

**SELECTED EXERCISE AND SKELETAL MUSCLE CHARACTERISTICS
OF AFRICAN DISTANCE RUNNERS**

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Thesis presented for the degree of

DOCTOR OF PHILOSOPHY

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*To Africa ...
... and all her people*

DECLARATION

I, Adele Robyn Weston, hereby declare that the work contained in this thesis is my own in conception, design and execution except where otherwise acknowledged. Furthermore, this work is original and has not been previously presented for a higher degree at this institution or elsewhere.

Signed

Signed by candidate

Date 6 September 1996

Place Durban

BRIEF ABSTRACT

Selected exercise and skeletal muscle characteristics of African distance runners.

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African runners dominate distance running both in South Africa and internationally. Therefore, the aim of this thesis was to compare selected exercise and skeletal muscle characteristics in well-trained African and Caucasian 10 km runners to determine if evidence exists of differences between these groups with respect to these physiological and biochemical characteristics. Furthermore, the relationship between exercise and skeletal muscle characteristics was investigated. Sedentary individuals from each population group were also studied to determine if differences existed in untrained skeletal muscle between groups.

Maximal oxygen consumption and peak treadmill speed were measured using an incremental treadmill protocol whilst submaximal exercise characteristics were measured during a specifically designed protocol consisting of four sequential submaximal workloads relative to the peak treadmill speed of the individual. The final workload was maintained until fatigue with resistance to fatigue defined as total test time. Running economy was measured at a treadmill speed of 16.1 km/hr. Race pace characteristics were measured directly at race pace. Characteristics measured during exercise tests were oxygen uptake, minute ventilation, respiratory exchange ratio and heart rate whilst plasma lactate concentration was determined immediately after exercise. Skeletal muscle characteristics were determined by needle biopsy of the vastus lateralis muscle. Skeletal muscle enzymes citrate synthase, phosphofructokinase, 3-hydroxyacyl CoA dehydrogenase, hexokinase and carnitine palmitoyl transferase were assayed spectrophotometrically. Skeletal muscle buffering capacity was measured using titration and fibre type proportions were analysed histochemically. Comparisons between groups were made with the Student's t-test for unpaired data whilst the relationships between variables were analysed using the Pearson's correlation coefficient.

The first major finding was that when exercising at the same relative percentage of individual maximal treadmill velocity, African distance runners were able to exercise for longer than the Caucasians (1376 ± 227 vs 1137 ± 126 sec, $p < 0.01$) with lower plasma lactate accumulation (4.8 ± 3.2 vs 7.7 ± 2.8 mmol/l, $p < 0.05$). Time to fatigue was significantly related to a lower plasma lactate concentration ($r = -0.63$) and a lower respiratory exchange ratio ($r = -0.53$). The second major finding indicated that African runners were able to race 10 km at a higher percentage of their maximal oxygen uptake (93.5 vs 86.0%, $p < 0.005$), whilst eliciting only a comparable plasma lactate concentration and respiratory exchange ratio. The third main finding was that the African runners were more economical than the Caucasian runners ($p < 0.05$).

The fourth main finding is that the African runners had a 50% greater activity of citrate synthase ($p < 0.005$) and 3-hydroxyacyl CoA dehydrogenase ($p < 0.01$) in the vastus lateralis than the Caucasians and this could not be explained by fibre type proportions, because the proportion of type I fibres was lower in the African runners ($p < 0.05$). Citrate synthase activity, was related to the runners' ability to resist fatigue at high intensity relative to their individual peak treadmill velocity ($r = 0.70$, $p < 0.05$). A higher CS activity was related to a lower plasma lactate concentration and a lower RER. The sixth main finding of this thesis was that skeletal muscle buffering capacity of the Caucasian runners was higher than that of the African runners ($p < 0.05$). A methodological study of buffering capacity in rats showed the buffering capacity was largely dependent upon fibre type and protein concentration, however these parameters could not explain the difference observed between the African and Caucasian runners. Furthermore, despite the differences in skeletal muscle characteristics observed between African and Caucasian runners in the current thesis, there was no evidence of these differences being inherently present in sedentary African and Caucasian individuals.

In conclusion, the current series of studies do provide evidence of differences in selected exercise and skeletal muscle characteristics between African and Caucasian distance runners, with the African runners possessing exercise and skeletal muscle profiles that are considered to be more advantageous for endurance performance.

ABBREVIATIONS

3-HAD	3-hydroxyacyl CoA dehydrogenase	AcCoA	acetyl Co-enzyme A
ADP	adenosine diphosphate	AMP	adenosine monophosphate
ATP	adenosine triphosphate	BCAA	branch chain amino acids
βm	skeletal muscle buffering capacity	βm_{titr}	βm by titration
$\beta m_{\Delta La/\Delta pH}$	βm by ratio of ΔLa to ΔpH	BSA	bovine serum albumin
CK	creatine kinase	CS	citrate synthase
CSA	cross-sectional area	cv	coefficient of variation
dH_2O	distilled water	DNA	deoxyribonucleic acid
DTNB	5,5' dithiobis 2-nitrobenzoic acid	dw	dry weight
EDTA	ethylenediaminetetraacetic acid	F-6-P	fructose-6-phosphate
GAPDH	glyceraldehyde phosphate dehydrogenase	HK	hexokinase
HR	heart rate	IMP	inosine monophosphate
La	lactate	La_{Ra}	rate of lactate appearance
La_{Rd}	rate of lactate disappearance	LDH	lactate dehydrogenase
MDH	malate dehydrogenase	ME	mercaptoethanol
min	minutes	MVC	maximal voluntary contraction
NH_3	ammonia	NAD	oxidised nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide	OBLA	onset of blood lactate accumulation
OAA	oxaloacetate	OPLA	onset of plasma lactate accumulation
OGDH	oxoglutarate dehydrogenase	PCr	phosphocreatine
P-NMR	nuclear magnetic resonance (^{31}P)	pH_i	initial pH of muscle homogenate
PFK	phosphofructokinase	P_iO_2	inspired partial pressure of oxygen
P_i	inorganic phosphate	PO_2	partial pressure of oxygen
PNC	purine nucleotide cycle	RER	respiratory exchange ratio
PTV	peak treadmill velocity	SDH	succinate dehydrogenase
RFLP	restriction fragment length polymorphism	SR	sacroplasmic reticulum
sec	seconds	TTF	time to fatigue
TEA	triethylamine	TRIS	tris(hydroxymethyl)aminomethane
TPI	triosephosphate isomerase	V_E	minute ventilation (expired)
UCT	University of Cape Town	$vVO_2\text{max}$	velocity @ $VO_2\text{max}$
VE/VO_2	ventilatory equivalent	ww	wet weight
$VO_2\text{max}$	maximal oxygen consumption		

CHAPTER ONE

INTRODUCTION

The endurance performance phenotype is characterised by a large degree of inter-individual variation. Previous research has determined that the endurance performance phenotype is at least partially dependent upon an individual's genotype although the magnitude of this dependence is less clear (Bouchard & Lortie, 1984; Bouchard et al, 1993). Research is continuing to further elucidate the degree to which genes determine endurance performance. It is currently believed that the magnitude is of the order of 25% for VO_2 max, but may be substantially higher for a submaximal endurance test (Bouchard et al, 1993). Acceptance of a genetic component to the endurance performance phenotype raises the possibility that racial differences in endurance capacity may exist (Boulay et al, 1988) although the evidence is far from conclusive.

With regard to the different African populations, there exists considerable anecdotal evidence suggesting an advantageous endowment for *sprint/power* performance in individuals of West African origin, and an advantageous endowment for *endurance* performance in individuals of East African origin. However, conclusive scientific documentation of physiological advantage in these different populations is scarce:

Athletes of West African origin, including North American Africans, dominate Olympic and World Championship athletic sprint events. Exercise capacity and skeletal muscle characteristics of West African sedentary individuals have been investigated by Ama and colleagues (Ama et al, 1986; Ama et al, 1990), who concluded that West Africans are "in terms of skeletal muscle characteristics, well endowed for sport events of short duration". Specifically, these untrained West Africans had a higher ratio of skeletal muscle glycolytic to oxidative enzyme activity and a higher percentage of type II skeletal muscle fibres than their untrained Caucasian (Canadian) counterparts. In addition, exercise tests indicated a higher peak power generation but a more rapid decay in power generation (fatiguability) in the West Africans. That these racial differences were apparent in this group of untrained individuals who had very similar lifestyles, suggests genetic rather than environmental causes and certainly warrants further investigation.

In contrast, runners of East African origin now dominate international mens distance running. At the 1996 World Cross Country Championships, this superiority was profound, with African runners claiming 14 out of the first 15 places in the senior men's event. African runners currently hold the World Records at 5 km, 10 km, 21.1 km, and at the 42.2 km marathon distance (as at July 1996). There is an equivalent dominance of distance events by Africans

within South Africa despite the considerably smaller number of African participants compared to Caucasians (Coetzer et al, 1993). Recent success of South African distance runners in the international arena are also apparent.

To date there are few studies to elucidate the physiological characteristics that may contribute to the apparently superior endurance capacity of runners of East (or South) African origin (Bosch et al, 1990; Coetzer et al, 1993; Saltin et al, 1995a; Saltin et al, 1995b). These studies have shown higher fractional utilisation of VO_2max during a simulated treadmill marathon (Bosch et al, 1990), better running economy (Saltin et al, 1995b) and greater fatigue resistance (Coetzer et al, 1993) in the African runners. However, all of these studies have had certain methodological limitations that are difficult to avoid in racial comparisons.

Furthermore, only one study has investigated the biochemical characteristics of skeletal muscle in these runners (Saltin et al, 1995a). The results indicate a higher level of the β -oxidation enzyme, 3-HAD, and a lower skeletal muscle buffering capacity in Kenyan runners when compared to Scandinavians of a similarly high standard. A potentially confounding factor, was the habitual residence of the Africans at altitude, compared to the acute acclimatisation of the low-land Scandinavians to altitude. To date, no study has investigated the exercise performance, skeletal muscle enzyme activity and buffering capacity of skeletal muscle in African and Caucasian long distance runners of East African origin who are habitually resident in the same sea level environment.

The possibility of a genetic basis for the differences between Africans and Caucasians is indicated by a recent study analysing DNA restriction fragment length polymorphism (RFLP) (Adjoa et al, 1996). This study compared Caucasians from Canada and South Africa with South Africans of African origin, and showed racial differentiation in selected muscle enzyme allele frequencies. All groups had similar RFLP for muscle adenylate kinase, phosphorylase and subunit V_a of cytochrome c oxidase genes, but the allele frequencies of the Africans were quite distinct. In addition, a small sub-sample of the Africans possessed a unique DNA sequence variant. Therefore, it would appear from this preliminary RFLP work, that there is evidence for racially distinct variability with respect to skeletal muscle *genotype*, that justifies investigation of skeletal muscle phenotypes in Africans and Caucasians.

Therefore, the primary aim of the current series of studies is to determine if there is evidence of racially distinct *phenotypes* in South African runners, with reference to selected exercise and skeletal muscle characteristics that are potential determinants of distance running ability. In addition, relationships between skeletal muscle characteristics and selected metabolic characteristics during exercise will be investigated in these runners.

Scope and limitations of the studies:

The runners and sedentary individuals investigated in this group of studies are all resident at sea level in Cape Town, Western Cape, South Africa. The African runners were all Xhosa or Zulu and the African sedentary individuals were Xhosa, Zulu, Tswana or Sotho. All of these ethnic groups are Bantu-speaking (the Eastern African language group) and share common genetic origins, having migrated from Eastern Africa at least as early as the 15th century (personal communication, Professor Copeland, Department of Anthropology, University of Cape Town). However, subsequent to settlement in South Africa, there has been some intermixing of the gene pool with local, and physically smaller, Khoikhoi and San people and within the different ethnic groups mentioned above, but very little genetic mixing with people of Caucasian origin as a result of colonial attitudes and the subsequent apartheid regime. Runners of obviously mixed race, who would have traditionally been classified as "coloured", have been deliberately excluded. This exclusion has only scientific justification in that the differences under investigation may be of a genetic origin, and inclusion of these individuals may have seriously confounded the findings. Therefore, the genetic make-up of the African people investigated in the current series of studies, is a derivation rather than a replication of that of the African people of East Africa. Hence, the results of this group of studies should be interpreted only with reference to the Africans of the Cape Town urban area in South Africa and extrapolations to the successful athletes from Kenya, Tanzania and Ethiopia would be purely speculative. In addition, the number of subjects in these studies is small, particularly with respect to volunteers for the muscle biopsy, and whilst the investigator believes that these runners are suitably representative of the 10 km runners in the Western Cape, caution must be exercised in the finality of the conclusions and this remains a limitation of this thesis.

Personal statement

The comparison of individuals of African and Caucasian origin in this thesis is one of scientific enquiry into the biological basis of exercise performance and relates only to the physiological and not sociological determinants of exercise performance. As a result of enforced social and sexual segregation in South Africa in the past, and entrenched social attitudes, there has been little intermingling of the Caucasian and African gene pools. However, this thesis and the investigator in no way support or condone this segregation or such attitudes.

CHAPTER TWO

REVIEW OF LITERATURE

INTRODUCTION TO THE REVIEW OF LITERATURE

The following chapters review the literature that has contributed significantly to our knowledge of factors that may be involved in determining endurance performance, specifically factors that may affect high intensity endurance performance such as 10 km running performance.

The first three sections (2.1, 2.2, 2.3) review the physiological factors that have been implicated as important determinants of endurance performance and which can be modified by training, in order to understand the implications for performance of the exercise and muscle biochemical characteristics that were investigated in African and Caucasian runners. These sections will concentrate primarily on the characteristics that were investigated in this thesis and that are presented in the experimental chapter.

A further determinant of performance is that which is inherent to an individual and is encoded by virtue of his or her genes. Groups of individuals may have small portions of their total genes that encode for specific characteristics (genotype) in common. In other words, a group of individuals may have specific genotypes that are distinct from other groups of individuals, which in turn result in the occurrence of distinct phenotypes between groups. An example are bearers of the genes encoding for a specific disease. Although, these fellow sufferers have only a minuscule proportion of their total genes in common with each other, these are solely responsible for the ensuing phenotype. Similarly, other groups of individuals, such as a race group may have genotypes that are distinct, or occur with higher frequency, than other race groups. The literature that has investigated the genetic determinants, and any racial distinctions, of phenotypes that may be related to endurance exercise performance is reviewed in the latter section (2.4).

CHAPTER 2.1

SKELETAL MUSCLE CHARACTERISTICS AND THEIR RELEVANCE TO ENDURANCE PERFORMANCE

2.11 SKELETAL MUSCLE FIBRE TYPE COMPOSITION

Human skeletal muscle fibres can be differentiated and classified by a variety of methods. Perhaps the most common of these is by histochemical staining for myofibrillar ATPase activity. The variation in activity remaining in myofibrils after preincubation of serial sections at pH 4.3, 4.6 & 10.3 allows for classification as type I, IIA, IIB and IIC fibres (Brookes and Kaijser, 1970). It is clear that this system is probably an over-simplification of the diversity of skeletal muscle fibres and investigators have suggested that a continuum of skeletal muscle fibre types exists (Pette and Staron, 1993). Also, muscle fibres should be considered as dynamic structures, able to adapt to the functional requirements placed upon them. The myosin ATPase is one of the proteins able to adapt to such a stimulus. For the purpose of this thesis, the more traditional classification is utilised because of technical restrictions, but the possible limitations of this simplification should be recognised.

There has been considerable interest in the fibre type composition of both sedentary individuals and various athletic populations as researchers attempt to elucidate the mechanisms important in successful endurance performance. A summary of some of the published data for fibre type composition and morphometry of the human vastus lateralis muscle is presented in Table 2.21. These early studies indicated that in most sedentary individuals there is a fairly equal proportion of type I and type II fibres whilst trained endurance athletes tend to have a higher percentage of type I fibres. However, trained individuals, in particular, are reluctant to have a muscle biopsy. Therefore, the number of subjects in most studies is small and hence the total data base of skeletal muscle fibre type information on well-trained individuals is relatively small, which may explain some of the apparent inconsistent results reported in the literature. Furthermore, by far the majority of studies have investigated the vastus lateralis muscle and even less information is available on other muscles of the body.

Table 2.11: Fibre type composition in the vastus lateralis muscle of sedentary and athletic populations

n	% type I	CSA ($\mu\text{m}^2 \times 10^2$)	% type II	CSA ($\mu\text{m}^2 \times 10^2$)	Reference
Sedentary:					
6	57	45.6	43	48.9	Edstrom et al, 1969
26	36	40.2	64	52.2	Gollnick et al, 1972
8	48	45.6	52	50.7	Edstrom et al, 1972
69	54	48.4	32/13*	52.7	Jansson et al, 1977
11	60	29.4	40	36.6	Larsson et al, 1979
10	63	28.5	37	35.1	Larsson et al, 1979
70	54		32/13*		Saltin et al, 1977
10	47	44.7	38/14*	45/36	Simoneau et al, 1985
Athletic:					
8 orienteers	68		25/3*		Saltin et al 1977
8 dist runners	59		41		Gollnick et al, 1972
11 cyclists	57	63.3	43	61.2	Burke et al, 1977
14 dist runners	79	83.4	21	64.9	Costill et al, 1976
18 middle dist	62	63.8	38	62.8	Costill et al, 1976

(CSA = cross sectional area; * = % type IIA / %type IIB; dist = distance)

A recent study summarising the skeletal muscle fibre type of more than 400 Caucasian men and women from North America, has shown that in fact there is considerable heterogeneity (Simoneau et al,1989). Twenty-five percent of all individuals studied had less than 35% or more than 65% type I fibres (i.e. only 75% of all the subjects fell within the middle "normal" range) with a range in the percentage of type I fibres from 15% to 85%.

As discussed later (chapter 2.43), twin studies have shown that the fibre type phenotype is primarily genetically determined (Komi et al, 1977; Komi et al, 1979). These authors conclude from twin studies (n=15 monozygotic pairs, n=16 dizygotic pairs) that fibre type composition was highly genetically determined (heritability coefficient = 0.93, see chapter 2.31). More recent studies (Lortie et al,1986; Bouchard et al, 1986) have refuted this finding showing considerably lower heritability coefficients. In their recent review of the human and animal experiments regarding the heritability of fibre type, Simoneau & Bouchard (1995) concluded that the genetic component accounts for between 40 - 50% of the variability in the

proportion of type I muscles in human muscles, implying that an individual's fibre type proportion is at least partially pre-determined by one's genotype.

Highly endurance trained athletes tend to exhibit a higher percentage of type I fibres (Costill et al, 1976) but there remains considerable debate whether those individuals with a high proportion of type I fibres have selected endurance sports because of success at this type of activity, or whether the high proportion of type I fibres is the result of an adaptation to this form of training.

Cross innervation and electrical stimulation of skeletal muscle in animals and transcutaneous electrical stimulation in humans has been shown to change type II fibres to type I fibres (Barany and Close, 1971; Pette and Schnez, 1977, Pette & Vrbova, 1992; Munsat et al, 1976). However, there seems little evidence for gross changes from type II fibres to type I fibres as a result of normal endurance training in humans. Gollnick et al (1973) investigated the effect of a 5 month training programme on fibre type composition and cross-sectional area. Although $VO_2\text{max}$ increased by 13%, no change occurred in the percentage of the type I or type II fibres although slow twitch fibres were larger after training. No differentiation was made between type IIA and IIB and therefore it was not possible to investigate changes between the sub-groups of fibre types.

Saltin et al (1976) reported that fibre type proportions did not change after either endurance or sprint training in human subjects using the one-leg training model. Similarly, Henriksson & Reitman (1976) reported no change in triceps brachii type I fibre percentage after a 50 day endurance regime of cross-country skiing. Type IIA and IIB fibre populations were reduced in favour of type IIC. Support for the concept of plasticity of muscle fibre type is apparent by the "hybrid" or "transitional" fibres containing more than one myosin heavy-chain isoform. These fibres have become obvious in experimentally-induced transformation of fibre types (Pette & Vrbova, 1992; Termin et al, 1989). It has been suggested that the type IIC is a transitional fibre type. This fibre type has been observed in both rats (Staron & Pette, 1993) and humans (Staron & Hikida, 1992).

Jansson et al (1978) showed a 17% reduction in type I fibres after the inclusion of high intensity "anaerobic" training (90-100% $VO_2\text{max}$) in previously well-trained subjects accompanied by an 11% increase in type IIC fibres. Simoneau et al (1985) investigated the response of fibre type proportions to 15 weeks of strenuous high intensity training. Training resulted in an increase in the proportion of type I fibres (from 41 to 47%) and a decrease in the proportion of type IIB fibres (from 17 to 11%) (both $p < 0.01$) whereas the proportion of type IIA fibres remained essentially unchanged. Jansson et al (1990) showed a decrease in

type I fibres and an increase in type IIA fibres after sprint training but this was only in one of the two groups in the study population. Individual data were not given to determine the number of individuals who changed their fibre type proportions.

One of the reasons for less than conclusive results may be the nature of the muscle sample obtained from a biopsy site. The sample obtained from the muscle biopsy is frequently assumed to be representative of the fibre type of the whole muscle. Several studies have attempted to quantify the reliability and reproducibility of muscle biopsy samples from the same individual and the general conclusion is that the technique has fairly high variability (coefficient of variation = 6%, Blomstrand & Ekblom, 1982; Simoneau et al, 1986). Such a high coefficient of variation must be taken into consideration when utilising the muscle biopsy technique.

In summary, the early cross-sectional studies comparing non-trained with endurance-trained athletes do indicate a higher proportion of type I fibres in the latter which suggests that a high proportion of type I fibres is a contributor to success in endurance events. Whether the proportion of type I fibres is a predictor of endurance performance in a homogenous group of well-trained athletes is unknown. However, it is difficult to say with confidence whether the proportion of relative fibre types is adaptable with endurance training although from the available data this seems more likely following high-intensity training. More longitudinal studies are required with advanced techniques. In particular, gel electrophoresis of myosin heavy chain isoform distribution may be more sensitive to change.

2.12 SKELETAL MUSCLE GLYCOLYTIC AND OXIDATIVE ENZYME ACTIVITIES

Skeletal muscle oxidative enzyme capacity is one of the potential determinants of endurance performance. Previous studies have found a relationship between oxidative enzyme activities and maximal whole body aerobic capacity in populations relatively heterogeneous for exercise capacity.

Hoppeler et al (1973) report a significant correlation between $VO_2\text{max}$ and human skeletal muscle mitochondrial volume density ($r=0.82$). Recalculating the data presented by Henriksson and Reitman (1976) of a group of human subjects heterogeneous with respect to $VO_2\text{max}$ values (35.9 - 61.3 ml/kg/min), reveals a significant correlation between $VO_2\text{max}$ and SDH activity in the vastus lateralis muscle ($r=0.59$, $r=0.64$; $p<0.05$ before and after training respectively). Booth & Nahara (1975) reported a relationship ($r=0.75$) between $VO_2\text{max}$ and cytochrome oxidase activity in the vastus lateralis of untrained men. However, when one studies a more homogenous population, this relationship does not exist. Calculating from the reported $VO_2\text{max}$ (range 71.3 - 84.4 ml/kg/min) and SDH (range 15.8 - 31.9 $\mu\text{mol/gm ww/min}$) values of the elite runners in the study of Costill et al (1976), no relationship was present ($r=0.26$).

However, endurance training does result in adaptations of the skeletal muscle enzymes suggesting that a higher activity may have a functionally important role to play during endurance exercise. The increase in the activities of mitochondrial enzymes of fat and carbohydrate metabolism is the result of an increase in enzyme protein concentration and an increase in both the size and the number of mitochondria (Hoppeler et al , 1973). Such adaptations are well documented in endurance athletes.

Gollnick et al (1972) compared oxidative (SDH) and glycolytic (PFK) enzyme activities in groups of untrained and trained individuals, clearly showing higher mean SDH activity in endurance trained athletes (60% higher) than untrained controls. The mean activity of the glycolytic enzyme, PFK, was actually lower in the trained subjects.

Table 2.12 Glycolytic and oxidative enzyme activities in untrained and endurance trained individuals

		PFK	SDH
		$\mu\text{mol/g ww/min}$	$\mu\text{mol/g ww/min}$
untrained	n=26	25.3 ± 0.1	4.35 ± 0.7
endurance	n=39	21.5 ± 0.1	6.98 ± 2.2

(summarised from the data of Gollnick et al, 1972)

Costill et al (1976) reported an even greater difference between the endurance trained and untrained subjects. This group reported that SDH activities in elite distance runners and well-trained middle-distance runners were 3.4- and 2.8-fold greater respectively than activities in untrained individuals. Similarly, Jansson & Kaijser (1987) report values for SDH and 3-HAD activities in trained subjects that were twice the values of untrained individuals. Sjogaard et al (1984) showed muscle enzyme activities (CS, 3-HAD and HK) they were 30-60% higher in elite, when compared to merely competitive, cyclists again highlighting the magnitude of the trainability of these enzymes. Differences in the magnitude of the difference between well-trained and untrained in the studies mentioned above may reflect *how* untrained the untrained subjects were. In both of the above studies the trained individuals had similar or lower values than untrained for glycolytic enzymes.

However, it is necessary to consider the results of longitudinal studies, to confirm that enzyme differences are an adaptation to endurance training and not a pre-determined selection requirement for successful endurance performance. A five month longitudinal training study (Gollnick et al, 1973) showed that oxidative enzyme activity (SDH) is adaptable and that the increase can indeed be sizeable (95% increase, $p < 0.01$). Subsequently, many studies have supported the findings of this study although inter-study comparisons of absolute values are difficult because of variance in methodology (Costill et al, 1976; Costill et al 1979; Chi et al 1983; Bylund et al, 1977; Mole et al, 1971; Morgan et al, 1971).

Henriksson and Reitman (1976) attempted to isolate the adaptations with respect to fibre type after either continuous or high-intensity training, using single fibre techniques. In a continuous training group, SDH activity increased only in type I fibres (by 32%), whilst in the higher intensity training group, SDH activity increased only in type II fibres (by 49%). These results imply that the recruitment pattern during training is important in stimulating oxidative adaptations.

In the study of Costill et al (1976), the individual with the lowest proportion of type I fibres in fact had the highest total SDH activity and there was no relationship between type I percentage and SDH activity for the whole group. Furthermore, the study of Essen & Henriksson (1984) determined enzyme profiles in pools of single fibres in endurance trained and control subjects. The oxidative capacity of the type II fibres of the endurance trained was *higher* than the oxidative capacity of the type I fibres of the controls. Citrate synthase activity was 2.1x, 2.3x and 2.0x higher in the endurance-trained subjects than in the non-endurance trained subjects in type I, IIA and IIB pooled fibres respectively. Clearly, the

oxidative capacity of even type II muscle fibres can be increased considerably and it is incorrect to classify type I fibre 'oxidative' and type II fibres 'glycolytic' as has traditionally been the case. Other single fibre studies have shown that considerable overlap exists between enzyme activities in the fast and slow fibre populations (Lowry et al, 1978; Pette & Spamer, 1986). This indicates that all fibre types have the ability to considerably increase their oxidative capacity not just type I fibres as perhaps traditionally thought.

Surprisingly, the single fibre studies of Essen & Henriksson (1984) indicated a greater glycolytic enzyme activity (PFK) in endurance trained individuals in all fibre types than in the non-endurance trained individuals. This appears contrary to the findings noted in Table 2.12 and the text above, but the above-mentioned studies measured PFK activity in muscle homogenate and the disparity therefore arises as a result of the higher proportion of type I fibres in the endurance trained individuals. Thus, it appears that endurance training may enhance glycolytic activity within the constraints of an individual's given fibre type composition. Detailed investigations of the response of enzyme activities in single fibres that have been pooled according to fibre type, to different types of training, are not yet available.

What is clear from the many training studies is that the changes in enzyme activities are disproportionately higher than the changes in $VO_2\text{max}$ (Henriksson & Reitman, 1976; Gollnick et al, 1973; Connett et al, 1990). This suggests there is a non-causal relationship between the two variables. In addition, Sjogaard et al (1984) followed a group of competitive cyclists throughout a season and observed considerable further improvement of CS, 3-HAD and HK activity (40-70%) whilst $VO_2\text{max}$ remained the same. In further support of the dissociation between mitochondrial enzyme activity and $VO_2\text{max}$, is the work of Henriksson and Reitman (1977) who investigated the time course of changes in $VO_2\text{max}$ and skeletal muscle enzyme activities, showing that that adapted asynchronously. They suggest that changes in skeletal muscle enzyme activities may be more relevant to submaximal endurance performance. Certainly the magnitude of submaximal endurance capacity changes after training are similar to the magnitude of the enzyme changes (50% or greater) although this type of relationship is not unequivocally causal (Davies et al, 1981; Gollnick et al, 1973; Karlsson et al, 1974).

Because athletes rarely race at $VO_2\text{max}$, time to fatigue during a submaximal exercise test may be a more applicable exercise test to determine whether change in endurance performance can be related to changes in skeletal muscle enzyme activities. But, to date, this has not been investigated in well-trained athletes. Animal studies have shown a significant relationship between submaximal fatigue resistance and skeletal muscle enzyme activities. In isolated muscles of the cat and rat, increases in the ability to resist fatigue

during repeated contractions have been associated with an increase in the activity of oxidative enzymes (Peckham, 1973; Hudlicka, 1977) suggesting that the ability of the muscle to sustain submaximal contractile activity is dependent upon its mitochondrial oxidative capacity.

The exact mechanism by which increased skeletal muscle enzyme activities improve submaximal endurance performance is somewhat unclear but suggestions include enhanced fat oxidation and decreased carbohydrate utilisation (Henriksson & Reitman, 1977) and tighter regulation of substrate flux (Gollnick & Saltin, 1982).

Firstly, the ability to regulate the relative utilisation of fats and carbohydrate probably has a more important role in submaximal endurance performance than in determining VO_2max . The intramuscular metabolic changes occurring after endurance training are increased fat utilisation (Karlsson et al, 1974; Costill et al, 1979), less disturbance to the intracellular ATP/ADP ratio and less reliance on glycolysis and therefore decreased lactate accumulation (Gollnick & Saltin, 1982; Karlsson et al, 1974; Dudley et al, 1987; Connett et al, 1990). Thus, the accumulation of metabolic by-products is reduced. The relationship between skeletal muscle oxidative enzyme activity and fatigue may be related to the accumulation of these metabolic by-products and the effects of these on excitation or contraction or on excitation-contraction coupling. For example, the recent study by Favero et al (1995) has clearly shown the effect of various metabolites on muscle sarcoplasmic reticulum, which has an integral role in the generation of muscle contraction. This investigation of SR vesicles showed that increasing the concentration of Mg^{2+} , La and H^+ , detrimentally affected the function of the sarcoplasmic reticulum with respect to channel opening and Ca^{2+} release.

However, in contrast, one recent study has indicated that the increase in fat utilisation that occurs with training, may occur before the increase in mitochondrial oxidative capacity, and therefore the two may not be causally related. Cadefau et al (1994) showed that the decreased lactate and decreased glycogen depletion in response to short-term endurance training preceded the changes in oxidative capacity suggesting that oxidative enzyme activity changes may not be a prerequisite for all of the metabolic changes previously proposed.

Secondly, Gollnick & Saltin (1982) suggest the increased enzyme concentration assists by reducing the required substrate concentration for a given velocity of reaction and therefore improving metabolic control. This is preferable to a simple increase in substrate concentration which might be potentially detrimental to the intracellular osmolality.

In summary, the plasticity of enzyme activities, in all fibre types, with training is undisputed. Similarly, it seems clear that the oxidative enzyme activities are more closely related to submaximal endurance performance than to $VO_2\text{max}$. However, what remains unclear is the exact mechanism by which this increase in oxidative enzyme activities acts to augment submaximal endurance performance. In addition, the stimulus for the increase in oxidative enzyme activity remains unclear. It is also clear that other skeletal muscle properties are important, some of which may not yet have been elucidated. In particular, if the intracellular accumulation of metabolic by-products is important, as discussed above, the ability to buffer such substances may be important. This is reviewed in the following section.

2.13 SKELETAL MUSCLE BUFFERING CAPACITY

One proposed mechanism of impaired contractile function during high intensity exercise is the lowering of the cytosolic pH. As a result of high intensity exercise, cytosolic H⁺ ion production is increased and those protons not lost to the circulation, or buffered, will cause a decrease in the muscle pH. This has been shown to inhibit skeletal muscle contraction (Donaldson et al, 1978; Fabiato & Fabiato, 1978; Hainault & Duchateau, 1989) particularly at the site of the actomyosin interaction (Cooke et al, 1988). H⁺ ions may directly compete for Ca²⁺ binding sites on troponin C (Fabiato & Fabiato, 1978). To attenuate the fall in pH, muscle cells have a large capacity to buffer H⁺ ions with cell constituents that have pK_a values in the physiological range.

Muscle buffering capacity (β_m) can be determined *in vitro* in skeletal muscle homogenates and the contributing buffers are dependent upon the experimental method used (Marlin & Harris, 1991; Mannion et al, 1993). The titration method ($\beta_{m_{titr}}$) measures the contribution of physico-chemical buffering by proteins, dipeptides and phosphates but excludes 'dynamic metabolic buffering' such as rephosphorylation of ADP by PCr. Alternatively, muscle buffering capacity can be calculated from the ratio of the change in intramuscular lactate to the change in intramuscular pH from rest to exhaustion ($\beta_m_{\Delta La/\Delta pH}$) (Sahlin et al, 1976).

Perhaps not surprisingly, some studies have shown buffering capacity to be elevated in athletes who are involved in high intensity exercise but the results are by no means conclusive:

Sahlin & Henriksson (1984) reported a significantly higher $\beta_{m_{\Delta La/\Delta pH}}$ in trained team sportsmen compared to untrained individuals. Nevill et al (1989) showed a significant relationship ($r=-0.57$, $p<0.05$) between 200m sprint time and $\beta_{m_{titr}}$. Parkhouse et al (1985) reported a higher β_m in sprinters and rowers than in untrained controls. Marathon runners were not different from controls suggesting that intensity of training may be important. However, Weston et al (1996), reported a significant correlation ($r=-0.82$, $p<0.05$) between $\beta_{m_{titr}}$ and 40 km time trial (~1 hour duration) in cyclists. Thus, the exact nature of the relationship between β_m and performance, particularly *sustained* high intensity performance, remains unclear.

Longitudinal training studies are also somewhat inconclusive. Some sprint training studies have shown an increase in β_m , as has aerobic training at altitude (Mizuno et al, 1990; Saltin et al, 1995a). In contrast, others have reported no changes in β_m after sprint training (Bevan

et al, 1986; Nevill et al, 1989) or high intensity isokinetic exercise training (Mannion et al, 1994).

The buffering capacity of different fibre types has not been directly investigated and variation in fibre type composition between athletes may be responsible for the inconsistencies in the literature. Some authors have attempted to address this issue by relating β_m to the proportion of muscle cross-sectional area occupied by specific fibre types. Parkhouse et al (1985) found a positive relationship between β_m and the proportion of the cross-sectional area occupied by type II muscle fibres. Mannion et al (1995) reported a similar trend although Sahlin & Henriksson (1984) found no such relationship.

Buffering capacity has been predominantly investigated in sprint athletes. Only two studies to date have investigated buffering capacity in distance runners despite certain distance events (for example, 10 km) being raced at very high intensities with the generation of considerable lactate with the subsequent potential for a decrease in intramuscular pH. Parkhouse et al (1985) found that the buffering capacity of marathoners was not different from that of untrained individuals. Saltin et al (1995a) reported that Scandinavian distance runners had a higher buffering capacity than Kenyan distance runners, but no data were available for less trained individuals for comparison. The Kenyan athletes showed a small trend toward a higher percentage of type I fibres than the Scandinavian group but this was not statistically significant and no correlation was reported to investigate whether the β_m was related to the proportion of fibre types (72.5% vs 67.7%). The paper reports that the Kenyans were predominantly distance (cross-country) runners, but it is not clear whether the Scandinavians were middle or long distance runners, although they were all of a high standard. Based on the findings of the studies discussed above, this may have affected the result.

In summary, the preceding three chapters indicate that skeletal muscle fibre type proportion, enzyme activities and buffering capacity exhibit considerable diversity in the human either as a result of the inherent properties of the muscle or as a result of adaptation to training.

However, it is not always possible to obtain muscle biopsies, in particular from well-trained athletes. Therefore, the measurement of the accumulation of metabolites in the blood is also of interest to exercise physiologists and many have attempted to correlate performance with the concentration of metabolites in the blood, and to establish links between the skeletal muscle characteristics discussed above and the level of metabolites in the blood. The current thesis also investigates the relationship between muscle characteristics and blood-borne metabolites and therefore this literature is reviewed in the following sections.

CHAPTER 2.2

BLOOD-BORNE METABOLITES AND THEIR RELEVANCE TO ENDURANCE PERFORMANCE

2.21 BLOOD LACTATE ACCUMULATION

Many studies have investigated the relationship between workrate and plasma lactate accumulation during exercise and several of the more pertinent studies are discussed here.

Jervell (1929) first described a curvilinear relationship between blood lactate concentration and exercise workrate and since that time there has been considerable debate regarding the exact nature of that relationship. The first indication of the existence of a threshold or critical power output at which blood lactate concentration begins to increase was made by Owles (1930) and later refined by Wasserman et al (1973). The relevance of this threshold concept to the determination of race pace has been thoroughly investigated. Athletes tend to race at the speed that allows the greatest oxygen consumption without a continuing rise in plasma lactate concentration (Farrell et al, 1979).

With respect to prediction of endurance performance, variables associated with lactate kinetics have shown a closer correlation with endurance performance than has the $VO_2\text{max}$. This is particularly evident in a more homogeneous group, for example already well-trained athletes. Farrell et al (1979) correlated two lactate variables with performance a) treadmill velocity at OPLA (onset of plasma lactate accumulation) and b) VO_2 at OPLA and found these to be better predictors of endurance performance (both $r=0.91$, $p<0.01$) than the $VO_2\text{max}$ ($r=0.83$, $p<0.01$). Subsequently, the presence of a distinct "threshold" has been questioned and some investigators have preferred to report the workload that elicits an absolute plasma concentration of lactate, usually 4 mmol/l. Fohrenbach et al (1987) correlated marathon velocity with running velocity at a plasma lactate of 4 mmol/l and obtained a very close correlation ($r=0.98$). Other investigators have found similar results (Kumagai et al, 1982, Williams & Nute, 1983, Coyle, 1995). Therefore, it appears that variables related to lactate may be better indicators of current performance, than is $VO_2\text{max}$.

