Angiotensin converting enzyme (ACE), Actinin (ACTN3), Tumour necrosis factor (TNF) gene polymorphism associated with biomarkers and physical characteristics in young African cricket players of Zulu origin

By

Ramakoaba Abigail

(20053333)

Dissertation submitted to the Department of Biochemistry and Microbiology, Faculty of Science and Agriculture. University of Zululand in partial fulfilment of the requirements for the Masters (MSc) degree in Biochemistry.

Supervisor: Prof T Djarova

Co- supervisors: Dr. J Grace

Dr. G. Watson

2012

Abstract

Current research in biochemistry and genetics focuses on finding a relationship between genes and biomarkers that are playing a fundamental role in physical performance. A possible link between these genes, molecular adaptation to exercise training and various markers of physical performance has been established in the past decade. A handful of genes have been studied by scientists, among them are the Angiotensin Converting Enzyme (ACE), ACTN3 (Actinin) and the Tumor Necrosis Factor (TNF). The ACE gene which encodes the angiotensin converting enzyme has been studied in detail and is known for influencing human physical performance and trainability. ACTN3 gene encodes the actinin-3 protein, that is known to form part of the sarcomeric Z-line, anchoring the actin filaments together and maintaining the mechanical integrity of the muscles. The TNF gene is among less studied genes, it encodes the Tumor Necrosis Factor protein which initiates the production of interleukins and that results in the production of the inflammatory biomarker C- reactive protein (CRP). Cricket is like any other sport in whereby there are energy requirements, physical characteristics that have been accepted as indicators of good performance in the game (body mass index, hand grip, quadriceps and hamstring strength) which are assessed for the purpose of the study. This study therefore sought to explore the ACE I/D, ACTN3 R/X and TNF G/A gene polymorphism, biomarkers (uric acid (UA) lactate (LA) and CRP) changes and the association with physical tests in a previously unexplored cohort of African Zulu cricketers. The participants were 31 Africans males aged 20-27 years (n=14 cricketers and n=17 controls). The genotyping (blood spots) was performed by PCR amplification followed by restriction digestion. After ANOVA the

association was examined using Chi^2 maximum likelihood test and Fisher's exact test. ACE genotyping for the whole group displayed a complete absence of II genotype, 67.7% DD and 32.3% ID genotypes. The frequency of D allele was 83.8% and I allele 16.2%. In cricketers DD and ID genotypes were 50% each compared to controls-83% DD and 17% ID. The D allele is associated with power/sprint performance and the I allele with endurance. ACTN3 genotype frequencies for the cohort were 90.3% RR and 9.7% RX. The XX genotype was absent. The R allele is linked to sports that require power/sprint and the X allele is related to endurance. No differences in genotype frequencies between the two groups were noted. R allele at extremely high frequency (100%) was associated with CRP (<3.0 mg/L) in cricketers (p=0.0001) and controls (p=0.0140). TNF genotyping displayed 42% GG, 45% GA and 13% AA for the whole cohort, but no differences between both groups. CRP (<3.0 mg/L) was associated (p=0.0001) with low A allele frequency (18% in controls and 40% in cricketers). Interestingly, a null homozygosis of both the ACE II and the ACTN3 XX genotypes was found for the first time in the cohort of Zulu cricketers. This research demonstrates high ACE D allele frequency and a strong ACTN3 R allele association with low CRP, UA and LA levels. This study provides evidence about the genotype distribution of previously unexamined cohort of African athletes.

Key words: ACE, ACTN3, TNF genes, polymorphism, allele frequency

DECLARATION

The experiential work described in this dissertation was conducted in the Department of Biochemistry and Microbiology, Faculty of Science and Agriculture, the Department of Biokinetics and Sports Science, University of Zululand and the Department of Biochemistry, Molecular Biology and Genetics, University of Kwa-Zulu Natal, Pietermaritzburg Campus between April 2009 and November 2010, under the supervision of Prof. T. Djarova, Dr. G. Watson and Dr. J. Grace.

This study represents the original work by the author. Where use was made of the work of others, it has been duly acknowledged in the text.

I declare the above statement as true.

Ramakoaba Abigail

Prof. T. Djarova

Dr. J. Grace

Dedication

This dissertation is dedicated to my late mother and aunt,Nomfundo and Pulane Ramakoaba, my siblings Vuyo, Mbabazeni,Karabo and Lesego, my grandmother Caroline Vapi and to my mentor Mr. Bonginkosi Dlamini.

Acknowledgements

I would like to thank God almighty for carrying me this far, this endeavour will not be possible if I did not have him by my side. I would also like to thank my supervisors Prof. T. Djarova and Dr. J Grace for always pushing me in the right direction and for the intensive support they gave me throughout the study. I would also like to thank Mr. S.C Mugandani for the input he has made in this work. Sincere gratitude goes to the University of Zululand cricket team and the students from the department of Biochemistry and Microbiology for their participation. We would also like to thank the University of Kwa-Zulu Natal Molecular Unit for the assistance in genotyping. I am grateful to the National Research Foundation (R.S.A) and the University of Zululand Research Committee for the financial support. Lastly, my family and friends for the love and support.

Table of Contents

List of Figures

List of Tables

List of symbols

Symbol	Interpretation
α	alpha
%	percent
µl	microlitre
bp	base pair
˚C	degrees Celcius
Mmol	milli moles
L	Litre
w/v	weight per volume
kg	Kilogram
cm	centimetre
m^2	metre squared
mm	millimetre
mg	milligrams
Hg	mercury
F	Forward
R	Reverse
‘	Prime
<	Less than
>	Greater than
±	Plus minus

List of Acronyms

ACE	Angiotensin Converting Enzyme
gACE	germinal Angiotensin ConvertinEnzyme
sACE	somatic Angiotensin Converting Enzyme
ACTN3	α-actinin 3
ADP	Adenosine Diphosphate
AMP	Adinosine Monophosphate
ATP	Adinosine Triphosphate
BP	Blood Pressure
BPM	Beats per minute
BIA	Bioelectric impedance analysis
BK2	Bradykinin 2
BMI	Body Mass Index
BMR	Basal metabolic rate
CRP	C-Reactive protein
DEXA	Dual-energy X-ray absorptiometry
DNA	Deoxyribose nucleic acid
cDNA	Complementary DNA
D	Deletion
DD	Deletion/Deletion
FBM	Fat Body Mass
HDL	High Density Lipoprotein

HR	Heart Rate
I	Insertion
I/D	Insertion/Deletion
I/I	Insertion/Insertion
IL	Interleukin
IMP	Inosine Monophosphate
LA	Lactate
LDL	Low Density Lipoprotein
LBM	Lean Body Mass
LV	Left ventricular
pCO2	Partial pressure of carbon dioxide
RAS	Renin Angiotensin System
RBC	Red blood cells
RHR	Resting heart rate
SID	Strong Ion Difference
STT	Systolic Tension Time
TNF- α	Tumor necrosis Factor- α
UA	Uric acid
WBC	White blood cells

CHAPTER ONE

INTRODUCTION

Recent scientific research has concentrated on possible associations between physiology, biochemistry and genetics in the field of physical exercise. It has investigated the inheritance of several traits of performance focusing on the genetic and molecular basis of adaptation to training and on various markers of sport performance (Calo et al., 2008). This has seen an influx of research that focuses on finding the relationship between the genes that are playing a substantial role in the performance of elite athletes. These studies have indicated that the tendency to engage in physical activities to some extent may be rooted in our genes. The adaptation to exercise is a complex process resulting in the ability of the athlete to produce highly coordinated movements, including tissue and cellular changes, which in turn might involve gene expression (Rankinen et al*.,* 2003).

