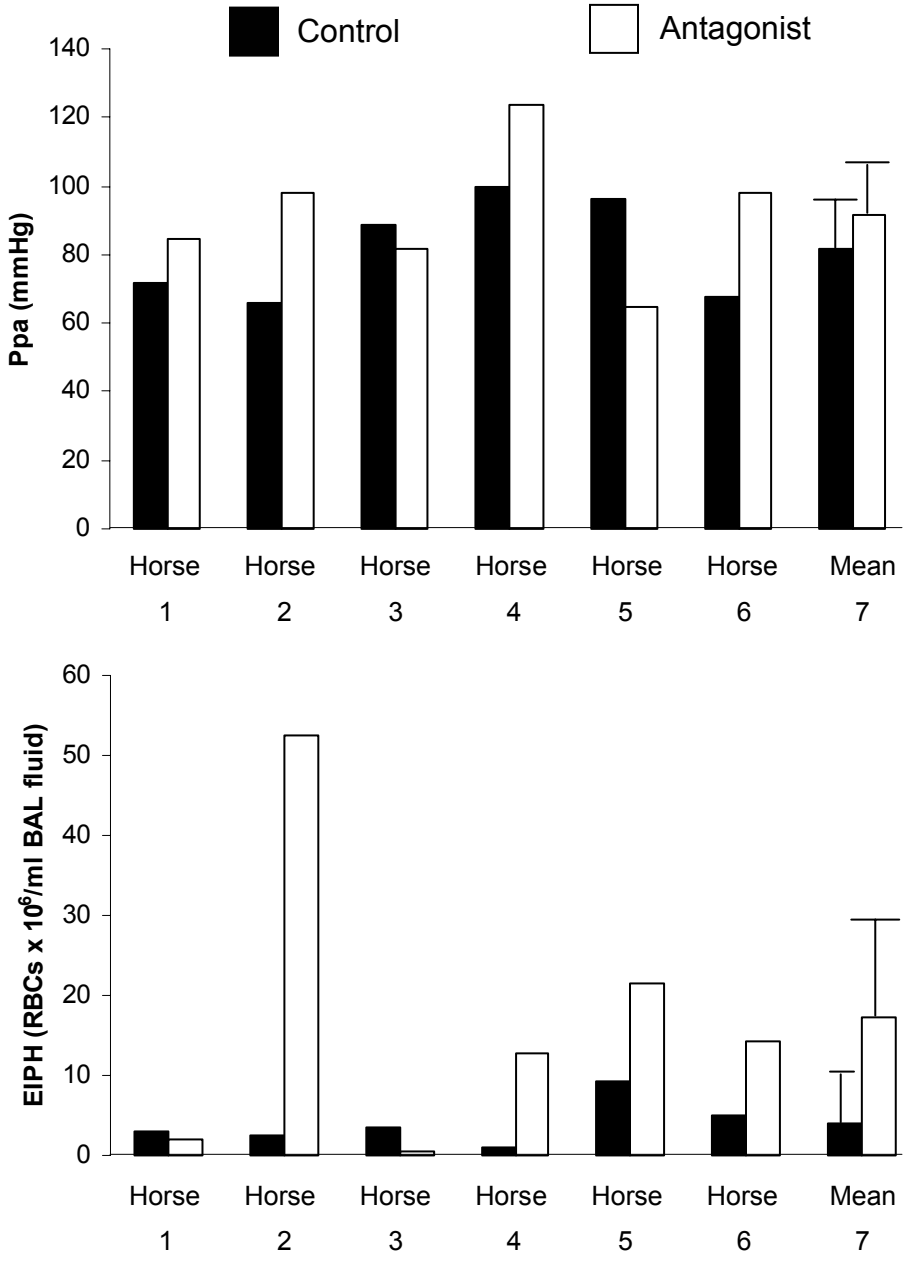

Figure 9. Individual and mean pulmonary artery pressures (Ppa; Top Panel) at fatigue and exercise-induced pulmonary hemorrhage (EIPH; RBCs/ml bronchoalveolar lavage (BAL) fluid (Bottom Panel) in horses administered saline (i.e. Control; n = 6) or the ET-1_A antagonist, TBC3214 (n = 6). $P > 0.05$ for Control vs. Antagonist.