Despite the clear relationship between lactate accumulation and exercise intensity, the mechanism and kinetics of this accumulation remains the topic of debate. Lactate formation has been shown to be augmented by hypoxia (Knuttggen et al, 1973; Linnarson et al, 1974; Katz & Sahlin, 1987) and reduced by hyperoxia (Linnarson et al, 1974; di Prampero, 1981) supporting the theory that increasing lactate accumulation is related to decreasing

mitochondrial partial pressure of oxygen. However the O_2 availability theory has been questioned and in fact cytosolic O_2 levels can be quite high despite the concurrent production of lactate. In addition, O_2 consumption is no higher in trained individuals at submaximal absolute workloads despite lower plasma lactate concentrations (Holloszy, 1973). More direct measurements have shown that the local PO_2 during supramaximal electrical stimulation induced contraction is above the minimum PO_2 for maximal mitochondrial ATP production.

An alternative school of thought is that increased lactate production merely represents an increased flux through the glycolytic pathway in response to increased carbohydrate metabolism secondary to increased workload and higher ATP utilisation rate. The requirement for reoxidation of NADH to NAD^+ to allow continued oxidation of 3-phosphoglycerate necessitates the conversion of pyruvate to lactate. With training an increased reliance on fat oxidation results in less glycolytic flux and therefore a reduction in blood lactate accumulation.

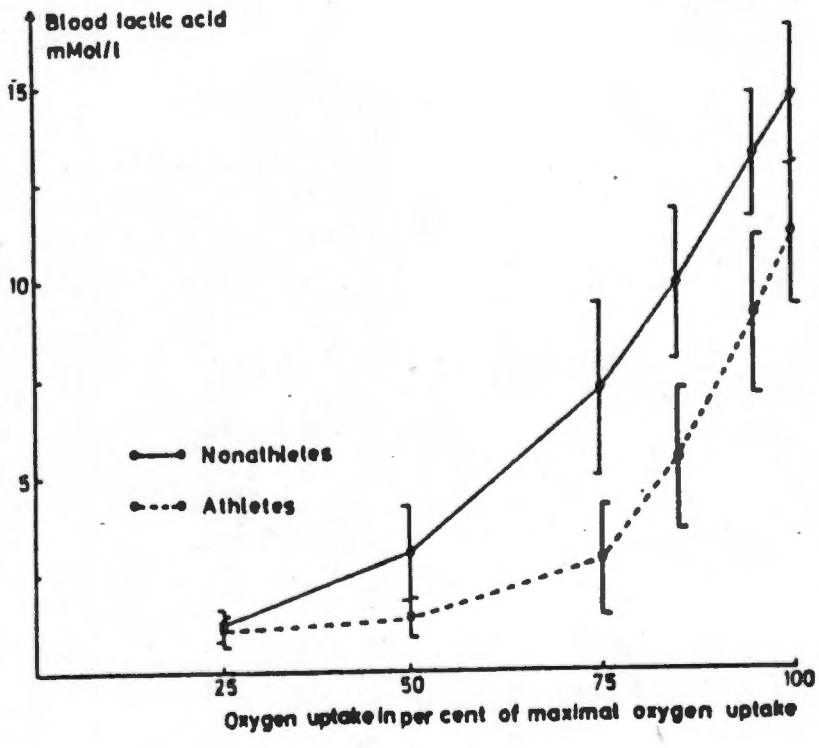
The longitudinal effect of endurance training on the plasma lactate vs workload relationship was determined over 50 years ago (Bang, 1936) and has been the topic of a number of studies since then (Wyndham et al, 1969; Saltin, 1969; Saltin et al, 1976; Karlsson et al, 1972, Donovan & Brooks, 1983). Fig 2.21 summarises this relationship.

As in cross-sectional studies mentioned above, longitudinal studies indicate that lactate kinetics may be a more sensitive indicator of longitudinal training adaptations than changes in VO_{2max} both in elite athletes (Sjodin et al, 1982) and less trained individuals (Denis et al, 1984). VO_{2max} often shows a lack of further adaptation to altered training in well-trained athletes (Daniels et al, 1978). This is discussed in more detail in Chapter 2.31.

The accumulation of lactate in the plasma is not only a function of lactate production but also of lactate clearance. This too is influenced by training status. A limited number of studies have attempted to individually quantify lactate production and lactate clearance rates. This can be achieved using radio-isotope tracer techniques in which the subjects are infused with radio-labelled lactate and this is allowed sufficient time to equilibrate through their lactate reservoir. During subsequent exercise, arterial or arterialized samples are obtained from which the resultant specific activity allows the calculation of the rate of appearance of lactate (La_{Ra}) and the rate of disappearance of lactate (La_{Rd}). Plasma lactate accumulates during exercise when the Ra exceeds the Rd (Stanley et al 1985).

Historically, a reduction in blood lactate accumulation after training has been ascribed to a decreased lactate production but lactate kinetics studies have highlighted the importance of

Fig 2.21 Blood lactate concentration in relation to workload expressed as %VO₂max



(Saltin, 1969)

lactate removal. Studies using tracer methodology support the importance of lactate removal (Stanley et al, 1984, MacRae et al, 1992) and have indicated that oxidation is the fate of the majority of lactate (Mazzeo et al, 1982; Mazzeo et al, 1986) lactate is now recognised as an important metabolic substrate. A large portion of this oxidation occurs in the exercising muscle and the extent is dependent on workload (Stanley et al, 1986; Brooks et al, 1986).

More recent studies have investigated lactate turnover before and after endurance training in previously untrained individuals. Using non-steady state equations, MacRae et al (1992) measured La_{Ra} and La_{Rd} during a ramp protocol with 6 min stages. They concluded that the decrease in lactate accumulation after training in previously sedentary subjects is primarily the result of a reduced appearance of lactate at the lower workloads ($VO_2 < 1.48$ l/min). However, at higher workloads ($VO_2 > 2.27$ l/min) an increase in Rd also became a contributory factor.

This may be related to the facilitated lactate transport mechanism recently reported in well-trained cyclists (Juel et al 1991). When results of MacRae et al (1992) were considered at relative intensities as is more appropriate, it became clear that the magnitude of the improvement in Rd was more profound than the magnitude of the reduction in Ra . For example at 50 % VO_2max , lactate Ra was 13% lower whilst lactate metabolic clearance rate was 38% higher. Non-steady state data must however be viewed with caution because of the assumptions with respect to lactate distribution volume and plasma volume. Furthermore, the extent to which the former component alters with endurance training is not known.

Accumulation of lactate in the plasma has previously been shown to be related to various characteristics of skeletal muscle. Plasma lactate accumulation during exercise in groups of trained individuals is inversely related to determinants of skeletal muscle oxidative potential namely a) percentage type I fibres (Ivy et al, 1980; Farrell et al, 1979; Sjodin et al 1981, Tesch et al, 1981) b) capillary density (Jacobs et al, 1983a; Tesch et al, 1981) c) skeletal muscle oxidative enzyme activities (Sjodin et al 1981, 1982;) and d) substrate utilisation (Karlsson et al 1974, Jansson & Kaijser, 1987, Kiens et al, 1993). In one study, capillary density and % type I fibre area together explained 92% of the variance in OBLA in active men (Tesch et al, 1981).

An increase in capillarisation may be an important contributor to changes in La_{Rd} . In addition, a recent study (Bangsbo et al, 1993) has indicated that lactate transport across the muscle membrane, measured by net femoral venous-arterial lactate differences, appears to

be partially saturated at higher muscle lactate concentration in humans. At higher muscle lactate concentrations, lactate transport did not maintain a linear relationship with the muscle lactate gradient, as was the case at lower muscle lactate concentrations. Therefore, an improvement in lactate transport with training could profoundly affect the clearance of lactate from the muscle cell. Of note is that at these higher levels of muscle lactate, the elimination of protons is approximately 50% more rapid than the efflux of lactate (Bangsbo et al, 1993), indicating that the transport mechanism of lactate is not synonymous with that of protons as traditionally thought.

A further blood indicator of muscle metabolism that is closely related to the plasma lactate concentration is the plasma concentration of ammonia. This gives an indication of the activity of the purine nucleotide cycle and is reviewed in the following chapter.

2.22 PLASMA AMMONIA ACCUMULATION

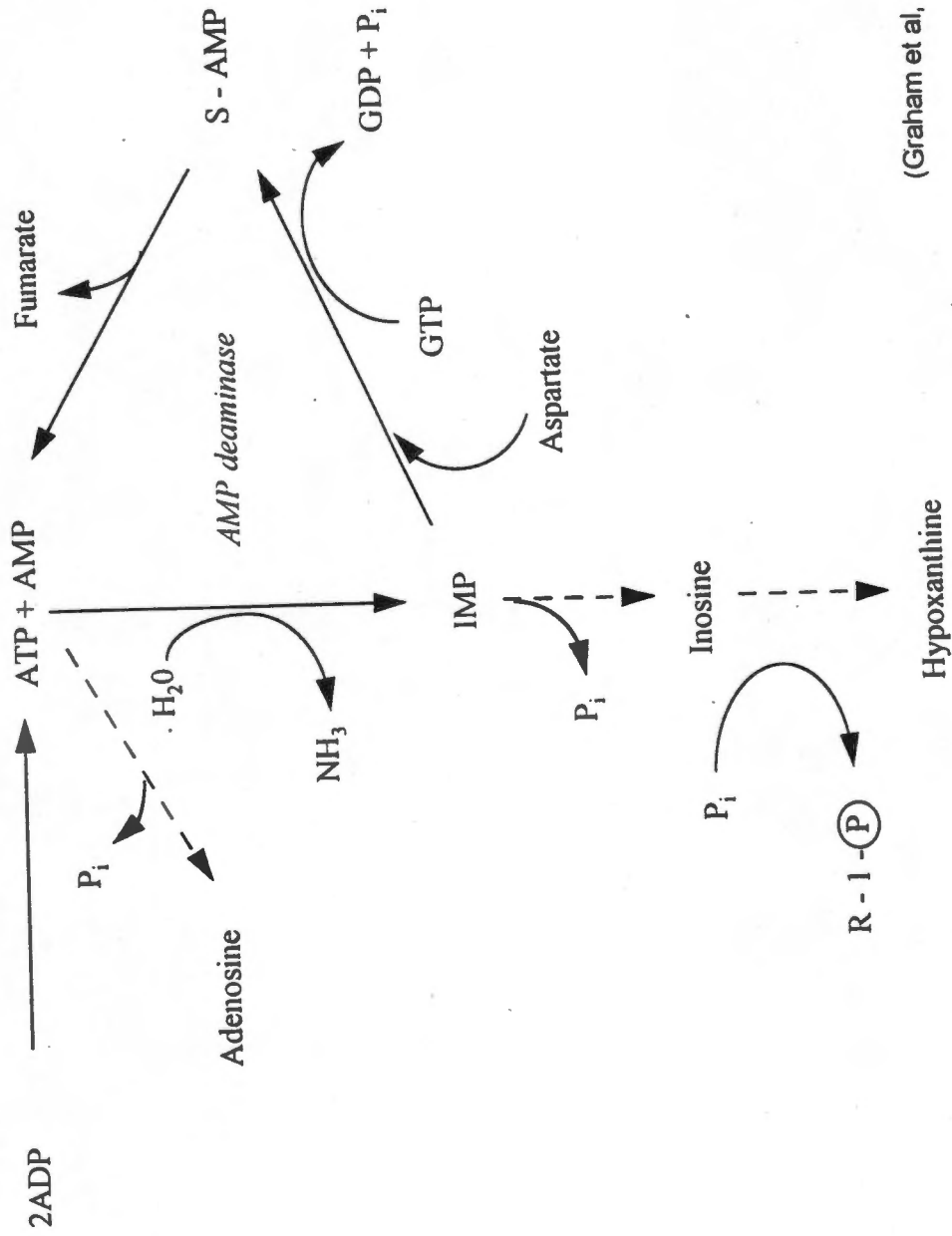
There are two major sources of ammonia (NH₃) in skeletal muscle 1) the deamination of branch chain amino acids (BCAA) and 2) the deamination of AMP to IMP by AMP deaminase in the purine nucleotide cycle (PNC) (Fig 2.22). In intense exercise the latter is by far the greater contributor (Lowenstein, 1990; Tullson & Terjung, 1990).

In human subjects, increases in plasma ammonia concentration are apparent at exercise intensities greater than 40% VO₂max and at intensities above 80% VO₂max, the plasma ammonia values can exceed 100 μmol/l (Dudley et al, 1983; Buono et al, 1984; Graham et al, 1990). Plasma ammonia concentration during maximal exercise may exceed 300 μmol/l (Graham et al, 1990). Recent field investigations by this laboratory (Weston, unpublished data) have found post-championship (12 km cross country running) plasma ammonia values ranging from 77 - 180 μmol/l and correlating strongly with plasma lactate (r=0.83, p<0.01). Whilst plasma lactate and ammonia are clearly related, the findings of Urhausen & Kindermann (1992) indicate that the accumulation of ammonia begins at a lower intensity, below the workload at which lactate begins to accumulate and may be dependent on the exercise duration. It has been suggested that this is somewhat determined by skeletal muscle fibre-type with ammonia accumulation being greater in individuals with a greater proportion of type II fibres (Tullson & Terjung, 1990).

The functions of AMP deamination have been suggested as:

- 1) the maintenance of ATP:ADP ratios to maintain muscular contraction. In situations of large ADP production, AMP deamination provides a method of decreasing ADP levels and therefore maintaining ATP:ADP ratios. ADP is hydrolysed to AMP by adenylate kinase. The rapid subsequent deamination of AMP maintains the higher ratio of ADP:AMP requiring the adenylate kinase reaction to continue in the direction of ATP production. The maintenance of a high ATP:ADP ratio facilitates cross-bridge turnover and muscular contraction.
- 2) the prevention of net adenine nucleotide loss. The degradation of adenine nucleotides to purine nucleosides and bases should be prevented as these compounds rapidly leave the cell and therefore represent a reduction of the adenine nucleotide pool. Therefore, the deamination of AMP to IMP and its subsequent recycling via the purine nucleotide cycle, represent a method of conservation of adenine nucleotides. However, the rapid IMP production at very high intensity exercise overloads this relatively slow two-step process, so some IMP is broken down to inosine and xanthine, so that nucleotide loss cannot always be prevented by the PNC.
- 3) as a proton buffer. Because the formation of ammonium from ammonia consumes a H⁺ ion, and ammonia is predominantly found in the form of ammonium, this reaction was

Fig 2.22 Purine nucleotide cycle



(Graham et al, 1993)

considered as a potentially important intracellular buffer. However, at closer examination this seems unlikely because of a quantitative mismatch between the large amount of lactate production (in mmol/l range) and the small amount of NH_3 production (in $\mu\text{mol/l}$ range) and therefore its rather insignificant potential as a buffer (Graham et al, 1990).

4) the regulation of carbohydrate metabolism. Although ammonium has been shown to activate PFK (Abrahams & Younathon, 1971) and IMP to activate phosphorylase b (Aragon et al, 1980) the temporal difference (i.e. later accumulation of NH_3) between the accumulation of these compounds and the requirement for the stimulation of glycolysis and glycogenolysis makes it unlikely that they are major regulators of carbohydrate metabolism in intense exercise although they may have a more significant regulatory role in longer duration exercise.

It is suggested that trained muscle experiences a lower NH_3 production as a result of a lesser increase in AMP with intense exercise that occurs as a result of the increase in skeletal muscle mitochondrial capacity (Graham et al, 1993). Lo and Dudley (1987) investigated the NH_3 metabolism after endurance training in humans and found a lesser plasma accumulation of NH_3 at the same absolute workload after training. Snow et al (1992) showed a similar decrease after sprint training. These findings are supported by further studies (Green et al, 1991, Denis et al, 1989) and therefore plasma NH_3 concentration may be a useful marker of training status.

In summary, both plasma lactate concentration and plasma ammonia concentration are related to the ability to sustain submaximal, but high intensity, endurance performance. As discussed in the previous two sections, their concentration in the blood is both related to each other and to the characteristics of skeletal muscle.

CHAPTER 2.3

EXERCISE LABORATORY INDICATORS OF ENDURANCE PERFORMANCE

2.31 MAXIMAL OXYGEN CONSUMPTION/PEAK TREADMILL VELOCITY

Maximal oxygen consumption ($VO_2\text{max}$) is the maximal rate at which oxygen can be consumed during exercise and it is traditionally utilised as a laboratory indicator of endurance capacity. Throughout this century many researchers have investigated the determinants of $VO_2\text{max}$. Several of the more meaningful studies are briefly outlined below. However, to discuss the controversies concerning $VO_2\text{max}$ in more detail is not an aim of this thesis.

Early studies by Hill et al (1923) suggested that $VO_2\text{max}$ was higher in endurance-trained athletes when compared to less trained controls and they also suggested that $VO_2\text{max}$ increased with training. These investigators suggested that exercise performance was limited by oxygen delivery which reached a maximum level as a result of limitations of the cardiovascular and/or respiratory systems. It was suggested that above this exercise intensity, oxygen delivery was insufficient for the demand and anaerobic metabolism was required and due to the production of anaerobic by-products, exercise was rapidly terminated. Thus, the individual with the highest $VO_2\text{max}$ was able to maintain higher levels of exercise without requiring significant contribution from anaerobic metabolism, and therefore had the better endurance performance capacity.

Subsequent to these early studies, attitudes have changed somewhat based on several experimental observations. Firstly, if indeed oxygen uptake was limited one would see a plateau in oxygen consumption with increasing workload. However, the occurrence of a plateau in oxygen consumption is disputed (Wyndham et al, 1959; Cumming & Borisyk, 1972; Noakes, 1986). Secondly, endurance training does result in adaptations in skeletal muscle characteristics (Holloszy & Booth, 1976) that, in the case of a purely central limitation, would be unlikely.

However, if the extraction of oxygen in the periphery was limiting then the addition of arm-work to leg-work would result in a further increase in $VO_2\text{max}$, which it does not. In actual fact, later studies have shown that the addition of arm work to leg work causes a reduction in leg blood flow by vasoconstriction to maintain systemic blood pressure in the face of the increased requirement for blood flow to the arms (Clausen et al, 1977). This suggests that cardiac output could not be substantially elevated to meet the demands of the additional arm-work. Similarly, if peripheral limitation to $VO_2\text{max}$ occurs, two-legged exercise would elicit an almost double $VO_2\text{max}$ than one-legged exercise which is not the case. In addition,

Clausen et al (1973) showed that training the legs alone increased the VO_2max determined by arm ergometry alone. Thus oxygen consumption could be increased without *any* adaptation to the skeletal muscles in question. Interestingly though, the reverse does not apply. This is probably because arm ergometry alone is insufficient to maximally tax the heart.

Other factors supporting O_2 delivery limitation are the increase in haemoglobin concentration that occurs with training and the ergogenic effect of blood doping. Blood doping has been shown to increase VO_2max (Buick et al, 1980) but it is not possible to conclude that this is due to improved oxygen delivery *per se*. Other factors such as acid-base status, plasma volume or lactate kinetics may be important. In his review, Gledhill (1982) concludes that at least 900 ml of blood need be given to elevate VO_2max . Similarly, some investigators have shown an increase in VO_2max with hyperoxia but others have not (Linnarsson et al, 1974; Welch, 1982) However, previous hyperoxic studies have had their inherent problems, primarily the difficulty in measuring VO_2 accurately when P_iO_2 is high, and the inability to measure cardiac output during maximal exercise. In addition, recent studies (Dempsey et al, 1984; Chance et al, 1992) have reported arterial oxygen desaturation in very elite runners and rowers respectively. This certainly suggests the possibility of an oxygen delivery limitation to maximal performance. Whilst these results are not conclusive, and there is probably a large amount of individual variation in oxygen saturation response to extreme exercise, they certainly warrant consideration.

Recently, there have been further investigation of a central limitation to VO_2max theory. Studies of blood flow and catecholamine levels during exercise (Anderson & Saltin, 1985; Holmqvist et al, 1986; Rowell et al, 1986, Savard et al, 1989) have suggested that if maximal blood flow is elicited by all the active muscles during whole body exercise, this would far outweigh the capacity of the heart to pump this volume, i.e. the cardiac output, is insufficient to meet the needs of whole body maximal exercise. Therefore, vasoconstriction in some areas is required to reverse vasodilation and to maintain blood pressure. It has been calculated (Saltin & Strange, 1993) that only 10 kg of muscle need to be exercising to maximally stress the cardiac system. These data are based on the assumption that calculations of maximal blood flow are correct, but even if overestimated somewhat, tend to suggest a cardiovascular limitation to maximal oxygen uptake.

In summary, it appears from the literature briefly reviewed above that there may be more than one single factor limiting VO_2max , although the current literature suggests that there is a role of the cardiovascular system in determining the maximal oxygen consumption of a large exercising muscle mass.

VO₂max as a predictor of performance:

If VO₂max is as much of a determinant of endurance performance as has been traditionally accepted by lay and scientific persons alike, one would expect it to emerge as a statistically significant predictor of endurance performance. In general, VO₂ max is a good predictor of performance in a heterogeneous group. Reports of the relationship between VO₂max and performance in a range of individuals from less-trained to well-trained individuals result in high correlations ($r= 0.88$, in runners with a marathon range from 2 hr 19 min to 4 hr 53 min, Maughan & Leiper, 1983).

Even in groups of trained athletes with a reasonable range of race performances, the correlations remain high. Costill (1967) found a strong relationship between VO₂max and endurance performance in collegiate cross-country athletes ($r=-0.82$) and in distance runners ($r=-0.91$) (Costill, 1973). Farrell et al, 1979; Foster et al, 1978; Maughan & Leiper, 1983; Hagan et al, 1981; and Sjodin & Svedenhag, 1985, all report similar correlations ($r=-0.91$, -0.86 , -0.88 , -0.63 , -0.78 respectively) for their studies of this relationship in trained individuals.

However, this is not the case in populations with more homogeneous race performances. In a group of very well-trained runners (10 km race time: 30-33 min) there was no relationship between VO₂max and race performance ($r=0.12$) indicating that factors other than VO₂max are responsible for the variance in 10 km race performance (Conley & Krahenbuhl, 1980). Similarly, the documented VO₂max of elite runners shows that the best runner does not necessarily have the highest VO₂max (Noakes, 1986). Some very good runners have very average VO₂max test results (for example, Derek Clayton marathon time 2:08:33, VO₂ max = 69.7 ml/kg/min; Zithulele Sinqe marathon time 2:08:04, VO₂max = 72.0 ml/kg/min; Willie Mtolo marathon time 2:08:15, VO₂max = 70.3 ml/kg/min). This may be compensated for by greater running economy or a high fractional utilisation (higher % of VO₂max sustained during a race), both of which are discussed in the following section.

It is apparent that a VO₂max of a reasonable level is a prerequisite for elite performance, but other physiological parameters are important in determining ultimate endurance performance.

Some investigators have suggested that "velocity variables" may be more predictive of running performance. Morgan et al (1989) suggested that the predicted treadmill velocity at VO₂max (vVO_{2max}) is a better predictor of 10 km performance time amongst well-trained runners than is the VO₂max. In that study, vVO_{2max} was extrapolated from a regression

equation calculated from submaximal oxygen consumption versus running velocity data. Therefore, this variable is effectively a "product" of both VO_2max and running economy because to obtain a high $v\text{VO}_2\text{max}$ a runner must have both a high VO_2max and be economical. Subjects ($n=10$) were well-trained and homogeneous with respect to race performance (mean 10 km time = 32.29 ± 1.27). VO_2max showed very little difference between subjects and therefore there was no significant relationship between VO_2max and 10 km time. However the relationship between $v\text{VO}_2\text{max}$ and 10 km time was highly significant ($r=-0.87$, $p<0.01$) as was velocity at a blood lactate concentration of 4 mM ($r=-0.82$, $p<0.01$).

In a similar vein, Noakes et al (1990) investigated the predictive power of peak treadmill running velocity obtained from an incremental test to exhaustion at zero gradient (1 km/hr increments every minute) on race performance over a variety of distances. The runners tested had a range of 10 km times from 29.5-42 min and a VO_2max range of 53.6-84.2 ml/kg/min. Peak treadmill velocity predicted 10 km running performance ($r=-0.94$, $p<0.01$). This was compared with the VO_2max which had a weaker correlation with 10 km time ($r=-0.55$, $p<0.01$).

The runners in the study of Morgan et al (1989) were considerably more homogeneous with respect to 10 km race time than those of Noakes et al (1990) which may explain the difference in the strength of the correlation between their respective maximal treadmill parameters (which are essentially the same) and 10 km race performance.

Recently, Hill & Rowell (1996) compared five different treadmill tests that investigate the running velocity associated with VO_2max , in 22 subjects. They concluded that two of the tests, those described by DiPrampo (1986) and Lacour et al (1991) were purely aerobic tests whilst the other three described by Billat et al (1994), Daniels et al (1977) and Noakes et al (1990) have a considerable anaerobic component. Regardless of the theoretical differences, the correlation between the tests was high ($r = 0.68 - 1.00$), and the predictive power of these tests of velocity at VO_2max with respect to performance, is generally high. This is undoubtedly because of the combination of both the VO_2max component and the running economy component. Running economy is discussed in more detail in the following section.

2.32 RUNNING ECONOMY AND FRACTIONAL UTILISATION OF VO₂MAX

As discussed previously, VO₂max does not adequately predict running success amongst athletes of similar ability. Perhaps more importantly the better athletes are able to

- 1) race at a higher percentage of their maximal oxygen consumption (%VO₂max) and
- 2) consume less oxygen for a given running speed.

Early studies with relatively few subjects investigated fractional utilisation of VO₂max and running economy (Costill, 1970, Costill, 1971), however the first thorough, systematic investigation was that of Costill et al (1973) in which laboratory data were related to 10 mile running performance. Surprisingly, the results indicated no relationship between VO₂ at 16.1 km/hr (range 48.5 - 54.8 ml/kg/min) and 10 mile running time in this heterogeneous group, but when VO₂ at this speed was expressed as %VO₂max for each individual the relationship became highly significant ($r=0.94$). Percentage of HRmax at 16.1 km/hr was even more closely related to performance ($r=0.98$). In contrast, Farrell et al (1979) found a significant relationship between VO₂ at 16.1 km/hr and 20 km race pace ($r=-0.56$, $p<0.05$). A later study of Conley & Krahenbuhl (1980) found a much stronger relationship ($r=0.82$, $p<0.01$) between submaximal running economy determined at 16.1 km/hr (VO₂ range 45-54 ml/kg/min) and 10 km running performance. The athletes in this study were considerably more homogeneous with respect to 10 km race time and VO₂max. It appears that in a group of elite runners with similarly high VO₂max, economy becomes a larger determinant of the variance in race time. However, as can be seen by the earlier study, good running economy alone is not a guarantee of good performance unless accompanied by a suitably, but not necessarily excessively, high VO₂max.

Differences in excess of 30% in running economy have been reported (Daniels, 1977) and we do not fully understand the factors that cause such large interindividual differences. Generally, longer distance runners (e.g. marathoners, ultramarathoners) are more economical but one can not ascertain whether this is cause or effect. Factors that may influence economy are a) years of training, b) training distance (Scrimgeour et al, 1986), c) fatigue (Daniels, 1985), d) biomechanical factors such as stride length (Cavanagh & Williams, 1982; Svedenhag & Sjodin, 1994), e) running technique and f) anthropometric characteristics (Svedenhag & Sjodin, 1994, Fredericks, 1987).

Certainly it appears that mass is an important determining factor. Economy is described as the metabolic power required to perform a particular mechanical task (Fredericks, 1987). Furthermore, as metabolic power has been proposed to dependent upon mass^{-0.66}

(Fredericks, 1987), then it follows that the metabolic power required to perform a specific task, reflected by the oxygen consumption, should also be standardised per $\text{kg}^{-0.66}$ in individuals of differing mass. Other authors have proposed that $\text{mass}^{-0.75}$ may be more appropriate (Bergh et al, 1991; Svedenhag & Sjodin, 1994), but in practice there is probably little difference between -0.66 and -0.75

During a laboratory 10 mile race, runners have been shown to race at a mean of 86% of their VO_2max (Costill et al, 1973). Bosch et al (1990) reported that African marathoners ran a simulated treadmill marathon at a mean value of 76% of VO_2max . However, the runners in this study ran the treadmill marathon considerably slower than their current marathon racing speed (87%). Therefore, the % VO_2max that they run in an actual race was not directly determined, but is likely to be considerably higher than 76%. Maughan & Leiper (1983) reported a much lower fractional utilisation of VO_2max (60%) in slower marathon runners than in the other studies and they report a significant positive correlation between fractional utilisation of VO_2max and average racing speed during a marathon ($r=0.74$ for males and $r=0.73$ for females). One should be aware that estimation of oxygen consumption during treadmill running can not directly be transferred to overland running. Factors such as air resistance and running surface suggest that laboratory measurements are almost certainly an underestimate of oxygen consumption. Costill et al (1973) estimated that the measured value of 86% VO_2max corresponds with an overland value of 91% VO_2max . A recent study (Loftin & Warren, 1994) showed that one group of cyclists completed a 16.1 km cycling time trial 28% faster than another group of cyclists despite no difference on VO_2max , as a result of a higher fractional utilisation of their VO_2max .

In summary, both running economy and fractional utilisation of VO_2max are important determinants of successful endurance performance, and are necessary for the optimal utilisation of an individual's VO_2max .

CHAPTER 2.4

GENETIC FACTORS AS DETERMINANTS OF ENDURANCE PERFORMANCE

2.41 GENETIC VARIATION IN ENDURANCE PERFORMANCE

A further determinant of performance is that which is inherent to an individual and is encoded by virtue of his or her genes. No two individuals have the same genetic material (excluding monozygotic twins) and the degree of variation is vast. However groups of individuals may have small portions of their total genes that encode for specific characteristics in common. In other words, a group of individuals may have specifics of genotype that are distinct from other groups of individuals, or that occur more frequently, which in turn result in the occurrence of distinct phenotypes between groups. An example are bearers of the genes encoding for a specific disease. Although, these fellow sufferers have only a minuscule proportion of their total genes in common with each other, these are solely responsible for the ensuing phenotype. Similarly, other groups of individuals, such as a race group may have genotypes that are distinct, or occur with higher frequency, than other race groups.

The genotype is responsible for the expression of the phenotype and may also be responsible for a degree of the adaptation and interactions that occurs to a phenotype. The endurance performance phenotype is characterised by large inter-individual variation. This is undoubtedly due to the contribution of a combination of both genetic and environmental factors. The extent to which phenotype is determined by genotype, rather than environmental factors, has been the subject of debate but there is now considerable evidence, both scientific and anecdotal, that one's endurance exercise capacity is at least partially determined by one's genes. The magnitude of the genetic effect and its role in the determination of endurance performance are unknown. As discussed below, earlier studies concluded that maximal aerobic capacity was highly genotype dependent. However these studies were often poorly controlled for environmental factors and later studies have indicated a more moderate dependence on genotype.

Scientific laboratory-based studies that have attempted to quantify endurance performance, have invariably utilised maximal oxygen consumption ($VO_2\text{max}$) as their yardstick. Unfortunately, because of its relatively short duration and maximal nature, this may not be the most functionally appropriate test of endurance capacity (see chapter 2.31) but convention has resulted in it being the most frequently reported test. A limited number of studies have also utilised a longer duration submaximal work test, which is perhaps more relevant to endurance performance.

Early studies investigated familial resemblance of HR during exercise and $VO_2\text{max}$ (ml/kg/min). Montoye et al (1975) investigated submaximal heart rate in a large group of parents and their children showing that the heart rate response of the children was clearly related to that of their parents ($p < 0.01$). Similarly, Montoye & Gayle (1978) showed a strong relationship ($r = 0.66$) between younger fathers and their sons with respect to $VO_2\text{max}$, but the relationship did not hold for fathers in the older age group. There was no significant relationship between spouses or siblings. In contrast, Lortie et al (1982) reported a much lower correlation for $VO_2\text{max}$ between parents and their children and one that is similar to that between spouses and between siblings. As spouses have no genetic link, but considerable environmental (or lifestyle) links, this tends to indicate a degree of environmental determination of aerobic capacity rather than genetic. Lesage et al (1985) investigated familial resemblance of HRmax and $VO_2\text{max}$, adjusted for gender and age. Maximum HR was significantly correlated between siblings and between mother and child. $VO_2\text{max}$ was not significantly related in either of the above combinations although the relationship between mother and child approached significance ($r = 0.28$).

Since these early familial resemblance studies, investigators have utilised a different approach by studying twin pairs. Twin studies allow a comparison to be made between the intra-twin pair correlation of monozygotic twins and the intra-pair correlation of dizygotic twins as well as correlations between pairs of siblings. Similarity between monozygotes that is over and above that of dizygotes and siblings is likely to predominantly reflect genetic dependence. One must keep in mind that environmental and dietary conditions may also be slightly more similar between monozygotic pairs than between dizygotic pairs, which would result in a slight over-estimation of the genetic component using the conventional heritability equations (see below). Engstrom & Fishbein (1977) investigated the physical capacity of twins and found a relationship within twin pairs ($r = 0.70$). In contrast, Howald (1976) reported no significant difference between dizygotic and monozygotic intra-pair differences as would have been expected if there was a genetic influence on the exercise test performance. However, as discussed by the authors, these data may be biased by the diverse physical activity history of two of the sets of monozygotic twins, thus reducing the intra-pair resemblance in the monozygotic group. The analysis excluding these pairs and one dizygotic pair with very different body mass, results in a significant intra-pair difference in the monozygotic pairs when compared to the dizygotic pairs and a high heritability index. Heritability index is a numerical estimate of the variance caused by genetic factors, expressed as a ratio of one (see below).

More recently Bouchard et al (1986) conducted a study with a large number of siblings ($n = 42$), dizygotic twins ($n = 66$) and monozygotic twins ($n = 106$). The study aimed to control

bias by using siblings of the same gender or twins who were living together in an attempt to isolate and quantify the genetic influence on VO₂ max, HRmax and V_Emax. There was a trend for VO₂max (ml/kg/min) within-pair correlations to decline from monozygotic (r=0.85) to dizygotic (r=0.74) to brothers (r=0.55) although statistically significant to the 1% level in all cases.

In addition to VO₂max, the above investigators included a test of endurance performance, namely a 90-min work output test. This test was previously described and validated by Boulay et al (1984) who showed a 2% variation in results between the first and second trial. This test was used in subsequent studies by this group and involves measuring total work during a continuous maximal effort on the cycle ergometer for 90 min. There was a strong within-pair correlation for the 90-min work output test expressed per kg body weight for monozygotes (r=0.66, p<0.001). It appears this test was not undertaken in the brothers. Intra-class coefficients for dizygotes were, when adjusted for gender and age, smaller but still significant.

The within-pair estimate of genetic variance was significant for both VO₂max and the 90-min work-output test but considerably more so for the latter (p<0.05 vs p<0.001 respectively).

The measure of genetic effect is expressed by the heritability fraction:

$$\text{eg } \frac{r_{\text{monozygotic}} - r_{\text{dizygotic}}}{1 - r_{\text{dizygotic}}}$$

(Newman et al, 1937)

where r = the relationship (correlation) between the twin pair for the given variable

Heritability fraction

VO ₂ max/kg	0.39
90-min work/kg	0.66
HRmax	0.53
V _E max	0.72

However, these values should be interpreted with caution, because the dizygotic intra-class correlation was slightly higher than that for brothers. This implies that a portion of the correlation is environmental and therefore the genetic effect may be slightly inflated. However, because of the long duration of the 90-min test, substrate availability may be a considerable determinant of the results (Karlsson et al, 1974) and a shorter submaximal test may produce an even tighter finding.

In summary, it would appear that the heritability fraction for $VO_2\text{max}$ is probably significant but smaller than first thought (somewhere between 10-30%) and may be overestimated by twin studies because of more similar environmental conditions for twins than for siblings. However, the 90-min test of work-output seems considerably more genetically-dependent than $VO_2\text{max}$, with heritability around 60-70%. Even if the identical environmental conditions of monozygotic twins has contributed to this high heritability coefficient, there is still likely to be a sizeable underlying genetic basis for that component of endurance performance that is evaluated by this test at least.

Trainability:

There is a marked variability between individuals in their *response* to identical training regimes (range in improvement in $VO_2\text{max}$ between 5 and 88%) and it has been postulated that this response may be genetically determined. Therefore, more recent studies have focused on genetic differences in the sensitivity to aerobic training, commonly referred to as *trainability*.

Lortie et al (1984) utilised a standardised 20 week cycle ergometer training programme in previously sedentary subjects (4-5x/week, 40-45min/session at moderate intensity with periodic higher intensity intervals). The investigators reported resultant changes in both $VO_2\text{max}$ and 90-min endurance performance. The degree of variability in response to the training was quite remarkable. Improvements in $VO_2\text{max/kg}$ ranged from 5-88% whilst 90-min work/kg increased from 16% - 97%. After analysing change with respect to the pre-training values, the initial training status was estimated to explain 30% of this variation. It was suggested by the authors that the remaining 70% may be attributed to variations in the response of genotypes to the training stimulus. It should be noted that other potential causes of variation such as motivation and experience at maximal efforts were not considered, so this may be an overestimate of genotype effect. However, this study did stimulate further direct research aimed at estimating the variability in the response to training and the extent to which this is genotype dependent.

Subsequently, ten pairs of monozygotic twins were involved in a 20 week aerobic training programme of a similar nature (Prud'homme et al, 1984). The results indicated that twin pairs responded to training with similar magnitudes of change with an intraclass correlation for change in $VO_2\text{max}$ (ml/kg/min) of 0.74 ($p < 0.01$). This value was not adjusted for gender, age or baseline training status but the authors report little heterogeneity between different twin pairs with respect to these variables. Correlations for change in ventilatory and anaerobic thresholds showed a significant, but not strong, similarity within twin pairs ($r = 0.43$, $r = 0.24$, respectively, both $p < 0.05$).