Amongst the candidate genes that have been studied, Angiotensin Converting Enzyme (ACE) and α-Actinin3 (ACTN3) genes have been of most interest in studies of different cohorts of athletes and conclusions were made that they are associated with physical performance. Other genes such as the Tumor Necrosis Factor-α (TNF-α) gene have been less studied. Other less exploited possibilities include studies involving the association of ACE gene or other muscle and metabolism related genes (ACTN3, AMPD1, ADRB2, TNF) with blood biochemical parameters and markers of inflammation and exercise induced oxidative stress (Lakka et al*.*, 2006). The expression of these genes leads to the synthesis of the respective proteins that

possibly influence the response of some biomarkers (C-reactive protein, uric acid, lactate and others) to physical exercise and exercise-induced oxidative stress (Montgomery et al*.,* 1999).

While other genes associated with human performance have been studied, none has created so much controversy and received as much scientific validation as the Angiotensin Converting Enzyme gene (ACE gene). The ACE gene is one thoroughly studied genetic factor that may influence human physical performance and trainability (Montgomery et al., 1999; Wolfarth et al., 2005). The ACE gene encodes for the Angiotensin Converting Enzyme (ACE) which is a key enzyme in the Renin-Angiotensin System (RAS), a system involved in the regulation of blood pressure.

Another gene of significance among polymorphism studies has been the ACTN3 gene, through which the synthesis of the protein α actinin-3 is encoded. It is specifically expressed in fast-twitch (type II) myofibers responsible for generating force at high velocity. The actinin-3 protein plays a structural role as a component of the Z-line of the sarcomere anchoring together actin filaments and maintaining the mechanical integrity of muscles (MacArthur and North, 2007). Research on the association of ACTN3 and biomarkers of oxidative stress in various sports has not been done thus far. Taking into consideration that ACTN3 protein might be related to muscle signalling and metabolism (MacArthur and North, 2007) and the role of some endogenous antioxidants such as uric acid and lactate in scavenging free radicals (Djarova et al., 2009) it would be noteworthy to explore the possible relationship.

A gene that has not received much attention in studies that seek to discover genes that are playing a pivotal role in the performance of elite athletes is the Tumour Necrosis Factor-α (TNF-α) gene) (Lakka et al., 2006). Its principal function is to encode for the tumour necrosis factor-α (TNF-α) protein, which induces the production of interleukins thereby inducing the hepatic production of the inflammatory biomarker C-reactive protein (CRP)(Lakka et al*.,* 2005)

Cricket is one of the most popular sports in South Africa; it has a long history and global appeal. Ironically there is a limited body of knowledge about the physiological and other requirements of cricket (Noakes et al., 2000). It has been suggested that the physiological demands of cricket are relatively mild. Contrary to this, cricket is currently undergoing a phase of transformation at an international level as it seeks to attract a more global audience. This leads to athletes being exposed to greater physical and physiological demands (Noakes et al*.,* 2000).

According to Martens (2004) the estimated energy and muscular fitness demands of cricket are as follows: aerobic capacity low to moderate, anaerobic capacity- moderate, flexibility- moderate, strength- moderate, endurance- low to moderate, speed- moderate to high. Fast bowlers have an ectomorphic somatotype, greater percentage of type II muscle fibers and a superior phosphagenic and glycolytic metabolic pathway together with eccentric muscle strength (Glazier et al., 2000). The speed of the ball at release was seen to determine success in bowling (Stulchen et al., 2007). Hand grip strength was also accepted as an indicator for a good performance in cricket (Koley and Yadar, 2009) whilst a shorter stature and

isokinetic knee and shoulder strength were seen to be contributory factors in the success of a batsman (Noakes and Durant, 2000; Nunes and Coetzee, 2007).

Recent research has focused on the analysis of genetic factors that influence world class athletic status in the European Union, Russia, Australia, Asia, Africa and in the United States of America. Studies of Nigerian athletes have been reported and research in Kenyan, Jamaican and American sprinters was conducted (Scott et al., 2010). In Russia, allele frequencies were studied in anaerobic power athletes. Yang et al*.,* (2007) studied Nigerian sprinters. Moran et al*.* (2007) and Pescatelo et al*.,* (2006) studied the ACE gene in relation to physical characteristics. These studies are of great importance in the field of physical exercise because they serve as a reservoir of information on the role of inheritance on fitness and performance traits. Studies of this nature could serve as an important tool in talent identification and prognosis of aerobic and anaerobic performance by using genetic testing (Calo *et al*., 2008).

1.1 Study Rationale

A number of scientists have investigated genes associated with physical performance in other sports and to the best of our knowledge no studies have looked at the association of ACE, ACTN3 and TNF genes within cricket. A handful of studies have also looked at the association of physical performance, polymorphism and biomarkers collectively but most of these studies were conducted with Caucasian populations with very few having been conducted among athletes of African origin. In South Africa particularly, there are no known studies which have looked at the

association among gene polymorphism, physical performance and biomarkers among African athletes of Zulu origin.

CHAPTER TWO

LITERATURE REVIEW

2.1.1 Angiotensin 1 Converting Enzyme (ACE) gene

Angiotensin converting enzyme (ACE) gene is located on the long arm of chromosome 17 (17q23.3). The complementary DNA (cDNA) sequences of the human germinal ACE (gACE) and somatic ACE (sACE) were determined in 1989, and the genomic sequence and structure in 1991(Corvol et al., 1995). The gene spans 21kilobases and includes 26 exons. In mammals, two isoforms of ACE exist, one expressed in somatic tissue (sACE, 1306 residues) and one in germinal cells in the male testes (gACE, 732 residues). This is illustrated in Figure 2.1. The two forms of the enzyme expressed by the ACE gene differ in that the gACE form has a single active site, whereas sACE has two active sites. The two forms arise as a result of two different promoter activities, with sACE being transcribed from a promoter region upstream of the duplication, and gACE from a promoter within intron 12 (Langford et al.,1991). This duplicated structure produces a protein with two domains, the N-domain and the C-domain.

