A STUDY OF CAPILLARIES IN EQUINE SKELETAL MUSCLE

A thesis presented in partial fulfilment

of the requirements for the degree of

Master of Philosophy

in

Anatomy and Physiology

at

Massey University

LYNN ELIZABETH SLASSOR

1993

ABSTRACT

Four horses of varying ages, breeds and sex were used to evaluate the variation in capillary supply and fibre type proportions in a range of equine skeletal muscles. A total of 100 muscles were sampled from the four horses. These samples were stained for myosin ATPase activity from which fibres were classified as ATPase low or ATPase high. Visualisation of capillaries was also achieved by the use of this stain. The 100 muscle samples were assessed for capillary/fibre ratio and for capillary density. From these muscles, 22 were chosen for more detailed analysis on the number of capillaries surrounding each fibre and the fibre area for ATPase low and ATPase high fibres individually.

Fibre type distribution was found to vary from 0% to 100% ATPase low fibres and therefore a complete range of muscle fibre type proportions were obtained. An extensive range was found within a muscle or between adjacent muscles.

There was no significant difference in mean fibre area between the two fibre types in the muscles examined.

The percentage of ATPase low fibres and capillary/fibre ratio showed a significant regression (regression coefficient = 0.36, $p < 0.05$)

For both the ATPase low fibres and the ATPase high fibres, larger fibres were surrounded by more capillaries.

It was concluded that, although fibre size is the primary determinant for capillary supply, the contractile properties of the muscle in which a fibre is found will also determine the number of capillaries it should have at its disposal.

ACKNOWLEDGEMENTS

My sincere thanks go to my supervisor Dr. A.S. Davies for his constant support and guidance throughout this study and for his careful scrutiny during the preparation of this thesis. I would also like to thank my co-supervisor Dr. R.W. Purchas for providing any advice required throughout this study.

Many thanks are extended to Mrs P.M. Davey and Mrs P.M. Slack for their help with histological preparations.

I would like to thank Mr R.I. Sparksman for his assistance with photography and the analysis of data.

A special thanks is extended to my family for their encouragement and support throughout the study.

TABLE OF CONTENTS

	PAGE
ABSTRACT	2
ACKNOWLEDGEMENTS	4
TABLE OF CONTENTS	5
LIST OF TABLES	9
LIST OF FIGURES	11
LIST OF APPENDICES	14
CHAPTER ONE : INTRODUCTION	15
CHAPTER TWO : REVIEW OF LITERATURE	18
2-1 Fuel Utilisation	18
2-1-1 Energy Supply	18
2-1-2 Factors Regulating Fuel Utilisation	19
2-1-2-1 Muscle Fibre Types	20
2-1-2-2 Intensity and Duration of Exercise	21
2-1-2-3 Physical Training	23
2-2 Capillaries	24
2-2-1 Capillary Structure	24
2-2-2 Measurement of the Spatial Distribution of Capillaries	31
2-2-3 Identification of Capillaries	34
2-2-4 Arrangement of Capillaries and Fibres in Transverse Sections of Muscle	36

2-2-5 Capillary Permeability 38

2-2-5-1 Models for Capillary Permeability 38

2-2-5-2 Oxygen Flow From Blood to Cells 41

2-2-6 Capillary Supply of Skeletal Muscle 50

2-2-6-1 Capillary/Fibre Ratio 50

2-2-6-2 Capillary Density 54

2-2-6-3 Number of Capillaries Surrounding Each Fibre 57

2-2-6-4 Relationship Between Oxidative Capacity and Capillary Supply 59

2-3 Adaptation to Exercise in Horses 62

2-3-1 Fibre Type Profiles 62

2-3-2 Fibre Size 68

2-3-3 Enzyme Levels 70

2-4 Objectives of this study 73

CHAPTER THREE : MATERIALS AND METHODS 74

3-1 Sampling 74

3-2 Method of Freezing and Storing 74

3-3 Cutting of Sections 74

3-4 Staining of Sections 75

3-5 Classification of Fibre Types 75

3-6 Measurement of Capillary Supply 75

3-7 Selection of Samples for Detailed Analysis 77

3-8 Statistical Analysis 77

CHAPTER FOUR : RESULTS 79

4-1 Identification of Capillaries 79

4-2 Selected muscles 79

4-3 Fibre Type distribution 79

4-4 Mean Fibre Area 80

4-5 Capillary/Fibre Ratio 80

4-6 Capillary Density 80

4-7 Number of Capillaries Surrounding Each Fibre 81

4-8 Relationship Between % ATPase low Fibres and Capillary/Fibre Ratio 81

4-9 Relationship Between Number of Capillaries Surrounding Each Fibre and Fibre Area 81