Hamel et al (1986) investigated the genetic determinants of trainability of both VO_2max and the 90-min work-output test when expressed per kg in only 6 twin pairs. This indicated a stronger genetic determination of the sensitivity of submaximal endurance performance to training, with a correlation coefficient of 0.81, ($p < 0.01$) after 15 weeks of training but also supported the earlier results with a VO_2max (ml/kg/min) trainability intraclass coefficient of 0.69, ($p < 0.05$).

Variations in mitochondrial DNA sequence can be responsible for differences in gene expression. Dionne et al (1991) investigated mitochondrial DNA sequence variants in sedentary males in an attempt to relate variants to either VO_2max or to the trainability of VO_2max . A high baseline VO_2max was prevalent in carriers of three particular mitochondrial DNA morphs whilst carriers of another morph exhibited a lower baseline VO_2max . Three carriers of a further variant had a significantly lower VO_2max response to training. This study further supports the notion that genetic factors may contribute to the magnitude of VO_2max as well as its response to training.

In summary, the evidence available suggests that genetic endowment may be a contributory factor in the determination of endurance performance although the extent of this endowment remains an issue of debate. More recent studies indicate a level of near 20% heritability of VO_2max with a higher heritability of submaximal performance (60-70%) and response to training (~70%).

Furthermore, groups of individuals that have been reproductively isolated from others have gene pools that are different from other groups. Therefore, the occurrence or relative frequency of a genotype may be different within this gene pool. Reproductive isolation may occur as a result of geographical, cultural, social or juridical barriers. Reproductive isolation may occur between racial groups or subgroups of racial groups. This may occur secondary to an of the previous methods of isolation, which maybe more likely to occur *between* populations than *within* populations. Groups that have been reproductively isolated from one another may then develop somewhat distinct genotypes.

2.42 RACIAL VARIATION IN ENDURANCE PERFORMANCE

The concept of race remains one of the more contentious and emotive issues of our time. In 1775, Immanuel Kant recognised four "varieties" of human, namely white, negroid, mongolian and hindu. Early characterisation was almost solely based upon visually evident traits. However more recently, with advances in the science of genetics, the human population is described in terms of gene frequencies. The occurrence of mutations, natural selection, geographical/cultural isolation and migration, amongst other forces, have resulted in the formation of different gene pools, which has established a genetic basis for the concept of race. Individuals of the same race are more likely to share common genetic determinants of phenotype than individuals from different races. This is particularly the case if the gene pools have been subject to little reproductive interaction, for example if geographically, sociologically, culturally, religiously or politically isolated.

As racial groups blend together there is an exchange of gene characteristics between groups. This flux is referred to as 'gene flux' (Bouchard, 1988). In some countries even constitutional laws have acted to unnaturally prevent such a flux. It is in these countries that the racial gene pools remain most unique.

But how unique is unique? It is now believed that most human genetic variation is common to all humans and that perhaps 10% is specific to population or racial groups. A degree of that percentage encodes for skin or facial characteristics. However, if this 10% was to include any of the exercise performance-determining genes it would be very significant indeed. There is certainly ample anecdotal evidence to suggest the presence of racial differences in sporting ability. In athletics alone, it is now evident that the sprint distances at Olympic and World Championship events are almost totally dominated by Africans of West African ancestry. In contrast, long distances (3000m and longer) are dominated by Africans of East African origin. In the 1996 World Cross Country Championships, African runners claimed 14 of the first 15 places in the senior men's event, and at the 1996 Olympics, the majority of men's distance running gold medals were won by East/South Africans.

It is tempting to explain these racial differences in exercise performance by cultural, sociological and environmental conditions which in many cases are profoundly different. In many early studies and some recent ones there was no effort made to control for variables such as diet, habitual daily activity, altitude of residence and socioeconomic factors:

In 1941, Robinson found no difference in $VO_2\text{max}$ (ml/kg/min) between Africans and Caucasians however submaximal economy (the amount of oxygen required to carry out a standardised task) was greater in Africans. Wyndham (1963) compared South African Bantu and Bushman with Caucasians but found no difference in $VO_2\text{max}$ (ml/kg/min) or ventilatory equivalent (V_E/VO_2). When a larger sample of subjects was considered, the Bantu were found to have greater mechanical efficiency in the stepping task than the Caucasians.

Leary and Wyndham (1965) investigated $VO_2\text{max}$ of African and Caucasian athletes in South Africa. Again, when expressed per body weight there was no difference between the races (63.2 vs 61.1 ml O_2 /kg/min respectively). As in the 1963 study above, mechanical efficiency was significantly greater in the Bantu athlete whilst stepping. However, ventilatory equivalent was not different. It should be noted that in neither of these studies were the groups matched for size. The data available indicated that the Bantu athletes were shorter and lighter. This may be of concern in measurements of economy but if the stepping height was constant for all, one would expect it to be relatively more difficult for the shorter athletes, contrary to the results. Thus improved efficiency of the Bantu athletes may, in reality, be even more pronounced than the results indicate.

Davis et al (1973) and DiPrampo & Ceretelli (1969) both found lower $VO_2\text{max}$ in untrained Africans (Tanzanians and Kenyans respectively) compared to Europeans, however when this was corrected for their smaller muscle mass, this difference was no longer apparent emphasising the importance of matching subjects for mass where possible.

These early studies made no attempt to control for environment or habitual activity. The more recent work of Ama et al (1990) has done so indirectly by recruiting West African and Caucasian subjects who were living under similar environmental conditions in Canada. Of course this makes no allowance for previous conditions or environment. Sedentary subjects were recruited in order to remove any possible effect of training status and therefore to further isolate racial differences. These studies were undertaken in West Africans. West Africans are considered to be power/sprint athletes and therefore the emphasis of this paper was on anaerobic performance. This was found to be significantly different between the racial groups. Peak cycling power was greater in the African subjects than in the Caucasian subjects, but fatigue was more rapid and therefore the African subjects had a lower total work output in 90 sec. Differences between the two groups in aerobic variables are summarised in Boulay et al (1988). Mechanical economy (efficiency) results were contrary to the studies of East Africans. Using a cycle ergometer test, African subjects were reported to require more oxygen for a given workload, implying poorer economy. This may help explain their considerably poorer performance in the 90-min work-output test. However, possible

explanations for the poorer economy of the African subjects include lack of familiarity with the mode of testing and also a lower training status than the Caucasians, who had a higher VO_2max . This was despite all subjects reportedly being sedentary.

Boulay et al (1988) reviewed the available data comparing work capacity in different races and concluded that small differences in maximal aerobic power between racial groups can usually be explained by factors such as size and habitual activity but that differences in submaximal endurance performance and economy warranted further investigation.

Bosch et al (1990) compared African and Caucasian marathon runners resident in South Africa. As in the earlier South African studies, the Caucasian runners were considerably taller and heavier than the African runners. When corrected for body mass, VO_2max was not different between groups. Runners ran a simulated marathon at the same percentage of their best marathon time (mean 87%) on an indoor treadmill. The main finding was that the African runners ran at a higher percentage of VO_2max (76% vs 68%) and had a higher RER (0.96 vs 0.91). Plasma lactate concentration was insignificantly lower in the African runners (1.3 mmol/l) when compared to the Caucasians (1.6 mmol/l). Running economy was not different between the two groups, with Africans and Caucasians utilising 49.9 and 51.0 ml $\text{O}_2/\text{kg}/\text{min}$ respectively at 16 km/hr. However, this measurement was made after only 90 sec and it is doubtful whether a steady state had been achieved.

Coetzer et al (1993) compared elite African and Caucasian athletes in South Africa. In an attempt to recruit the best athletes, the resultant two groups were quite diverse with respect to primary running distance. Caucasian runners tended to be middle distance runners whilst the African runners were longer distance runners. This may explain some of the findings. VO_2max , V_E and submaximal economy were not different between groups when corrected for body mass. Nor was there a difference in percent fibre type composition in the vastus lateralis although type II muscle fibres were not differentiated into type IIA and IIB. However, African athletes did exhibit a lesser plasma accumulation of lactate during exercise and were able to resist fatigue longer during a repeated isometric test (6 sec MVC interspersed with 4 sec rest). These results may be explained by the fact that the African athletes were more 'endurance' athletes. Surprisingly, despite this the African athletes were reportedly training at high intensity more often than the Caucasians (36 vs 13% of weekly training at $>80\%$ VO_2max , respectively), although with similar training volumes. This alone may explain some of the results of this study.

Recently, Saltin et al (1995b) measured aerobic capacity in 42 Kenyans (active boys and junior and senior runners) and 12 elite Scandinavian runners. When comparing the seniors

of Kenya and Scandinavia, there were some interesting results. $VO_2\text{max}$ was not different, however the Kenyans accumulated less plasma lactate and ammonia during submaximal and maximal work. Plasma lactate concentration was also lower after a cross-country race. At a treadmill running speed of 16 km/hr and inclination of 2.8%, the Kenyan runners were 5-15% more economical with respect to oxygen consumption (i.e. required less oxygen to carry out the given workload). This speed elicited a VO_2 near maximal in the Scandinavians but only amounted to 85-90% of $VO_2\text{max}$ in the Kenyans. Therefore, one would expect that the speed required to elicit the same percentage of $VO_2\text{max}$ would be higher in the Kenyans. In summary, the superior endurance performance of the Kenyans may be related to either greater economy or to decreased plasma lactate and ammonia accumulation, or both.

Differences in work capacity have also been reported between Andean natives and Caucasians (Hochachka et al, 1991). These are reviewed in more detail together with their skeletal muscle characteristics in chapter 2.44.

In summary, there appears to be very little racial difference in maximal aerobic power when values are corrected for body size. However, there is some, although not entirely convincing, evidence for better economy and a lesser accumulation of plasma lactate in Africans of East African origin. At this point, systematic studies of these parameters in sea level African and Caucasian athletes of similar performance and body mass are still lacking.

2.43 GENETIC VARIATION IN SKELETAL MUSCLE

Acknowledging that genotype is at least a partial determinant of endurance performance, particularly when measured by sub-maximal work capacity (chapter 2.41), the next question is to isolate which physiological mechanisms contributing to endurance performance, may have an underlying genetic predisposition. One area of particular relevance to this thesis is the biochemical composition of skeletal muscle, which has previously been implicated in endurance performance (Gollnick & Saltin, 1982, chapter 2.1).

Skeletal muscle characteristics have long been recognised to contribute to endurance performance. In particular high oxidative enzyme activities, and to a certain extent a high percentage of type I fibres, have been associated with superior endurance performance (Lortie et al, 1985; Gollnick et al, 1972; Holloszy and Coyle, 1984) These characteristics and their association with endurance performance were discussed in more detail in Chapters 2.11 & 2.12.

Limited studies have investigated the heritability of skeletal muscle characteristics. Howald (1976) investigated the ultrastructure and biochemical function of skeletal muscle in 17 pairs of twins. The investigators statistically compared mean intra-pair differences between dizygotic and monozygotic twins. There was no statistical difference between monozygotic and dizygotic twins for mitochondrial volume density or mitochondrial membrane surface densities nor for the activity of enzymes HK and SDH. However, GAPDH and 3-HAD were more closely correlated within monozygotic pairs than within dizygotic pairs ($p < 0.05$, $p < 0.01$ respectively). This finding implies that the activities of the latter two enzymes may be considerably more genetically determined than the others. As mentioned in the previous chapter 2.41, two sets of monozygotic twins had particularly diverse lifestyles which may have exaggerated the intra-pair difference. Although these two pairs were excluded from the analysis for $VO_2\text{max}$, this is not reported for the muscle variants. It is possible therefore that the results under-estimate the level of genetic determination of the enzyme activities measured in this study. CS and PFK activities were not measured.

Komi and co-workers (1977) investigated the heritability of fibre type and skeletal muscle enzymes in 31 pairs of twins and reported a very high heritability coefficient for fibre type ($r=0.93$). In contrast, there was no evidence of heritability of any of the enzyme activities measured. However, subsequent studies have provided conflicting evidence:

Bouchard et al (1986) combined the monozygotic and brother data of Lortie et al (1986) with their own dizygotic data to calculate the heritability coefficient for skeletal muscle fibre type

distribution and enzyme activities. Notably, data were adjusted for age and gender where appropriate. Using the Newman et al (1937) calculation of heritability coefficient described earlier, the following enzyme activities were significantly influenced by genetics:

Table 2.12 Heritability of skeletal muscle enzyme activities

Variable	Heritability Coefficient
PFK	0.30
OGDH	0.42
PFK/OGDH	0.26
3-HAD	0.56
MDH	0.63
LDH	0.67

Subsequent comparison of brothers and dizygotes showed that brother intra-class coefficients were higher than those of dizygotes for some variables and in some cases the dizygote resemblance was near zero. Hence these data must be interpreted with caution. There was little evidence for any genetic effect on fibre type proportions or fibre cross-sectional area. The suggested plasticity of fibre type between type IIA and IIB may be a factor. Similarly, relatively large variation between biopsy sites may be important as was discussed in more detail in Chapter 2.11. In summary, this study provides some evidence for a limited measurable genetic effect on enzyme activities. This inheritability of skeletal muscle enzyme activities does not appear to be of the magnitude of the inheritability of submaximal work capacity and therefore is unlikely to be solely responsible, however it certainly can not be ruled out as one of the contributory mechanisms.

The authors of a recent NMR spectrometry study suggest some indirect, but inconclusive, evidence for genetic endowment of muscle characteristics. Park et al (1988) showed the quantitative properties of muscle metabolism (ATP, phosphocreatine, P_i and pH) were advantageous even in the *non-trained* muscles (wrist flexors) in world-class endurance athletes when compared to sedentary controls during a submaximal exercise test involving wrist flexion. Although this tends to indicate that the qualities of endurance trained muscle are inherently present even in the untrained muscle, it can not categorically be recognised as a genetic predisposition since the study design could not control for systemically-induced adaptations to the habitual endurance training.

With respect to the genetic determination of the *trainability* of skeletal muscle biochemical characteristics, two studies predominate:

Simoneau et al (1986) investigated the genotype dependence of response to training of the skeletal muscle in 14 pairs of monozygotic twins. Dizygotic twins and siblings were not investigated. The training programme involved high intensity continuous and interval training. The results showed marked inter-individual variation in response to training with significant intra-twin correlations for change in CK, HK, LDH, MDH, OGDH and PFK/OGDH ratio. Interestingly, this study also indicated a transformation between type II and type I fibres (see chapter 2.11).

Hamel et al (1986) also investigated the trainability of skeletal muscle enzyme activities in twins (n=6). The changes in enzyme activities in the later half of the endurance training programme (7-15 weeks) appeared to be more genotype dependent than the changes in the first 7 weeks, with change in PFK, MDH, HAD and OGDH showing sizeable intra-pair correlations coefficients. Over the entire training programme, 3-HAD showed the greatest degree of intra-pair correlation ($r=0.69$) with this being as high as $r=0.89$ over the second 7-week period. It is difficult to explain why the intra-pair variability is so vastly different between the two 7-week periods, however, absolute change in 3-HAD activity did occur during both periods. No genotype dependence was observed with respect to change in fibre type and contrary to the findings of Simoneau et al (1986) there was no evidence of fibre type transformations.

The results of this study must be interpreted with caution for 2 reasons a) the small number of twins and b) the absence of dizygotic twin data for comparison.

In summary, there appears to be some evidence of heritability of baseline skeletal muscle enzyme activities but this is by no means conclusive or sizeable. It appears that for the enzyme activities measured in the studies mentioned above, that 3-HAD in particular, is significantly genetically determined. There also appears to be genetic determination of the response of some skeletal muscle enzyme activities to training. A relatively high coefficient of variation for enzyme activity analysis from repeated biopsies remains an inherent problem in the interpretation of this data. In their recent review regarding the heritability of baseline fibre type, Simoneau and Bouchard (1995) conclude that the genetic component accounts for as much as 40 - 50% of the variability in the proportion of type I muscles in human muscles with as much as 15% of the variance explained by sampling variance (see chapter 2.11).

2.44 RACIAL VARIATIONS IN SKELETAL MUSCLE

Very few studies have investigated racial variation in skeletal muscle characteristics and the two most meaningful studies were carried out in individuals of West African origin.

Ama et al (1986) investigated the differences in skeletal muscle characteristics in sedentary West Africans and Caucasians living in Canada. The results indicated a slightly higher percentage (8%) of type II fibres (67.4 vs 59.1, $p < 0.01$) and higher anaerobic enzyme activities (~30-40%) in the West Africans. Oxidative enzyme activities were no different. Without single fibre analysis, it is not possible to know to what extent the great difference in enzyme activities was due to the difference in fibre type, but because of the disproportionate difference, the difference in enzyme activities is unlikely to be explained by fibre type alone. The fact that these differences occurred in the untrained populations, in individuals with similar current lifestyles, tends to suggest a genetic component. The authors concluded that these results "suggest that Black individuals are in terms of skeletal muscle characteristics, well endowed for sport events of short duration". This statement is, in fact, only true of Africans of West African origin, similar to those in the sample, and does certainly not apply to individuals of East African origin!

Results reported by Levesque et al (1994) are somewhat to the contrary, showing no difference between West African and Caucasian subjects with respect to percentage type I, IIA and IIB fibres prior to training (Caucasian 60%, African 62% type I). Following a 12 week training programme (knee flexion/extension), there was a transition from IIB to IIA in both groups with both responding similarly.

Only two studies to date have documented skeletal muscle characteristics in endurance athletes of East/South African origin:

Coetzer et al (1993) measured fibre type proportions in vastus lateralis in South African (East African origin) African and Caucasian distance runners and found no statistically significant difference (63.4 ± 13.3 , 53.3 ± 4.5 % type I respectively). Unfortunately cross-sectional area and the relative IIA and IIB fibre type proportions were not measured.

Saltin and co-workers (1995a) recently undertook the only study to date to investigate skeletal muscle biochemical characteristics in Africans and Caucasians. These investigators compared Kenyan and Scandinavian runners after the Scandinavians had spent 2 weeks at altitude, and the results of the senior runners are considered here. Fibre type proportion of vastus lateralis was not different between Kenyan and Scandinavian runners (72.5 vs 67.7%

type I fibres) and neither was cross sectional area (4.96 vs $4.94 \times 10^3 \mu\text{m}^2$) or capillarisation different. In the vastus lateralis, CS activity was similar whilst 3-HAD activity was 20% higher in the Kenyans (55.8 vs $45.0 \mu\text{mol}/\text{min}/\text{gm d.w.}$, $p < 0.05$). In the gastrocnemius, 3-HAD activity was 50% higher in the Kenyans. Skeletal muscle buffering capacity was 11% higher in Scandinavians prior to their altitude training when compared to Kenyans, and this was increased to 15% higher ($p < 0.05$) after their training camp at altitude. The authors do not report if there was any relationship between any of the measured enzyme activities and skeletal muscle buffering capacity, or whether skeletal muscle buffering capacity was related to fibre type proportions.

Recent DNA polymorphism studies comparing Caucasians from Canada and South Africa and Africans from South Africa have yielded some interesting results. While all groups had similar restriction fragment length polymorphism, the allele frequencies were distinctly different. Canadian and South African Caucasians had virtually identical allele frequencies for muscle adenylate kinase, phosphorylase and subunit Va of cytochrome c oxidase genes whilst the South African Africans had markedly different frequencies and in fact a unique DNA sequence variant was present in two subjects (Adjoa et al, 1996). This serves as an example of racial, not geographical distinction in a muscle genotype.

In addition to the studies detailed above comparing African and Caucasian individuals, several studies have compared the Andean natives with Caucasians. The Andeans are high altitude dwellers who have displayed differences in work capacity and metabolism when compared to Caucasians and these observations warrant further investigation. Specifically, the Andeans accumulate less lactate for a given work rate at altitude than lowlanders exercising at the same work rate at sea level (Hochachka et al, 1991). This could not have been due to a higher training status of the Andeans as in fact they were less trained than the lowlanders, although habitually active. This paradox did not disappear after the Andeans had been removed from altitude for six weeks and retested at sea level, and therefore the authors concluded that this paradox "is a developmentally or genetically fixed characteristic of these Andean natives". Furthermore, their work capacity during plantar flexion was significantly higher than Caucasian sedentary individuals, power athletes and endurance athletes. In addition, the change in PCr from rest to exercise was less for a given amount of work in the Andean natives (Matheson et al, 1991). The metabolic changes (measured using NMR) have a controlling role in the relative use of different metabolic pathways and are likely to inhibit the activation of glycolysis, resulting in a lower production of lactate. Thus it appears that a given amount of work results in a lesser perturbation of the intramuscular environment due to a tighter control of ATP synthesis with respect to ATP utilised. It is tempting to speculate that the above findings may effectively represent a "case study" of the

"tighter metabolic control" theory described by Gollnick & Saltin (1982). The cause of the difference between the Andean Indians and the Caucasians was assumed to be in response to the chronic hypobaric hypoxia experienced by the Andeans but may be a racially-based difference. Alternatively, many generations of a race group who have been habitually exposed to hypobaric hypoxia may have developed a racially-distinct adaptation to the environmental stress, ultimately resulting in a unique genotype feature.

In summary, it appears from the available literature summarised in the preceding four sections that genetic factors do contribute to endurance performance. Furthermore, it appears that certain differences may occur between groups of individuals of different racial origins although whether these occur as a result of genetic or environmental causes is not yet clear. Very few studies have investigated racial differences under similar habitual environmental conditions.

CHAPTER 2.5

SUMMARY OF LITERATURE AND STATEMENT OF AIM

It is clear from the literature summarised above that many factors influence endurance performance. The extent to which these factors are genetically determined is still unclear despite numerous studies. There is sufficient evidence to say that at least a component of endurance performance is genetically determined. In recent years the dominance of East African athletes in distance running events has become increasingly evident but the physiological mechanism for this superiority is unknown. Nor do we know if this ability is genetically determined. In addition, the relationship between mitochondrial oxidative enzyme activities and submaximal resistance to fatigue is clearly not well understood, particularly in well-trained individuals.

Therefore, the aim of the current series of studies is to compare selected metabolic and skeletal muscle characteristics in well-trained African and Caucasian distance runners, to determine if evidence of racial distinction is present in the individuals investigated. A racial comparison may serve to highlight differences between the racial groups that warrant more detail genetic investigations. In an effort to overcome the methodological problems of previous studies, great care was taken to 1) test only subjects within a relatively narrow range of 10 km performance, 2) to ensure adequate and similar current training status 3) to recruit subjects living in the same geographical area at sea level and 4) to minimise body mass differences when appropriate.

CHAPTER THREE EXPERIMENTAL WORK

CHAPTER 3.1

FATIGUE RESISTANCE AT A HIGH PERCENTAGE OF PEAK RUNNING VELOCITY IN AFRICAN AND CAUCASIAN DISTANCE RUNNERS

RATIONALE AND AIM

The findings of Coetzer et al (1993) indicated a greater resistance to fatigue in African runners compared with Caucasian runners, despite comparable VO_{2max} . The test used to quantify resistance to fatigue was repeated isometric contractions of the quadriceps muscles but fatigue during this type of test may not be representative of fatigue occurring during distance running. At two submaximal running workloads (17 km/hr and 21 km/hr), oxygen consumption was no different between the groups although plasma lactate concentrations were significantly lower in the African runners. This suggests that there may be a difference between South African African and Caucasian runners in the plasma accumulation of some of the metabolites involved in fatigue. However, the African and Caucasian groups compared in that study were not well matched for peak force developed during a maximum voluntary contraction (a measurement critical to the fatigue test). Furthermore, a partial explanation of the greater resistance to fatigue in the African group may be that they were predominantly long distance runners compared with the Caucasian athletes who were predominantly middle distance track athletes. Thus, either pre-selection for the event in which they were successful, or subsequent adaptations as a result of different training methods may have biased the results.

Therefore, the primary aim of this study (3.1) was to investigate fatigue resistance using a dynamic, running test protocol in groups of African and Caucasian sub-elite runners. Subject groups were matched for primary running distance with a similar mean level of performance in 10 km races. In South Africa, it is not possible to recruit sufficient elite Caucasians for comparison with elite Africans.

Secondly, the study also aimed to investigate plasma lactate concentrations at the same relative running intensities. Some previous studies have indicated that Africans accumulate less plasma lactate than their Caucasian counterparts (Coetzer et al, 1993; Saltin et al, 1995b) whilst others have shown no difference (Bosch et al, 1990). However these studies have had inherent methodological limitations as previously discussed and therefore this matter warrants further investigation.

The hypothesis to be tested in the current study, was that African 10 km runners have an enhanced ability to resist fatigue during high intensity running when compared to Caucasian 10 km runners, and that this ability is related to selected metabolic characteristics.

METHODS

Subjects:

Fifteen sub-elite distance runners were recruited from local running clubs for this study. All considered 10 km to be their primary racing distance. Subject characteristics are listed in Table 3.11. All subjects were seasoned competitors (> 3 years competitive running). All subjects were informed of the possible risks of the experimental procedures and all gave their written informed consent. The study was approved by the Ethics and Research Committee of the Faculty of Medicine, University of Cape Town.

Table 3.11 **Subject characteristics**

Subject	Racial group	Age (yrs)	10 km time (min)	Training volume (km/wk)
1	Xhosa	29	34.0	120
2	Xhosa	31	34.6	100
3	Xhosa	36	32.5	120
4	Xhosa	18	32.5	80
5	Zulu	28	34.0	60
6	Xhosa	20	30.6	100
7	Xhosa	18	32.0	120
8	Xhosa	16	35.3	60
Mean		24.5	33.2	95
SD		7.4	1.6	25
9	Caucasian	27	32.0	120
10	Caucasian	25	32.3	100
11	Caucasian	34	34.9	65
12	Caucasian	33	37.3	70
13	Caucasian	26	33.2	100
14	Caucasian	25	34.3	95
15	Caucasian	29	35.5	62
Mean		28.4	34.2	87
SD		3.7	1.9	22

Procedure:

Prior to the study, all subjects came to the laboratory to familiarise with the testing procedures, in particular the use of the treadmill and breathing mask.

Anthropometry:

Height and weight were measured and four skinfold measurements were made (tricep, bicep, suprailliac, subscapular) and % body fat was calculated using the formula of Durnin and Wolmersley (1974).

VO₂max/Peak treadmill velocity test:

Subjects completed a peak treadmill velocity test (Powerjog EG30, Birmingham, England) with concurrent measurement of oxygen consumption (VO₂), minute ventilation (V_E), respiratory exchange ratio (RER) and heart rate (HR). Gas analysis equipment (Oxycon Alpha, Jaeger Mijnhardt, Wuertzburg, The Netherlands) was calibrated prior to each test using a certified known gas mixture and the pneumotach was calibrated with 3 litre calibrated syringe. After a 5 minute warm up at 14 km/hr, the testing protocol commenced at 14 km/hr with 1km/hr increments in velocity every minute until volitional exhaustion. Peak treadmill velocity (PTV) was designated as the last velocity that was maintained for a full 60 sec. At exactly 3 minutes after exercise, a venous blood sample was obtained for determination of plasma lactate concentration. Samples were immediately centrifuged at 4°C at 3000 rpm and plasma was obtained and frozen for later spectrophotometric analysis (Beckman DU-62, Beckman Industries Inc, USA) (see Appendix 6.2). A pilot study was carried out to determine the most suitable time for sampling blood to ensure that the peak plasma lactate concentration was obtained (see Appendix 6.1). Results were in agreement with the earlier findings of Bassett et al (1991), indicating that the most suitable time to determine peak plasma lactate concentration in trained individuals, is 3 min after maximal exercise.

Fatigue resistance test:

On a third visit to the laboratory, subjects completed the following discontinuous test, comprising four workloads. A 20-gauge cannula (Jelco, Critikon, Florida, USA) was inserted into a forearm vein and was flushed with heparinised saline. Subjects warmed up for 5 minutes at 14 km/hr prior to completing 4 consecutive workloads. These workloads were selected after pilot trials to determine those workloads most suitable for runners of the calibre expected for this study. The aim was that the first three workloads could all be sustained for five minutes each, prior to proceeding to the fourth workload which was continued until fatigue. Subjects exercised for 5 minutes at 72% (workload 1), 5 minutes at 80% (workload 2) and five minutes at 88% (workload 3) of the pre-determined peak treadmill velocity (PTV).

VO₂, V_E, RER and HR were measured throughout exercise. Running economy was calculated during the last minute of each workload and expressed as ml of oxygen utilised per kg of body mass at the measured speed in km/hr. Between workloads, the subject ceased running for 1 minute while a venous blood sample was obtained at 30 seconds for subsequent analysis of plasma lactate concentration (see Appendix 6.2). Exactly one minute after the completion of the third workload, the subject began the final workload in which he ran at 92% of his peak treadmill velocity until exhaustion (workload 4). A venous blood sample was obtained at 30 sec after exercise for determination of plasma lactate concentration. Total "time to fatigue" (TTF) is reported as the total exercise time (ie. 15 min + time sustained at workload 4).

The repeatability of the total time to fatigue achieved using the above-mentioned exercise protocol was estimated by calculating the coefficient of variation following repeated trials in three individuals. The mean coefficient of variation was 2.0%. The individual results are reported in Appendix 6.12.

Statistics

The Student's unpaired t-test was used to compare African and Caucasian runners and the Pearson's correlation coefficient was used to investigate relationships between time to fatigue and metabolic variables for all individuals. Correlations were not calculated *within* groups due to insufficient numbers.

RESULTS

African runners were shorter and lighter but had a similar level of body fat as their Caucasian controls. Anthropometric data are given in Table 3.12.

Table 3.12 Anthropometric results

	African	Caucasian	significance
Height (cm)	171.3 ± 5.7	180.6 ± 9.4	p<0.05
Mass (kg)	60.1 ± 6.1	69.3 ± 6.2	p<0.05
% body fat	11.5 ± 2.9	12.8 ± 2.6	ns

Results of the maximal treadmill exercise test are given in Table 3.13.

Table 3.13 Maximal exercise test results

	African	Caucasian	significance
Peak treadmill velocity (km/hr)	21.3 ± 2.0	22.0 ± 1.5	ns
VO ₂ max (ml/kg/min)	61.7 ± 6.3	63.9 ± 6.7	ns
VO ₂ max (ml/kg ^{0.66} /min)	247.8 ± 21.1	269.6 ± 28.4	p=0.06
HRmax (beats/min)	190 ± 7	190 ± 6	ns
Plasma [lactate] (mmol/l)	10.3 ± 3.2	11.7 ± 3.1	ns
RERmax	1.10 ± 0.06	1.14 ± 0.06	ns

Considering this homogeneous group of runners as a whole, peak treadmill velocity was correlated with personal best 10 km time ($r = -0.61$, $p < 0.05$, Fig 3.11) but not at all with VO₂ max ($r = -0.24$, ns).

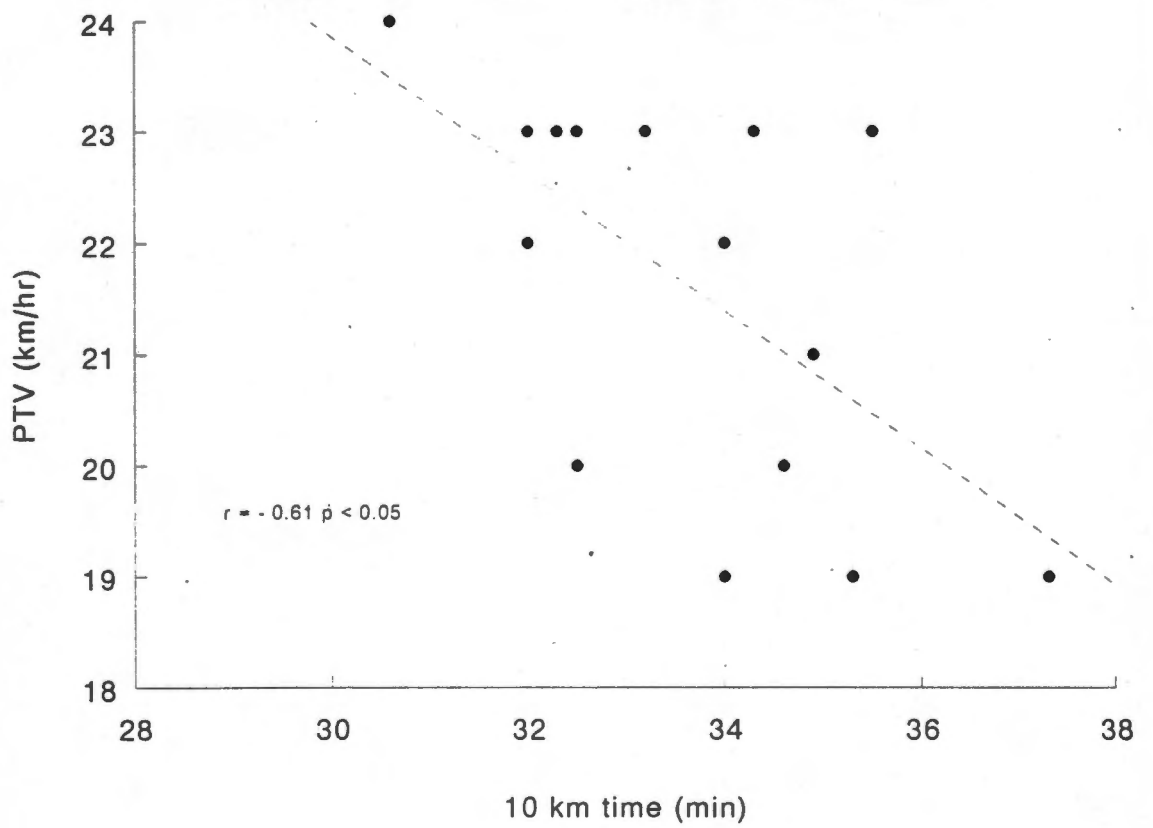
The results of the first three submaximal workloads are presented in Table 3.14.

Table 3.14 Submaximal exercise test results

	African (n=8)	Caucasian (n=7)	significance
<i>Workload 1 (72%):</i>			
HR (beats/min)	152 ± 18	154 ± 19	ns
RER	0.91 ± 0.04	0.89 ± 0.06	ns
Plasma [lactate] (mmol/l)	2.3 ± 0.9*	2.4 ± 0.7	ns
<i>Workload 2 (80%):</i>			
HR (beats/min)	163 ± 15	166 ± 15	ns
RER	0.94 ± 0.05	0.93 ± 0.06	ns
Plasma [lactate] (mmol/l)	2.9 ± 2.5**	4.4 ± 2.0	ns
<i>Workload 3 (88%):</i>			
HR (beats/min)	176 ± 13	175 ± 12	ns
RER	0.97 ± 0.07	0.98 ± 0.07	ns
Plasma [lactate] (mmol/l)	4.8 ± 3.2	7.7 ± 2.8	p<0.05

* and ** denotes n=7 and n=6 respectively as a result of insufficient blood obtained within the one minute interval between workloads.

Fig 3.11 Relationship between 10 km race time and Peak Treadmill Velocity



African runners had considerably lower plasma lactate concentrations at workloads 2 & 3 although the difference was more pronounced and statistically significant only at workload 3. RER was not different at any of the workloads.

When relative economy was calculated at each submaximal workload, the Africans showed a tendency to be more economical at all workloads than their Caucasian counterparts, with the mean running economy values being remarkably consistent over the range of workloads. Although this did not reach significance at any individual submaximal workloads, when oxygen uptake was normalised to $\text{kg}^{-0.66}$ rather than per kg as suggested by some authors (Astrand & Rodahl, 1976; Fredericks, 1987), the economy was significantly better in the Africans at all workloads. Calculated running economy is presented in Table 3.15.

Table 3.15 Running economy at submaximal intensities

Workload	African	Caucasian	significance
1*	3.03 ± 0.40	3.20 ± 0.20	ns
2*	3.00 ± 0.42	3.18 ± 0.26	ns
3*	2.97 ± 0.31	3.19 ± 0.25	ns
1#	12.1 ± 1.5	13.5 ± 0.9	p<0.05
2#	12.1 ± 1.5	13.4 ± 1.1	p<0.05
3#	11.9 ± 1.1	13.5 ± 1.1	p<0.01
*	Economy calculated from VO_2/speed (ml/kg/min)/(km/hr)		
#	Economy calculated from VO_2/speed (ml/kg ^{0.66} /min)/(km/hr)		

There was a marked difference between the two groups in the mean time sustained at workload 4 with African runners continuing to exercise at this high intensity for considerably longer than their Caucasian counterparts ($p<0.01$). This four minute difference represents a 21% greater resistance to fatigue with respect to total exercise time (1376 vs 1137 sec, $p<0.01$) and a 98% greater resistance to fatigue with respect to the time for final high intensity workload alone. Mean results for workload 4 are listed in Table 3.16 and for the total fatigue test in Figure 3.12.