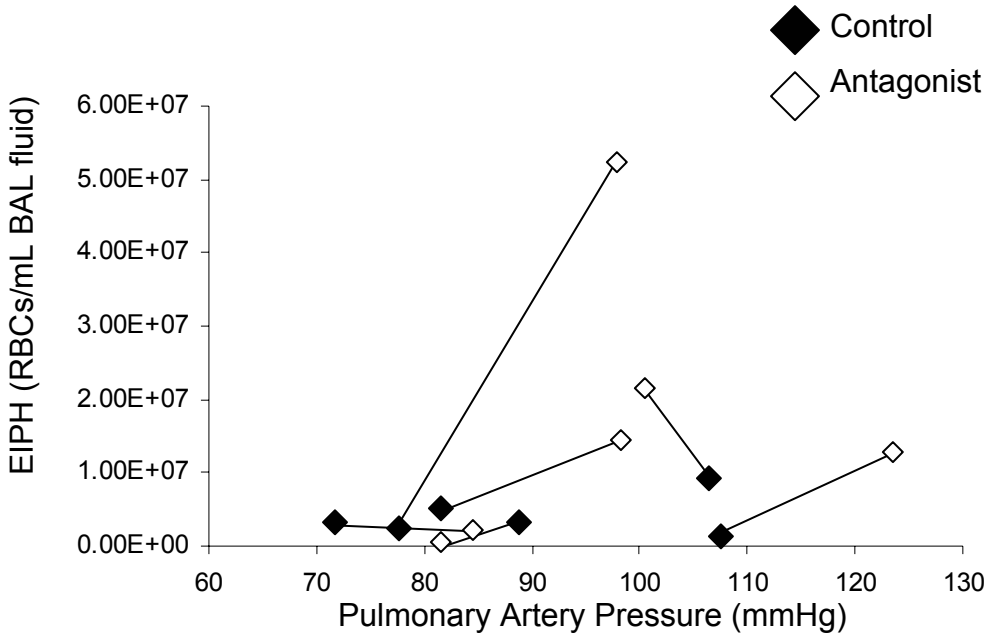

Figure 10. No relationship ($P > 0.05$) was found between EIPH and maximal pulmonary artery pressure in horses administered either saline (i.e. Control; n = 6) or the ET-1_A antagonist, TBC3214 (n = 6). Lines connect control and antagonist treatments between individual horses.