CHAPTER FIVE : DISCUSSION 94

5-1 Methods for the Identification of Capillaries 94

5-2 Fibre Type Distribution 95

5-3 Mean Fibre Area 99

5-4 Capillary Supply 100

5-4-1 Capillary/Fibre Ratio 102

5-4-1-1 Relationship Between %ATPase Low Fibres and Capillary/Fibre Ratio 105

5-4-2 Capillary Density 107

5-4-3 Number of Capillaries Surrounding Each Fibre 108

CHAPTER SIX : CONCLUSIONS 112

APPENDICES 114

REFERENCES 117

LIST OF TABLES

Table **Page**

2-1-1 Total energy stores available in the horse (From M^{c}Miken, 1983) 19

2-1-2 Summary of the muscle fibre type classification systems shown as they correspond to the classification system described by Peter et al (1972) 20

2-1-3 Contribution of glucose, glycogen and fatty acids to oxygen consumption of leg muscle of man (From Goodman, 1986) 23

2-2-1 Reported values for some capillary densities in the guinea-pig, cat and mouse. (Modified from Plyley and Groom, 1975) 32

2-2-2 Reported values for capillary/fibre ratios in striated muscle of the rabbit, rat, mouse, guinea pig, cat and dog. (Modified from Plyley and Groom, 1975) 52

4-1 % ATPase low fibres, capillary/fibre ratios (C/F) and capillary densities (CD) of the muscles from Horse 1 82

4-2 % ATPase low fibres, capillary/fibre ratios (C/F) and capillary densities (CD) of the muscles from Horse 2 83

4-3 % ATPase low fibres, capillary/fibre ratios (C/F) and capillary densities (CD) of the muscles from Horse 3 84

4-4 % ATPase low fibres, capillary/fibre ratios (C/F) and capillary densities (CD) of the muscles from Horse 4 85

4-5 % ATPase low fibres, capillary/fibre ratios (C/F) and capillary densities (CD) of 22 muscles from 4 horses 86

4-6 Mean fibre areas for ATPase low (AL) and ATPase high (AH) fibres in 22 muscles from 4 horses 87

4-7 Mean number of capillaries surrounding each ATPase low (AL) and ATPase high (AH) Fibre in 22 muscles from 4 horses 88

4-8 Regression coefficients for the relationship between the number of capillaries surrounding each fibre (CAF) and fibre area (FA) 89

LIST OF FIGURES

Figure	Page
2-2-1 Traditional concept of the structure of a capillary (From Mayerson, 1962)	25
2-2-2 An electron micrograph showing the current concept of a capillary (From Weibel, 1984)	26
2-2-3 Cell junctions in the endothelium of rat tongue capillaries (From Bruns and Palade, 1968a).	27
2-2-4 More complex intercellular junction in the endothelium of a rat capillary (From Bruns and Palade, 1968a).	27
2-2-5 Pericyte in a capillary of rat myocardium (From Bruns and Palade, 1968a)	30
2-2-6 Possible models for the description of capillary:fibre geometry in striated muscle (From Plyley and Groom, 1975)	37
2-2-7 Relationship between capillary:fibre ratio and mean number of capillaries surrounding a fibre (From Plyley and Groom, 1975)	38
2-2-8 Oxygen flow from the capillary to the muscle cell (From Weibel, 1984)	41

2-2-9 Simple model of a slab-like cell supplied by a capillary to show the profile of PO_2 as a function of distance into the cell (From Weibel, 1984) 43

2-2-10 Model for oxygen delivery into the Krogh cylinder and a plot of the relationship between PO_2 and oxygen saturation (From Weibel, 1984) 45

2-2-11 Schematic diagram of the macro model of *The Krogh Tissue Cylinder* (From Quistorff et al, 1977) 47

2-2-12 A hexagonal fibre is completely supplied by 6 capillaries. Each capillary participates in the supply of three adjacent fibres. The equilateral triangle (fine stippled area) is divided by bisectors into 6 triangular micro-units (coarse stippled area). (From Akmal et al, 1977) 49