Fig 3.12 Time to Fatigue in African and Caucasian runners

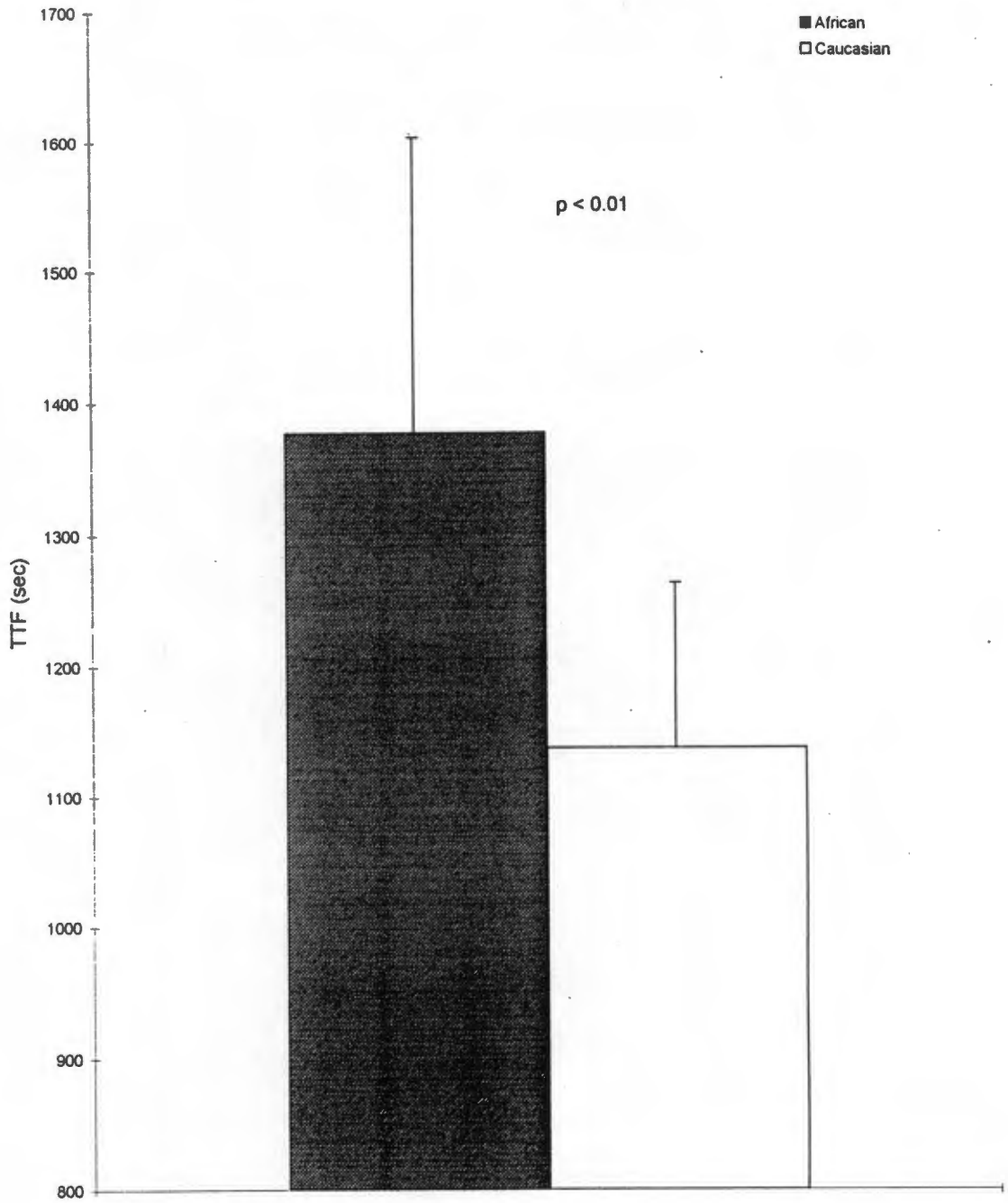


Fig 3.13 Relationship between Time to Fatigue and plasma [lactate] at 88% PTV

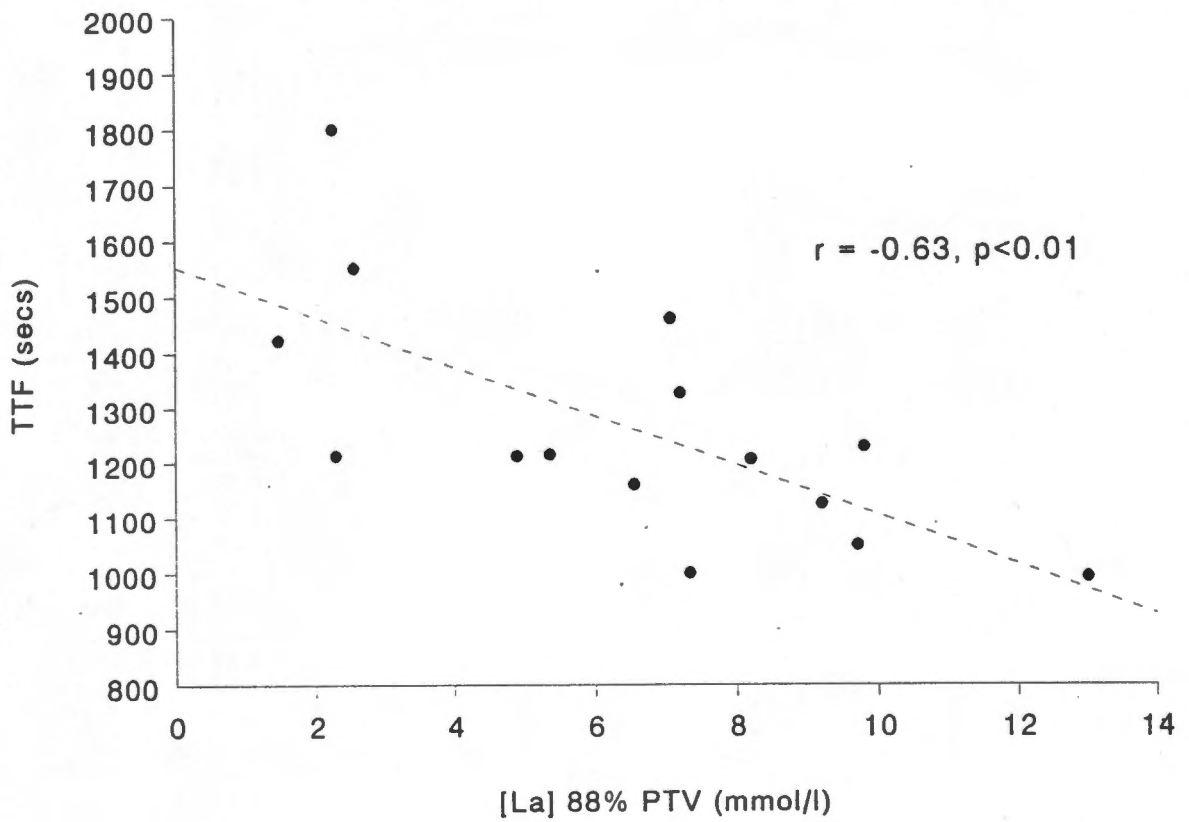


Table 3.16 Final workload of fatigue resistance exercise test (92%)

	African	Caucasian	significance
Heart Rate (beats/min)	188 ± 7	185 ± 7	ns
%max HR	99.2 ± 3.8	97.4 ± 4.4	ns
Plasma [lactate] (mmol/l)	7.2 ± 4.8**	11.1 ± 3.5	p=0.06

** denotes n=6 as a result of insufficient blood obtained within one minute after cessation of workload 4. The HR reported above was obtained within the final 15 secs of this workload.

The relationship between total time to fatigue and metabolic parameters was investigated using the data from both the 88% PTV exercise bout, which was of equal duration for all subjects, and the metabolic data of the 92% PTV bout which was of variable length for all subjects and far from steady state. In addition, plasma lactate concentration was available on all individuals at the 88% PTV workload and therefore those results are reported here. The 88% PTV exercise bout immediately preceded the time to fatigue run. In the fifteen subjects studied, total time to fatigue was significantly correlated with plasma lactate concentration ($r = -0.63$, $p < 0.01$, Fig 3.13) and RER ($r = -0.53$, $p < 0.05$) during the 88% PTV. The plasma lactate concentration result obtained immediately after cessation of the 92% PTV workload showed a similar relationship to that at 88% PTV ($r = -0.76$, $p < 0.01$, $n = 13$).

DISCUSSION

Previous reports of elite African distance runners (including South African world class marathoners, Zithulele Sinqe and Willie Mtolo), have indicated that some have achieved considerable success despite relatively low $VO_2\text{max}$ values (Noakes, 1986). This dissociation between $VO_2\text{max}$ and performance has been previously reported in homogeneous groups of well-trained runners (Conley et al, 1980; Morgan et al, 1989) emphasising that whilst a high $VO_2\text{max}$ is required for elite distance running performance it is certainly not the sole determinant of performance. Indeed, in the only published investigation of the highly successful Kenyan distance runners to date, $VO_2\text{max}$ was reported to be very similar to that of less successful Scandinavian runners. Hence, $VO_2\text{max}$ alone can not explain the superior performance of the Kenyans (Saltin et al, 1995a). The lack of correlation between $VO_2\text{max}$ and 10 km race time in the current study further supports this conclusion. Thus, other factors must contribute to successful distance running performance in these populations. The metabolic results of the current study suggest mechanisms that may be responsible for these findings.

The main finding of the intergroup comparison in the current study was that African runners had a higher resistance to fatigue at the same percentage of peak treadmill velocity than did

Caucasian runners with similar VO_2 max values. This finding is in line with those of Coetzer et al (1993) who found greater isometric fatigue resistance in African South African distance runners. The advantage of the current study is that it investigated fatigue resistance during a running task designed to closely represent the fatigue occurring during a high intensity distance running event. The cumulative time of the running test was between 17 and 30 minutes, which is approximately equivalent to the duration of a 6-10 km race. Furthermore, subjects were all distance runners currently in training for a 10 km race and were relatively homogeneous with respect to their range of 10 km performances (30 -37 min). Therefore, several of the limitations of the previous study (Coetzer et al, 1993) have been overcome whilst strengthening their conclusions.

The difference in fatigue resistance between the African and Caucasian runners in the current study was pronounced, with mean time sustained at the final workload being 98% longer in the African runners when compared to the Caucasian runners. Since the workload was set relative to the Peak Treadmill Velocity (PTV), it could be argued that the maximal test of the African runners was not a truly maximal effort, in which case 92% PTV would represent a somewhat lower relative intensity in the Africans when compared to the Caucasians. However, this is unlikely since all maximal test results (RER_{max} , HR_{max} , $[La]_{max}$) were similarly high in both groups.

An alternative explanation, and indeed one which proposes a physiological mechanism that could account for the improved resistance to fatigue at the same percentage of peak velocity, was the lesser accumulation of plasma lactate which we observed. The African runners showed a lower accumulation of plasma lactate at all steady state workloads although this was more profound at the higher relative workloads. Furthermore, time to fatigue was significantly correlated with plasma lactate concentration at 88% PTV ($r = -0.63$, $p < 0.01$, Fig 3.13) and 92% PTV ($r = -0.76$, $p < 0.01$). This relationship suggests that the lower accumulation of plasma lactate by the African runners at least partially explains their longer time to fatigue, or is, at least a marker of a metabolic difference that explains their superior running ability in this test.

Plasma lactate accumulation during submaximal work at a given relative intensity has previously been shown to be related to training status (Saltin, 1969; Jacobs, 1986). In the current study however, all runners were of a similar standard, had all been in regular training with local running clubs for many years and there was no difference in mean weekly training distance between groups. Therefore, it is unlikely that the lesser accumulation of plasma lactate of the African runners is the result of a difference in current training status *per se*.

Regardless of the cause of lower plasma lactate accumulation, there are functional consequences of this metabolic characteristic for muscle function. The recent study of Favero et al (1995) in rabbit muscle shows that increasing the concentration of lactate inhibits SR Ca⁺⁺ release, inhibits 3[H]ryanodine binding to the SR and reduces the channel opening probability. These factors have all previously been related to the onset of muscular fatigue *in vitro* (Rosseau & Pinkos, 1990; Westerblad et al, 1991) and therefore the accumulation of lactate *in vivo* may facilitate fatigue by dysfunction of excitation-contraction coupling. The ability to resist fatigue has been related to mitochondrial oxidative capacity in animal models (Davies et al, 1981; Mayne et al, 1991). As we have seen in the current study that fatigue resistance is also related to plasma lactate concentration, it could be speculated that plasma lactate concentration is causal linked to the mitochondrial oxidative capacity. The relationships between fatigue resistance, plasma lactate accumulation and selected oxidative enzymes in these human subjects is described in Chapter 3.4.

When race pace was considered as a percentage of peak treadmill velocity, race pace represented a considerably higher percentage of PTV in the African runners when compared to the Caucasian controls ($85.6 \pm 5.4\%$ vs $80.1 \pm 4.3\%$, $p < 0.05$). Therefore, it appears that the African runners were able to race at a workload approximating workload 3 of the laboratory testing protocol, whilst the Caucasian athletes race at a workload more similar to workload 2. The plasma lactate concentration of the Caucasians after workload 2 was similar to the plasma lactate concentration of the Africans after workload 3. This suggests that the ability to resist fatigue at high relative intensities which enables the African runners to sustain a higher fractional utilisation of their peak treadmill velocity during a race, may be related to plasma lactate accumulation. This warrants further direct systematic investigation during a submaximal exercise bout at race pace.

A further mechanism that may explain the Africans' ability to continue to exercise at a high intensity for longer is an enhanced running economy at the submaximal workloads. This would result in a lesser metabolic demand at any given velocity than in a less economical runner, enhancing the ability to sustain this workload for a longer duration. The ability to sustain exercise could be the result of either less glycogen depletion or lesser metabolic by-product accumulation, dependent upon the duration and intensity of the event. The results indicated a tendency for the African runners to be more economical but this was not statistically significant at individual workloads. As with the lactate results, there was a tendency for this difference to become more pronounced at the higher workloads.

In this study, the groups of subjects were not matched for size which may be a limitation in the comparison of running economy. The exact effect of mass and height on running

economy is not known and the preferred design for comparison would be to match groups with respect to size. However, as economy is regarded as the metabolic power regarded to carry out a particular task, and metabolic power is proportional to $\text{mass}^{-0.66}$ (Fredericks, 1987), it seems appropriate to scale oxygen consumption to $\text{kg}^{-0.66}$, rather than kg^{-1} , as has been suggested by previous authors (Astrand & Rodahl, 1976; Fredericks, 1987). This enhanced the magnitude of the difference to significance at all individual workloads in the current study. In addition, comparison of running economy between groups was made at comparable *relative* intensities, which did not always constitute the same absolute running speed. These limitations are addressed in the investigations conducted in the following chapter.

The importance of the current study is that it unequivocally confirms superior fatigue resistance in African distance runners, during a sustained running task, and strongly suggests several potential mechanisms for further investigation.

CHAPTER 3.2

FRACTIONAL UTILISATION OF MAXIMAL OXYGEN UPTAKE AND RUNNING ECONOMY IN WELL-TRAINED AFRICAN AND CAUCASIAN DISTANCE RUNNERS

RATIONALE AND AIM

Previous studies have indicated that African distance runners race at a higher percentage of VO_2max (Bosch et al, 1990; Coetzer et al, 1993), accumulate less plasma lactate (Chapter 3.1, Coetzer et al, 1993; Saltin et al, 1995b) and less plasma ammonia (Saltin et al, 1995b) than their Caucasian counterparts at comparable workloads. Bosch et al (1990) investigated physiological characteristics of African and Caucasian distance runners whilst they ran a simulated marathon on the treadmill. When running at the same percentage (~87%) of their best marathon time, the African runners ran at a higher percentage of VO_2max (76% vs 68%, $p < 0.05$), a higher heart-rate and a higher RER. Plasma lactate levels were marginally lower in the Africans but this was not significant. In addition, Coetzer et al (1993) extrapolated race VO_2 from the VO_2 vs treadmill speed relationship obtained during the non-steady state incremental exercise test and expressed this as a percentage of maximal oxygen consumption. On this basis, the African runners raced 10 km at a higher percentage of their VO_2max . However, no studies to date have directly measured VO_2 , RER and plasma lactate and ammonia concentrations in the laboratory in African and Caucasian distance runners whilst running *at current race pace*.

In addition, as reviewed in chapter 2.42, some investigators have reported greater economy of movement in African individuals albeit predominantly during stepping tasks (Robinson et al, 1941; Wyndham et al, 1963; Leary & Wyndham, 1965). However, investigations of *running* economy have produced inconclusive results. Coetzer et al (1993) found no difference in economy when expressed relative to body weight. However, these subjects were not matched for primary race distance since the Caucasian athletes were predominantly middle distance runners and the African runners were longer distance runners. Furthermore, the difference in mean body mass was very large (56 kg vs 70 kg, Africans and Caucasians respectively). Saltin et al (1995b) found that Kenyan runners were more economical than Scandinavian runners when there was little difference in mass between the two groups. When VO_2 was normalised per $\text{kg}^{-0.75}$ rather than per kg, as suggested by Svedenhag & Sjodin, 1994, the difference is accentuated. The difference between the groups was larger at the faster speeds. Results reported in the first chapter of this thesis also tended to suggest a difference in running economy that was more profound at higher relative workloads but again these athletes were not matched for size, with the mean height and mass of the Caucasians being greater. Furthermore, comparisons were made at the same relative, not absolute, running velocities. Therefore, it appears that the current literature does not clearly indicate

whether or not there is a difference in running economy between African and Caucasian distance runners. The reasons for the inconsistencies may lie in the use of non steady state oxygen consumption values and poor matching of groups. Clearly it would be preferable to have groups matched for body mass rather than correcting mathematically.

Therefore, the aim of this study was to investigate fractional utilisation of maximal oxygen consumption, running economy and blood lactate and ammonia concentrations, at a steady state absolute workload (16.1 km/hr) and at 10 km race pace in well-trained African and Caucasian groups of runners matched for current 10 km performance. In addition, to minimise the potential effects of body mass on running economy, the groups were matched for body mass.

The hypothesis to be tested by the current study, was that African 10 km runners are able to utilise a higher fraction of their VO_2max when racing when compared to Caucasian runners, and that this ability is related to selected metabolic characteristics. Furthermore, it is hypothesised that African runners are more economical than Caucasian runners.

METHODS

Subjects

Fourteen well-trained distance runners (6 African, 8 Caucasian), with a 10 km race time range of 29.2 - 37.0 min, were recruited for the study from local running clubs. Mean ages were 31.0 ± 4.9 for Africans and 25.9 ± 5.3 for Caucasians. All reported that their primary running distance to be 10 km. The two groups were of the same performance standard with no difference in current 10 km race time (African 33.3 ± 3.1 min; Caucasian 32.0 ± 2.5 min). Every effort was made to recruit African and Caucasian athletes of similar body mass. All subjects were seasoned competitors (at least three years of competitive running) and were currently in training for a 10 km race (108 ± 43 km/wk). All subjects were informed of the possible risks of the experimental procedures and all gave their written informed consent. The study was approved by the Ethics and Research committee of the Faculty of Medicine at the University of Cape Town.

Procedures

Anthropometry:

Height, weight, thigh length and mid-thigh circumference were measured. Five skinfold measurements were made (tricep, bicep, suprailiac, subscapular, mid-thigh) and % body fat calculated using the formula of Durnin and Wolmersley (1974). Lean thigh volume was calculated using the assumption of the thigh as a truncated cone and taking into account the thigh skinfold measurement (Katch & Katch, 1974).

VO₂max/Peak treadmill velocity test:

All subjects had undertaken at least one familiarisation session prior to testing. This included treadmill running at low and high speeds and with and without the face mask. Further sessions were undertaken if deemed necessary in the opinion of the investigator. Subjects then completed a peak treadmill velocity test with concurrent measurement of VO₂, V_E, RER and HR as described in detail in Chapter 3.1. At exactly 3 minutes post exercise a venous blood sample was obtained for determination of plasma lactate concentrations. Samples were immediately centrifuged at 4°C at 3000 rpm and plasma was obtained and frozen for later analysis (see Appendix 6.2).

Submaximal exercise testing:

On a separate day, subjects returned to the laboratory 3 hours after eating. Subjects did not ingest caffeine overnight or prior to the test. Subjects again warmed up at 14 km/hr for 5 minutes. Subjects then completed two submaximal workloads, one at 16.1 km/hr and the other at current 10 km race pace. Each workload was undertaken for 6 minutes separated by a rest period of 5 minutes. VO₂, V_E, RER and HR were measured continuously and results averaged over 15 sec intervals. Mean values of the last 60 sec were designated as steady state values for data analysis. A venous blood sample was obtained exactly 1 minute after exercise for each workload for determination of plasma ammonia and plasma lactate concentrations. Samples were immediately centrifuged at 4°C at 3000 rpm and plasma was obtained. Plasma ammonia was assayed within 2 hours (see Appendix 6.3) and plasma for lactate analysis was frozen for later analysis (see Appendix 6.2).

In additional calculations, running economy was defined as the steady-state oxygen consumption in ml/kg/min obtained at each workload. VO₂ was also normalised per kg^{0.66} (Astrand & Rodahl, 1976; Fredericks, 1987). The fractional utilisation of maximal oxygen consumption (%VO₂max) was calculated.

Statistics

The Student's unpaired t-test was used to compare African and Caucasian runners and the Pearson's Correlation coefficient was used to investigate relationships between variables for all individuals. Correlations were not calculated *within* groups due to insufficient numbers.

RESULTS

Anthropometric results showed no significant difference between the groups in body mass, % body fat or lean thigh volume although the Caucasian subjects were 6 cm taller (Table 3.21).

Table 3.21 Anthropometric results

	African	Caucasian	significance
Height (cm)	172.2 ± 5.7	178.3 ± 5.3	p<0.05
Mass (kg)	62.0 ± 8.0	64.9 ± 3.0	ns
% body fat	13.6 ± 3.0	12.1 ± 3.3	ns
Lean thigh volume (cc)	2152 ± 770	2568 ± 295	ns

Maximal treadmill test results are listed in Table 3.22.

Table 3.22 Maximal treadmill test results

	African	Caucasian	significance
Peak treadmill velocity (km/hr)	21.2 ± 1.5	23.0 ± 1.6	p<0.05
VO ₂ max (l/min)	3.74 ± 0.45	4.55 ± 0.51	p<0.01
VO ₂ max (ml/kg/min)	60.7 ± 7.5	69.9 ± 5.4	p<0.01
VO ₂ max (ml/kg ^{0.66} /min)	245.9 ± 27.0	289.2 ± 25.5	p< 0.005
HRmax (beats/min)	193 ± 4.1	189 ± 5.7	ns
RERmax	1.09 ± 0.05	1.13 ± 0.05	ns
Plasma [lactate] (mmol/l)	9.9 ± 2.6	12.6 ± 2.9	ns

All subjects achieved a RER value of higher than 1.0 and a maximal heart rate of above 90 % of their age-predicted HRmax (220-age). VO₂max was considerably higher in the Caucasian athletes, even when corrected for body mass (15%, p<0.01), however this was *not* transposed to a faster mean 10 km race time. Mean peak treadmill velocity (PTV) was also significantly higher in the Caucasian runners than in the African runners.

Steady state results at 16 km/hr and at calculated 10 km race pace are listed in Table 3.23. At 16.1 km/hr there was no significant difference between the two groups in any of the measured variables although there was a tendency for the African athletes to utilise less oxygen for the same speed (p=0.07). When expressed as kg^{-0.66}, this difference reached significance. Running economy at 16.1 km/hr did not correlate with ten km performance in this homogeneous group. However, the African runners were able to utilise a considerably higher percentage of their maximal oxygen uptake and to exercise at a higher heart rate at 10 km race pace.

Table 3.23 Steady state exercise test results

	African	Caucasian	significance
<i>16.1 km/hr:</i>			
HR (beats/min)	173 ± 13	160 ± 15	ns
RER	0.93 ± 0.03	0.94 ± 0.04	ns
VO ₂ (ml/kg/min)	47.5 ± 3.5	49.9 ± 2.4	p=0.07
VO ₂ (ml/kg ^{0.66} /min)	192.6 ± 13.2	206.2 ± 8.2	p<0.05
VO ₂ /(km/hr)	2.95 ± 0.22	3.10 ± 0.15	p=0.07
%VO ₂ max	77.5 ± 8.6	71.8 ± 7.5	ns
V _E (l/min)	89.4 ± 7.9	89.8 ± 12.1	ns
<i>10 km race pace:</i>			
HR (beats/min)	187 ± 10	174 ± 11	p<0.05
RER	0.98 ± 0.05	0.99 ± 0.06	ns
VO ₂ (ml/kg/min)	56.9 ± 7.8	59.5 ± 5.9	ns
VO ₂ (ml/kg ^{0.66} /min)	230.3 ± 27.2	245.8 ± 26.0	ns
VO ₂ /(km/hr)	3.16 ± 0.43	3.17 ± 0.31	ns
%VO ₂ max	93.5 ± 3.4	86.0 ± 4.8	p<0.005
V _E (l/min)	110.9 ± 17.9	119.2 ± 19.3	ns
% HRmax	97 ± 4	92 ± 7	p=0.09

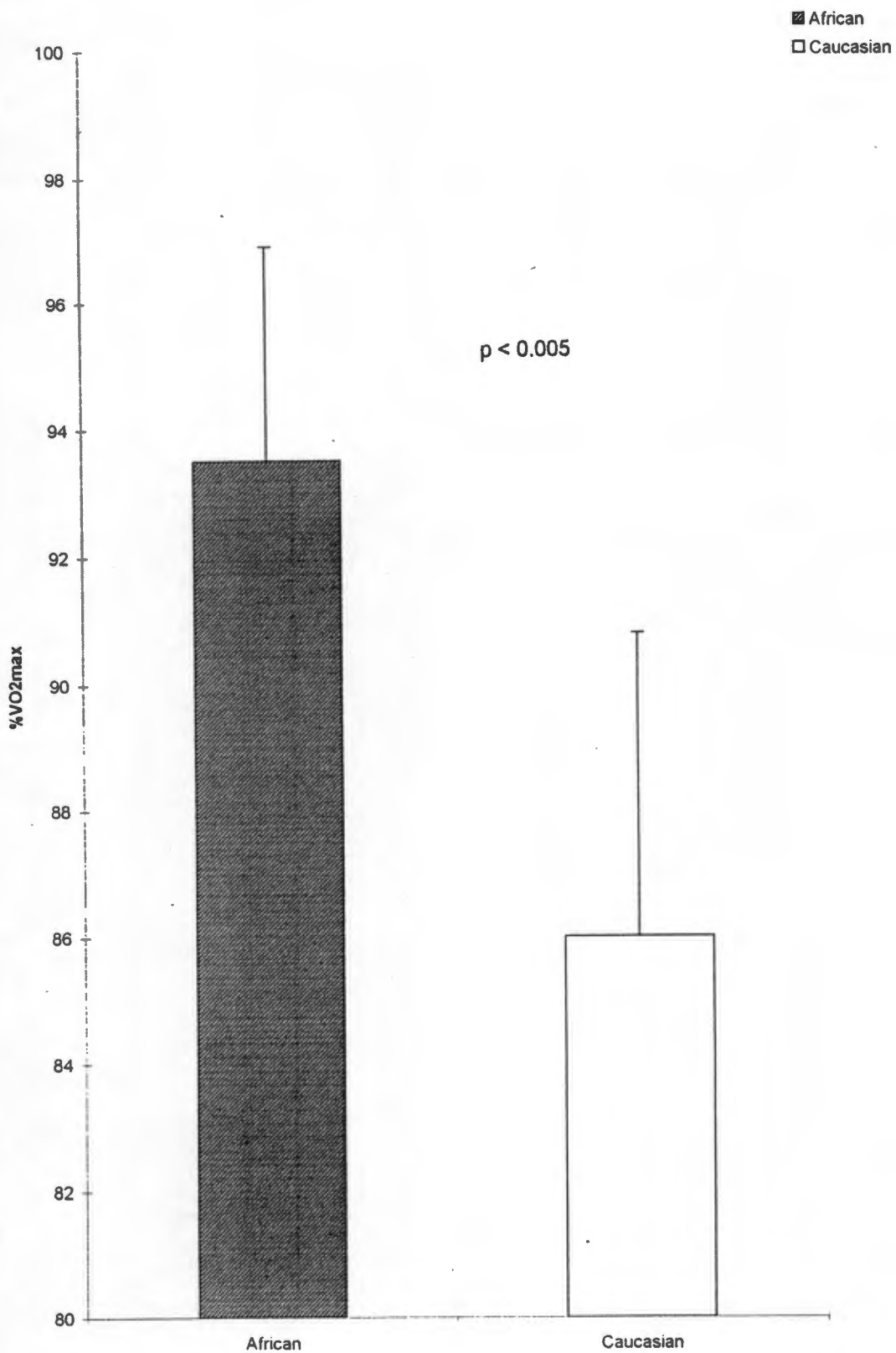
Considerable accumulation of ammonia occurred at race pace (Table 3.25) and this was higher in the African runners than in the Caucasian runners. However, accumulation of lactate in the plasma was not higher in the African runners despite the higher relative exercise intensity.

Table 3.24 Plasma ammonia and lactate concentrations

	African	Caucasian	significance
16.1 km/hr NH ₃ (μmol/l)	71.8 ± 45.6	54.9 ± 6.1	ns
Race pace NH ₃ (μmol/l)	82.9 ± 9.4*	60.3 ± 16.9	p<0.05
16.1 km/hr lactate (mmol/l)	2.8 ± 1.9	2.1 ± 1.6	ns
Race pace lactate (mmol/l)	5.0 ± 2.3	4.2 ± 1.7	ns

* One African individual had a particularly high plasma ammonia (> 2 SD from the mean of the remainder of the group (217 μmol/l)) and therefore has been excluded from the analysis the African mean

Fig 3.21 Fractional utilisation of VO₂max at 10 km race pace



DISCUSSION

In support of the findings of the first chapter, the results of the current study more clearly indicate that the African athletes were able to achieve the same performance as the Caucasian group over 10 km, despite having a considerably *lower* maximal oxygen uptake (15% lower, $p < 0.01$). This was accentuated when scaled to $\text{kg}^{-0.66}$ (18% lower, $p < 0.005$). Factors that may explain such a finding are a) greater running economy or b) the ability to sustain a higher percentage of their VO_2max throughout the event, or a combination of both. These two factors have been systematically investigated in the current study.

The oxygen consumption at 16.1 km/hr (268 m/min) of the subjects investigated in the current study (range 42.7 - 52.6, mean 48.9 ml/kg/min) was comparable to that of other runners of this calibre. Daniels et al (1977) reported a mean VO_2 of 50.5 ml/kg/min in ten highly trained distance runners and Costill et al (1973) a mean of 51.7 ml/kg/min in sixteen highly trained distance runners (range 48.4 - 54.8 ml/kg/min) when running at 16.1 km/hr. The mean VO_2 in the Conley & Krahenbuhl study (1980) was 50.3 ml/kg/min with a range of ~45-54 ml/kg/min.

When VO_2 was normalised per kg, the findings of the current study showed a trend toward greater running economy in the African runners when compared to the Caucasian runners ($p = 0.07$). The difference in mean body mass between the two groups in the current study was relatively small and statistically insignificant (2.9 kg). Running economy is regarded as the metabolic power required to run at a particular velocity and metabolic power has been reported to be proportional to $\text{mass}^{-0.66}$ (Fredericks, 1987). Therefore, when calculating running economy it seems appropriate to scale oxygen consumption to $\text{kg}^{-0.66}$, rather than kg^{-1} . In the current study, when VO_2 is normalised per $\text{kg}^{-0.66}$, the results are accentuated and running economy is significantly better in the Africans than the Caucasians ($p < 0.05$). A significant difference in running economy is in agreement with the findings of Saltin et al (1995b) in Kenyan runners, but contrary to previous less well-controlled studies in South African runners (Bosch et al, 1990; Coetzer et al, 1993) where there was a sizeable difference in mean mass between the subject groups (12 kg and 13 kg, respectively) and results were not scaled to $\text{mass}^{-0.66}$.

It may be that treadmill running in the laboratory does not adequately reflect running during a race. In general, the African subjects were considerably less experienced with treadmill running than the Caucasians and every effort was made to overcome this with considerable treadmill familiarisation. If any deviation from "normal" relaxed running style persisted, this could only have been *detrimental* to the measured economy of the African runners, so it is

possible that the difference in economy between the two groups when running outside of the laboratory, may be even more pronounced than measured here.

The results further indicate that the African runners evaluated in this study were able to race 10 km at a significantly higher relative intensity than Caucasian runners. At 10 km race pace, the Africans utilised a considerably higher percentage of their maximal oxygen uptake than the Caucasians (93.5% vs 86.0% respectively, $p < 0.005$). Running at a higher relative intensity elicited a higher HR (187 vs 174 beats/min, $p < 0.05$) and a higher concentration of plasma ammonia (82.9 vs 60.3 $\mu\text{mol/l}$, $p < 0.05$). This could potentially be the result of either a physiological or motivational advantage. However, if this was solely the result of a difference in motivation, one would expect the RER and plasma lactate concentrations to also be higher, but this was not the case. Despite higher relative intensity at race pace, the African athletes had similar RER values to the Caucasian group. By extension, one might argue that if both groups exercised at the same standardised relative intensity, the RER of these Africans would probably be lower, indicating a greater oxidation of fat and lesser reliance on glycolytic pathways. Thus, further lactate accumulation would be prevented and less perturbation of the cellular environment would occur. This would enable a further increase in running speed and relative intensity before the same carbohydrate flux is achieved, an argument that the results of the current study support. Despite the higher racing intensity, the African athletes accumulated only the same concentration of plasma lactate as the Caucasians. This is in support of the findings of the previous chapter, that indicated both that the Africans accumulated less lactate than the Caucasians but also that this was significantly related to their ability to resist fatigue ($r = -0.63$, $p < 0.01$). It is tempting to speculate that race pace is in fact determined by the ability to minimise carbohydrate flux and subsequent plasma lactate accumulation. However, these two factors are not directly proportional, since plasma lactate accumulation is a function of lactate disappearance as well as appearance. It is not possible at this stage to elucidate whether the current lactate results are due to a lesser production of lactate or a greater clearance. A knowledge of skeletal muscle biochemical properties such as enzyme activities and buffering capacity may assist in the explanation for the increased fractional utilisation and altered lactate accumulation in the African runners.

Despite the significant intergroup differences in fractional utilisation of VO_2max in this study, the range of fractional utilisation of VO_2max reported for both African and Caucasian runners is similar to those previously reported for runners (Costill et al, 1973; Scrimgeour et al, 1986) and in recent investigations in cyclists in this laboratory (Bellinger, 1996). The mean value of ~89% for all runners in the current study, is slightly higher than that reported by Scrimgeour et al (1986) and Costill et al (1973). These authors reported the mean % VO_2max sustained

during 10 km and 10 mile races respectively was 85.8% and 86.1%. The values reported by Davies and Thompson (1979), of four ultra-distance world record holders, are more similar to those in the current study (~89%). Therefore, the sub-elite African runners in the current study are not outside the normal range for well-trained distance runners, but rather are representative of the higher end of the range.

The fractional utilisation of HRmax (mean 95%) is in agreement with recent reports. Selley et al (1995) indicated that runners of a similar standard as those in the current study, raced at 90% HRmax during a ten kilometre race. Similarly, the mean percentage of time spent at heart rates between 90-100% HR max (field) during a 16 km cycle time trial may be as much as 92% of the race (Palmer et al, 1995).

At race pace, both plasma lactate and plasma ammonia concentrations were considerably elevated, with mean plasma lactate concentrations above the classically described "onset of blood lactate accumulation" value of 4 mmol/l (see chapter 2.21). This is in line with other findings in this laboratory that have indicated significant plasma accumulation of lactate in shorter endurance events (eg < 1hour). The findings of Bellinger & Bold (personal communication) showed plasma lactate of all subjects was 6-8 mmol/l after ten minutes and remained so for the duration of a 40 km cycling time trial that lasted approximately 50 minutes. This indicates that even exercise of this relatively long duration results in substantial hydrogen ion production.

Ammonia accumulation in the plasma was higher in the African runners than the Caucasians. This may be related to two factors: either the higher relative exercise intensity in the African runners or to differences in the proportion of skeletal muscle fibre types. Type II skeletal muscle fibres produce ammonia more readily than type I fibres (Tullson & Terjung, 1990), hence skeletal muscle characteristics need to be investigated in an attempt to explain the metabolic differences between these two groups of runners.

In summary, the results of the current study indicate that the African runners in this study were more economical than the Caucasian runners. Furthermore, the African runners raced 10 km at a higher percentage of their VO_2 max and HRmax, with only a similar accumulation of lactate and whilst eliciting only a similar RER despite accumulation of more plasma ammonia. These characteristics may explain the ability of these African runners to achieve the same race performance despite a 15% lower VO_2 max.

The following chapters investigate skeletal muscle characteristics that are known to influence plasma lactate and ammonia accumulation, and the RER during exercise.

CHAPTER 3.3

SKELETAL MUSCLE ENZYME ACTIVITIES IN AFRICAN AND CAUCASIAN RUNNERS

RATIONALE AND AIM

Potential explanations for the differences observed in the previous two chapters include skeletal muscle characteristics. Both a high percentage of type I fibres and high activities of skeletal muscle oxidative enzymes have been shown to be advantageous for endurance performance in a heterogeneous population (Ivy et al, 1980; Sjodin et al, 1982) although in a homogeneous group of well-trained runners, these parameters appear to be less predictive of performance (see review of literature, Chapters 2.11 & 2.12). The results of the previous study (Chapter 3.2) indicated that the African runners were able to exercise at a significantly higher relative intensity during races whilst eliciting only the same mean RER and plasma lactate concentration, and therefore were able to achieve a similar mean 10 km performance despite a significantly lower mean VO_2 max. The ability to elicit the same RER and plasma lactate concentration despite a higher exercise intensity is remarkable and is likely to have a metabolic basis such as up-regulation of oxidative enzymes in the skeletal muscle (Gollnick & Saltin, 1982).

Ama et al (1986) investigated the skeletal muscle characteristics of sedentary individuals of West African origin and showed evidence of differences with respect to fibre type and glycolytic enzyme activity compared with sedentary Caucasians. It was concluded by the authors that West African individuals "are, in terms of skeletal muscle characteristics, well-endowed for sport events of short duration". Only one study has reported skeletal muscle characteristics in African endurance runners (of East African origin) (Saltin et al, 1995a). Skeletal muscle characteristics were investigated in four Kenyan and five Scandinavian senior runners. Fibre type distribution and capillarisation were no different between the two groups. However, whilst there was no difference in citrate synthase activity in the vastus lateralis muscle or in the gastrocnemius muscle, the 3-hydroxyacyl CoA dehydrogenase (3-HAD) activity was considerably higher in the Kenyan vastus lateralis and gastrocnemius muscles ($p < 0.05$) when compared to the Scandinavians. However, dissociation between relative activities of 3-HAD and CS activity, is unusual suggesting that further confirmation of this finding is warranted.