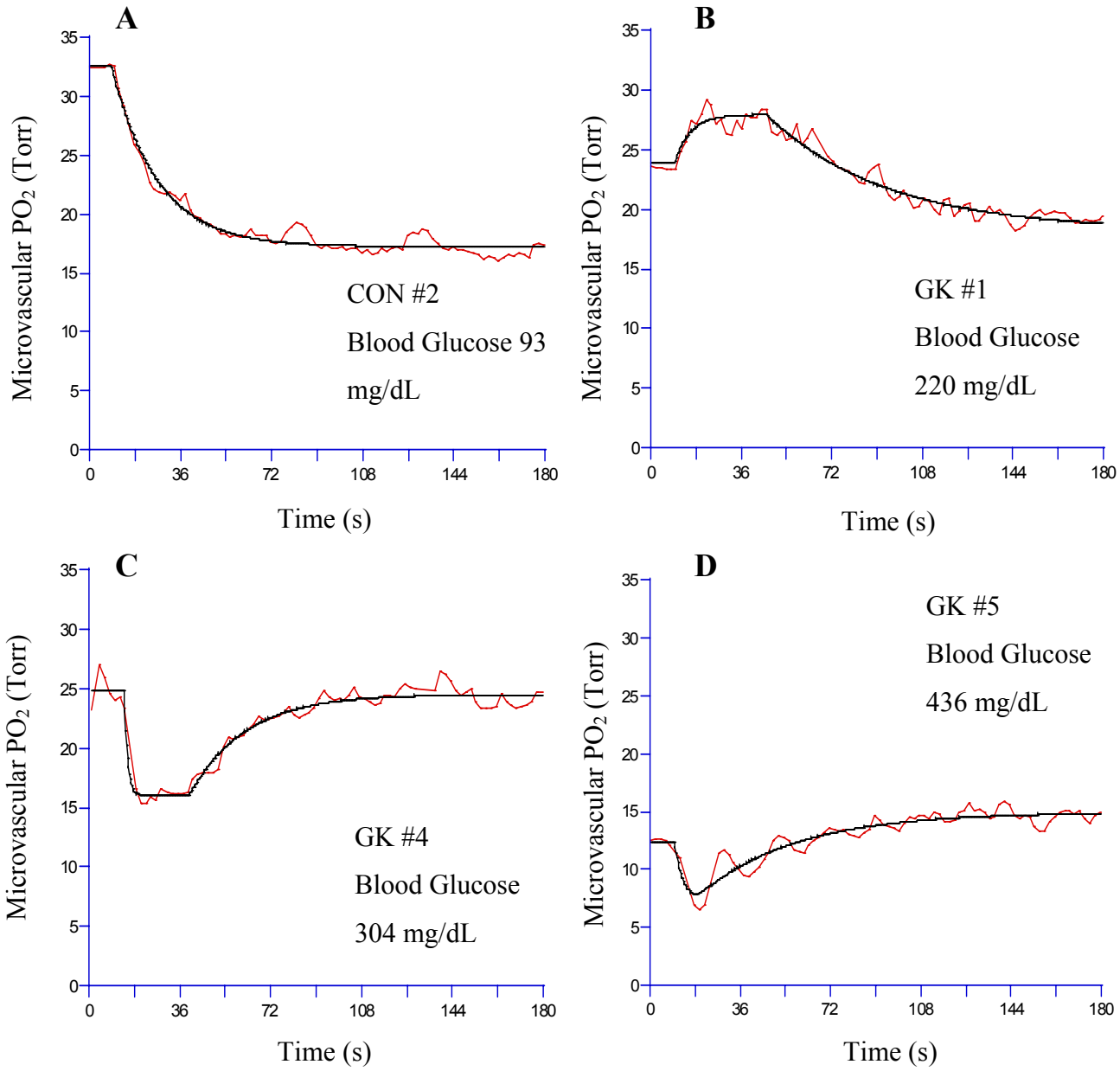

Figure 11. Dynamic microvascular PO_2 (PO_2mv) profiles for the stimulation period in a representative control (CON; Panel A) and for three Goto-Kakizaki (GK) diabetic rats (Panels B,C, & D). Time 0 represents the start of 180 s of electrical stimulation (1 Hz, 3-5 V). Blood glucose concentrations are provided for each rat. Note the variability in the responses among the GK rats. A lower PO_2mv in the GK rats is indicative of a lower ratio of $\dot{Q}O_2/\dot{V}O_2$.