3-1 An example of the type of illustration used to calculate the parameters of capillary / fibre ratio, capillary density and number of capillaries surrounding each fibre 76

4-1 Sections of horse muscle stained for myosin ATPase activity at pH 7.2 90

4-2 Graph of the relationship between capillary density (capillaries/mm^2) and mean fibre area for the 22 selected muscles 92

4-3 Graph of the relationship between the percentage of ATPase low fibres and capillary/fibre ratio for 100 muscles from 4 horses 93

LIST OF APPENDICES

Appendix		Page
One	Description of Horses	114
Two	Muscles Sampled From Each Horse	115
Three	Myosin ATPase Staining Technique	116

CHAPTER ONE : INTRODUCTION

This introduction represents an uncritical discussion of the role of exercise physiology in the horse. It outlines the areas which are to be covered in this thesis and the reasons why these areas are relevant to the study of the physiology of equine athletes.

The effect of exercise on the structural and metabolic characteristics of skeletal muscle has become a subject of interest. Studies on a range of species including humans have shown that there are a number of physiological changes associated with the improvement of performance as the result of training.

Horses compete in a wide range of disciplines from endurance rides through to races over short distances as well as performing the more precise movements required for disciplines such as jumping and dressage. The way in which energy is produced for these activities differs depending on the intensity and duration of the exercise. The changes which occur in response to training also differ depending upon the type of exercise that is performed and therefore the training program an individual undertakes is of vital importance. Exercise of submaximal intensity that is maintained over a relatively long period is fuelled by aerobic respiration during which ATP is formed from the breakdown of glycogen in the presence of oxygen. Event horses use aerobic metabolism during the lengthy 'roads and tracks' section of the cross-country phase during a competition while it is also the major form of energy production for endurance horses. In contrast, during sprinting and jumping which involve short bursts of maximal power, the muscles require energy much more rapidly than the aerobic pathway can manage. In this case the individual must utilise anaerobic respiration which also involves the breakdown of glycogen to ATP but this breakdown is not complete and results in the production of lactate which builds up and finally results in fatigue. Training programs which involve a large amount of endurance type work will result in an increase in the individuals aerobic capacity while those involving sprinting over short distances will increase the horses anaerobic capabilities. The design of a training program must therefore take into account the needs of each individual horse.

The rate of diffusion of oxygen into working muscles is one factor that affects performance. An increase in oxygen diffusion is facilitated by an increase in capillary supply and it has been shown that an increase of aerobic capacity in an individual is associated with an increase in capillary supply during training. Capillary supply differs between muscles depending on the function of the muscle. For example, muscles which contract slowly and constantly such as the heart and diaphragm require large amounts of oxygen and therefore have a more extensive capillary supply than those muscles which contract very quickly but only occasionally and therefore rely mostly on anaerobic metabolism and consequently do not have a large requirement for oxygen.

In this study the variation in capillary supply from muscle to muscle in the horse is investigated in order to obtain a range of muscles varying from those with an extremely low capillary supply to those which have a high capillary density.

Not all muscles within an animal are the same colour, some being redder and others whiter with many variations between these two extremes. Through the use of histochemical methods, it has been shown that most mammalian skeletal muscle consists of a mosaic of fibres with distinct variations in metabolic and functional characteristics. Fibres may be differentiated into two distinct types on the basis of contractility through staining for myosin adenosine triphosphatase (ATPase) activity. ATPase low or 'slow twitch' fibres have relatively slower contraction and relaxation times and are considerably more fatigue resistant than ATPase high or 'fast twitch' fibres.

The colour of various muscles has been related to capillary supply with the general belief being that the red (slow contracting) muscles are better supplied with blood vessels than the white (fast contracting) muscles. Studies have also extended into the capillary supply of individual fibres and the difference in supply between ATPase low and ATPase high fibres. It would be expected that because ATPase low fibres are slower contracting and have a high oxidative (aerobic) capacity they would require a greater oxygen supply and would therefore be surrounded by more capillaries than the ATPase high fibres which have more anaerobic capabilites and require less oxygen.

In the following chapter the literature available on capillary supply to various skeletal muscles in all species will be reviewed. The objectives of the present study will be given at the conclusion of this chapter.