In addition, the Scandinavian athletes were normally resident at sea level and travelled to altitude for only 14 days prior to the trial, whilst the Kenyan athletes were born, resident and trained at high altitude. The effect of altitude on skeletal muscle oxidative enzyme activities is not clear. Exposure to hypoxia in animals has been shown to augment these enzymes and high altitude residents who are active also appear to have a higher activity of *both* CS and

HAD (Saltin et al, 1980) which was not the case in the recent study of Saltin et al (1995a). However, acute exposure of sea level natives to altitude generally does not cause an increase in oxidative enzymes, although this may be related to the reduction in physical activity that is normally experienced (Saltin & Gollnick, 1983). There was no difference in the oxidative enzymes in the Scandinavians as a result of the acute exposure to altitude when comparing pre- and post-altitude results in the study of Saltin et al (1995a). Furthermore, it is not clear from the description of the subjects whether the Scandinavians were also distance runners or whether they were middle distance runners. If the two groups have different primary race distances, it may not be appropriate to compare their skeletal muscle enzyme activities. Therefore, the skeletal muscle enzyme activities of that study are difficult to interpret but certainly justify further investigation. A preferable comparison of skeletal muscle enzyme activities in African and Caucasian runners would be in distance runners who all live and train at sea level.

Indeed, a recent DNA restriction fragment length polymorphism (RFLP) analyses of Caucasians from Canada and South Africa and Africans from South Africa does provide evidence of racial distinction in muscle enzyme genotype. While Africans had similar RFLP to the Caucasians, the allele frequencies were very distinct. Canadian and South African Caucasians had identical allele frequencies for muscle adenylate kinase, muscle phosphorylase and subunit Va of cytochrome c oxidase genes, whilst the South African Africans had markedly different frequencies and in fact a unique DNA sequence variant was present in two subjects (Adjoa et al, 1996). Therefore, it would appear that there is a degree of racially distinct genetic variability in skeletal muscle characteristics that warrants further investigation.

Therefore, the aim of this study was to investigate selected enzyme activities and fibre type proportion in a biopsy of the vastus lateralis muscle in African and Caucasian 10 km runners who are resident at sea level at the same geographical location.

METHODS

Subjects. Twelve athletes (5 African, 7 Caucasian) were recruited to take part in the study. All subjects had previously taken part in studies in this laboratory (either 3.1 or 3.2). All consented to have a muscle biopsy performed. Subjects gave their written informed consent to all procedures which had previously been approved by the Ethics and Research Committee, Faculty of Medicine, University of Cape Town. All subjects were resident at sea level in Cape Town, South Africa. African runners were all of the Xhosa ethnic group. All athletes were seasoned sub-elite runners who considered their primary competitive distance

to be 10 km, with no difference in mean 10 km performance. Subject characteristics are presented in Table 3.31.

Exercise testing. All subjects visited the laboratory prior to testing to ensure familiarisation with the exercise testing protocol. The runners were tested on a treadmill to determine VO_2 max using the protocol described in detail in Chapter 3.1. A venous blood sample was obtained three minutes post exercise for the determination of blood lactate concentration (see Chapter 3.1). Plasma lactate was determined in duplicate by spectrophotometric assay (Appendix 6.2).

Muscle biopsy. On a separate day in the rested condition, a needle biopsy of the vastus lateralis was obtained using the technique described by Bergstrom (1962) with suction applied as described by Evans et al (1982). The site was first anaesthetised using 2% lignocaine. A small incision was made prior to insertion of the biopsy needle. The biopsy sample was immediately divided into two portions. Half was immediately frozen in liquid nitrogen and stored at -70°C for later enzyme assays. For all subjects, the remaining portion was orientated and embedded in Tissue-tek and frozen in n-Pentane cooled in liquid nitrogen and stored at -20°C for later histological analyses (see below for details).

Enzyme assays. The techniques for the determination of the enzyme assays required by this thesis were not currently available in our laboratory and had to be redeveloped by the author with the assistance of a visiting researcher, based on methods available in the literature. Further notes concerning developmental procedures, final procedure and reproducibility are provided in the Appendices 6.4 - 6.8.

Muscle samples (~20 mg w.w) were homogenised in a potassium phosphate buffer, pH 7.4. Phosphofructokinase (EC 2.7.1.11) (PFK) and citrate synthase (EC 4.1.3.7) (CS) were assayed spectrophotometrically in duplicate at 25°C (Beckman DU-62, Beckman Instruments, Inc., U.S.A.) in all athletic and sedentary subjects. CS, a key oxidative enzyme in the tricarboxylic acid cycle, was assayed using a modified Srere technique (Srere, 1969). The reaction mixture consisted of 80 mM Tris-HCl, pH 8.4, 0.1 mM 5,5'-dithiobis[2-nitrobenzoic acid] (DTNB), 0.3 mM acetyl-CoA, 0.5 mM oxaloacetate and 5 μl of homogenate. Changes in optical density were recorded at 412 nm using a DTNB mM extinction coefficient of 13.6. PFK, a key glycolytic enzyme, was assayed using the method of Ling, Marcus & Lardy (1965). The assay mixture contained 50 mM Tris, pH 8.2, 2 mM EDTA, 5 mM MgCl_2 , 20 mM 2-mercaptoethanol, 2 mM F6P, 40 μg aldolase, 10 μg of TPI and GlyPDH, 0.16 mM NADH, 10 μl of homogenate and 2 mM ATP. The disappearance of NADH was monitored at 340 nm using an NADH mM extinction coefficient of 6.22.

In addition, 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) (3-HAD), hexokinase (EC 2.7.1.1) (HK), and carnitine palmityl transferase (CPT) were assayed in just seven athletes (4 African, 3 Caucasian). Insufficient muscle sample was available to assay this group of enzymes in all subjects. 3-HAD and HK were assayed in duplicate spectrophotometrically using the methods described in Bass et al (1969) and CPT using the method described in Di Mauro (1973). Due to the large amount of sample required it was not possible to assay CPT in duplicate.

Test-retest reproducibility by this investigator utilising the above methods, was estimated by Pearson's correlation as 0.98 and 0.94 for CS and PFK respectively and 0.94, 0.94 and 0.95 for 3-HAD, HK, and CPT respectively with no uniform shift from the line of identity (see Appendix 6.8). Test-retest raw absorbance data of the CS and PFK reproducibility trials are presented graphically in Fig 3.30a & 3.30b respectively. Enzymes are expressed in μ mol/gram of muscle wet weight/minute (μ mol/g ww/min).

Histology. Routine histology was carried out using myosin ATPase stain at pH 4.3 and 9.4 to differentiate type I and type II fibres (Dubowitz, 1973). An example is reproduced in Appendix 6.14. All the fibres viewed in one field were counted to calculate the relative fibre type proportions. In all individuals this was more than 150 fibres.

Statistics. Comparisons between Africans and Caucasians were made using a Student's t-test for unpaired data. Relationships were investigated using the Pearson's Correlation coefficient. Results are expressed as mean \pm standard deviation.

RESULTS

African runners were lighter when compared to the Caucasian runners but had a similar level of body fat. Anthropometric data are displayed in Table 3.31.

Table 3.31: Subject characteristics and anthropometric results

	African (n=5)	Caucasian (n=7)	significance
Age (yrs)	29.4 \pm 6.9	28.4 \pm 3.7	ns
Height (cm)	172.2 \pm 5.9	179.0 \pm 7.8	ns
Body mass (kg)	61.0 \pm 7.6	67.9 \pm 4.9	p<0.05
% body fat	12.3 \pm 1.7	12.6 \pm 3.0	ns
10km time (min)	32.6 \pm 1.7	33.8 \pm 2.6	ns

Fig 3.30a CS reproducibility: raw data

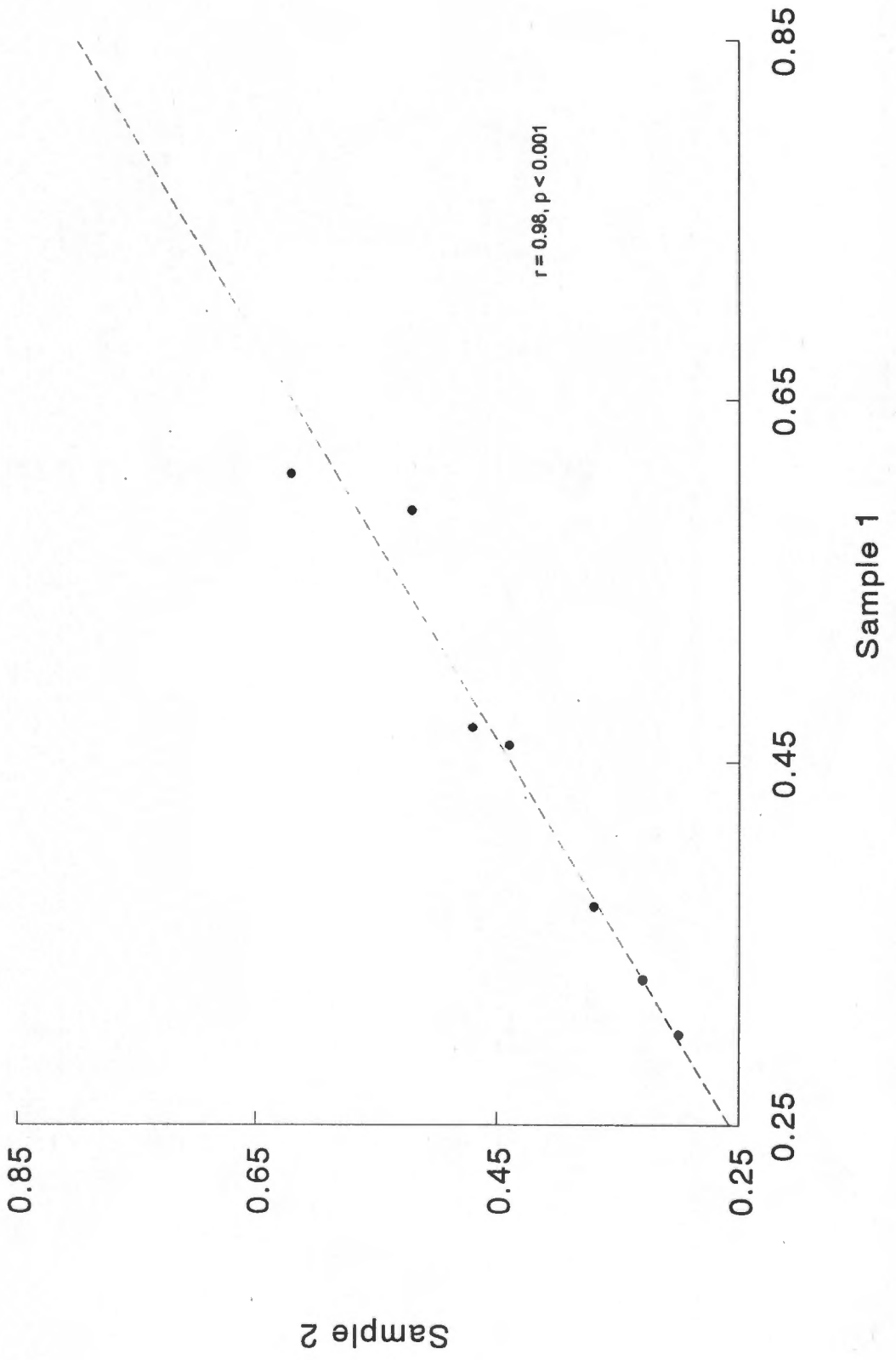
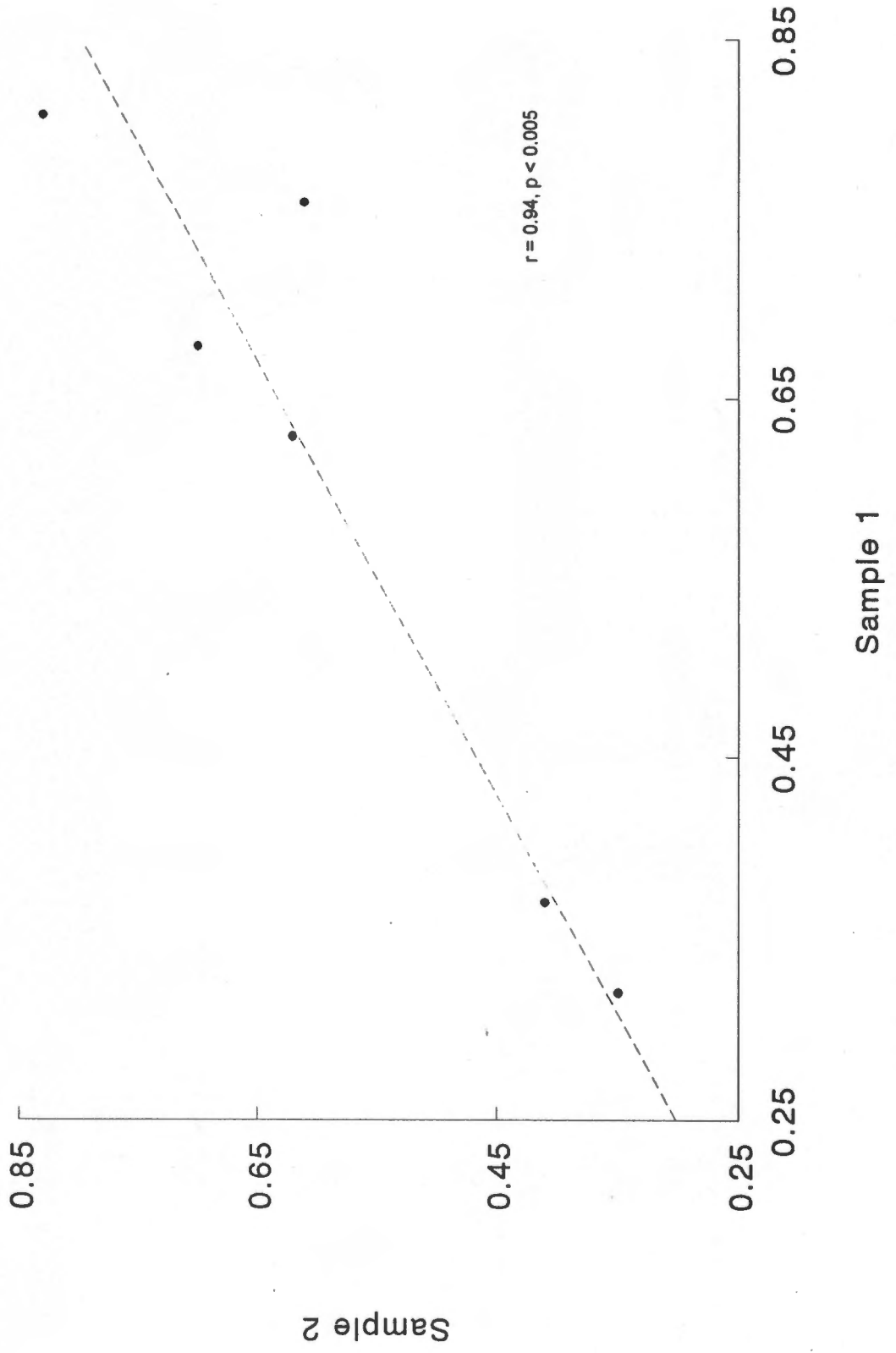


Fig 3.30b PFK reproducibility:raw data



Results of the maximal treadmill test are given in Table 3.32.

Table 3.32 Maximal exercise test results

	African	Caucasian	significance
Peak treadmill velocity (km/hr)	20.8 ± 1.6	22.4 ± 1.9	ns
VO ₂ max (ml/kg/min)	59.3 ± 6.6	65.1 ± 7.8	ns
HRmax (beats/min)	192.6 ± 7.8	190.6 ± 5.7	ns
Blood lactate (mmol/l)	9.7 ± 2.4	12.4 ± 2.1	p<0.05
RER max	1.08 ± 0.07	1.14 ± 0.06	ns

Considering the group of runners as a whole, peak treadmill velocity was significantly correlated with personal best ten kilometre time ($r=-0.55$, $p<0.05$) although VO₂max did not correlate with 10 km time.

Citrate synthase and phosphofructokinase activities are displayed in Figure 3.31. The activity of CS was 50% higher ($p<0.005$) in the African runners when compared to the Caucasian runners. Mean PFK activity was 28% higher in the African runners but this did not reach significance. Although not measured in all subjects, 3-HAD activity was also significantly higher in the African runners ($n=4$) when compared to the Caucasian runners ($n=3$) (54%, $p<0.01$) but CPT and HK activity were not different in this smaller sample (see Table 3.33). The African:Caucasian ratio was similar for both CS and 3-HAD activity (1.50:1 & 1.54:1 respectively).

Table 3.33 Enzyme activities in the vastus lateralis of African and Caucasian distance runners ($\mu\text{mol/g ww/min}$)

	African	Caucasian	significance
CS	27.9 ± 7.5	18.6 ± 2.1	$p<0.005$
PFK	50.9 ± 7.6	39.9 ± 16.0	$p=0.09$
3-HAD	23.9 ± 4.7	15.5 ± 5.1	$p<0.01$
HK	5.1 ± 1.1	4.4 ± 0.1	ns
CPT	4.1 ± 0.4	4.8 ± 0.4	ns

Enzyme activities measured in the current study did not correlate with maximal exercise test parameters (VO₂max or PTV) or with 10 km performance time. A subsample of these subjects also completed the fatigue resistance test at the same *relative* percentage of

Fig 3.31 CS and PFK activity in African and Caucasian runners

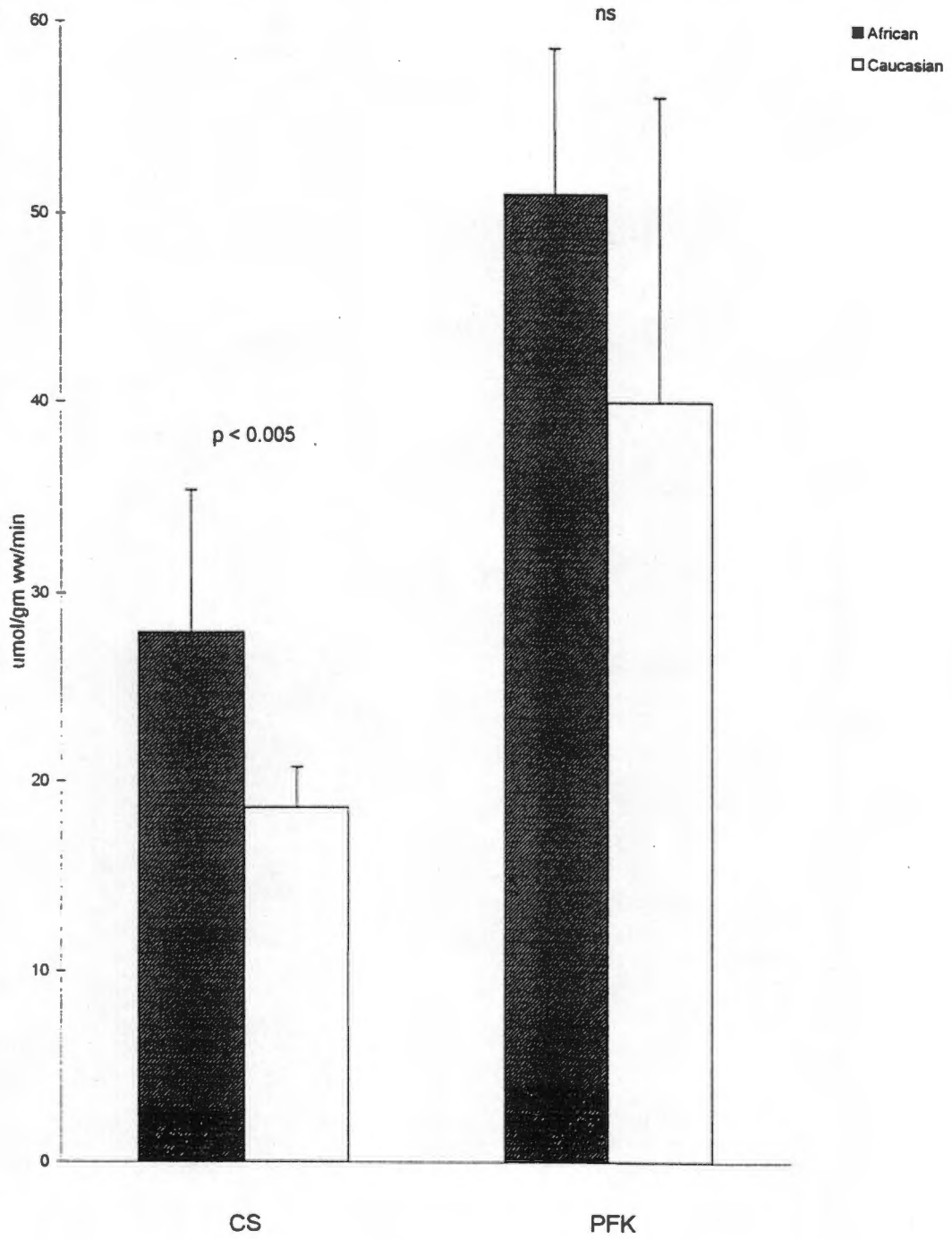
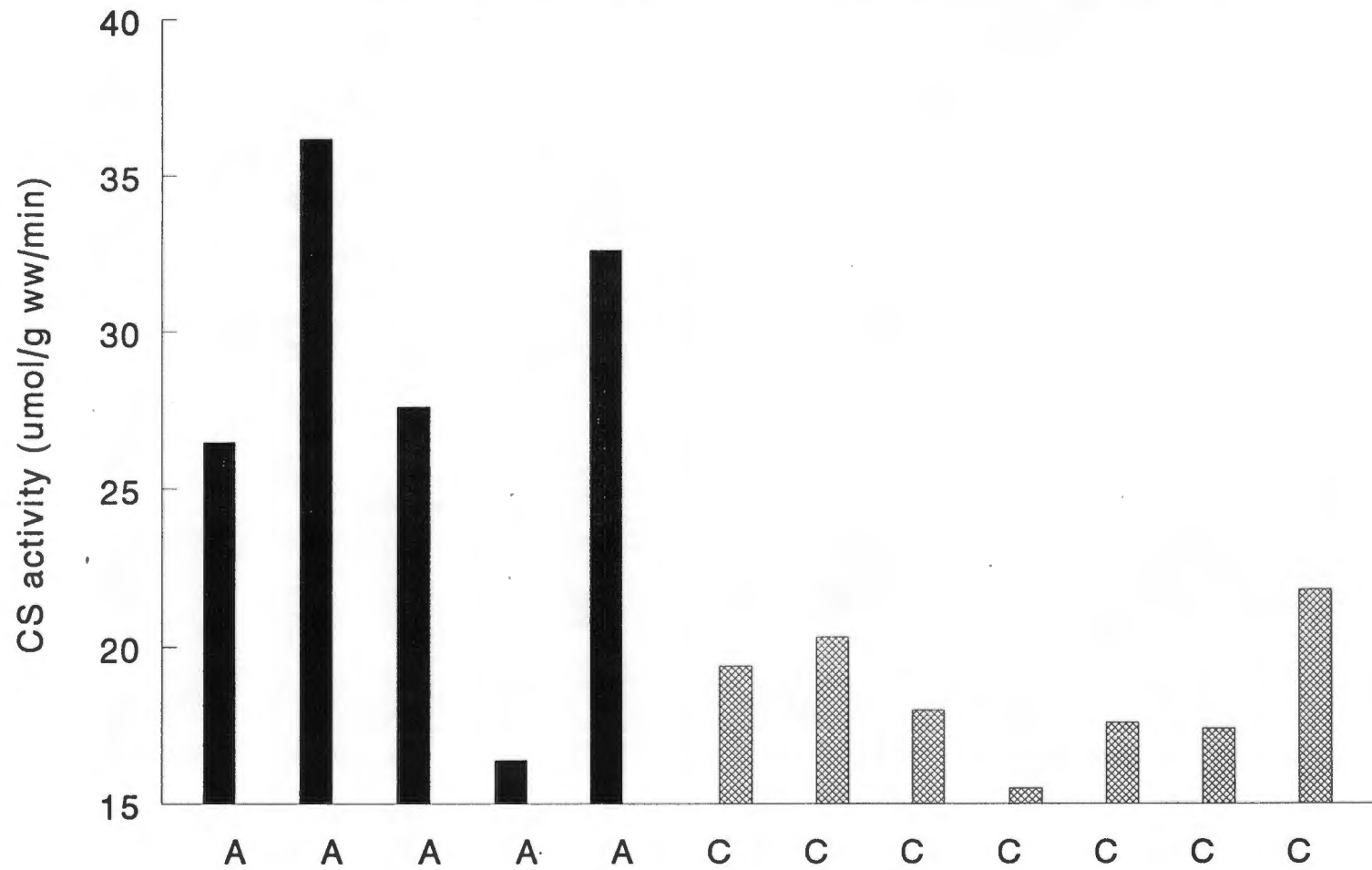


Fig 3.32 CS activity in individual African and Caucasian distance runners



individual peak treadmill speed described in chapter 3.1 and these results are collated and presented in the following chapter (3.4).

Table 3.34: Fibre type proportions

	African	Caucasian	significance
% type I	49.6 ± 17.3	67.1 ± 17.5	p=0.05

When considered as a total group, CS activity was not related to the percentage of type I fibres and PFK activity was not related to the proportion of type II fibres. There was no significant association between 10 km time (min) and percentage of type I fibres in this homogenous group of well-trained runners although the direction of trend was toward a *lower* percentage of type I fibres being advantageous ($r=0.38$, ns).

DISCUSSION

The main finding of the current study was that the activities of citrate synthase (CS) and 3-hydroxyacyl CoA dehydrogenase (3-HAD) were considerably higher in the vastus lateralis of African distance runners when compared to their Caucasian counterparts. Mean activities of these key oxidative enzymes were 50% and 54% greater respectively in the African runners compared to the Caucasian runners. Furthermore, only one African runner fell within the range of the seven Caucasian runners (Fig 3.32). The mean activity of the glycolytic marker enzyme, phosphofructokinase, was also higher in the African athletes (28%) but this did not reach significance.

When citrate synthase activity is compared with that reported by other investigators, the mean of the Caucasian runners (18.6 $\mu\text{mol/g ww/min}$) is similar to that reported by others in well-trained athletes (Saltin & Gollnick, 1983; Mizuno et al, 1990), but the mean of the African runners (27.9 $\mu\text{mol/g ww/min}$) was considerably higher. These data are partially in agreement with the findings of the only previous investigation of enzyme activities in African distance runners, made in muscle biopsies of elite Kenyan distance runners (Saltin et al, 1995a). These authors also reported a higher activity of 3-HAD in the African athletes but found CS activity to be the same as in the Caucasian runners. The athletes in the current study all lived and trained at sea level, therefore excluding any possible effects of altitude that may have confounded the interpretation of the results of the study of Saltin et al (1995a) discussed in the introduction. In addition, it is not clear whether the runners in the previous study were all distance runners or whether the Kenyan runners were longer distance runners than the Caucasians. However, even if that was a partial explanation of the findings of that study, it can not explain the difference observed in the current study in which all runners were primarily 10 km runners. Therefore, if the subjects investigated are representative of

African distance runners, the combined data of these two studies provide convincing evidence that African distance runners do have enhanced oxidative enzyme capacities.

In the current study, the African and Caucasian runners were of a similar performance level with no difference in mean current 10 km race time. However, African runners achieved comparable mean race performances, if not slightly better, despite a tendency toward a lower mean VO_2max . Their higher skeletal muscle oxidative capacity may have made this possible.

Previous authors have postulated that higher skeletal muscle oxidative enzyme activity is likely to have a more pronounced advantageous effect during submaximal activity than during maximal activity (Gollnick & Saltin, 1982). In fact, the relevance of mitochondrial oxidative capacity to VO_2max in trained individuals has been questioned (Henriksson & Reitman, 1977; Holloszy & Coyle, 1984). This suggested dissociation is further supported by the findings of the current study in which there was no association between VO_2max and oxidative enzyme capacity amongst this homogeneous group of runners.

The tendency ($p=0.09$) toward a higher activity of the glycolytic enzyme PFK in the African runners is interesting although the values of both groups are similar to those in a large study (Simoneau & Bouchard, 1989). Endurance athletes usually have PFK activities that are similar to, or even lower than, sedentary subjects, as do the runners in the current study (Gollnick & Saltin, 1982; Jansson & Kaijser, 1987). This would imply that endurance training normally provides no stimulus for the augmentation of this glycolytic enzyme. But the findings of Essen & Henriksson (1984) showed that, in fact, glycolytic enzyme activity measured in pooled dissected single fibres was elevated in both fibre types with training, but that because of the lower proportion of type II fibres, this would not be apparent if analysed in a muscle homogenate. Furthermore, the findings of Costill et al (1976) reported that the activity of glycolytic enzyme phosphorylase was less in elite distance runners than in untrained men. However, when scrutinised more closely, the activity was only 7% less than of untrained individuals, *despite* the fact that the elite runners had only 50% of the type II fibres of the untrained men. Therefore, it is likely that the glycolytic capacity in those fibres was probably considerably higher than in the type II fibres of the untrained subjects.

If present *in conjunction with* a high oxidative capacity, then a high glycolytic capacity may be beneficial. The higher the relative intensity of exercise the more profound the contribution of glycolytic metabolism to energy liberation. Previous studies have shown indirectly (Bosch et al 1990; Coetzer et al, 1993) or directly (Chapter 3.2) that African distance runners are able to race at a higher % VO_2max than Caucasian distance runners and this may be related to their ability to enhance activities of enzymes of both glycolytic and oxidative pathways.

The integration of glycolytic and oxidative pathways, specifically during *high intensity* exercise, has been emphasised by the results of recent radioisotope studies. These have indicated that during high intensity activity the oxidation of lactate is an important contributor to the metabolic clearance of lactate and an important source of substrate (MacRae et al, 1995; Stanley et al, 1986). With high glycolytic and oxidative capacity, an athlete may be able to both supply more rapid energy via glycolysis to enable higher intensity activity and to rapidly oxidise the by-product, therefore resulting in lesser perturbation of cellular homeostasis.

A possible explanation of the differences in enzyme activities could be a difference in fibre type proportions. Type I fibres are traditionally considered oxidative fibres with a higher CS activity. However, in the current study, the African runners had a *lower* percentage of type I fibres. Therefore differences in fibre typing clearly do *not* explain high CS activity in the African runners in the current study. A possible explanation for the higher oxidative capacity of the muscle homogenate in the current study is a higher oxidative capacity of the type II fibres - in particular the type IIA fibres, in the African subjects.

Unfortunately, technical difficulties prohibited the differentiation of type IIA and IIB in all subjects and therefore this is excluded from the analysis. Differentiation was possible in some samples and several of the African runners had < 2% type IIB fibres. It may be that the cause of difficulties in differentiation of type IIA and type IIB fibres is the result of there being very few type IIB fibres in these subjects. In order not to bias the results with this assumption, it was thought prudent to report only type II fibres as a whole.

As discussed in chapter 2.12, it is possible for type II fibres to enhance their oxidative capacity considerably. In fact, in cross-sectional comparisons, the oxidative capacity of type II fibres in one individual may be higher than the oxidative capacity of type I fibres in another individual (Essen & Henriksson, 1984). Analyses of the enzyme profiles in single fibres pooled for type would be useful as this would allow quantification and comparison of the enzyme concentration in pooled fibres of distinct fibre types. Many authors have shown in longitudinal studies that the oxidative capacity of type II fibres is plastic and that a continuum of fibres may be a more correct description (Pette and Staron, 1993). However, it is clear that any differences in the finer classification of fibre type *per se* that were not measured here would certainly not explain the 50% greater activity of CS in the Africans reported here. Thus the analyses of enzyme activity in muscle homogenate is more likely to be affected by the oxidative capacity of the fibres *per se*, irrespective of classification.

Hence, the underlying *reason* for the apparently advantageous skeletal muscle enzyme profile of the African runners reported here was not determined by this study. The sample sizes in the current study and the only other previous study (Saltin et al, 1995a) are small. The reported differences could be the result of a genetic difference or an environmental/lifestyle difference such as training, or a combination of both. Training distance was not different between groups in the current study but training intensity was not quantified. However, Coetzer et al (1993) showed that African *distance* runners trained for more time at a greater than 85%VO₂max than did Caucasian *middle-distance* runners. It is not possible to isolate genetic and environmental determinants from the data presented here, although the substantial difference in the current study between African and Caucasian well-trained runners suggests that training alone is unlikely to be the only explanation unless there is a difference in the genetic *response to training*. Certainly these data warrant follow-up with a larger epidemiological study of both distance runners and sedentary individuals and the training responses of the later. These proposed follow-up studies would only be feasible as a large, lengthy multi-centre trial to enable recruitment of sufficient subjects. However, because of the magnitude of the difference observed in the runners in the current study, a smaller study of sedentary subjects was completed as part of the current thesis and is presented in Chapter 3.5.

The degree of genetic contribution to skeletal muscle enzyme activity has been previously investigated in twin studies. There appears to be evidence of a limited genetic determination of 3-HAD in some studies (Howald, 1976; Bouchard et al, 1986; Hamel et al, 1986) but not in others (Komi et al, 1977). There is no substantial evidence of a significant genetic component with respect to other oxidative enzymes. The racial difference in RFLP allele frequencies reported by Adjoa et al (1996) for other muscle enzymes does indicate the presence of genetic differences with the potential for different phenotypic expression. The current study provides evidence of racial differentiation of muscle phenotype with respect to CS and 3-HAD activity, the origin of which is presently unclear.

CS activity was not related to VO₂max in the current group of subjects. However, it is possible that an advantageous oxidative enzyme profile assists with the optimal utilisation of the subject's VO₂max by facilitating a higher fractional utilisation, enabling them to optimise their 10 km race performance. This hypothesis is investigated in the following chapter which integrates the skeletal muscle enzyme activity results with the results of the fatigue resistance exercise test described in chapter 3.1, in those subjects who completed both investigations.

CHAPTER 3.4

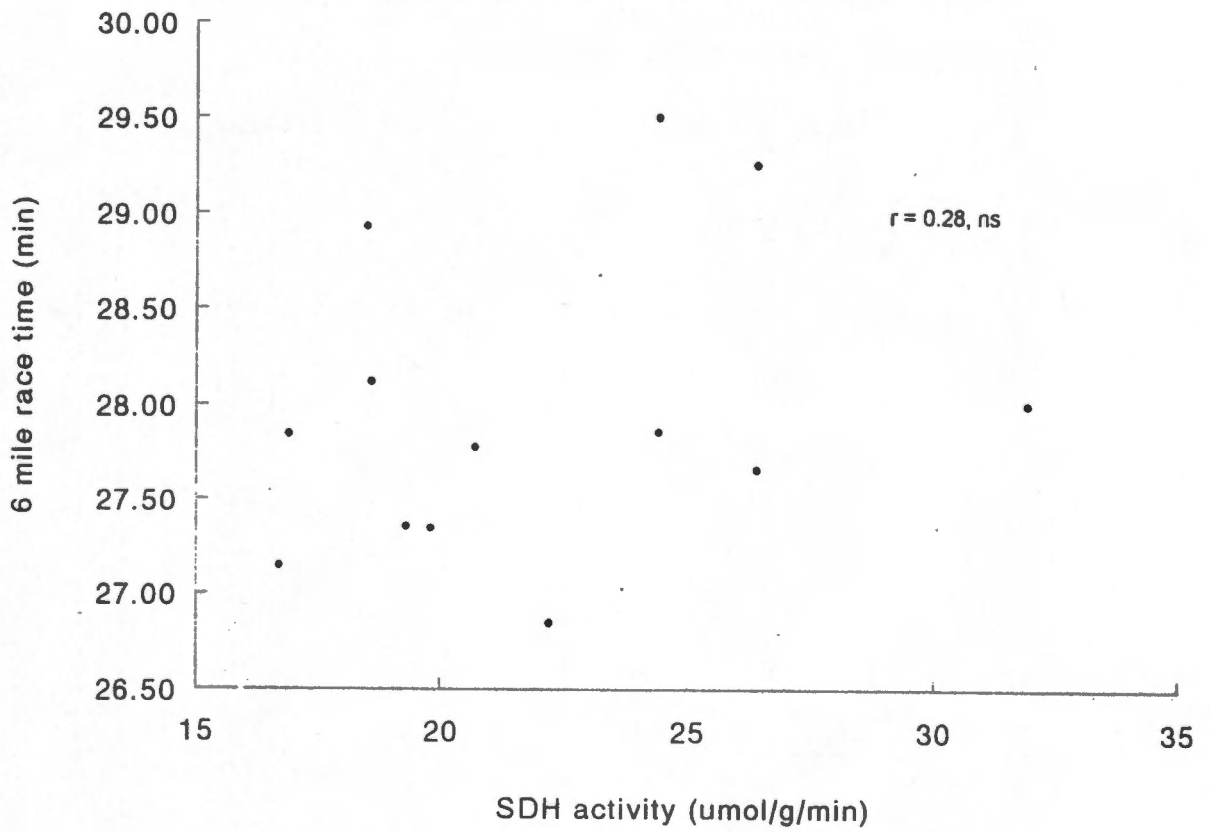
INTEGRATED DATA ANALYSIS: RELATIONSHIP BETWEEN FATIGUE RESISTANCE AND SKELETAL MUSCLE ENZYME ACTIVITIES IN DISTANCE RUNNERS

RATIONALE AND AIM

It is now generally accepted that the activity of oxidative enzymes is not a good predictor of $VO_2\text{max}$ in a homogeneous *well-trained* group of athletes although invasive studies of muscle properties in this population are rare. Studies that have been undertaken show no relationship between skeletal muscle CS (or SDH) activity and $VO_2\text{max}$ in highly trained cyclists and runners (Foster et al, 1978; Holloszy and Coyle, 1984; Costill et al, 1976, Weston et al, 1996). It has been suggested that the activity of oxidative enzymes may in fact play a larger role in determining performance during *submaximal* exercise (Gollnick & Saltin, 1982). However, the relationship between oxidative enzyme capacity and fatigue resistance during a submaximal task, has not been directly measured in a group of well-trained humans. Calculating from the data of Costill et al (1976), in a very homogeneous group of elite distance runners, there was no relationship between SDH activity and 6-mile race time within the group ($r=0.28$, Fig 3.40). Coyle et al, (1988) *did* investigate both time to fatigue (at 88% $VO_2\text{max}$) and oxidative enzyme activities in well-trained cyclists divided into those with a high lactate threshold and those with a low lactate threshold. However, the study focuses upon the differences between the two groups and the relationship between the two variables mentioned above is not reported. As a result of the considerable difference in time to fatigue between the two groups of subjects with no difference in oxidative capacity between the two groups, it is unlikely that the two variables were significantly correlated. In rats and cats, several investigators have directly shown a significant relationship between submaximal fatigue resistance during repeated muscle contractions and oxidative enzyme activity (Peckham, 1973; Hudlicka, 1977; Davies et al, 1981). Therefore, studies in humans are warranted to elucidate the relationship between skeletal muscle oxidative enzyme activity and the ability to resist fatigue during submaximal exercise at a workload relative to the subject's maximal workload. This relationship has considerable relevance to the current thesis.