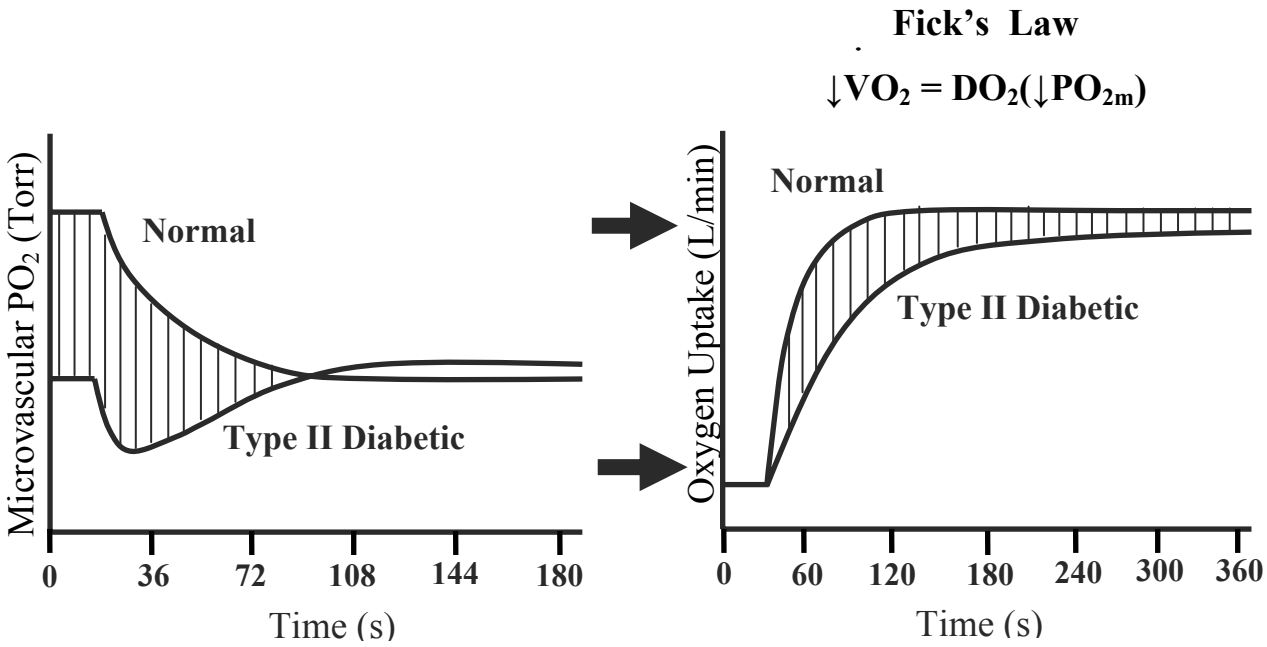

Figure 12. A reduced microvascular PO_2 (PO_2mv), in theory, can account for the slowed $\dot{V}O_2$ kinetics exhibited by Type II diabetic patients.