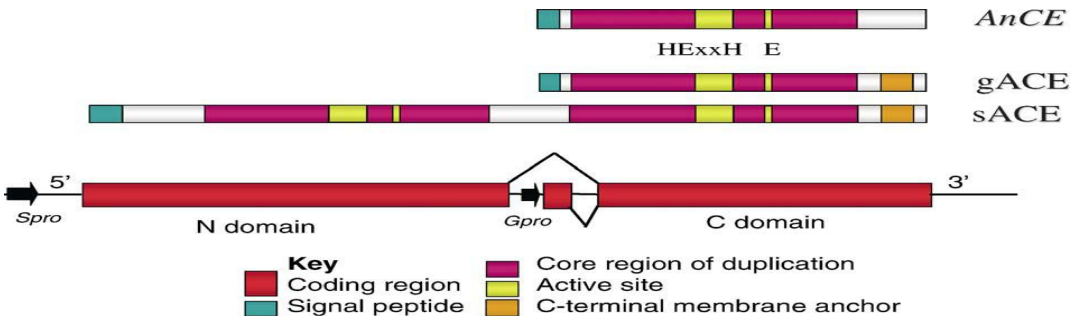


Figure 2.1: **The gene and protein structure of ACE. Adapted from Coates et al., (2000)**

The somatic form of ACE is coded for by a tandem duplication forming a two-domain protein with an N- and a C-domain and transcribed from a somatic promoter upstream of the gene. The germinal form (gACE) is expressed from a specific internal promoter (Gyro), and includes a germinal specific exon. Sequences within the core region (purple), show very high sequence homology, delimiting the extent of the duplication. The active site(s) (yellow) are arranged around the residues which act as zinc ligands. All forms of ACE have a N-terminal signal peptide (green). Vertebrate forms have a C-terminal hydrophobic region (orange) which acts as the C-terminal membrane anchor whereas invertebrate isoforms (exemplified by AnCE) lack the membrane anchor and are thus extracellular soluble (Coates et al., 2000)

Angiotensin I-converting enzyme (ACE) is a circulating enzyme that takes part in the body's renin angiotensin system (RAS), though which in association with rennin mediates extracellular volume (that is, the volume of the blood plasma, lymph and interstitial fluid) and arterial vasoconstriction. It is secreted by pulmonary and renal endothelial cells and catalyses the conversion of angiotensin I to angiotensin II, this conversion makes up the RAS system.

2.1.2 The RAS (renin–angiotensin system) and blood pressure regulation

The renin-angiotensin system (RAS) has been shown to play a key role in the regulation of blood pressure and influences the cardiovascular system (Van Berlo et al., 2003). Blood pressure is regulated by the RAS system through which homeostasis is maintained. This system is present in the kidney and other tissues

and it involves a series of biochemical reactions in which renin cleaves angiotensinogen to yield angiotensin I, acted upon by the key enzyme angiotensin converting enzyme (ACE) (Joans & Woods, 2003).

Angiotensin I is converted into angiotensin II, whose effects are mediated predominantly through two specific human receptors (AT1 and AT2). The stimulation of the AT1 receptor by Angiotensin II mediates a hypertensive response through primary vasoconstriction, salt and water retention secondary to adrenal aldosterone release as it is illustrated in Figure 2.2. The enzyme also degrades bradykinin, whose action on the type 2 bradykinin 2 (BK2) receptor results in vasodilatation regulating the contraction and relaxation of blood vessels, hence blood pressure regulation. The enzyme activity might lead to elevated angiotensin II concentrations (Rigat et al., 1990). The local RAS that exists in the skeletal muscle may influence functional performance.

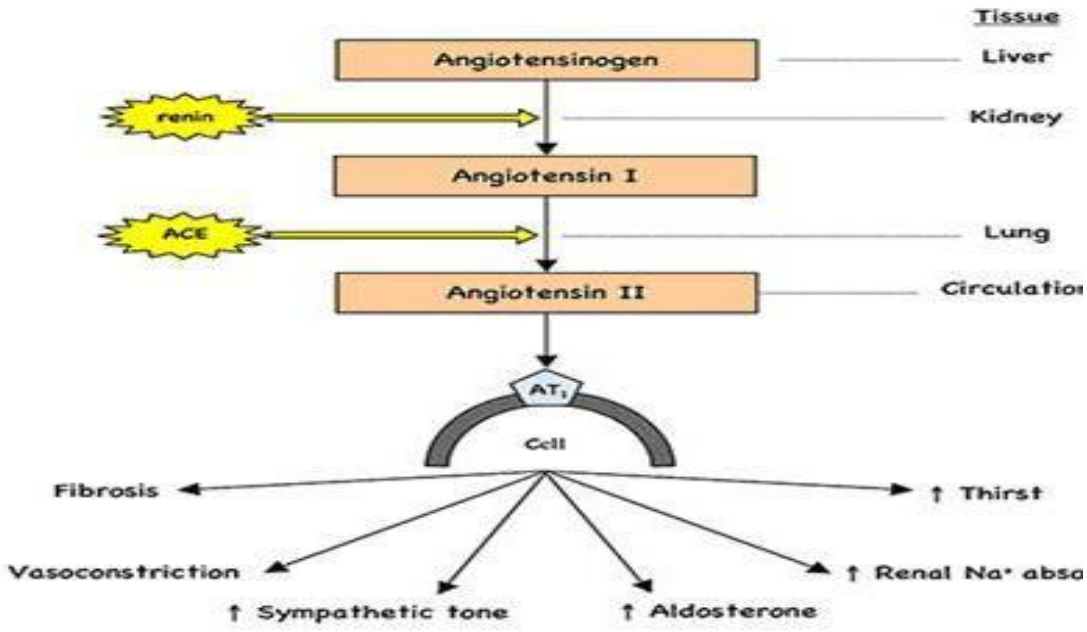


Figure 2.2: A schematic representation of the Renin Angiotensin System (RAS) showing angiotensin converting enzyme (ACE) as a component of RAS (Lubel et al*., 2008).*

The ACE gene polymorphism not only accounts for half the variation in serum angiotensin converting enzyme but is also associated with local enzyme activities in tissue systems including the human myocardium (Costerousse et al.,1993).

2.1.3 Ace gene polymorphism

ACE gene polymorphism is based on insertion (I) or deletion (D) within an intron of a 287 base-pair nonsense DNA domain resulting in three genotypes namely the homozygous (II), heterozygous (ID) and homozygous (DD). In general the insertion I allele of the ACE gene is associated with elite endurance performance and improvement in performance as a result of training while the D allele is associated with success in power events (Wang et al., 2008). The protein angiotensin converting enzyme, which converts angiotensin I to angiotensin II, was shown to have higher levels in D homozygote than in those with ID or II genotypes (Scott et al., 2005). Williams et al., (2008) examined the ACE ID genotype associations with quadriceps muscle strength in 81 young Caucasian men, 44 of whom completed an 8 weeks strength-training program. Baseline isometric strength was significantly associated with the ACE ID genotype.

The frequencies of the I allele were found to be higher in Lithuanian elite athletes than in controls (Gineviciene et al*.,* 2010). In East African distance runners, no association between ACE genotypes and elite endurance athletic status was found

(Scott et al., 2005). South African born athletes genotypes frequencies of the ACE gene were observed during the ironman triathlons and not much difference in allele distribution was reported (Collins et al., 2004). The distribution of the ACE I/D polymorphism was investigated in Korean male elite athletes but no difference in genotype or allele distribution was found between these groups and unrelated non athletes (Oh et al., 2007).