Not all the runners subject to investigation in the exercise study presented in Chapter 3.1 volunteered to undergo a muscle biopsy of the vastus lateralis. Therefore, it was not possible to relate exercise performance data with the muscle characteristics in all subjects. However, a group of ten subjects took part in both the fatigue resistance trial (Chapter 3.1) and the determination of skeletal muscle enzyme activities (Chapter 3.3) and their data are considered here as a group. The inter-relationships between the exercise and muscle characteristics for these individuals are outlined below.

Fig 3.40 SDH activity vs 6 mile run time (from Costill et al, 1976)



METHODS

Ten runners with a range of 10 km race performance of 32.5 - 37.3 min took part in both studies. Within this group there were 6 Caucasian runners and 4 African runners, considered here as a group. Further details of the fatigue resistance test are given in Chapter 3.1. It is important to note that the workloads utilised were all of the same *relative* intensity for all subjects, rather than the same *absolute* workload. Details of the muscle biopsy of the vastus lateralis and its subsequent analysis are given in Chapter 3.3.

RESULTS

There was no relationship between CS activity in the vastus lateralis and VO_2 max in this group of subjects. The relationship between CS activity and current 10 km performance did not reach significance ($r=-0.41$). However, the total time that the runner was able to resist fatigue during the fatigue resistance running test was significantly related to the vastus lateralis CS activity ($r=0.70$, $p<0.05$, see Fig 3.41).

To investigate in more detail the mechanism by which CS activity is related to submaximal fatigue resistance, the relationship between the CS activity and the various submaximal metabolic responses measured during exercise at 88% PTV, was investigated. This workload was chosen for analysis because it was of the same duration for all subjects (5 min) and it immediately preceded the intensive, non-steady-state 92% PTV time to fatigue bout. At 88% PTV, a higher CS activity was significantly associated with a lower plasma lactate ($r = -0.73$, $p=0.01$; Fig 3.42), a lower RER ($r = -0.63$, $p<0.05$; Fig 3.43) and a lower heart rate ($r = -0.64$, $p<0.05$).

PFK activity showed no significant relationship with any of the above mentioned exercise variables.

DISCUSSION

The complex mechanisms by which higher skeletal muscle oxidative enzyme activities enhance endurance performance are not understood. It is now generally accepted that the activity of the skeletal muscle oxidative enzymes is not a determinant of VO_2 max in a homogeneous well trained group of athletes although there are relatively few such studies in good to elite athletes. Data reported by Holloszy and Coyle (1984) show no relationship between CS activity and VO_2 max in highly trained cyclists and runners. The same finding was reported by Costill et al (1976) in elite runners. The results of the current study also show no significant correlation in this fairly homogeneous group of runners. However, a higher skeletal muscle oxidative enzyme capacity may have implications for performance of sustained *submaximal* exercise, even though no such relationship exists with maximal

Fig 3.41 Relationship between CS activity and Time to Fatigue

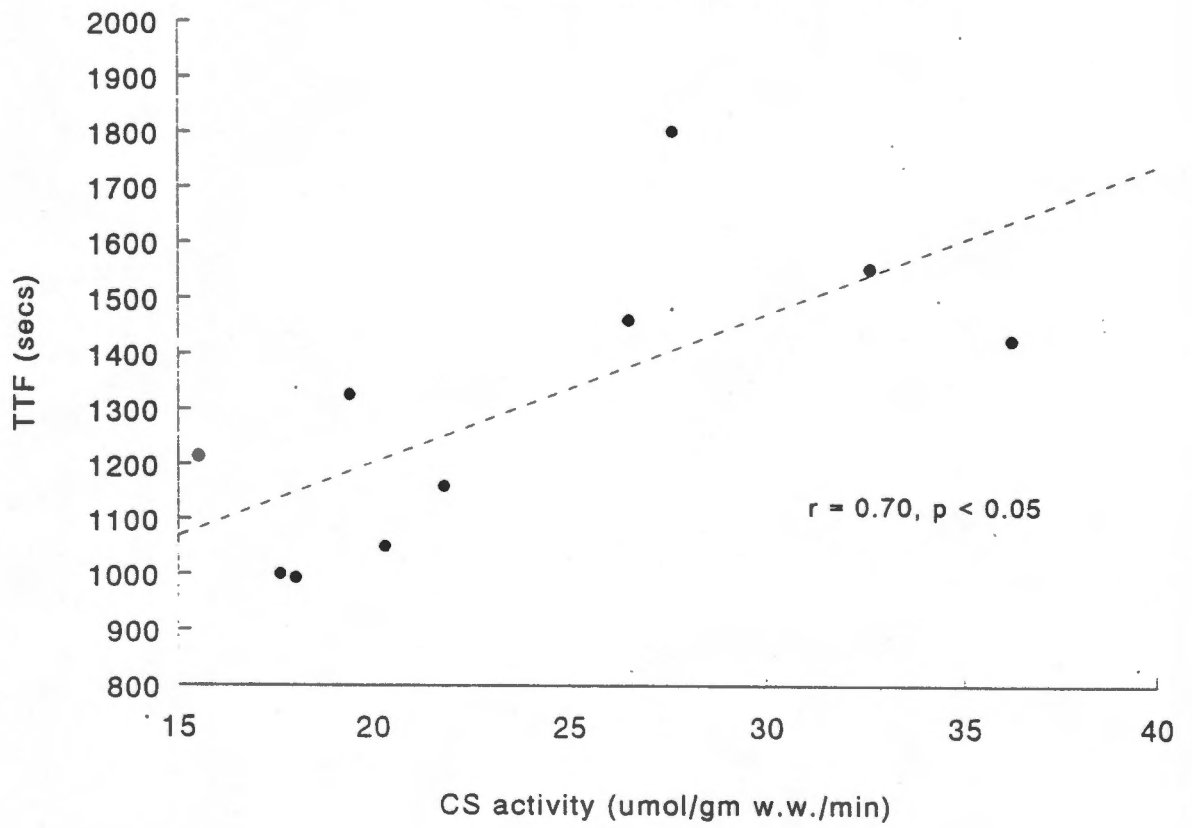


Fig 3.42 CS activity vs plasma [La]
at 88% of PTV

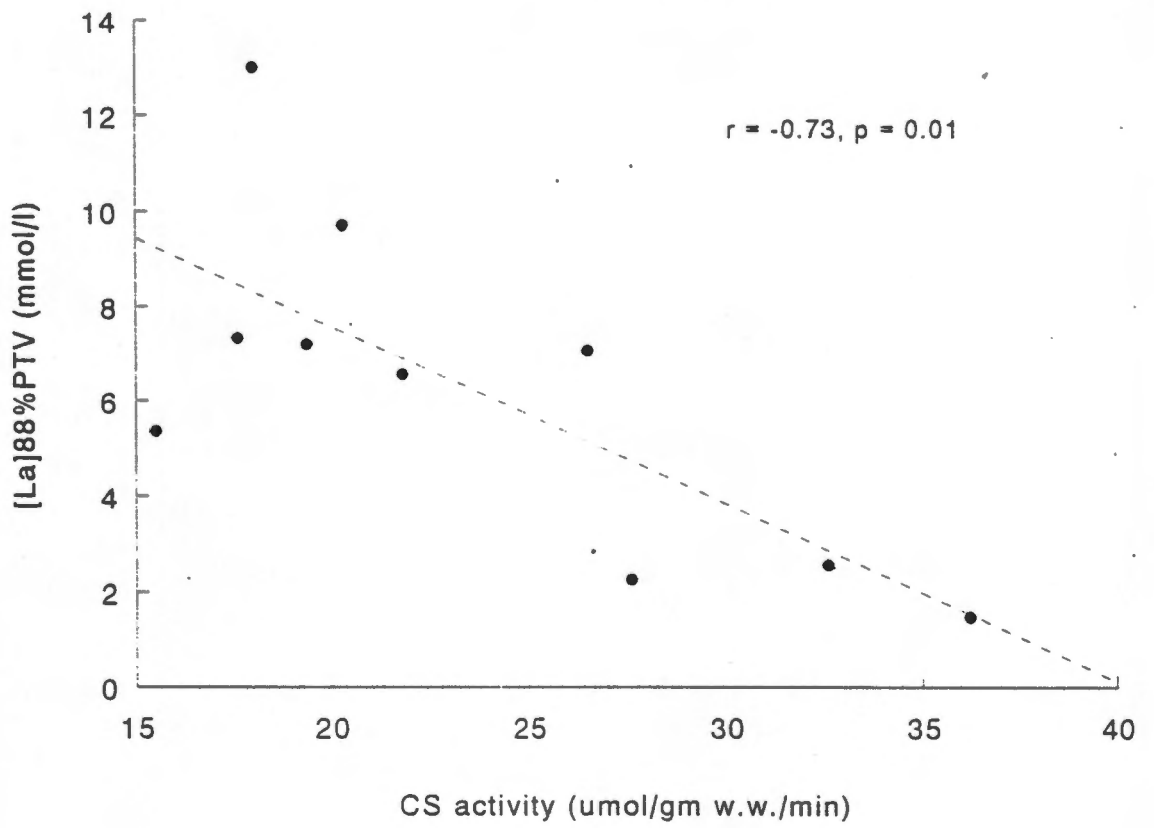
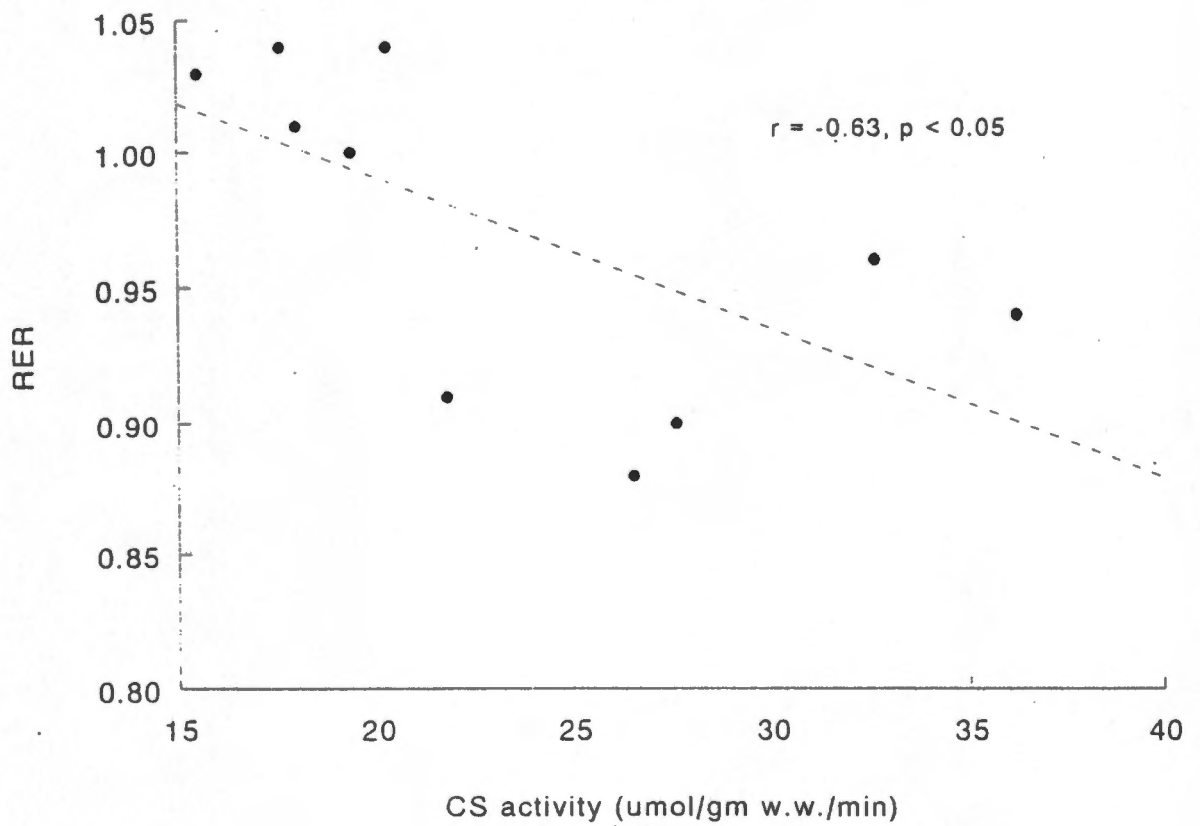


Fig 3.43 CS activity vs RER
at 88% of PTV



exercise performance as determined by $VO_2\text{max}$. It was hypothesised that a high mitochondrial oxidative capacity may be an advantage for sustaining exercise at a high intensity relative to one's current peak treadmill velocity in laboratory tests or to sustaining a fast race pace during shorter endurance events such as 10 km running.

The results of this study indicated that the ability to resist fatigue in the current running task at the same relative intensities (mean duration of total test = ~22 min) was significantly related to the level of skeletal muscle citrate synthase activity ($r=0.70$, $p<0.05$). However, the relationship between CS activity and 10 km race time was not significant. Therefore, it appears that CS activity is not predictive of 10 km time in a homogeneous group of well-trained runners, but is indicative of the ability to perform well, relative to one's own maximal aerobic capacity.

Citrate synthase activity represents the constant-proportion group of mitochondrial enzymes of the Krebs cycle and respiratory chain (Pette, 1966), increasing in proportion to mitochondrial density (Hoppeler, 1990; Reichmann et al, 1985). Increases in the ability to resist fatigue during repeated contractions in isolated muscles of the cat and rat, are associated with an increase in the activity of oxidative enzymes (Peckham, 1973; Hudlicka, 1977), suggesting that the ability of the muscle to sustain repeated contractions is partially dependent upon its oxidative enzyme capacity. However, more recent animal studies have questioned this relationship, suggesting that there may be a dissociation in the time course of the change in enzyme activity and the change in fatigue resistance during intervention trials (Kernell, 1987; Simoneau, 1993), with CS activity continuing to increase after fatigue resistance had tended to show a plateau. This may be due to other confounding influences on the resistance to fatigue.

For example, other prerequisite factors may adapt asynchronously in response to the stimulation. Or alternatively there may be a limit to the extent to which further enhancement of CS activity can continue to improve fatigue resistance, as tested by this particular task in animal studies. Nevertheless, whilst it is likely that oxidative enzyme activity is an important determinant of fatigue resistance, it is probably not the sole contributor, and is likely to be more closely related to some exercise performance tasks than others. To date, no other studies have directly related submaximal fatigue resistance to oxidative enzyme activity in well-trained human subjects or differentiated the subtle difference in the effect of oxidative enzyme activity simply on race performance versus a performance task set relative to each subjects' personal maximal capacity.

The delay in the onset of fatigue during high intensity treadmill running in the current study is also related to the minimising of the accumulation of blood lactate and an association with greater oxidation of fat shown by the negative correlation with RER. Both are significantly negatively related to the CS activity in the current study. Karlsson et al (1972) provided evidence that the lower blood lactate concentration in more trained individuals is secondary to a lower concentration of lactate in the exercising muscle.

The production of lactate does not necessarily imply hypoxia but results from a rate of NADH production from glycolysis that is higher than its rate of removal by oxidation in the Krebs cycle and electron transport chain. Excessive NADH is removed by the pyruvate to lactate reaction. Thus, decreasing the cytosolic NADH/NAD ratio by increasing oxidation or by decreasing flux through the glycolytic pathway (not necessarily as a result of changes in the availability of oxygen) can decrease skeletal muscle lactate production. Increased mitochondrial volume, as evidenced by an increase in oxidative enzyme activity, is one way to achieve this. Valberg et al (1985) showed a significant negative correlation between oxidative enzymes and post-race muscle lactate concentration in well-trained horses. An increase in mitochondrial oxidative capacity would assist by both increasing oxidation and by a tighter control of glycolysis by inhibition of the rate limiting steps. PFK is inhibited by a high cytosolic ATP/ADP x P_i ratio or regulated by the [ADP] per se (Jacobus, 1982). In addition, the capacity for transfer of reducing equivalents into the mitochondria via shuttle systems would be enhanced (Kobayashi & Neely, 1975) by an increase in the relative concentration of shuttles.

The relationship between lower RER and delayed fatigue is indicative that greater utilisation of fat may increase fatigue resistance. Greater utilisation of fat has previously been shown to occur with improvements in endurance performance (Saltin & Gollnick, 1983; Gollnick & Saltin, 1982). An increase in mitochondrial oxidative capacity and the associated decrease in flux through the glycolytic pathway as a result of the enzymatic inhibition detailed above, results in an increased ratio of acetyl CoA units of fatty acid origin with respect to those of carbohydrate origin. Thus, the result is an increase in the net utilisation of fatty acids with respect to carbohydrate and subsequent reduction of the RER value.

Jansson & Kaijser (1987) and Kiens et al (1993) have investigated the effect of endurance training on both substrate utilisation and skeletal muscle enzyme activities in cross-sectional and longitudinal studies, respectively. In the former study (Jansson & Kaijser, 1987), the endurance trained subjects had lower RER values than less trained subjects during submaximal exercise at the same relative %VO₂max. That this observation was indeed an effect of training, was confirmed by the longitudinal study (Kiens et al, 1993) which showed a

decreased RER at the same absolute workload in the trained leg when compared to the untrained leg. The shift towards greater oxidation of free fatty acids was accompanied by a reduction in carbohydrate oxidation, especially by a profound reduction in glycogenolysis. Following Henri-Michaelis-Menten kinetics, the rate of the reaction at any given substrate concentration, is dependent upon the total enzyme concentration. Thus a doubling of enzyme concentration at the same substrate concentration causes a large increase in the velocity of reaction, thus immensely benefiting the metabolic control (Gollnick and Saltin, 1982). In addition, a lesser requirement for substrate results in a lesser perturbation in osmotic pressure in the muscle cell.

In general, the ultimate result of increased oxidative enzyme capacity would be greater utilisation of fat and lesser production of lactate and therefore less disturbance to the homeostasis of the muscle environment (eg pH/osmotic pressure) for a given relative workload. Our data show that the muscle and metabolic factors discussed above have a distinct functional advantage, with respect to optimising one's fatigue resistance during high relative intensity exercise.

Furthermore, the oxidation of lactate within the active muscle cell and less active muscle cells, has been proposed as an important energy substrate (Mazzeo et al, 1982, Mazzeo et al, 1986, Stanley et al, 1986, MacRae et al, 1995). This pathway provides for intracellular catabolism of lactate and this pathway in itself may reduce muscle lactate concentration and consequently reduce blood lactate concentration and may therefore be an additional contributor to the enhanced resistance to fatigue shown in this study. If the transport of lactate from the cell proves to be a saturable process as suggested by Bangsbo et al (1993), then this mechanism may indeed prove important for determination of endurance performance.

To the best of my knowledge, the current study is the first to show a direct association between oxidative enzyme activity and fatigue resistance during a high intensity submaximal running task in well-trained humans. In addition, the negative correlations between CS activity and both plasma lactate concentration and RER, elucidate the metabolic consequences of the enhanced oxidative capacity which become evident during high intensity but submaximal exercise in humans. It has long been accepted that increased fat utilisation is more important in longer endurance events (several hours) with the hypothesis that fatigue in this type of activity was delayed due to glycogen sparing. Therefore, an important suggestion of the current study is that fat oxidation is important in the resistance of fatigue even during a shorter endurance exercise task of higher intensity, lasting less than 35 minutes where glycogen sparing is unlikely to be important.

CHAPTER 3.5

SKELETAL MUSCLE ENZYME ACTIVITIES IN SEDENTARY AFRICANS AND CAUCASIANS

RATIONALE AND AIM

Chapter 3.3 indicated a higher activity of skeletal muscle citrate synthase and 3-hydroxyacyl CoA dehydrogenase in the African runners studied compared to the Caucasian runners. This partially supports the findings of Saltin et al (1995a) who reported higher 3-HAD activity in Kenyan when compared to Scandinavians runners although CS activity was not different. Saltin et al (1995a) commented that a genetic factor may explain the finding of a high 3-HAD activity in the Kenyan runners but that the evidence is weak, partially because no sedentary samples were obtained. However, these enzymes were also measured in nine junior runners with considerably fewer years of running training. 3-HAD activity was as high in these juniors as in the seniors and although CS activity was significantly lower, which may indicate that 3-HAD activity was inherently higher in the Kenyans.

In contrast, Ama et al (1986) reported an enzyme profile more advantageous for short duration exercise (sprinting/power events) in Africans of West African origin. Glycolytic enzyme activity was 30-40% greater in the West Africans than the Caucasians. When compared to the mean value of PFK obtained in a large sample of men (n=218, Simoneau & Bouchard, 1989), the results of the West African sedentary subjects was more than one standard deviation higher than the mean, indicating a considerable difference. An important feature of the study was that these subjects were all sedentary yet had enzyme profiles similar to that of the top West African sprinters, leading the authors to conclude that West African individuals were genetically endowed to succeed in this form of activity.

In order to negate the effects of training and to investigate genetic endowment, in this study skeletal muscle enzyme activities were investigated in a sample of sedentary African and Caucasian individuals resident at sea level, who have similar current lifestyles. Therefore, the aim of this study was to determine whether the difference in oxidative enzyme activity observed in the runners in Chapter 3.3, were also observed in sedentary members of the same population groups.

METHODS

Subjects

Twelve sedentary subjects volunteered to undergo a muscle biopsy (6 African, 6 Caucasian). All were students attending the University of Cape Town and had similar current lifestyles and diet. All were undergraduates who lived in university residence/communal student

housing. A three-day dietary record confirmed similar macronutrient intake (% protein, % carbohydrate, % fat, % alcohol) although the mean percentage of energy consumed as fat was higher in the Africans (Table 3.51). All were currently sedentary having not exercised regularly (exercised < 1 hr/wk) for the previous six months as determined by a modified Blair questionnaire (Blair et al, 1985) and interview. Subject characteristics are displayed in Table 3.51. All procedures were approved by the Research and Ethics Committee of the University of Cape Town Medical School.

Exercise testing

All subjects visited the laboratory prior to testing to ensure familiarisation with the exercise testing protocol. The subjects were tested on an electronically-braked cycle ergometer (Lode, Groningen, The Netherlands). In our previous experience, sedentary subjects feel more comfortable and are more likely to achieve maximal oxygen uptake on the cycle ergometer than on the treadmill. The cycle ergometer protocol began at 2 W/kg body mass and increased by 25 W every 2.5 min until the subject could no longer maintain a cadence of 50 revs/min. This protocol is a modification of the Peak Power Output (PPO) test utilised in this laboratory in both athletic and patient populations (Kuipers et al, 1985; Hawley & Noakes, 1992). The pilot studies indicated that utilisation of this modified protocol in sedentary subjects resulted in a test of a similar length to use of the original protocol in athletes. Oxygen consumption (VO_2), minute ventilation (V_E) and respiratory exchange ratio (RER) and heart rate (HR) were measured continuously. A venous blood sample was obtained two minutes after exercise (Bassett et al, 1991) for the determination of plasma lactate concentration (Appendix 6.2)

Muscle biopsy. On a separate day in the rested condition, a needle biopsy of the vastus lateralis muscle was obtained with suction using the technique described by Evans et al (1982) as described in Chapter 3.3. The sample was immediately divided into two portions. Half was immediately frozen in liquid nitrogen and stored at -70°C for later enzyme assays. Due to indications of a drift in ultra-low freezer temperature, to -65°C , it was deemed prudent to freeze-dry this portion and to store until time of analysis (Virtis Sentry, The Virtis Co Inc., NY, USA). A 1:4 ratio between dry weight and wet weight was assumed to determine the correction factor for expressing the enzyme activities of these samples per unit wet weight (Rawn, 1989). The remaining portion was orientated and embedded in Tissue-tek and frozen in n-Pentane cooled in liquid nitrogen and stored at -20°C for later histological analyses.

Enzyme assays. Muscle samples (~20 mg w.w) were homogenised in a potassium phosphate buffer, pH 7.4. Phosphofructokinase (EC 2.7.1.11) (PFK) and citrate synthase (EC 4.1.3.7) (CS) were assayed spectrophotometrically in duplicate at 25°C (Beckman DU-

62, Beckman Instruments, Inc., U.S.A.) in all subjects as described in Chapter 3.3. For further detail, see Appendices 6.6 & 6.7. Test-retest reproducibility using the above methods was 0.98 and 0.94 for CS and PFK activities respectively (see Fig 3.30a & 3.30b, Appendix 6.8). Enzyme activities are expressed in $\mu\text{mol}/\text{gram}$ of muscle wet weight/minute ($\mu\text{mol}/\text{g ww}/\text{min}$).

Histology. Routine histology was carried out using myosin ATPase stain at pH 4.3, 4.6 and 9.4 to differentiate type I, IIA & IIB fibres (Dubowitz, 1973).

Statistics Comparisons between Africans and Caucasians were made using a Student's t-test for unpaired data. Relationships between variables were investigated using the Pearson's Correlation coefficient.

RESULTS

African sedentary subjects were shorter and lighter than Caucasian sedentary subjects but the percentage of body fat was not different. Means anthropometric data are displayed in Table 3.51.

Table 3.51 Anthropometric data and dietary composition

	African	Caucasian	significance
Age (yr)	21.3 \pm 1.5	21.8 \pm 1.7	ns
Height (cm)	173.3 \pm 6.9	180.5 \pm 5.7	p<0.05
Weight (kg)	60.8 \pm 1.0	75.0 \pm 7.7	p=0.01
% body fat	11.3 \pm 3.6	13.2 \pm 2.6	ns
<i>Dietary composition:</i>			
% carbohydrate	41.7 \pm 4.1	44.8 \pm 6.3	ns
% fat	38.9 \pm 3.7	32.1 \pm 4.4	p<0.05
% protein	16.7 \pm 1.4	15.7 \pm 6.1	ns

Results of the maximal exercise test are given in Table 3.52.

Table 3.52 Maximal cycle ergometer exercise test results

	African	Caucasian	significance
Peak power output (Watts)	186.7 \pm 25.1	257.3 \pm 42.5	p<0.005
PPO/kg	3.08 \pm 0.22	3.46 \pm 0.60	ns
VO ₂ max (ml/kg/min)	45.0 \pm 5.0	53.9 \pm 6.5	p=0.01
HRmax (beats/min)	187.5 \pm 10.9	192.5 \pm 6.8	ns
Blood lactate (mmol/l)	11.8 \pm 4.5	14.0 \pm 1.8	ns
RERmax	1.10 \pm 0.09	1.14 \pm 0.04	ns

Fig 3.51 CS and PFK activity in sedentary African and Caucasian subjects

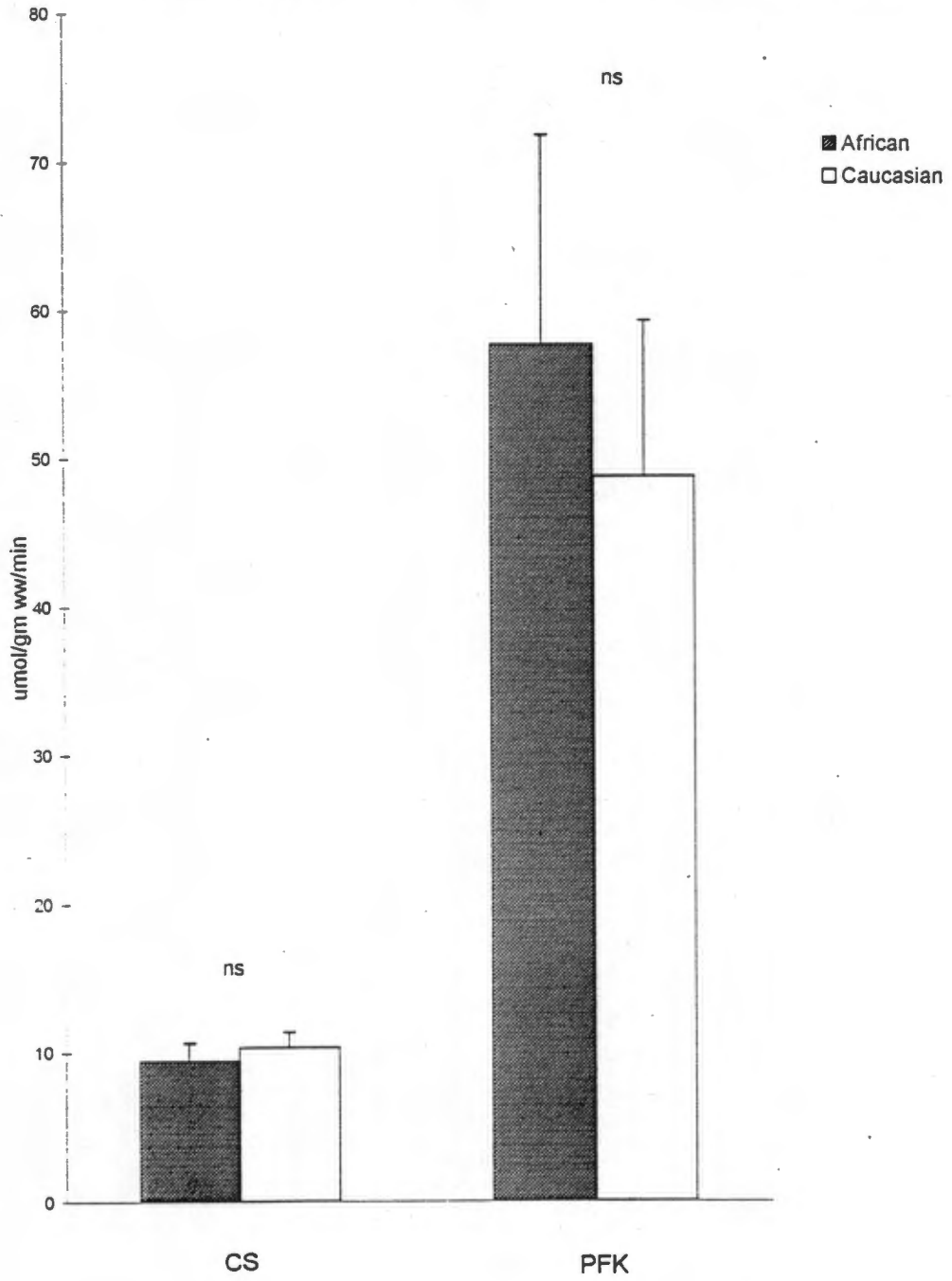
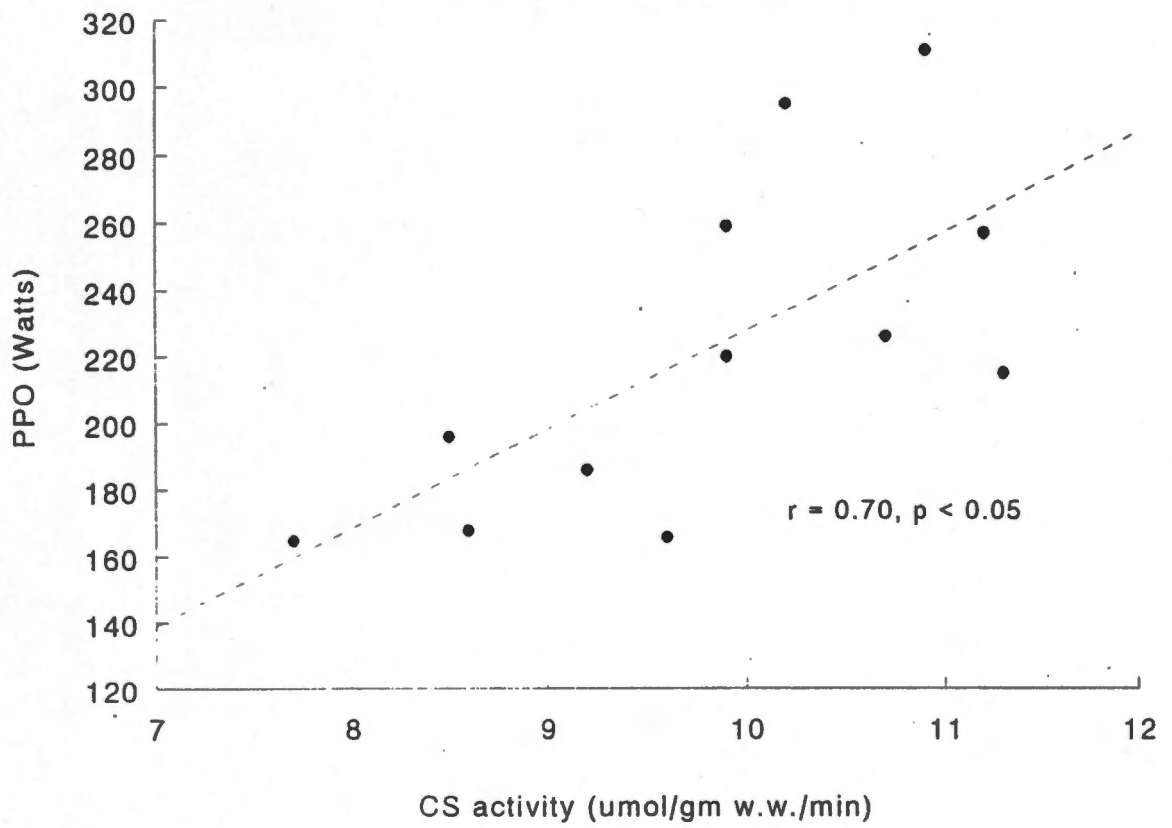


Fig 3.52 CS activity vs Peak Power Output in sedentary subjects



The sedentary Caucasians had a considerably higher peak power output than the sedentary Africans, but this difference was statistically negated when differences in body mass were taken into account. Nevertheless, even when corrected for body mass, $VO_2\text{max}$ was different.

The range of fibre type proportions was large and there was no significant difference between the groups.

Table 3.53 **Fibre type proportions**

	Sedentary Africans	Sedentary Caucasians	significance
% type I	40.1 ± 10.0	48.3 ± 14.3	ns
% type IIA	27.5 ± 3.8	24.2 ± 6.2	ns
% type IIB	32.3 ± 8.7	27.5 ± 10.6	ns

Skeletal muscle citrate synthase (CS) and phosphofructokinase (PFK) activities are displayed in Figure 3.51. There was no significant difference between the African and Caucasian sedentary subjects in this study. Citrate synthase activity correlated significantly with Peak Power Output ($r = 0.70$, $p < 0.05$, Fig 3.52) and PPO/kg ($r = 0.55$, $p = 0.06$) but not with $VO_2\text{max}$ ($r = 0.45$) or % type I fibres ($r = 0.42$). Phosphofructokinase activity was not related to fibre type proportions in these sedentary subjects.

DISCUSSION

The skeletal muscle enzyme activities measured in the current study were not different between the sedentary Africans and the sedentary Caucasians and therefore this study found no evidence of an inherent endowment of Africans with respect to oxidative enzyme activity. The results indicated considerable overlap between sedentary subjects from these two population groups with respect to the oxidative enzyme activity measured, that was not the case in the runners. As the difference between the African and Caucasian runners was very large (50% higher oxidative enzyme activity in the Africans, see Chapter 3.3) with little overlap (only one African runner fell within the range of the seven Caucasian runners), we hypothesised that if higher oxidative enzyme activity was an inherent characteristic of the muscles of this population group, that this may be apparent in a relatively small sample of sedentary individuals. However, the results indicated no apparent difference in the sedentary group.

Nevertheless, it is possible that a superior endowment of skeletal muscle oxidative enzyme activity may exist in only a small subgroup of the population, and that successful runners

come from this portion of the population. Therefore, from the current study it is not possible to conclusively exclude that the higher level of oxidative enzymes present in the African runners investigated in the previous chapter is genetically determined.

If not inherently present, higher oxidative enzyme levels could be the result of a different training regime or other environmental/lifestyle factors such as habitual diet. The current study attempted to minimise the differences between the two groups by selecting students with similar lifestyle who were not trained.

Alternatively the *response* to an equivalent training stimulus may be different. This would result in a difference only becoming apparent in trained individuals. Investigators have shown a large degree of variability in response to a given training stimulus (Lortie et al, 1984) and studies have indicated that this response *per se* may be partially genetically determined. Comprehensive studies by Simoneau et al (1986) and Hamel et al (1986) have indicated a genetic determinant of the *response* of skeletal muscle enzyme activities to endurance training. Both studies indicated significant intra-twin correlations in the response of the oxidative enzymes to training. Therefore, it is possible that whilst sedentary Africans and Caucasians have similar skeletal muscle enzyme profiles, if a similar training stimulus were to be applied, the resultant enzyme profiles may differ. Longitudinal training studies are required to test this possibility.

In summary, this investigation of skeletal muscle enzyme activities in a small number of sedentary Africans and Caucasians did not provide evidence of inherent endowment of the favourable enzyme profile for endurance performance that was present in the African runners. Despite this, for the reasons outlined above, a genetic endowment can not be discounted at this point. More detailed studies of the genetic basis of the differences between these groups are warranted.

CHAPTER 3.6

SKELETAL MUSCLE BUFFERING CAPACITY DETERMINED BY TITRATION IN TYPE I AND TYPE II MUSCLES OF RATS

RATIONALE AND AIM OF CHAPTERS 3.6, 3.7 & 3.8.

As reported in Chapter 3.1, the African runners are able to run at the same high relative intensity for close to four minutes longer than the Caucasian runners ($p < 0.01$). Also, as reported in Chapter 3.2, the African runners are capable of racing over 10 km at a significantly higher fraction of their $VO_2\text{max}$ ($p < 0.005$). Two intramuscular factors which may contribute to these exercise performance characteristics, are different enzyme activities and fibre type. Both of which were investigated in Chapter 3.3. Another possible mechanism which would allow the continuation of exercise at high intensity is an enhanced ability to buffer intracellular protons (skeletal muscle buffering capacity, βm), thereby delaying the decline in intracellular pH. As reviewed in Chapter 2.13, intracellular pH has been closely linked to skeletal muscle fatigue during high intensity contractions although the exact mode of action is still an area of debate (Hainault & Dacheteau, 1989; Donaldson et al, 1978; Fabiato & Fabiato, 1978 ; Cooke et al, 1988).