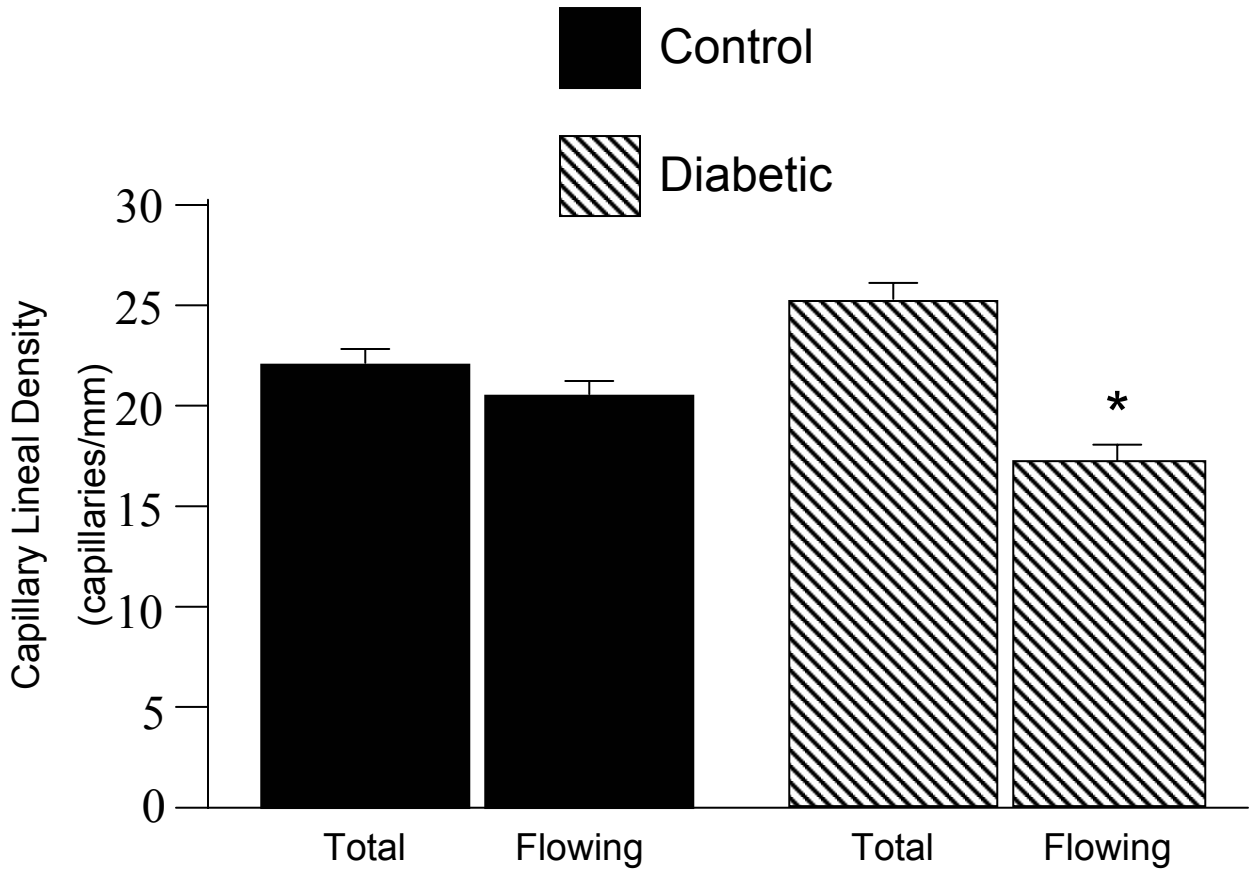

Figure 13. Total (i.e. perfused and non-RBC perfused capillaries) and flowing (only RBC perfused capillaries) capillary lineal density in Control and Diabetic GK rats. Total capillary lineal density did not differ between the two groups of animals, but flowing capillary lineal density was significantly lower in the Diabetic rats ($P < 0.05$).