Some studies have sought to find an association between the ACE gene polymorphism and the standard measure of aerobic capacity (VO_{2max}) to assess endurance performance. Although VO_{2max} in this study was not considered, it is documented in literature that a high level of aerobic fitness is an important prerequisite but not a final determinant of elite performance. VO_{2max} is influenced by genetic and environmental factors with marked inter-individual responses to training (Woods, 2009). Several studies have concluded that there is no consistent effect of the ACE I/D polymorphism on VO_{2max} reported (Sonna et al., 2001 and Woods et al., 2002). However an increase in VO_{2max} associated with the I allele was found and this increase was due to an increase in maximal arteriovenous oxygen difference (Hagberg et al., 1998).

ACE gene polymorphism influences human physical performance. However, it was not found to influence the aerobic capacity, but associations of I allele or II genotype with endurance performance, oxygen consumption and fatigue index were observed (Montgomery et al., 1999). Angiotensin converting enzyme activity is consistently highest in DD subjects, intermediate in ID and lowest in II subjects. It is considered that gene polymorphism might contribute to the improvement of endurance

performance via an increase in myosin heavy chain gene expression (Vescovo et al., 1998). High D allele frequencies were reported in Nigerians (Woods et al., 2009), Afro-Caribbean people (Bloem et al., 1996) and in Taekwondo athletes of Turkish and Azerbaijan origin (Gunay et al., 2010).

The I allele on the other hand was found to be overrepresented among elite mountaineers (Montgomery et al., 1998). The I allele was found at excess frequencies amongst rowers (Gayagay et al., 1998) and elite long-distance runners (Myerson et al., 1999 ; Hruskovicova et al., 2006). In studies conducted over a decade ago the I allele was found with excess frequency in elite distance runners and rowers in which Caucasians and Australians were examined for their frequency of the ACE I/D polymorphism. A clear excess of the I allele and the II genotype was reported (Gayagay et al., 1998). On the other hand Taylor et al., (1999) studied Australian national athletes from sports which demand a high level of aerobic fitness and this cohort included hockey players, cyclists, skiers, track and field athletes, swimmers, rowers and gymnasts. No difference in ACE genotype and allele frequency was found when compared with the controls. From these findings there was a demand for scientists to delve into ACE I/D polymorphism studies.

2.2 Actinin- 3 (ACTN3) gene

In humans, two genes (ACTN2 and ACTN3) encoding skeletal muscle proteins called α-actinins were found. The contractile apparatus of the skeletal muscle is composed of repeating units called sarcomeres that contain ordered arrays of actin-containing thin filaments and myosin-containing thick filaments (MacArthur and

North, 2004). The actinin 2 and actinin 3 proteins are localized at the Z line of the sarcomers in muscle tissue and the Z lines are dense bands that run perpendicular to the myofibrils and anchor the thin filaments constituting a number of functional domains (Figure 2.4).

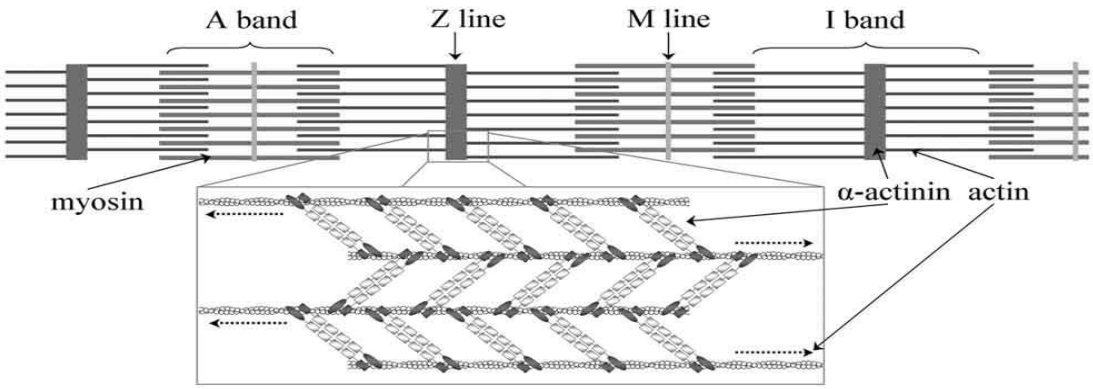


Figure 2.3: Localisation and domain structure of the sarcomeric α-actinins (MacArthur and North, 2004). The sarcomeric α-actinins are found at the Z line, where they anchor actin containing thin filaments from adjacent sarcomeres. Antiparallel dimers of α-actinin cross-link actin filaments and stabilize them against force generated by the contractile apparatus (top inset; dashed arrows indicate direction of force).

ACTN2 gene is expressed in all muscle fibers (slow twitch type I and fast twitch type II), whilst ACTN3 is restricted to fast twitch (type II) myofibers (North et al., 1996). A common human polymorphism of the ACTN3 gene was identified as a mutation and this mutation occurs whereby the cytosine – thymine (C-T) nucleotide transition in codon 577 of exon 16 leads to a stop-codon (R577X) resulting in α-actinin-3 protein deficiency in muscle fibres (North et al., 1996). Approximately one billion people globally are deficient in α-actinin-3 (Druzhevskaya et al., 2008). As a result of the mutation three genotypes which constitute three variants of allele combinations are found, namely the homozygotes RR, heterozygotes, RX and the homozygotes XX. There is a highly significant association between ACTN3 R577X genotypes and athletic performance (Yang et al., 2003).

The X allele is unable to encode a detectable α-actinin-3 protein, but because the ACTN2 gene is expressed in both type I and II myofibers, it can compensate for the loss of the ACTN3 protein in type II fibers in individuals who are homozygotes (XX). The nonsense allele is found in every human population, with a wide variation implying that balancing selection may have been involved in maintaining the polymorphism. This suggests that the ACTN3 genotype may be one of the factors that influence normal variation in muscle function and as a result, human performance (Yang et al., 2003).

Several studies have investigated the relationship between ACTN3 gene polymorphism and sport performance. Recent publications provided a substantial body of evidence regarding the association of ACTN3 with athletic performance and certain physical characteristics (Yang et al., 2003; MacArthur and North, 2007; Gomez-Gallego et al., 2008 and Norman et al., 2009).

Yang et al., (2003) found significant differences in ACTN3 allele frequencies between sprint athletes from Australia and the control group. From this research, it appears that the presence of the ACTN3 α- protein might be associated with greater success in activities requiring sprint or power performance parameters. There is strong evidence to suggest that ACTN3 has been maintained in the human genome because of functions independent of ACTN2. The ACTN3 sequence has remained highly conserved in evolutionary terms, since its divergence from 300 million years ago.

Some studies failed to support the relationship between ACTN3 R577X polymorphism and elite strength athletes and the association of XX genotype with endurance performance (Yang et al., 2005; Schneider and Rupert, 2009). On the other hand, the association of ACTN3 R577X polymorphism with power performance in athletes was reported (Moran et al., 2007, Druzhevskaya et al., 2008; Andonov et al., 2008). However, later findings suggested that heterozygotes with RX and XX genotypes are not disadvantaged in elite sprint/power performance (Yang et al., 2007). Cohorts of sprint athletes of various origins have shown a very low frequency of XX genotype (Scott et al., 2010).