During this type of exercise task, Coetzer et al (1993) showed that African runners have a higher fatigue resistance than Caucasian runners, suggesting that their βm may indeed be different. Furthermore, the findings of Bangsbo et al (1993) indicated that the transport of lactate across the muscle membrane appears to be partially saturated at high intramuscular lactate concentrations. If this is indeed the case during high intensity exercise, the intramuscular lactate concentration would quickly rise resulting in a concomitant fall in intramuscular pH.

Most previous investigations of βm have shown that it is an important component of sprint or short duration performance but very few studies have investigated its role in sustained high intensity events lasting 30-60 minutes. A recent study (Weston et al, 1996) showed a positive correlation between skeletal muscle buffering capacity and 40 km time trial cycling performance (~55 min duration), highlighting the relevance of βm to endurance performance that is of an intense nature. It is possible that βm may also be a determinant of 10 km running performance, an event typically lasting ~30 min in elite performers. To date this has not been measured, and therefore the investigation of βm is warranted in this thesis.

As discussed in the review of literature, the muscles primarily investigated by muscle biopsy in human exercise studies are very heterogeneous with respect to fibre type. Therefore, it is conceivable that a degree of the inconsistency between previous cross-sectional studies of

β m in humans may be due to individual variation in fibre type composition of the muscles investigated, independent of any additional effect of training. There is also some evidence (chapter 2.13) for a weak relationship between % type II fibres and β m, although this is far from conclusive. The fact that we found the African runners to have a significantly higher proportion of type II fibres than the Caucasian runners in the chapter 3.3, provided additional impetus for a comparison of β m in samples from the two populations.

Therefore, prior to investigating β m in African and Caucasian 10 km runners (Chapter 3.7) and sedentary individuals (Chapter 3.8), it was decided to initially carry out a study utilising rat muscle. The aim of this study was to compare skeletal muscle buffering capacity of a type I muscle with that of a predominantly type II muscle, using the titration method. To do this it was necessary to use an animal model with muscles that are homogeneous with respect to fibre type. In addition, technical considerations and the reproducibility of the titration method of determining skeletal muscle buffering capacity in this laboratory were determined prior to its use in the human studies to follow.

METHODS

Animal care and exercise programmes:

Twenty-seven Long-Evans rats were involved in this study. The procedures were approved by the University of Cape Town Medical School Animal Research Review Committee. The rats were all from the same breeding stock and were aged 16 -18 weeks. Rats were randomly divided into two groups:

1. 16 rats were housed in live-in running wheels and ran spontaneously for four weeks. Live -in running wheels (Campus Industries, Cape Town, SA) had a diameter of 36 cm and a track width of 11 cm. Wheels were regularly checked for ease of rotation in either direction. Revolutions were recorded by a mechanical counter, irrespective of direction.
2. Sedentary control rats (n=11) were housed in normal rat laboratory cages (18 cm x 20.5 cm x 24.5 cm).

All rats had access to food and water ad libitum and were housed in a room maintained at 20°C and lighted between 0600 and 1800 hr.

Sample preparation:

After the four week experimental period, rats were weighed and humanely sacrificed with 1 ml/100 g body weight overdose of a 5% sodium pentobarbitone solution injected into the

peritoneum. Hindlimbs were immediately skinned and the soleus muscle and the superficial portion of the vastus lateralis muscle were removed and prepared as follows:

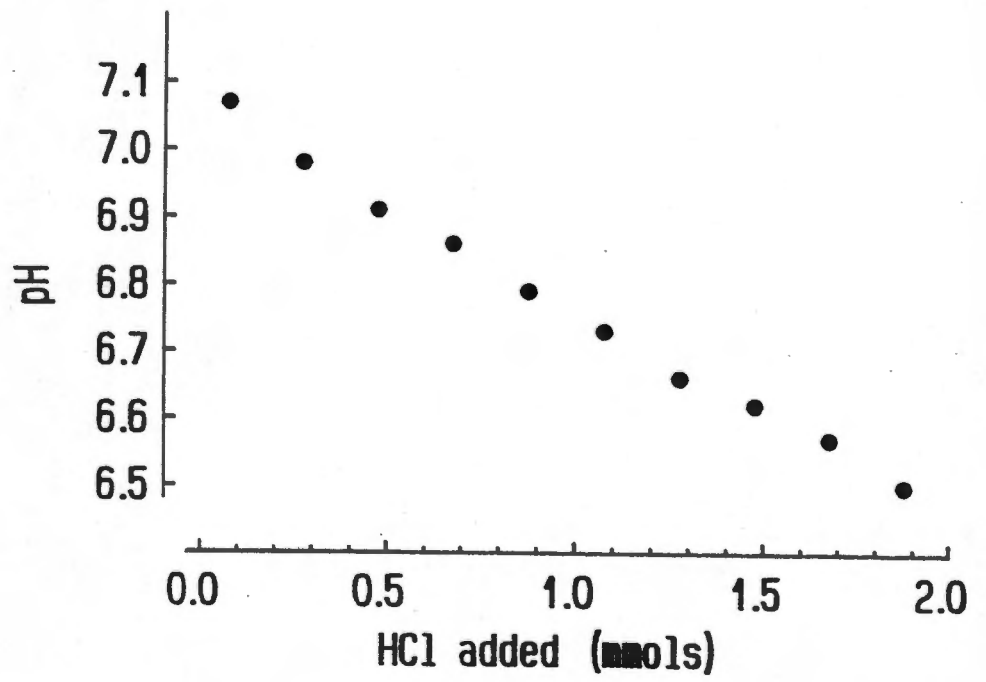
1. for buffering capacity determination, gross connective tissue was removed and the sample was immediately frozen in liquid N₂ and stored at -70°C until freeze-dried,
2. for protein assays and enzyme assays, the sample was frozen immediately in liquid N₂ and stored at -70°C for subsequent analysis,
3. for histology, muscle from a sample of rats was orientated within embedding medium (Tissue-tek) and rapidly frozen in liquid nitrogen-cooled n-Pentane and stored at -80°C prior to sectioning at -20°C.

βmtitr procedure:

Skeletal muscle buffering capacity had not previously been determined in our laboratory and therefore the author was responsible for the initiation and development of the technique based on the techniques available in the literature. A technician assisted the investigator in the development of the necessary equipment set-up (ie. circulating water bath) and helped with initial trials. Further detail of development procedures is given in Appendix 6.9.

The pH titration method used in the current study was based upon that of Marlin and Harris (1991). Care was taken to alternate the order in which samples were analysed (ie. superficial vastus then soleus, soleus then superficial vastus) and to use sample pairs of a comparable weight to prevent a systematic error. Weights ranged from 10 -15 mg dw. The sample was dissected free of any remaining connective tissue, blood and fat prior to homogenisation on ice with a hand-held glass homogeniser. Samples were homogenised in a 1 ml solution of 145 mmol/l KCl, 10 mmol/l NaCl and 5 mmol/l iodoacetic acid (Na salt), pH 7.0. Iodoacetic acid was included to inhibit glycolysis during homogenisation. The resulting range of dilution has been shown to have no effect on either buffering capacity or initial pH reading (Marlin & Harris, 1991). The homogenate was incubated at 37°C for 5 minutes prior to measurement of the initial pH (pH_i). If pH_i was below 7.1 this was adjusted upward with a 100 mmol/l NaOH solution. For these particular samples, the mean volume added was 12.2 μl which represents only a 1.2% dilution. Temperature was maintained at 37°C throughout the titration using a circulating water bath. Muscle homogenates were titrated with 20 μl aliquots of 10 mmol/l HCl whilst the homogenate was stirred continuously and pH measured with a microelectrode (Crison 52-08, Barcelona, Spain). In this study, the titration was performed from pH 7.1 to pH 6.5 as this is reflective of the change in muscle pH from rest to exhaustion during intense exercise. A typical titration curve is displayed in Figure 3.61 and illustrates that there may be slight deviation from linearity in pH change over our experimental range as previously reported (Marlin and Harris, 1991; Mannion et al, 1993). The effect of this

Fig 3.61 Titration curve of a typical rat muscle homogenate



deviation is minimised by titrating over less than a full pH unit, however, the results are expressed per full pH unit.

Ten samples of homogenate from the same muscle sample were analysed to assess the reproducibility of the titration technique in our laboratory. The coefficient of variation was calculated as 3.8%. Raw data are displayed in Appendix 6.10.

Enzyme assays:

Muscle samples (40-80 mg w.w) from all rats housed in the running wheels were homogenised in a potassium phosphate buffer, pH 7.4, using a 1mg:19 μ l dilution. Citrate synthase (CS) and phosphofructokinase (PFK) activities were assayed in duplicate at 25°C in a spectrophotometer (Beckman DU-62, Beckman Instruments, Inc., U.S.A.):

Citrate synthase activity was assayed using a modified Srere technique (Srere, 1969). The reaction mixture consisted of 80 mM Tris-HCl, pH 8.4, 0.1 mM 5,5'-dithiobis[2-nitrobenzoic acid] (DTNB), 0.3 mM acetyl-CoA, 0.5 mM oxaloacetate and 5 μ l of homogenate. Changes in optical density were recorded at 412 nm and using a DTNB mM extinction coefficient of 13.6.

Phosphofructokinase activity was assayed using the method of Ling, *et al* (1965). The assay mixture contained 50 mM Tris, pH 8.2, 2 mM EDTA, 5 mM MgCl₂, 20 mM 2-mercaptoethanol, 2 mM F6P, 40 μ g aldolase, 10 μ g of TPI and GlyPDH, 0.16 mM NADH, 10 μ l of homogenate and 2 mM ATP. The disappearance of NADH was monitored at 340 nm using an NADH mM extinction coefficient of 6.22.

Test-retest reproducibility using the above methods was 0.98 and 0.94 for CS and PFK activity respectively (see Appendix 6.8). Enzyme activity is expressed in μ mol/gram of muscle wet weight per minute.

Protein assay:

Protein concentration (both contractile and non-contractile) of the muscle homogenate of all running rats was assayed spectrophotometrically in triplicate with biuret solution (Kingsley, 1942, Appendix 6.11). Results are expressed in mg protein/100 mg of wet weight of muscle.

Histochemistry:

Routine histochemistry was carried out on muscle samples of a sub-sample of rats (n=11) using myosin ATPase stain at pH 4.3 and 9.4 (Dubowitz *et al*, 1973) in order to get a representative indication of fibre type in these rat muscles.

Statistics:

Group means of soleus and superficial vastus were compared using a paired Student's t-test. Relationships between distance run within the group of running rats and other variables were determined using a Pearson's correlation.

RESULTS

There was no significant difference in mean body mass between running rats and sedentary rats at the time of sacrifice (430 ± 67 vs 369 ± 89 g respectively). Runners had gained only a mean of 18 grams during the 14 weeks of spontaneous activity. There was a large variation in individual running distances within the group of running rats (range 0.1 - 26.7 mean km/week).

Histochemistry results were obtained on 11 soleus and 11 superficial vastus muscle samples. Soleus had no less than 73% type I fibres (mean 84.8 ± 6.9 %) whilst superficial vastus was in all cases 100% type II fibres.

Mean muscle buffering capacity ($\beta_{m_{titr}}$) was 18% higher in the superficial vastus muscle compared to the soleus muscle ($p < 0.001$). This was the case in both the running group and the sedentary group (24% and 10% respectively, Fig 3.62) but there was no difference between these groups. $\beta_{m_{titr}}$ was not related to mean weekly running distance amongst the spontaneously running rats. Initial muscle homogenate pH (pH_i) was lower in the superficial vastus muscle when compared to the soleus muscle in both groups (Table 3.61). There was a strong exponential relationship between running distance and pH_i in running rats in both the soleus ($y = 0.34 \exp[0.43x] + 6.4$, $r = -0.73$, $p < 0.01$) and in the superficial vastus ($y = 0.64 \exp[0.23x] + 5.95$, $r = -0.86$, $p < 0.001$) (Fig 3.63).

Table 3.61: Mean $\beta_{m_{titr}}$ ($\mu\text{mol H}^+/\text{g}$ muscle dry weight/pH) and initial muscle homogenate

pH (pH_i)

Group	soleus	s.v.	% diff	p value
<i>$\beta_{m_{titr}}$:</i>				
Runners	216 ± 30	268 ± 50	24	$p < 0.001$
Sedentary	230 ± 18	253 ± 17	10	$p < 0.005$
<i>pH_i:</i>				
Runners	6.63 ± 0.16	6.36 ± 0.25		$p < 0.001$
Sedentary	7.01 ± 0.17	6.80 ± 0.21		$p < 0.01$

s.v. superficial vastus

%diff difference between the soleus and s.v. expressed as percentage of the lower value

Values are expressed as mean \pm SD

Fig 3.62 Skeletal muscle buffering capacity of soleus and superficial vastus in rats

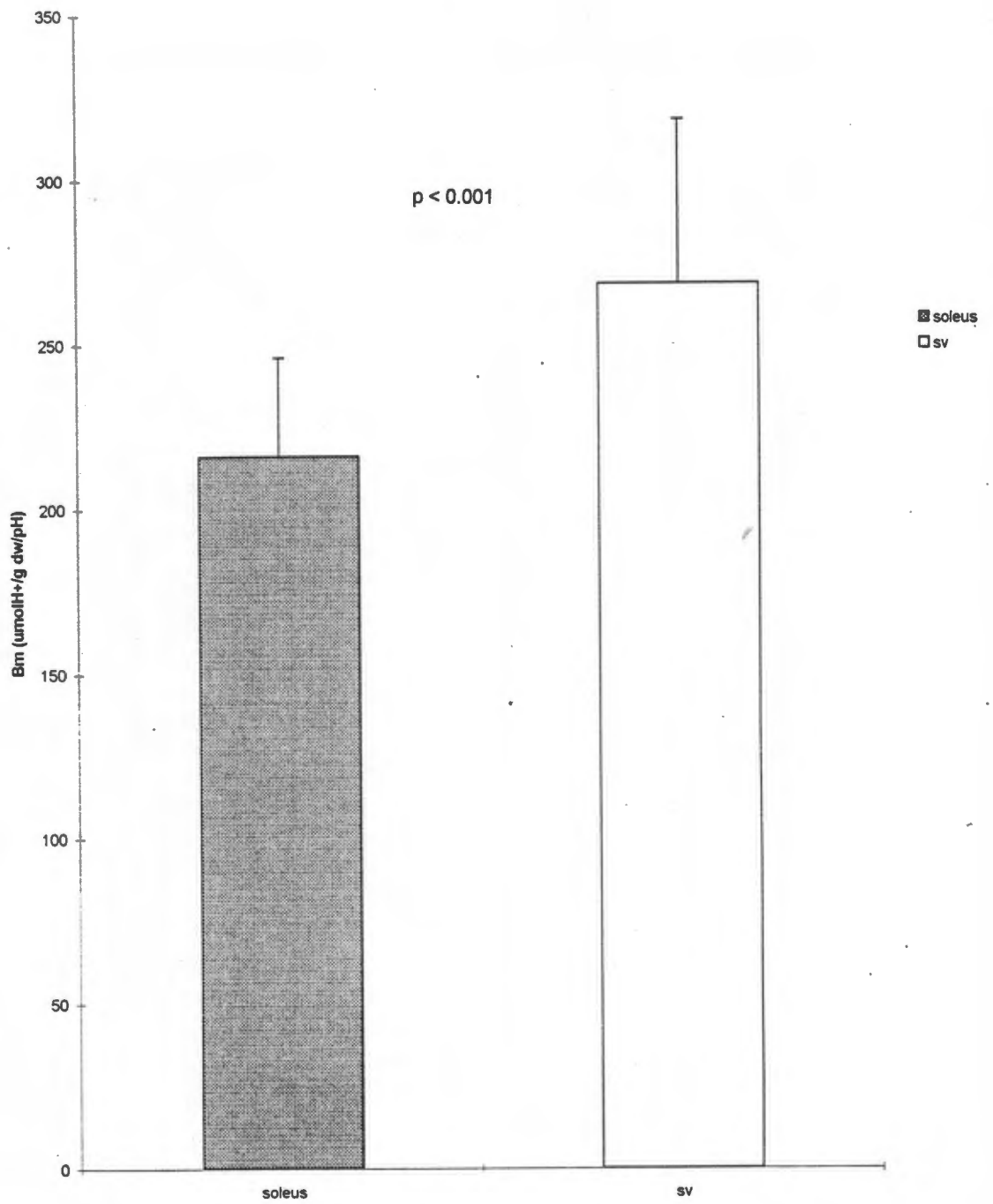
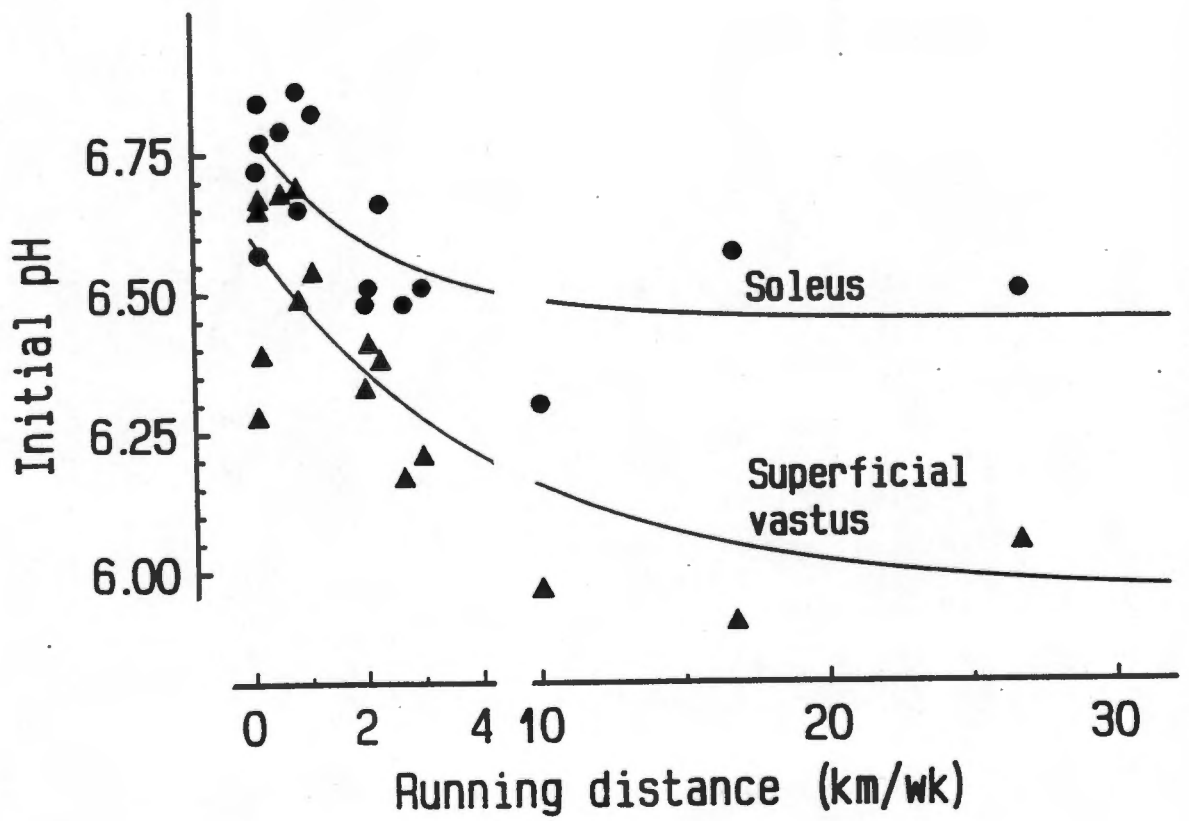


Fig 3.63 Relationship between weekly running distance and pH_i in soleus and superficial vastus muscle of rats



Citrate synthase activity was significantly higher in the soleus muscle than in the superficial vastus muscle ($p < 0.0001$), whilst phosphofructokinase activity was significantly higher in the superficial vastus muscle ($p < 0.001$) (Table 3.62). CS activity was significantly correlated with mean weekly running distance of the running rats in the superficial vastus ($r = 0.66$, $p < 0.01$) but not in the soleus muscle. PFK activity was not related to spontaneous running activity in either muscle.

Table 3.62: Mean enzyme activity of running rats ($\mu\text{mol}/\text{min}/\text{gram wet weight}$)

Enzyme	soleus	s.v.	p value
CS	28.6 ± 7.3	10.8 ± 3.5	$p < 0.0001$
PFK	34.9 ± 8.9	62.9 ± 29.2	$p < 0.001$

s.v. superficial vastus

Values are expressed as mean \pm SD

The percentage of muscle wet weight accounted for by protein was consistently greater in the superficial vastus muscle when compared to the soleus muscle (22.04 ± 3.7 vs 16.77 ± 3.0 respectively, $p < 0.0001$). This represents a 31% difference. There was no detectable relationship between protein to muscle wet weight ratio and spontaneous activity.

DISCUSSION

The results showed that the superficial vastus muscle of the rat, containing 100% type II fibres, has a greater ability to buffer changes in intramuscular pH than does the soleus muscle, which is predominantly type I muscle.

Most βm measurements in humans have been made in tissue from the vastus lateralis muscle. However, the fibre type composition of this muscle is extremely heterogeneous and variable (Johnson et al, 1973; Simoneau & Bouchard, 1989). Assuming a similar relative difference in βm between type I and II fibres in humans as has been observed between the soleus and superficial vastus in the current rat study (24% greater in type II), the calculated difference in βm is 10% when comparing two individuals, one with 70% type I and the other with 70% type II fibres. Since percentage fibre type varies considerably between individuals (Komi et al, 1979), more than it does as a result of any physiological intervention within one individual, this may partially influence the results of cross-sectional studies and may have contributed in part to the conflicting results in the current literature.

Protein density was 31% higher in the superficial vastus than in the soleus in the running rats. This difference was of a similar magnitude to the difference in βm (24%). Any

discrepancy could be the result of a higher level of non-buffering protein in the superficial vastus or a difference in the protein residues available for proton buffering between the two fibre types. This may explain the findings of Parkhouse et al (1985) and Mannion et al (1995) who found a trend toward higher βm as the proportion of cross-sectional area occupied by type II fibres increased.

In this study, pH_i values were considerably lower than physiological resting level of 7.05, indicating a continued lowering of the pH of the muscle after sacrifice. However, the apparent exponential decay plateau of pH_i versus weekly running distance was of the order of a half pH unit higher in the soleus when compared with the superficial vastus. Whilst it would be inappropriate to reach a conclusion from this methodological study, I hypothesise that the lower pH_i of the superficial vastus muscle homogenate may be due to a) mechanisms that sustain glycolysis in the superficial vastus or b) mechanisms that enhance oxidative potential and lactate/proton transport in the soleus. Rat type I fibres have previously been shown to have a 50% higher lactate transport capacity out of the muscle cell than type II fibres (Juel et al, 1991). Since the transport of lactate is coupled to the transport of hydrogen ions, this may be physiologically related to the lower buffering capacity *in vivo*.

In the current study there was no relationship between the level of spontaneous training and βm_{titr} in either muscle fibre type. However, this should not necessarily be interpreted as an inability of βm to adapt to voluntary wheel running. It has previously been shown that rats housed in similar wheels to those used in this study, exercise at approximately the same speed regardless of total distance (low mileage rats, 41 ± 3 metres/min vs high mileage rats, 45 ± 2 metres/min) (Rodnick et al, 1989). However, the rats utilised in the current study were, in general, older and heavier than in previous studies and their mean spontaneous running distance was considerably lower (Rodnick et al, 1989; Mondon et al, 1985). Therefore, one possible explanation for the lack of change in βm_{titr} is that the total training distances were simply insufficient to provoke this muscle adaptation. However, training was sufficient to elicit other biochemical adaptations in the muscle. Both CS activity and pH_i correlated significantly with mean running distance covered per week. Another possible explanation is that an enhancement of βm_{titr} requires high intensity, intermittent exercise with *short* rest intervals. In this study, rats exercised of their own volition with self-selected exercise and rest bouts. This is in contrast to human studies involving sprint training (Sharp et al, 1986; Bell & Wenger, 1988) or sustained submaximal high intensity training (Weston et al, 1996) that imposed set intensities and rest periods. It may be that the latter fatiguing portion of the exercise bout with resultant hydrogen ion accumulation is critical to the augmentation of βm_{titr} .

A third possible explanation is that the training load (resistance) of spontaneous wheel running is insufficient to provoke changes in βm_{titr} . If protein density is indeed an important determinant of βm_{titr} , then heavy resistance training may be required to provoke net protein synthesis. Alway et al, (1988) showed that the increase in more dense contractile protein may be disproportionate to the increase in less dense mitochondrial protein in strength-trained athletes.

CS activity was positively correlated with mean weekly mileage in the superficial vastus ($r=0.66$, $p<0.01$) but not in the soleus muscle. This indicates that predominantly type II muscle also has the ability to increase its oxidative capacity in response to training and *still* maintain significantly higher βm_{titr} . This ability to improve oxidative capacity without compromising buffering capacity may be of importance in athletes partaking in shorter endurance events of high intensity.

In conclusion, the results of this study have shown a higher βm_{titr} in the predominantly type II superficial vastus when compared to the predominantly type I soleus muscle in rats. I hypothesise that this is at least partially due to a higher ratio of protein to weight of muscle in the type II muscle. βm_{titr} was not related to the amount of spontaneous activity in this study but CS activity was positively correlated with mean running distance per week in type II muscle.

It is therefore recommended that future cross-sectional studies comparing βm_{titr} in human mixed muscle eg. vastus lateralis, should report fibre type percentage and protein concentration. Therefore, in the human studies to follow, fibre type and protein concentration were also investigated.

CHAPTER 3.7

SKELETAL MUSCLE BUFFERING CAPACITY AND FIBRE TYPE PROPORTION IN AFRICAN AND CAUCASIAN RUNNERS

BRIEF RATIONALE AND AIM

The only previous study to investigate β_m in African distance runners was that of Saltin et al (1995a) who reported a β_m value in Kenyan runners that was less than that of Scandinavian runners ($p < 0.05$). However, the African athletes in this study had a considerably, but not significantly, higher level of type I fibres. The results of the previous chapter indicated a difference in the β_m of type I and type II fibres in rats. Assuming a similar degree of difference between type I and type II fibres in human subjects, it is possible that this may have accounted for the higher β_m of the Scandinavian runners. Furthermore, the Kenyan runners were long distance and cross-country runners but it is unclear whether the Scandinavians were also long distance runners or whether in fact they were middle-distance track athletes. 800 m runners have previously been shown to have a higher β_m than distance runners (Parkhouse et al, 1985) and not knowing the subjects' primary racing distance, the higher β_m of the Scandinavian runners is difficult to interpret. In addition, the confounding effects of residence at high altitude of the Kenyans or acute exposure to altitude in the Scandinavians, may have influenced the results. Mizuno et al, (1990) reported an improvement in β_m in endurance athletes after training at altitude for 2 weeks. However, as this occurred in both the triceps and the gastrocnemius, despite different patterns of use, this was probably an effect of hypoxia and not the endurance training *per se*.

Therefore, the aim of the current study is to investigate skeletal muscle buffering capacity, together with fibre type proportions, in African and Caucasian runners who are living and training at sea level and who are predominantly 10 km runners.

METHODS

Subjects:

β_m and fibre type composition were determined in 10 runners (5 African, 5 Caucasian). Subjects were recruited from those who had taken part in the previous studies in the laboratory. All subjects were resident at sea level in Cape Town, South Africa and all were seasoned sub-elite runners who considered their primary competitive distance to be 10 km. Subject characteristics are displayed in Table 3.71.

Subjects gave their written informed consent to all procedures which had previously been approved by the Research Ethics Committee of the University of Cape Town Medical School.

Many of the subjects had previously had a muscle biopsy of the vastus lateralis for the measurement of skeletal muscle enzyme activities, detailed in Chapter 3.3. If necessary, the VO_2 max test was repeated to ensure it was tested within 3 weeks of the current muscle biopsy procedure.

Muscle biopsy:

A skeletal muscle biopsy was obtained from the vastus lateralis muscle with suction using the technique described by Bergstrom (1962) as modified by Evans et al (1982). The procedure is explained in more detail in Chapter 3.3. Two bites of muscle were obtained from the same incision. One was immediately frozen in liquid nitrogen, and later freeze-dried prior to the analysis of βm . For the analysis of βm , samples were coded and paired so that each pair contained an African and a Caucasian sample. Within the pair, the order of analysis was random and samples were blinded to the investigator. Skeletal muscle buffering capacity was determined by titration as described in more detail in the previous chapter (Chapter 3.6).

The second biopsy sample was embedded in Tissue-tek and frozen in liquid nitrogen-cooled n-Pentane. The sample was subsequently sectioned and the percentage of fibre types was determined using the myosin ATPase staining technique previously described (Chapter 3.3). All fibres in one field were counted, in all cases this was at least 150 fibres per sample.

Statistics:

Comparisons between African and Caucasian runners were made using a Student's t-test for unpaired data. Relationships between variables in the group as a whole were investigated using the Pearson's Correlation coefficient. Results are reported as mean \pm SD.

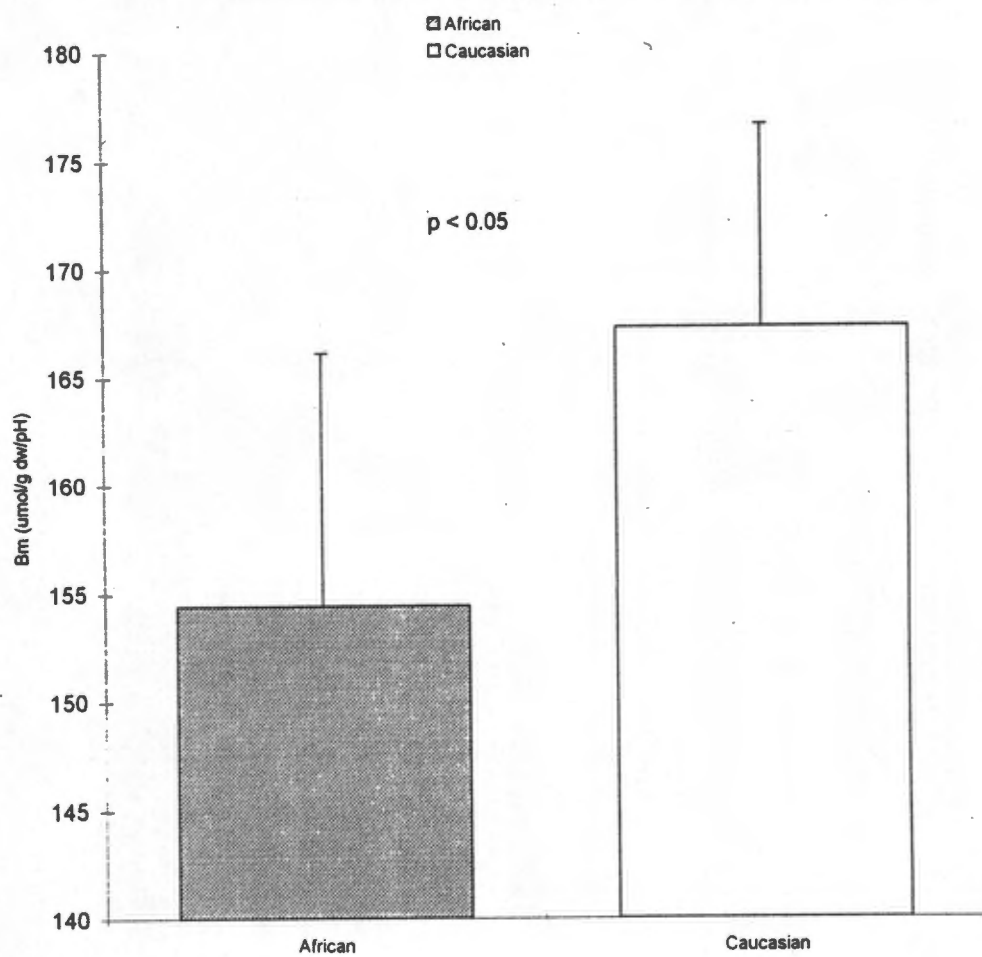
RESULTS

Mean anthropometric and exercise test data are presented in Table 3.71.

Table 3.71 Subject characteristics

	African runners	Caucasian runners	significance
Age (yrs)	31.4 \pm 3.2	28.6 \pm 4.4	ns
Mass (kg)	60.8 \pm 7.8	65.8 \pm 3.0	ns
Height (cm)	172.0 \pm 5.8	179.0 \pm 6.9	ns
VO_2 max (ml/kg/min)	59.1 \pm 6.3	67.9 \pm 7.5	ns
10 km time (min)	32.9 \pm 1.8	33.4 \pm 2.5	ns
Current weekly km	120 \pm 51	90 \pm 28	ns

Fig 3.71 Skeletal muscle buffering capacity in African and Caucasian runners



βm_{titr} was 8% higher in the Caucasian runners than in the African runners (Fig 3.71) but there was no difference in the initial muscle homogenate pH (pH_i) or the concentration of protein in the homogenate (Table 3.72).

Table 3.72 βm and fibre type proportions

	African runners	Caucasian runners	significance
βm_{titr} ($\mu\text{mol H}^+/\text{g dw}/\text{pH}$)	154.4 \pm 11.7	167.3 \pm 9.3	$p < 0.05$
pH_i	7.11 \pm 0.07	7.13 \pm 0.05	ns
% type I fibres	56.2 \pm 16.7	69.6 \pm 15.3	ns
% type II fibres	43.8 \pm 16.7	30.4 \pm 15.3	ns
Protein (mg/ml)	7.7 \pm 1.4	7.8 \pm 1.8	ns

There was no correlation between βm and VO_2max or race performance in this total group of runners. In the current study, βm was not related to the proportion of type II fibres or the concentration of protein measured in the sample. A high total protein concentration had a tendency to be associated with a high proportion of type II fibres ($r=0.46$, ns). Nine of the subjects in the current study had previously had CS activity of the vastus lateralis measured (chapter 3.3). The negative relationship between CS activity and βm approached significance ($r=-0.49$). There was no relationship between PFK activity and βm .

DISCUSSION

The βm results of the African and Caucasian runners investigated in this study are in agreement with those of Saltin et al (1995a). The βm of the Caucasian runners was 8% higher ($p < 0.05$) than African runners. In the previous study of Saltin et al (1995a) the result may have been explained by residence at altitude or primary race distance as discussed earlier, but in the current study all subjects were resident at sea level and were all primarily 10 km runners so these factors can not be explanatory.

Considering all individuals investigated in the current study, βm was not related to the proportion of type II fibres, although it should be acknowledged that the number of subjects is small. Furthermore, in contrast to the runners in the study of Saltin et al study (1995a) there was a tendency for the Caucasian runners in the current study to have a *higher* proportion of type I fibres than the African runners (69.6% vs 56.2%) although because of a large range of values this did not reach statistical significance. Making the assumption that the findings of the previous rat study hold for humans, with type II fibres having higher βm than type I fibres, it is unlikely that fibre type proportions could explain the difference between the Caucasian

and African runners in the current study. Therefore, there must be a further explanation for the difference in βm between Africans and Caucasians in the current study.

The higher βm of the Caucasians may be the result of a difference in training. However, training distance was measured in the current subjects, with no difference between groups, although training intensity was not quantified. Coetzer et al (1993) report that African runners train more intensely than Caucasians, spending a higher percentage of training time at 85% VO_2 max or above. Higher intensity training has previously been associated with higher βm (Sharp et al, 1989; Weston et al, 1996) and therefore if the African runners in the current study are assumed to be training more intensely or at the least at the same intensity as the Caucasians, this would certainly not explain the higher βm of the Caucasians, in fact one would expect the opposite result. A major determinant of βm is the concentration of protein available to take part in the intramuscular buffering of protons. The concentration of total protein was therefore measured in the current study and was not different between the Caucasians and Africans in this study, although was related to the proportion of type II fibres. However, more specifically than total protein concentration, the relative concentration of constituent proteins with pK_a values in the range of pH likely to be present during exercise is a major determinant of βm . Therefore, although speculative, a difference in the relative concentration of the constituent proteins that contribute to intramuscular buffering can not be discounted.

βm was not significantly related to 10 km race time. Therefore, in contrast with the findings of Weston et al (1996) in elite cyclists, the current results indicate that βm may not be an important factor in the determination of 10 km performance in sub-elite runners. The tendency toward a negative relationship between βm and CS activity in the vastus lateralis is difficult to interpret. In chapter 3.4, it was shown that CS activity is an important determinant of the individual runners ability to resist fatigue at a high percentage of their own peak treadmill speed. It is tempting to speculate that those individuals who do not have a high CS activity require an elevated βm to achieve a comparable race performance. Alternatively, training methods may differ because moderate intensity endurance exercise is likely to provoke an increase in mitochondrial oxidative activity (Gollnick et al, 1973) whilst higher intensity activity is likely to provoke an increase in βm (Sharp et al, 1989).

In summary, the results of the current study show a higher βm in the vastus lateralis of the Caucasian distance runners when compared to African distance runners that is not explained by fibre type proportions or total protein concentration of the muscle sample. At present, one can not rule out an inherent genetic difference as a cause of the higher βm in Caucasians. If such a difference is also apparent in sedentary African and Caucasian individuals with similar

lifestyles, this would point toward a genetic rather than a training cause. To the best of the investigator's knowledge, βm has never been compared in sedentary Africans and Caucasians. Therefore, βm is investigated in a sample of sedentary Africans and Caucasians in the following chapter.

CHAPTER 3.8

SKELETAL MUSCLE BUFFERING CAPACITY IN SEDENTARY AFRICANS AND CAUCASIANS

BRIEF RATIONALE AND AIM

The results of the previous chapter indicated that β_m was significantly higher in the Caucasian runners than in the African runners. This was in agreement with a previous comparison of β_m in Kenyan and Scandinavian runners (Saltin 1995a). The somewhat higher percentage of type II fibres in the Scandinavians appeared to be one possible explanation for the findings of that study, particularly in the light of the findings of chapter 3.6 of this thesis that type II muscle in rats has a higher β_m than predominantly type I muscle. However, that explanation is perhaps less plausible given the findings of chapter 3.7, which showed a higher β_m in the Caucasians runners *despite a lower* proportion of type II fibres. As both of these studies involved well-trained individuals, it is not clear whether a higher β_m is an inherent property of Caucasian skeletal muscle or whether Caucasian runners adapt their β_m to a greater extent than the African runners.