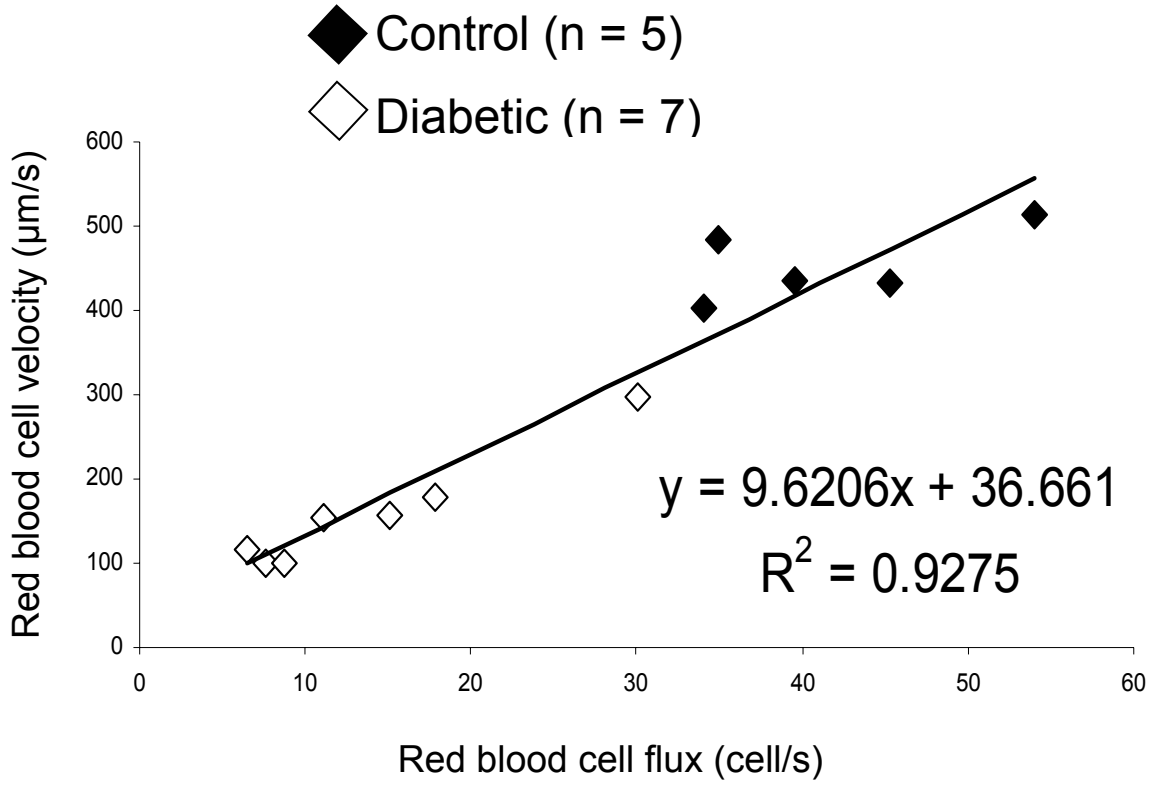

Figure 14. Correlation between RBC velocity and flux in capillaries supporting RBC flow within spinotrapezius muscles of Control and Diabetic rats. The average value for all capillaries within a single animal is represented by each data point.

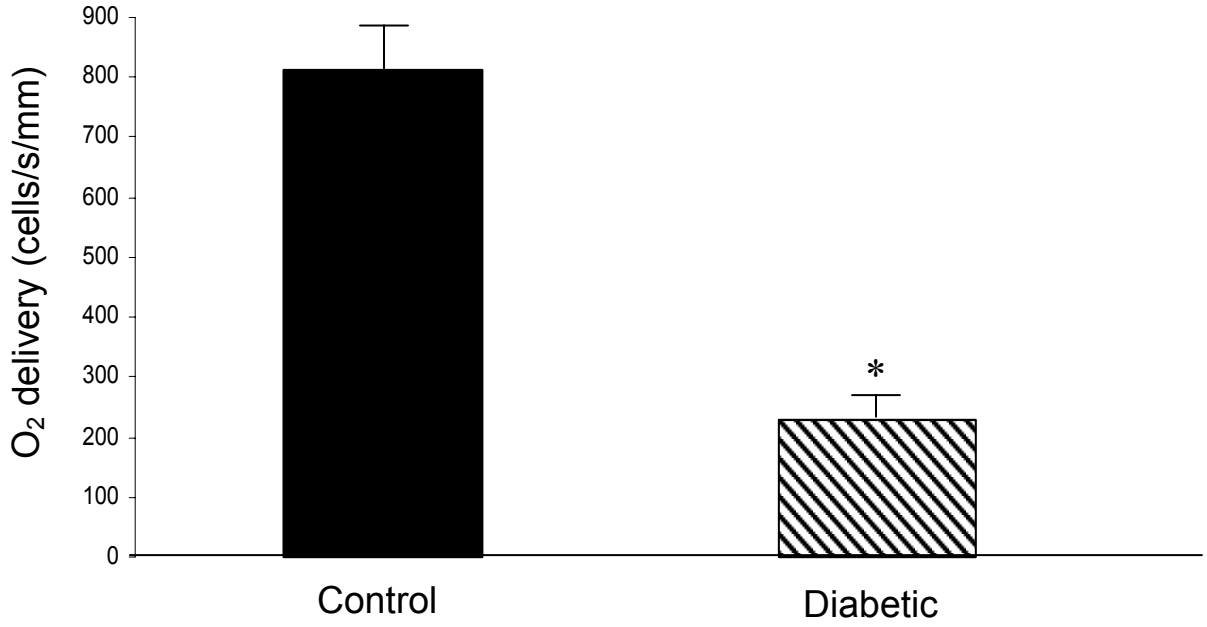

Figure 15. The estimated O_2 delivery (cells/s/mm; as calculated by the product of flowing lineal density and red blood cell flux) was significantly lower in Diabetic animals when compared to Control animals (* $P < 0.05$).

CHAPTER 5

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