From an evolutionary point of view it is difficult to explain the high frequency of the XX ACTN3 genotype given the power performance of the RR genotype. One possibility is that the power performance effect of the RR genotype only manifests in the extreme circumstances of athletic competition, outside the range of human activity (North et al., 1999). From this model it can be deduced that the X allele could have been selectively neutral during human evolution and became established in the human population by random genetic drift. However, this explanation is difficult to reconcile with the high level of evolutionary conservation that has been previously demonstrated for ACTN3 (Mills et al., 2001). It is possible that the X allele is selectively neutral but has reached its current frequency because of positive selection on a beneficial polymorphism at a nearby locus which refers to “genetic hitchhiking” (Kaplan et al.,1989).

It was found that α-actinin 2 and α-actinin 3 are differentially expressed, spatially and temporally, during embryonic development and ACTN2 gene expression does not

completely overlap the ACTN3 gene in mouse postnatal skeletal muscle. It is considered that the force-generating capacity of type 2 muscles fibres at high velocity, the speed and tempo of movements, and the capacity of the individuals to adapt to exercise training are all strongly genetically influenced (Rankinen et al*.,* 2002). Furthermore the frequency of the α-actinin-3 deficient genotype (577XX) varies from 25% in Asian populations to 0.1% in an African Bantu population and the frequency in Europeans is approximately 18% (Mills et al*.,* 2001).

2.3 Tumour Necrosis factor α - (TNF) gene

Tumour necrosis factor-α (TNF) gene is located on the short arm of chromosome 6 (6p21.3). The substitution of guanine (G) to adenine (A) at position 308 located at the promoter region stimulates the rate of transcription of TNF-α gene resulting in three genotypes (GG, GA and AA). The principal function of the TNF gene is to encode the tumour necrosis factor-α (TNF-α) protein. This protein is linked to a family of cytokines that possess pro-inflammatory properties including the Interleukin 1 (IL-l), Interleukin 6 (IL-6) and Interleukin 8 (IL-8). TNF-α protein stimulates the production of interleukin 6 (IL-6) thereby inducing the hepatic production of C-reactive protein (CRP). CRP is a sensitive biomarker of the inflammatory status of the individual and exercise-induced oxidative stress (Mattush et al., 2000; Pitsavos et al., 2003; Lakka et al., 2005; Murakami et al., 2009, Djarova et al., 2009, 2010).

It has been suggested that exercise causes a response similar to an immune reaction, albeit of a smaller degree as compared to some infectious diseases (Tuan et al., 2008). This has been postulated from increased plasma levels found. These events include infiltration by cells of the immune system like neutrophils within a few minutes of initiation of exercise and macrophages which follow subsequently. Activation of these cells is followed by a resultant release of acute phase reactants and proinflammatory cytokines like IL-1 and IL-2 (Tuan et al., 2008).

The TNF gene G308A polymorphism could affect downstream signalling of TNF- α protein and thus circulating CRP levels. To the best of our knowledge, only one paper establishing an association of TNF G308A polymorphism with CRP levels in response to an exercise-training programme has been published. This paper reports that the AA genotype is associated with increased plasma CRP levels in African American men and women reporting frequencies of 73.7% GG, 22.8% GA and 3.5% AA in African American males (Lakka et al., 2006). Djarova et al., (2011) reported 36% GG, 57% GA and 7% AA genotype frequencies in young South African cricket players of Zulu origin.

2.4 Biomarkers and Exercise-induced Oxidative Stress

During exercise the production of free radicals is believed to be the underlying mechanism for a series of biochemical and physiological changes that are indicative of free radicals (Li Li, 1994). Strenuous exercise generates excess free radicals followed by increased lipid peroxidation and resulting in extensive cell and tissue oxidative damage. Exercise can produce an imbalance between reactive oxygen

species (ROS) and the innate antioxidant defence system resulting in oxidative stress (Jackson, 2005).

Antioxidant defence system consists of glutathione, antioxidant vitamins and antioxidant enzymes. ROS production (superoxide anions, hydrogen peroxide and hydroxyl radicals) detected by electron spin resonance has been measured in the liver and muscle fibres in rats and human blood samples after sub maximal and maximal exercise to exhaustion (Jackson., 2005). Oxidative stress does not always result in oxidative damage to the tissues but it may lead to potential damage to lipids, proteins, heat shock proteins and DNA and consequently decreasing athletic performance (Djarova et al., 2009). The biomarkers that can be used for assessing the level of oxidative stress, amongst others, include C-reactive protein, uric acid and lactate.

2.4.1 C-Reactive Protein (CRP)

C - reactive protein (CRP) is a biomarker of sub clinical inflammation and a sensitive estimate of the risk factors for cardiovascular diseases (Pitsavos et al., 2003*)*. Regular physical exercise of moderate intensity was found to reduce CRP (Mattusch et al., 2000; Pitsavos et al., 2003). A physically active lifestyle is associated with lower CRP levels and a decreased cardiovascular disease (CVD) risk profile in former athletes (Jackson, 2005). Exercise induced inflammatory responses have been described by numerous authors (Fielding et al., 1993; Suzuki et al., 2000 and Margonis et al., 2007). During strenuous exercise of high intensity the production of

free radicals (ROS) can be generated by an influx of neutrophils and macrophages into the muscle and an activation of cytokines (Suzuki et al., 2000).

CRP is related to the glycaemic status in healthy subjects, insulin resistance, and obesity (Yudkin et al.,1999). The amount of CRP in humans is associated with the expression of the TNF gene. According to Pearson et al. (2003) circulating C-reactive protein concentrations reflect the inflammatory status of an individual. Inflammation triggers the production of pro- inflammatory cytokines such as interleukin 1 (IL-1) and tumor necrosis factor alpha (TNF-α), the stimulation of these cytokines stimulate the production of IL-6 which induces the hepatic production of acute phase reactants including CRP (Libby et al*.,* 2002*)* as shown in figure 2.4. Elevated levels of CRP increase the risk of acute myocardial infarction, ischemic stroke, peripheral artery disease (Koening et al*.*, 1999).

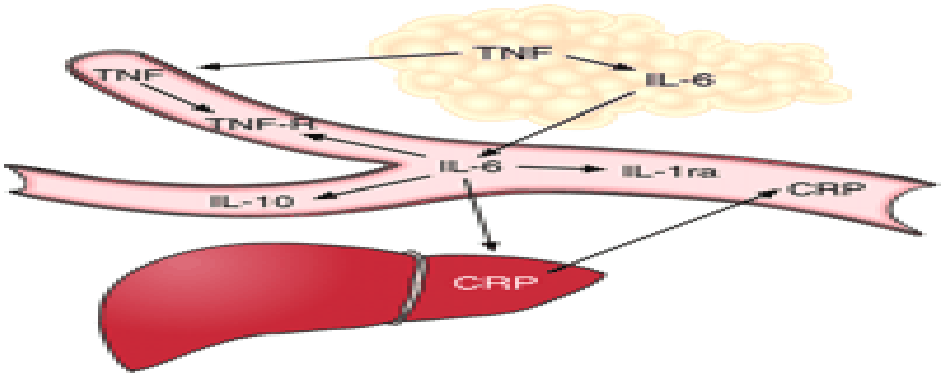


Figure 2.4: A diagrammatic presentation of the series of events that occur in the production of CRP, adapted from Peterson et al. (2005).