Buffering capacity can adapt to training. Studies have indicated improvements in skeletal muscle buffering capacity with *sprint* training although there are other studies which contradict this result. The literature is reviewed in Chapter 2.22. Weston et al (1996) reported an increase in skeletal muscle buffering capacity in endurance cyclists with addition of high intensity training to an endurance training regime. In addition, there was a substantial but not significant relationship ($r=0.74$) between the increase in β_m and the reduction in the time taken to complete a 40 km time trial after high intensity, but submaximal, interval training in this small group of well-trained endurance cyclists. This has not been investigated in endurance runners, so it is premature to attribute the differences in β_m in chapter 3.7 to differences in training regimes. To negate any confounding effects of training upon β_m , it is necessary to investigate β_m in sedentary African and Caucasian individuals.

METHODS

Subjects:

Twelve sedentary subjects who volunteered for the study were all students attending the University of Cape Town and had similar current lifestyles and diet (all undergraduates who lived in university residence/communal student housing). Dietary results are presented in Chapter 3.5. All were currently sedentary having not exercised regularly (exercised < 1 hr/wk) for the last six months as determined by a modified Blair questionnaire (Blair et al, 1985) and interview. The study procedures were approved by the Research Ethics Committee of the University of Cape Town Medical School.

Muscle biopsy:

A skeletal muscle biopsy was obtained from the vastus lateralis muscle with suction using the technique described Bergstrom (1962) and later modified by Evans et al (1982). The procedure is explained in more detail in Chapter 3.3. Two bites of muscle were obtained. The first was immediately frozen in liquid nitrogen and later freeze-dried prior to the analysis of β m. For the analysis of β m, samples were coded and paired so that each pair contained an African and a Caucasian sample. Within the pair, the order of analysis was random and blinded to the investigator. Skeletal muscle buffering capacity was determined by titration as described in more detail in Chapter 3.6.

The second biopsy sample was embedded in Tissue-tek and frozen in liquid nitrogen-cooled n-Pentane for determination of fibre type percentage using myosin ATPase staining as previously described (Chapter 3.3). All fibres in one field were counted, in all cases this being at least 150 fibres per sample.

Statistics:

Comparisons between sedentary Africans and Caucasians were made using a Student's t-test for unpaired data. Relationships between variables in the group as a whole were investigated using the Pearson's Correlation coefficient. Comparisons between sedentary subjects (African and Caucasians pooled) and runners (African and Caucasian pooled), were also made using an unpaired Student's t-test. Results are expressed as mean \pm SD.

RESULTS

Subject characteristics are presented in Table 3.81.

Table 3.81 Subject characteristics

	Sedentary Africans	Sedentary Caucasians	significance
Age (yrs)	21.3 \pm 1.5	21.8 \pm 1.7	ns
Mass (kg)	60.8 \pm 10.0	75.0 \pm 7.7	p=0.01
Height (cm)	173.3 \pm 6.9	180.5 \pm 5.7	p<0.05
VO ₂ max (ml/kg/min)	45.0 \pm 5.0	53.9 \pm 6.5	p<0.05

In contrast to the results in the runners, the sedentary Africans had a higher β m than the sedentary Caucasians (Table 3.82). There was no significant difference in fibre type proportions although there was a tendency for the Africans to have a higher percentage of type II fibres. There was also a tendency for the sedentary Africans to have a higher concentration of total protein than the sedentary Caucasians.

Table 3.82 β m and fibre type proportions

	Sedentary Africans	Sedentary Caucasians	significance
β m(μ mol H ⁺ /gm dw/pH)	169.1 \pm 7.2	159.1 \pm 11.4	p=0.05
pH _i	7.03 \pm 0.04	7.13 \pm 0.05	p<0.005
%type I	40.1 \pm 10.0	48.3 \pm 14.3	ns
%type II	59.9 \pm 10.0	51.7 \pm 14.3	ns
Protein conc (mg/ml)	7.1 \pm 1.2	6.4 \pm 0.7	ns

Considering the whole group, a high β m was associated with a high proportion of type II fibres, although this did not reach significance ($r=0.35$). The initial pH of the muscle homogenate was significantly lower in the muscle of the Africans and this was associated with a higher β m ($r=-0.51$, ns).

Results of the sedentary individuals in this study were compared with those of the runners in the Chapter 3.7. When considered as a total group, the runners had a significantly higher concentration of protein per dry weight of muscle than the sedentary individuals (7.8 \pm 1.5 vs 6.7 \pm 1.0 mg/ml respectively) however there was no significant difference in β m between the runners and the sedentary individuals. The mean percentage of type I fibres in the vastus lateralis of the runners was considerably higher than that of the sedentary individuals (63% vs 44.2% type I fibres).

DISCUSSION

Skeletal muscle buffering capacity was significantly higher in the sedentary Africans than the sedentary Caucasians. Therefore, the higher β m reported previously in Caucasian runners (Saltin, 1995a, Chapter 3.7) is not necessarily an inherent factor present prior to training.

This result in sedentary individuals may be partially explained by a tendency toward a higher percentage of type II fibres in the African individuals. The results of chapter 3.6 indicated that in rats the type II fibres have a higher β m when compared to type I fibres. Making the assumption that the same holds for human type I and II skeletal muscle fibres, then a higher percentage of type II fibres would result in a higher β m. Furthermore, the results of the current study in sedentary human individuals somewhat mimic the patterns reported in Chapter 3.6 in rats. In the sedentary individuals, a high β m was associated with a low initial pH of the muscle homogenate and a higher total protein concentration, as was the case in the rat muscle. However, the relationship between β m and fibre type was not seen in the runners in Chapter 3.7 and therefore there appears to be a discrepancy between the sedentary and the athletic human data. The discrepancy may be due to a higher oxidative

capacity of *all* fibre types and therefore less distinction between type I and type II fibres, or less type IIB fibres in the well-trained runners. This certainly warrants a larger study in the athletic population in general.

A comparison of βm in chapters 3.7 and 3.8 indicated that there was no significant difference between these distance runners and the sedentary individuals which is in agreement with the findings of Parkhouse et al (1985) who showed no difference between untrained individuals and marathoners.

In summary, the findings of the current study provide no evidence for an inherently higher βm in Caucasians when compared to Africans in sedentary individuals. Therefore, the βm findings are similar to those of the skeletal muscle oxidative enzymes investigated in this thesis. A significant and sizeable difference was apparent between the African and Caucasian well-trained runners despite no evidence of an inherent difference being apparent in the African and Caucasian sedentary individuals. Because of the small sample size and resultant low statistical power, it is premature to reach a definitive conclusion regarding buffering capacity in African and Caucasian runners.

CHAPTER FOUR

SUMMARY OF THE MAIN FINDINGS

The results of the current series of studies provide evidence of differences between the African and Caucasian long distance runners investigated in these studies with respect to selected exercise and skeletal muscle characteristics. The following is a summary of the main findings of the current series of studies with more detailed discussions to be found in the relevant chapters. After the exposition of the summary of the main findings, there follows a discussion of the limitations of the current series of studies, as well as an indication of future studies which are warranted as a result of the current findings.

The first major finding was that the African runners had the ability to delay the onset of fatigue during high intensity running when compared to Caucasian controls. When exercising at the same relative percentage of individual maximal treadmill velocity (which was not different between groups), African distance runners were able to exercise for a mean duration that was four minutes longer than the Caucasian distance runners ($p < 0.01$). A longer time to fatigue was significantly related to a lower plasma lactate concentration and a lower respiratory exchange ratio indicating that these variables are important markers of the ability to resist fatigue during high intensity running. We hypothesised that the ability to resist fatigue is important in determining the relative intensity that can be maintained throughout a race.

The second major finding confirmed this suggestion. When running at current 10 km race pace in the laboratory, the African runners were shown to run at a higher percentage of their maximal oxygen uptake when compared to the Caucasian runners (93.5 vs 86.0%, $p < 0.005$), and a higher percentage of their maximal heart rate (97% vs 92%, $p = 0.09$). However, this higher relative intensity, elicited only a comparable plasma lactate concentration and respiratory exchange ratio. These results and those of the first study indicate that the African runners accumulate less plasma lactate and elicit a lower RER than the Caucasians when exercising at the same relative intensity.

In general, the groups of African and Caucasian runners investigated in this series of studies were matched for their current 10 km race performances. But the Africans achieved this despite a lower $VO_2\text{max}$. Therefore, it appears that the greater ability to resist fatigue and the higher fractional utilisation of $VO_2\text{max}$, compensated for the lower absolute $VO_2\text{max}$ in the African runners, facilitating a similar race performance. These data more fully explain why $VO_2\text{max}$ is not always the best predictor of racing performance. In fact, it appears that other physiological factors can compensate for a slightly lower $VO_2\text{max}$ in the elite runner

(Morgan et al, 1989). One such factor is the ability to run economically, in order to minimise oxygen consumption.

The third main finding was that the African runners were more economical than the Caucasian runners. This was consistently apparent at a variety of workloads. The difference in economy was more profound when oxygen consumption was normalised per $\text{kg}^{0.66}$ as has been suggested to be more appropriate by other authors (Astrand & Rodahl, 1976, Fredericks, 1987). As discussed in chapter 3.2, the reason for this greater economy of movement during running in the Africans is not yet clear. Biomechanical analysis is required.

The fourth main finding was that the African group of runners with the same mean 10 km race performance as the Caucasian group of runners, had a significantly lower proportion of type I skeletal muscle fibres. Therefore, the fifth main finding of this series of studies was surprising. The African runners studied were found to have a 50% greater activity of key oxidative enzymes of carbohydrate and fat metabolism (CS and 3-HAD) in the vastus lateralis when compared to the Caucasian runners. Although the sample size in this invasive study is small, the magnitude and the significance of the difference between the two groups ($p < 0.005$) is unlikely to be purely a sampling effect. Furthermore, only one African runner fell within the range of the Caucasian runners with respect to CS activity. The mean % type I fibres was *lower* in the African athletes than in the Caucasians. Hence the percentage of type I fibres could not be an explanation of the higher oxidative enzyme activities. As discussed more fully in Chapters 3.3 & 3.4, skeletal muscle oxidative enzyme capacity, is no longer believed to be a predictor of maximal oxygen consumption, but may be important for optimising the utilisation of one's own maximum capacity during exercise of this duration and intensity. This hypothesis is supported by the fifth major finding of this thesis.

Skeletal muscle oxidative capacity, represented by CS activity, was significantly related to the runners' ability to resist fatigue at a high running intensity relative to their individual peak treadmill velocity ($r = 0.70$, $p < 0.05$). The mechanism by which CS activity is beneficial is elucidated by the association between a higher CS activity and a lower plasma lactate concentration ($r = -0.73$) and a lower RER ($r = -0.63$). These associations suggest that the African runners had a preferential utilisation of fat and lesser glycolytic flux with a resultant minimising of the perturbations of the intracellular environment during intense exercise. The mechanism is discussed in more detail in Chapter 3.4.

The sixth main finding of this thesis was that skeletal muscle buffering capacity of the Caucasian runners was higher than that of the African runners, and βm was not related to % type II fibres or to 10 km race time in this group of sub-elite runners.

Furthermore, despite the differences in skeletal muscle characteristics observed between African and Caucasian runners in the current thesis, there was no evidence of these differences being inherently present in sedentary individuals. However, it is possible that successful runners lie at the extremes of the normal population curve and that the randomly-selected sedentary individuals in the current study may be more reflective of the mean of the populations. Therefore, although this thesis provides no direct evidence of the observed differences between African and Caucasian runners being of purely genetic origin, this can not be ruled out. If it were feasible, an informative design would be the analysis of skeletal muscle characteristics in *detrained* African and Caucasian runners. This would establish if the oxidative enzyme capacity remain more elevated in the African runners than in the Caucasians without a training stimulus. However, a more valuable approach would be to elucidate the underlying genetic cause by identification of the responsible genes, and then an investigation of incidence of favourable genes in the respective populations.

In conclusion, the current series of studies do provide evidence of differences in selected exercise and skeletal muscle characteristics between African and Caucasian distance runners, with the African runners possessing characteristics that may be beneficial for optimising their endurance performance.

Limitations:

In the current thesis, exercise parameters were measured in the laboratory with the subject running on a motorised treadmill. Many subjects are unfamiliar with this sensation and considerable familiarisation is required. In particular the African runners have less experience running on a treadmill than the Caucasians and therefore considerable efforts were made by the investigator to ensure subjects were fully familiarised. Nevertheless, if familiarisation is considered to be a potential limitation, it is likely that continued familiarisation of the African runners would have served only to further improve running economy and time to fatigue, these *exaggerating* the observed differences.

Ideally these parameters should be measured in the field whilst racing. Unfortunately this is not possible. Not only is this technically difficult, but runners of this standard and their coaches are reluctant to partake in studies on race day.

The number of subjects in the invasive studies reported in the current thesis is small and therefore the results should be interpreted with due caution. Recruitment for invasive procedures is difficult and this is a limitation of many studies of this nature. Furthermore, the site of the muscle biopsy for the current investigations was the vastus lateralis, rather than

the gastrocnemius muscle of the lower leg. This is the site previously found to be the most acceptable to subjects in our laboratory and provides a lower risk of complications, however it should be acknowledged that this site may not be ideal for investigating the physiological and biochemical adaptations of runners. This may be a limitation in the comparison between trained and untrained individuals, but should have less impact on comparisons between Africans and Caucasians of a similarly trained state, and therefore is of minimal limitation to this thesis.

Skeletal muscle investigations in the current thesis were performed in muscle homogenates. As a result of equipment limitations, it was not possible to assess enzyme activities and buffering capacity in muscle fibres pooled according to fibre type.

The findings of the current study can not directly be extrapolated to explain the success of the very elite African runners. To study those athletes is difficult as elite runners are reluctant to be assessed and are certainly very reluctant to undergo invasive procedures.

Future directions:

The differences in oxidative enzyme activities and βm between the African and Caucasian runners could conceivably be due to different habitual training although when expressed as km/wk as in the current series of studies no difference was apparent. However, thorough quantification of training volume and particularly training intensity is required. But, the *response* to training has been shown to vary considerably between individuals *and* to be partially genetically-determined. Therefore, it would be ideal to carry out a study of skeletal muscle biochemical characteristics measured before and after an identical training regime in both African and Caucasian sedentary individuals.

Furthermore, the higher oxidative enzyme activities of the Africans despite a lower proportion of type I fibres certainly warrants further investigation. To do so it is necessary to a) pool isolated single fibres and quantify glycolytic and oxidative enzymes with respect to fibre type and b) to analyse substrate utilisation and depletion during exercise with respect to pooled single fibres.

In addition, the findings of the current series of studies indicate that substrate choice and subsequent lactate accumulation observed in the African runners, was related to the higher oxidative enzyme capacity. However, a further full inventory of extensive metabolic studies is required. Amongst these studies is a) habitual and pre-race nutrition and basal metabolism b) the determination of lactate production and lactate clearance (including lactate oxidation), and d) lactate transport.

In addition, further investigation of African and Caucasian runners in the field are required, however this requires considerable cooperation from both coaches, athletes and race organisers. These studies were attempted amongst the current series of studies and methodological difficulties have been highlighted. Financial incentives are required for the full cooperation of all involved. Furthermore, with respect to the origin of the greater running economy, it would seem that biomechanical analysis of gait is warranted.

Conclusion:

In conclusion, the current series of studies in sub-elite runners has provided further evidence of several differences between African and Caucasian runners with respect to their metabolic response to exercise. However, it is the first study to present details of skeletal muscle biochemical differences that provide an explanation for these metabolic differences and may provide an insight into the superior performance of African distance runners. If one does speculate that the characteristics observed here in African sub-elite runners (higher oxidative enzyme activity, greater fatigue resistance and fractional utilisation of VO_2 max, and greater running economy) are also present in elite Africans who have as high maximal oxygen consumption as their Caucasian counterparts, these would ultimately lead to greater success.

CHAPTER FIVE

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CHAPTER SIX

APPENDICES

APPENDIX 6.1

LACTATE PILOT STUDY RESULTS

A pilot study was undertaken by the investigator to determine the most appropriate time to draw blood in order to obtain the peak plasma lactate concentration following exercise to exhaustion during the incremental treadmill protocol to be utilised in Chapter 3.1.

Subjects:

Twelve distance runners were recruited for this pilot study (7 African & 5 Caucasian). All were sub-elite or elite runners of a similar standard to those recruited for the studies described in this thesis. Eight were indeed subjects for one or more of the studies that followed.

Procedure:

Prior to exercise, a 20-gauge Jelco cannula was inserted in a forearm vein and a three-way tap was attached. A pre-exercise blood sample was drawn to ensure patency and the cannula was then flushed with heparinised saline.

Subjects completed the warm-up and maximal exercise test (1km/hr increments/min) on the treadmill as described in Chapter 3.1. Following completion of the exercise test a blood sample was drawn at 1,2,3 and 4 minutes post exercise. Tubes were gently mixed and stored on ice until they were centrifuged at 4°C at 3000 rpm. Plasma was removed and stored for subsequent assay as described in Appendix 6.2.

Results:

The individual results are displayed in Table 6.11

Table 6.1 Plasma lactate concentration after maximal exercise

Subject	1 min	2 min	3 min	4 min	
1	11.6	13.1*	13.0	-----	
2	10.2	12.5	13.2	13.6*	
3	6.2	6.2	6.4*	6.3	
4	9.2	9.0	9.3*	9.0	
5	12.3	12.9	13.6*	13.0	
6	2.0	8.0	8.8*	8.1	
7	13.8*	13.8*	13.5	12.3	
8	2.3	4.2	4.4*	4.3	
9	8.2	9.1	9.7*	9.4	
10	-----	10.2	10.6*	10.0	
11	-----	8.0*	7.3	7.0	
12	8.0	8.3	8.6*	8.5	
Mean	6.9	9.3	9.7	8.5	(includes complete series only)
SD	3.6	3.2	3.2	3.1	

Peak values are denoted with *

In 9 out of the 12 subjects, the peak lactate concentration occurred 3 minutes post exercise, constituting 75% of the sample. In the individuals for whom the peak occurred elsewhere, their value at 3 min was not substantially different. Therefore, it was considered appropriate to sample blood for determination of peak plasma lactate concentration at 3 minutes post maximal exercise in runners for the purposes of this thesis.

APPENDIX 6.2

LACTATE ASSAY

1. Collect venous blood sample in a vacutainer containing potassium oxalate/sodium fluoride (grey top), invert and keep on ice.
2. Centrifuge and remove plasma as soon as possible then freeze plasma for future analysis
3. PIPES buffer (100mmol/l, pH 6.8) + 4-chlorophenol (5.4 mmol/l) + sodium azide and surface-active agents (from kit: bioMerieux Lactate PAP).
4. Add 10 ml of the above buffer to the enzyme solution (from kit). Shake gently. Stable ~ 6 weeks in fridge.
5. Set wavelength @ 505 nm
6. Zero with reagent blank
7. Make up a standard with 3 mmol/l (27 mg/100ml) in 1g/l of sodium azide. Use 10 ul of this in 1 ml of buffer as the standard.
8. Add 10 ul of sample to 1 ml of buffer.
9. Incubate 5 min (room temp) and read absorbance.

Calculation:

$$(\text{Abs sample} / \text{Abs standard}) \times n$$

where n = conc of std in mmol/l or mg/100ml)

APPENDIX 6.3

AMMONIA ASSAY

1. Collect venous blood sample in vacutainer containing K₃ EDTA (15%) (purple top).
Centrifuge and remove plasma as soon as possible (preferably within 5 mins).
Store on ice and cover with parafilm.
Can wait no longer than three hours to do assay.
2. Using ammonia kit (Boeringer Mannheim NH₃ kit):
Add 2.5 ml of buffer (bottle 1a) to bottle 1 (reagent).
Close bottles after use
Stable for ~ 24 hours

Add 0.5 ml of buffer (bottle 1a) to enzyme bottle (bottle 2).
Dissolve by allowing to stand at room temperature (21°C) and swirling for 10 min.
Close bottle after use.
Stable for 6 weeks in fridge (or longer).
3. Set wavelength at 340 nm
Temp 20-25.
Calibrate with distilled water
Prepare one reagent blank (everything except sample)
4. Add 125 µl of sample.
Add 625 µl of reagent in each cuvette
Mix with parafilm and allow to stand for 10 min.
Read initial absorbance (reading A)
5. Add 5 µl of enzyme. Mix and read at 10 min (reading B)
6. Add further 5 µl of enzyme. Mix and read at 10 min (reading C)

Calculation:

NH_3 ($\mu\text{mol/l}$) = $[(\text{A}-\text{B})-(\text{B}-\text{C}) - \text{R}] \times 959$ (959 will be specific for kit and wavelength)

where R = (A-B)-(B-C) for blank and then correct using chart (~measured x 0.67)

APPENDIX 6.4

NOTES FROM MUSCLE ENZYME ASSAY DEVELOPMENT

Using muscle tissue from the rat, the following work was undertaken prior to finalising the techniques that are described in this thesis for the assaying of human skeletal muscle enzyme activities :

1. Choice of homogenising buffer: Three buffers were compared. 100mM phosphate buffer, KH_2PO_4 , K_2HPO_4 , 2mM EDTA $2\text{H}_2\text{O}$, 5mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, pH 7.4 (described in more detail below) was compared with 50mM TRIS.HCl buffer, 2mM EDTA, 5mM MgCl_2 , 0.02% BSA, 20-30 mM 2-ME, pH 7.4-8.2 and a 50mM TEA buffer, 2mM EDTA, 5mM MgCl_2 , 0.02% BSA, 20-30 mM 2-ME, pH 7.4-7.5. The phosphate buffer was found to be the most suitable for all the enzymes with the exception of OGDH (the BSA causes the problem). OGDH was found to be the least reproducible and less reliable and was subsequently excluded from the studies. It was felt CS would adequately represent Krebs cycle oxidative enzymes for the purposes of this thesis. The phosphate buffer can be stored at 4°C until there are any signs of turbidity.

2. Time from homogenising to analysis: A pilot trial was undertaken to assess which assay should be completed first. This was done by assaying aliquots of the same sample at various time intervals subsequent to homogenisation (immed, + 6 hr, + 24 hr, + 48 hr). It was important to do this as it was not possible to do all assays in one day and some had to be stored until the next day. It was found that CPT was the most labile and should always be done first. HK and PFK assays should be done the same day. The homogenate for the CS assay could be stored for up to two days without activity loss. When only PFK and CS assays were to be done, homogenisation and the PFK assay occurred on the first day and the CS assay on the subsequent day with the homogenate being frozen overnight.

3. Method options for CS: Srere PA or Shephard D & Garland PB both in *Methods of Enzymology* (1969) or Sugden PH & Newsholme EA, *Biochem J* (1975). Selected Srere method because reaction rate was the most linear for the longest time. The principle involved here is $\text{Acetyl CoA} + \text{oxaloacetate} + \text{H}_2\text{O} \rightarrow \text{Citrate} + \text{CoASH} + \text{H}^+$. The CS activity is measured by linking the release of CoASH to the colourmetric agent DTNB which has a molar extinction coefficient of 13,600, at 412 nm. Starting absorbance should be around 1.000 - 1.300.

4. PFK assay: as per method of Ling et al (1965), but reduced the sample size from 20 μl to 5 μl . In early trials the reading was made every minute but we found it more suitable to read every 30 sec and multiple by two and this procedure was adopted for all assays in the thesis. Initial reading should be about 0.800. For assaying PFK in the soleus of the rats the amount of homogenate was increased from 5 μl to 10 μl and the calculation adjusted accordingly. PFK in the SV remained at 5 μl and it

should be noted to expect quite high readings. In the human samples it is not a problem although we didn't test any well-trained sprint athletes.

5. When working with freeze-dried muscle it is very advantageous to finely chop the sample in a weighing boat prior to homogenising. When this was not done we found it difficult to get a smooth homogenate which is crucial for accurate measurements. It is important to weigh on the basis of nett weight gain to your homogenising vessel.

6. Important to keep the sample on ice through-out homogenisation and sonication as this is when the temperature is most likely to rise and activity to be lost.

7. It was my experience that the hand-held glass homogenisers were preferable to the mechanical homogeniser. The latter had problems with retaining small pieces of the sample within its "teeth". Non-freeze dried samples were much easier to homogenise and this was decided to be the preferable approach - assuming this could be adequately stored at $< - 50^{\circ}\text{C}$ prior to analysis, otherwise freeze-drying is unavoidable.

8. Solutions containing NADH, NADPH and AcCoA should be prepared fresh each day. These are very labile and will jeopardise the accuracy of results if used after storage ± 24 hr. Had originally thought OAA was the same but have used it successfully the next day ie. within 36 hr.

9. DTNB is very light sensitive and must be stored wrapped in tinfoil. If the solution looks quite yellow make up again fresh. If even the powder has gone brighter yellow, it probably needs to be replaced.

10. Sample sizes used for other assays: CPT 100 μl (therefore can't often be done in duplicate); HK 20 μl ; HAD 10 μl .

APPENDIX 6.5

PREPARATION OF MUSCLE SAMPLES FOR ENZYME ASSAYS

Sample is dissected free of any remaining connective tissue or blood, chopped finely using a scalpel blade and then homogenised in the following homogenising buffer.

Homogenising buffer:

1. Make up PO₄ buffer 100 mM pH 7.4
KH₂PO₄ 572 mg
K₂HPO₄ 2752 mg
EDTA 2H₂O 2mM 149 mg
MgCl₂.6H₂O 5mM 203 mg
make up to 200 ml dH₂O

2. pH to 7.4

3. Store in opaque bottle in fridge

4. Later, before first use, add:

	to 25 ml PO ₄ buffer stock	to 12.5 ml PO ₄ buffer
2 Mercaptoethanol (ME) 20 mM	35 µl	17.5 µl
BSA (0.02%)	5 mg	2.5 mg
	(for ~ 30 samples of 8 mg dw)	(for ~15 samples)

This can be stored for less than one week.

To homogenise and sonicate sample:

1. Weigh sample

2. Add homogenising buffer at 1:20 ratio for ww
1:90 ratio for dw
eg. 25 mg ww x 19 = 475 µl homo buffer
8 mg dw x 89 = 712 µl homo buffer

3. Homogenise by hand using a glass homogeniser then use a homogeniser on ice, for 10 sec.
Wash homogeniser tip thoroughly with dH₂O in between samples.

4. Take on ice to sonicator (Heat systems, Ultrasonics). Wash sonicator tip with d_4H_2O and dab dry with paper towel.
Sonicate sample 3 x 10 sec on ice

6. Use plastic pipette to separate into 2 eppendorfs, one of which will be for PFK and the other for CS. Freeze the sample not being used that day (usually the CS sample).

APPENDIX 6.6

PHOSPHOFRUCTOKINASE (PFK) ASSAY

1. PFK buffer 100mM pH 8.2
50mM TRIS 50 ml of 100 mM TRIS (CS assay stock)
2mM EDTA 74.4 mg
5mM MgCl₂ 101.5 mg
make up to 100 ml dH₂O

2. Store in fridge.

3. Later add,

to 19.2 ml of PFK buffer stock to 38.4ml PFK buffer stock

BSA	2 mg	4 mg
2-ME	14 µl	28 µl
F-6-P	12 mg	24 mg
aldolase (10mg/ml stock)	80 µl	160 µl
TPI + GlyPDH	100 µl	200 µl

Can keep in the fridge for up to 2 days

4. Add fresh, before use;

NADH	2 mg	4 mg
------	------	------

5. Make up ATP

13 mg/210 µl TRIS

PFK assay

970 µl of reagent (with NADH) (or 975 µl)

10 µl muscle homogenate (5 µl)

incubate 1 minute @ 25°C

20 µl ATP

incubate 1 minute

read every minute @ 340 nm for 5 min

APPENDIX 6.7

CITRATE SYNTHASE (CS) ASSAY

1. TRIS buffer 100mM pH 8.3
1.514 g of base form (Fwt 121.1)
1.970 g of HCl form (Fwt 157.6)

store in opaque bottle in fridge
This is enough for ~ 250 assays
2. 1mM DTNB (Boehringer 104477, Fwt 396.4)
2mg in 5ml of the TRIS buffer ~ 30 assays
or 10mg in 25 ml
wrap vial in tin foil and put in fridge
3. 4mM AcCoA (Boehringer 101907, Fwt 827.4 (3H₂O salt))
Must prepare fresh
5mg/1.5 ml dH₂O ~20+ assays
or 6.67mg/2ml dH₂O ~30+ assays
4. OAA (Boehringer Mannheim 107999 free acid form, Fwt 132.1)
10mM 2.67mg/2ml of TRIS 100mM ~30 assays
stable for ~ 1 week in freezer

CS assay

795 µl of 1 TRIS
100 µl of 2 DTNB
50 µl of 3 AcCoA

Mix in cuvette

Add 5 µl of homogenate

Read at 412 nm

Wait 3 min

Add 50 µl of 4 (OAA) read 1st 5 sec and then

Read every 30 sec for 7 min

APPENDIX 6.8

REPRODUCIBILITY OF ENZYME ASSAYS (raw absorbance change)

Two samples of the same muscle homogenate were prepared and assayed to assess the reproducibility of the assay technique. Several homogenates were tested for each assay. Readings of raw absorbance changes are presented below:

Homogenate	Reading 1	Reading 2	
CPT:			
1	.037	.045	
2	.034	.040	
3	.055	.058	
4	.044	.046	
5	.053	.052	$r = 0.95, p < 0.01$
CS:			
1	.059	.052	
2	.047	.047	
3	.061	.062	
4	.046	.044	
5	.037	.037	
6	.033	.033	
7	.030	.030	$r = 0.98, p < 0.001$
HAD:			
1	.034	.039	
2	.032	.034	
3	.049	.042	
4	.033	.037	
5	.028	.031	
6	.020	.024	
7	.019	.023	$r = 0.94, p < 0.005$
PFK:			
1	.068	.070	
2	.076	.061	
3	.063	.062	
4	.081	.083	
5	.037	.041	
6	.032	.035	$r = 0.94, p < 0.005$

HK:

1	.013	.014
2	.013	.015
3	.023	.023
4	.012	.015
5	.013	.012
6	.013	.015

$r = 0.94, p < 0.005$

APPENDIX 6.9

NOTES ON DEVELOPMENT OF TECHNIQUE TO MEASURE MUSCLE BUFFERING CAPACITY BY TITRATION

1. When making up the buffer pH to 7.0. In practice the initial solution is considerably more acidic and therefore considerable NaOH needs to be added, so must allow for considerable volume to be added before making up to volume.
2. Pilot studies determined the loss of sample using the mechanical homogeniser and transferring the sample to another vessel for titration. This was as high as 17%, and therefore it was decided to utilise the hand-held homogeniser and to titrate in the same vessel.
3. Attempted then to use just sonication but this was not sufficient to adequately homogenise the sample. In addition, the sample must be kept cool throughout. Hand-held homogenisation was definitely preferable.
4. Pilot studies were undertaken to investigate the minimum sample size that could be used. Using the "effect of dilution" graph of Marlin and Harris (1991), the maximum conservative dilution allowable without affecting the result was calculated for a dry sample weight of 5 mg. However, this meant that the volume of buffer was only 0.7ml. When attempting to homogenise this sample, there was found to be insufficient fluid. For future reference, it was decided to utilise a minimum of 1ml of buffer.
5. Very important to remove connective tissue prior to weighing
6. Pilot studies showed that incubation of between 5 and 20 minutes at 37°C did not affect the result. If the incubation period was less than 5 minutes, the pH was not stable. Incubations of >20mins were not carried out as in practice all titrations were completed in ~10mins from the beginning of titration. During and after homogenisation and prior to beginning the titration, the sample should be kept on ice.

APPENDIX 6.10

REPRODUCIBILITY OF SKELETAL MUSCLE BUFFERING BY TITRATION

Based on the method of Marlin & Harris (1991) as described in detail in chapter 3.5, the reproducibility of the method was assessed in ten repeat aliquots from the same mixed muscle homogenate (rat gastrocnemius). The raw data are presented below, represent μl added:

Aliquot	Result
1	138.2
2	130.4
3	132.2
4	131.5
5	135.1
6	127.6
7	125.9
8	127.5
9	122.8
10	137.7
Mean	130.9
SD	5.0

Coefficient of variation = 3.8%

APPENDIX 6.11

PROTEIN ASSAY

Following measurement of βm , the sample was frozen and stored at -20°C until all samples were available to be assayed concurrently. The protein concentration was assayed in triplicate utilising the Biuret methodology (Kingsley, 1942).

Make up:

1. Protein standard

50 μl Validate (Versatol) 65 mg/ml protein

350 μl dH_2O

to give 8.13 mg protein/ml in standard sample

2. Biuret solution:

9 g NaK Tartrate dissolved in 0.2 M NaOH

3 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$

1 g KI

make up to 1 litre with 0.2M NaOH (8 g/litre)

(sometimes difficult to dissolve, must add in the order list and stir well)

Then dilute stock (5 vol stock:3 vol dH_2O)

Then:

Into each cuvette (triplicate) add:

450 μl Biuret solution

25 μl muscle homogenate (immediately after vortexing)

Into standard cuvette (quadruplicate):

450 μl Biuret solution

25 μl standard protein sample

Wait 30 min incubation at room temperature (21°C)

Zero spectrophotometer with dH_2O

Read @ 540 nm

Calculations:

Mean sample reading \times (8.13/standard sample reading) = [protein] in mg/ml

APPENDIX 6.12

REPRODUCIBILITY OF FATIGUE RESISTANCE EXERCISE TEST

The reproducibility of the time to fatigue achieved during the fatigue resistance test was estimated by calculating the coefficient of variation for total test time in three subjects. Testing was undertaken using the test protocol as described in Chapter 3.1 and the time was blind to both the subject and the investigator during the course of the test.

Subject 1 completed the test 3 times and the results were as follows:

970 sec

960 sec

980 sec

giving a coefficient of variation of 1.0%.

Subject 2 completed the test 3 times and the results were as follows:

810 sec

843 sec

850 sec

giving a coefficient of variation of 2.5%.

Subject 3 completed the test three times and the results were as follows:

1135 sec

1095 sec

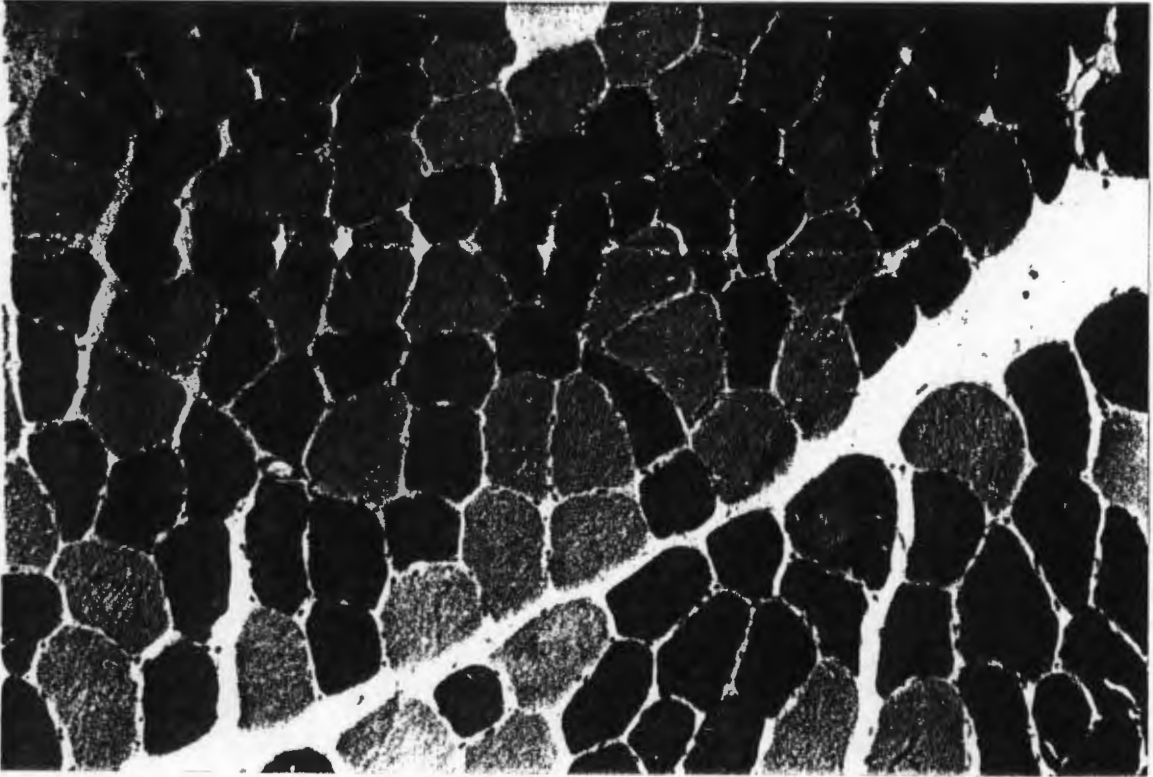
1150 sec

giving a coefficient of variation of 2.5%

Therefore, the mean coefficient of variation of 2.0%.

APPENDIX 6.13

EXAMPLE OF SKELETAL MUSCLE FIBRE TYPING BY MYOSIN ATPASE STAIN, pH 4.3



APPENDIX 6.14

EQUIPMENT UTILISED

Electronically braked cycle ergometer:

Lode, Groningen, The Netherlands

Treadmill:

Powerjog EG30, Birmingham, England

Running wheels (rats):

Campus Industries, Cape Town, SA

pH micro-electrode:

Crison 52-08, Barcelona, Spain

Sonicator:

Heat systems, Ultrasonics

Spectrophotometer:

Beckman DU-62, Beckman Instruments Inc, USA

Freeze-drier:

Virtis Sentry, The Virtis Co Inc., Gardiner, NY, USA

On-line gas analysis system:

Oxycon Alpha, Jaeger.Mijnhardt, Wuerzburg, The Netherlands

Heart rate monitors:

Polar Heartrate monitors, Munich, Germany

20-gauge cannula:

Jelco, Critikon, Tampa, Florida, USA