2.4.2 Triglycerides and Cholesterol

Athlete's food choices influence the athlete's biochemical responses during exercise and most importantly exercise performance (Maughan et al., 2002)**.** Dietary fats

provides a rich source of energy during exercise and studies have shown that chronic exposure to high fats diets increase use of fats as a fuel source at rest and during exercise. This is due to an increase in intramuscular triacylglycerol stores and induction of metabolic genes involved in fat oxidation (Helge, 2002). The increase in fat oxidation after exercise results in a decreased use of stored glycogen for fuel, which may play a role in athletic performance (Horowitz and Klein, 2000).

Elevated concentrations of lipoproteins have been shown to be an independent risk factor for atherosclerotic disease. Physical activity and physical fitness have been shown to improve lipoprotein metabolism and reduce the risk of coronary artery disease (Martin et al., 1996). Acute reductions in triglycerides and low density lipoprotein (LDL) cholesterol concentrations have been confirmed in endurance athletes after prolonged exercise.

These athletes have higher serum concentrations of high density lipoprotein (HDL) cholesterol and lower concentrations of triglycerides compared to sedentary subjects (Couillard et al., 2001). Lower concentrations of total cholesterol and low density lipoprotein (LDL) cholesterol have also been reported in physically active men (Cullinane et al., 1982). Exercise has shown to increase basal and exercise fat oxidation and studies have shown that habitual exercise may specifically improve use of stored lipids and inhibit the proliferation of adipose tissue (Heck et al., 2004).

2.4.3 Glucose

Exercise plays a crucial role in maintaining blood glucose levels and in regulating insulin within the body. Glucose is delivered to the muscles and broken down to yield Adenosine triphosphate (ATP) which is the fuel source for the working muscles

(Steenberg et al., 2002). With exercise, insulin is not readily released from the pancreas due to the lowered blood glucose levels. Moderate exercise does decrease glucose levels in the body. Vigorous exercise can cause the hormones epinephrine and glucagon to be released. Both act by stimulating the degradation of glycogen into glucose in the liver, resulting in an increase in blood glucose levels (Goodwin, 2010).

It is known for some time now that an individual who regularly exercises may experience improvements in insulin sensitivity, glucose metabolism and an increase in the use of fat as a fuel during exercise (Holloszy et al., 1998). Ritcher et al., (1982) reported that a single exercise session can alter glucose transport and insulin sensitivity in skeletal muscle of rats and subsequent work has confirmed these findings in humans (Heck et al., 2004).

2.4.4 Lactate

Lactate is a product from the metabolism of glucose and is considered as an important fuel during prolonged aerobic exercise and mainly in the recovery period after exercise. Other researchers suggest that lactate is taken up and oxidized by mild to moderately active skeletal muscle during recovery (Brookes, 1986; Thiriet et al., 1993). At some stage during high intensity exercise, when the rate of demand for energy is high, lactate is produced faster than the ability of the tissues to remove it and lactate concentration begins to rise. This is a beneficial process since the regeneration of NAD^+ ensures that energy production is maintained and exercise can continue. The increased lactate produced can be removed in a number of ways including oxidation to pyruvate by well-oxygenated muscle cells which is then directly

used to fuel the Krebs cycle, conversion to glucose via gluconeogenesis in the liver and released back into the circulation (Robergs et al., 2004).

The effect of lactate on acidosis has been the topic of recent research in the field of exercise physiology. Robergs et al*.,* (2004) have accurately investigated the proton movement that occurs during glycolysis, suggesting that $[H^+]$ is an independent variable that determines its own concentration. Lindinger et al. (2005) used the stoichiometric approach of Robergs et al. (2004) to discuss the role of causative factors such as strong ion difference [SID], partial pressure of carbon dioxide (pCO_2) and weak acid buffers on the concentration of hydrogen ions $[H^+]$. Lactate is a strong anion, and causes a reduction in [SID], which causes an increase in $[H^+]$ to maintain electroneutrality. PCO_2 also causes an increase in $[H^+]$. During exercise, the intramuscular lactate concentration and pCO_2 increases causing an increase in $[H^+]$ and thus a decrease in pH (Cooper et al., 2002).

Blood lactate response to exercise appears to be highly related to various types of endurance performance. Two mechanisms have been discussed in the control of lactate metabolism during exercise. The first one supports the view that lactate production during exercise is due to a restricted availability of oxygen at the level of mitochondria (Katz, 1990). In contrast, the second mechanism is based on experimental data that oxygen lack in mitochondria does not occur. It suggests that although an oxygen limitation to metabolism can increase muscle lactate production and raise circulating levels, hypoxia is only one cause of elevated lactate production. Another factor such as the β-adrenergic simulation of the skeletal muscle can accelerate the rate of glycolysis and play a pivotal role in lactate production (Stainsby and Brooks, 1990).

It is known that exercise lowers blood lactate concentration due to a combination of decreased rate of blood lactate appearance and increased blood lactate removal (Weltman, 1995).

2.4.5 Uric acid

Uric acid is produced from the breakdown of purine compounds in the muscle and its elevation is proportional to the exercise intensity and the training levels (Hellsten et al., 2006). Both lactate and uric acid increase concurrently with the increased intensity of exercise. During high intensity exercise, muscle phosphocreatine (PCr) and glycogen are used for the rephosphorylation of adenosine diphosphate (ADP). When PCr stores in the muscle are failing, ADP accumulation starts, increased ADP level triggers the myokinase reaction, where two ADP molecules form one molecule of adenosine triphosphate (ATP) and one of adenosine monophosphate (AMP). The latter is further deaminated to inosine monophosphate (IMP) and metabolized via inosine, hypoxanthine, xanthine to uric acid in humans.

During the degradation of purine nucleotides, oxidation of hypoxanthine takes place in the capillary endothelial cells of muscle, liver and other tissues where xanthine dehydrogenase / oxidase oxidizes it to xanthine and to uric acid (Heunks and Dekhuijen, 2000). Uric acid acts as a component of the innate antioxidant system. High uric acid concentrations found after submaximal and maximal exercise could be considered as an indicator of mobilization of the tissue antioxidant stores into the plasma (Djarova et al., 2009).

2.4.6 Erythrocytes, White Blood Cells and Haemoglobin

Oxidative stress is one of the factors proposed to be responsible for damaged erythrocytes observed during and after exercise (Senturk et al., 2001). Erythrocytes may accumulate molecular modifications and modifications of the membrane components because of their limited biosynthetic capacity and poor repair mechanisms whenever they are exposed to unusual oxidative stress (Margonis et al., 2007). During high intensity exercise, the production of free radicals can be generated by an influx of neutrophils and macrophages into the muscle and an activation of cytokines (Suzuki et al., 2000; Opara, 2006). The increase in cellular metabolism especially during strenuous exercise increases haemoglobin turnover and haemoglobin autoxidation that can result in an increased carbonyl content of the globin moiety.

2.4.7. Blood Pressure

Blood pressure is defined as the force or pressure exerted by the blood by any unit area of the blood vessel. Blood pressure is usually measured in millimetres of mercury (mmHg). The beating of the heart leads to two distinct pressures in the arteries, namely the systolic and diastolic blood pressures. Systolic blood pressure is the result of the constriction and forced emptying of the left ventricle of the heart into the aorta. The pressure within the arteries when the heart is between contractions is called the diastolic blood pressure (Waugh and Halligan, 2002).

Blood pressure is usually expressed as systolic over diastolic blood pressure with the average normal value being <120/80 mmHg (ACSM, 2010). Angiotensin I-converting enzyme gene (ACE) is a component of RAS which plays a role in

regulating blood pressure through cascades of biochemical reactions. An increase in plasma ACE activity may increase blood pressure through increased production of angiotensin II that is as a result of the cleavage of angiotensin I by ACE to form angiotensin II (Calo et al., 2008).

2.5 Muscular Strength

Muscular strength is generally defined as the ability to generate force at a given velocity of movement (Wemer et al., 2010). It is expressed in Newtons (N) although kilograms and pounds are also used and it is commonly expressed in terms of resistance lifted. Strength can be assessed either statically/ isometrically (no overt muscular movement or limb movement) or dynamically (movement of an external load or body part in which the muscle changes length). Static or isometric strength can be measured using a variety of devices, including cable tensiometers and handgrip dynamometers (ACSM, 2010). One method of measuring dynamic strength is by using isokinetic machines.

On the basis of the studies conducted and literature reviewed ACTN3 is one of the genes that is associated with muscle strength, based on the relationship of ACTN3 to exercise performance and the known physiological function of ACTN3 in muscle fibers. It is expected that the R577X genotype would be associated with baseline muscle strength (Clarkson et al., 2005).

2.5.1 Isokinetic strength

Although various studies have been conducted regarding the effect of isokinetic strength on the performance of various sports such as volleyball, soccer and swimming, as far as we know, to date no such studies have been conducted on African players of Zulu origin. It is, however, imperative that isokinetic testing forms part of cricket player's testing protocols due to the fact that it provides valuable information that may be used for performance enhancement. Isolated isokinetic strength testing allows sport related professionals to identify pre-existing muscle weaknesses and imbalances that can be addressed in strength and conditioning programmes (Davis et al., 2000). The analysis of cricket clearly shows that muscle strength is a requirement for batsman, bowlers and fielders who want to achieve success in the one day cricket format (Nunes et al., 2007).

2.5.2 Isometric strength

Isometric strength (handgrip strength) is referred to as the muscular strength and force that athletes can generate with their hands and is a term which is generally familiar among strength athletes. The strength is the result of forceful flexion of all finger joints, thumbs and wrists with the maximum voluntary force that the subject can exert. A handgrip dynamometer is commonly used to establish the isometric strength of the hand and forearm muscles.

According to Ross and Rosblad, (2002) strong correlations exist between grip strength and various anthropometric traits such as weight, height, hand length. The

assessment of grip strength assumes importance in a number of situations and is a significant predictor of performance in various sports such as lawn tennis (Lucki and Nicolay, 2007), club volleyball (Melrose et al., 2007) and ten pin bowling (Tan et al., 2001). Hitherto the information regarding the association of handgrip strength and anthropometric variables in cricketers is lacking (Koley et al., 2009).

2.6 Body composition

Body composition is used to describe the percentages of fat, bone and muscle in human bodies. Due to the fact that muscular tissue takes up less space in our body than fat tissue, our body composition, as well as our weight, determines leanness (Wemer et al., 2010). It is well established that excess body fat, particularly when located around the abdomen is associated with hypertension, metabolic syndrome, type II diabetes, stroke, coronary artery disease and hyperlipidemia (Rader et al., 2007).

Various methods can be used to determine an individual's body composition and for the purpose of this study body mass index (BMI) or Quetelet Index and fat percentage was used. BMI or Quetelet Index is a measure of body weight based on a person's weight and height. Though it does not actually represent the percentage of body fat, it is used to estimate a healthy body weight based on a person's height, assuming an average body composition. It is calculated by dividing body weight in kilograms by height in meters squared (kg/m^2) (ACSM, 2010).

Fat percentage is the amount of body fat in relation to the total body weight and it is the major aspect of body composition related to fitness and health (Franks and Howley, 1998). Essential body fat is necessary to maintain life and reproductive functions (Romeo-Corral et al., 2006). Fat percentage is estimated by measuring skinfold thickness at specific locations on the body. The thickness of these folds is a measure of the fat under the skin, also called subcutaneous adipose tissue. Skinfold thickness results rely on formulas that convert these numbers into an estimate of a person's percentage of body fat according to a person's age and gender (Deurenberg et al., 1991).

Lean body mass (LBM) is the mass of the body minus the fat (storage lipid). There are a number of methods for determining the lean body mass. Some of these methods require specialized equipment such as underwater weighing (hydrostatic weighing), BOD POD (a computerized chamber) and DEXA (dual-energy X-ray absorptiometry). Other methods for determining the lean body mass are simple such as skinfold, calipers and bioelectric impedance analysis (BIA) (Webster, 2008). Fat body mass (FBM) is the mass of the body minus the lean body mass (LBM). Due to differences in body composition, BMI is not necessarily an accurate indicator of body fat, for example individuals with greater than average muscle mass will have a higher BMI. The thresholds defining the line between 'normal' and “overweight” or “obese” are sometimes disputed for this reason (Deurenberg et al., 1991).

A concern in athletes is the determination of ideal body weight or ideal body composition for peak performances in a sport. It is difficult to define the ideal body composition for a specific sport because all other aspects of physique plus many

other factors including genes contribute to sport performance. Although ranges of body fat percentage for athletes are related to successful performance, athletic performance cannot be accurately predicted solely on the basis of body composition.

Persons who are heavier for example have an advantage over their lighter counterparts when activity demands that the inertia of another body or an external object must be overcome and persons who are lighter with regards to body weight have an advantage when the goal is to propel the body (Jacqueline and Berning, 2006).

2.7 Heart Rate

Heart rate (HR) is the number of heartbeats per unit of time and is expressed as beats per minute (bpm) and can vary as the body's need to utilise oxygen and excrete carbon dioxide changes, such as during exercise or sleep. According to ACSM (2010), several techniques can be used to determine heart rate and these include radial or carotid pulse palpation, auscultation with a stethoscope, or the use of HR monitors. The pulse palpation technique involves feeling the pulse by placing the first and second fingers over the radial artery which is located near the thumb side of the wrist or the carotid artery located in the neck near the larynx. In the auscultation method, the bell of the stethoscope should be placed to the left of the sternum just above the nipple. This method is most accurate when the heart sounds are clearly audible and the subject's torso is relatively stable. The measurement of the heart rate is used by individuals such as athletes who are interested in monitoring their heart rate to gain maximum efficiency from their training.

Several studies have reported associations between heart rate variability and ACE II genotype (Busjahn et al., 1998; Thayer et al., 2003 and Wang et al., 2004) whereas the DD genotype may play a role in the severity of the decrease in left ventricular (LV) systolic performance, progressive LV dilation (Haung et al., 2003). The heart rate is measured to calculate the Systolic Tension Time (STT). STT is a measure of myocardial oxygen consumption (Wilson, 1992). It is the tension exerted by the myocardial fibers expressed as the product of the duration of ventricular systole times the heart rate.

2.8 Metabolic Rate and Energy expenditure

Basal metabolic rate (BMR) measured in kilojoules (kJ) is the energy required by an individual during physical, emotional and digestive rest. It is the minimum amount of energy required to maintain life or sustain vital functions. BMR is affected by age, gender, body composition and nutritional and health status. It is also known to be highest in people who are growing and in those with considerable lean body mass such as physically fit people and males. One way to increase BMR is to participate in endurance and strength training activities regularly to maximise lean body mass (Shils et al., 2006).

Energy expenditure refers to the amount of energy that a person uses to breathe, circulate blood, digest food, and be physically active. Meeting energy needs is a nutritional priority for athletes. Optimum athletic performance is promoted by adequate energy intake (Manore et al., 1995). Athletes need to consume enough energy to maintain appropriate weight and body composition while training for a

sport (Rodrigues et al., 2004). Inadequate energy intake relative to energy expenditure compromises performance and negates the benefits of training. With limited energy intake, fat and lean tissue will be used for fuel by the body. Loss of lean tissue mass results in the loss of strength and endurance as well as compromised immune, endocrine and musculoskeletal function (Mougios, 2006).

The direct effects of physical activity interventions on energy expenditure are of importance when placed in the context of total daily energy demands. Hence the suggestion has been made that exercise produces energetic benefits in other components of the daily energy budget, thus generating a net effect on energy balance much greater than the direct energy cost of the exercise alone (Speakman et al., 2003).

Another component of a person's energy output is physical activity and is a considerable variable and the most exchangeable component of energy expenditure. During physical activity the muscles need extra energy to move and the heart and the lungs need extra energy to deliver nutrients and to dispose waste material. The amount of energy needed for any activity depends on muscle mass, body weight and activity (Eleanor et al., 2003). The activity duration, frequency and intensity also influences energy expenditure. Increased physical activity augments energy expenditure; hence athletes have higher than normal energy requirements.

Studying genes that are playing a considerable role in athletic performance is a new development in the field of sports science, genetics and biochemistry. Not much research in different sport disciplines has been conducted. In the South African

context particularly, studies of this nature are still scarce. Thus far, Payne et al., (2007) conducted a study on the Xhosa population and Milander et al., *(*2009) studied athletes during the South African ironman triathlon. This is an indication of how far behind South Africa is when compared to western countries and also signifies that there is more demand for these genotype studies in South Africa.

2.9 Aims and objectives of the study

The aim of this study was to assess genetic and biochemical markers associated with physical characteristics in Zulu cricket players.

The objectives of the study were:

a) To establish the polymorphism in ACE, ACTN3 and TNF genes.
b) To assess the changes at rest in C-reactive protein, uric acid, lactate, glucose, triglycerides, cholesterol, haemoglobin, red and white blood cells in control group and in cricket players before and after cricket training exercise.
c) To evaluate the anthropometrical and physiological characteristics (stature and body mass, body mass index (BMI) fat percentage (Fat %), fat body mass (FBM),lean body mass (LBM), resting heart rate (RHR) and blood pressure (BP) as well as physical characteristics (isometric strength of the hand and forearm muscles and isokinetic strength of quadriceps and hamstring muscles) in cricket players and controls.
d) To estimate the daily energy and metabolic rate of cricketers and control group.

CHAPTER 3

METHODOLGY

This chapter presents a description of the materials and methods that were used to recruit experimental subjects, measure physical indices and to give account on how genotyping was performed for ACTN3, TNF and ACE genes respectively. A description of the materials and methods that were used is included.

3.1 Materials

FTA® Classic cards, TBE Buffer, distilled water, weighing boats, DNA loading dye, Pipettes, and pipette tips, microcentrifuge tubes (1,5 µl), 1.2 % agarose and spatula.

3.2 Methods on PCR protocol and reagents preparation (See appendix A for details)

3.2.1 Equipment

Rotor-Gene 6000 (Corbett Research, Australia), Dimension Xpanada (Siemens, Germany), BioCare professional stethoscope, $ALPK_2$ sphygmomanometer, Large skinfold calliper, Leicester height measure, Seca Robusta 813 scale, Takei Kiki Kogyo handgrip dynamometer, Isoknee-α, isokinetic dynamometer, votexer, Flexigene, thermocycler and a computer linked Kodak EDAS 290 gel imaging system.

3.2.2 Chemicals and reagents

FTA® purification reagent, TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0), NEB3 buffer, the following primers were used:

ACE F (forward): 5´-CTGGAGACCACTCCCATCCTTTCT-3´,

ACE-R (reverse):5´GATGTGGCCATCACATTCGTCAGAT3,

ACTN3 (forward):5, CTGTTGCCTGTGGTAAGTGGG-3´

ACTN3 R (reverse): 5´-TGGTCACAGTATGCAGGAGGG–3´,

TNF-α (forward):5´ - AGGCAATAGGTTTTGAGGGCCAT - 3´

and TNF-α (reverse):5´- TCCTCCCTGCTCCGATTCCG - 3´.

SensiMix (with Mg^{2+} concentration of 3 mM), loading buffer, agarose powder, Tris borate- EDTA (TBE) gel, *NcoI* and *DdeI* Restriction endonucleases.

3.3 Experimental Participants

Fourteen cricket players (experimental group) and seventeen students (control group) from the University of Zululand aged between 19-26 years were recruited. All participants were volunteers and they received details on the objectives of the study during an informational meeting. Written consent was obtained prior to the study. The participants were questioned about previous diseases, current health status and medication by a professional with medical and health background. The exclusion criteria included history of chronic disease, current infection, use of antibiotics, herbal, antioxidant and steroids containing supplements. Experimental protocols were approved by the Ethic Committee of the University of Zululand and were

conducted in accordance with the Helsinki Declaration for Ethical Treatment of Human Subjects. The cricket players participated in regular 2 hours training sessions 5-6 times a week and played club matches in the Uthungulu District in Kwa-Zulu Natal over the weekend and inter-university matches during the season. The students of the control group reported leisure physical activities such as playing volleyball, basketball or soccer for short periods not more than once or twice weekly.

3.4 Blood Samples Collection and Biochemical analysis

Blood was collected from the antecubital vein into vacutainers by trained phlebotomists from an accredited pathology laboratory (Lancet, Richardsbay) at the Department of Biokinetics and Sports Science Laboratory, University of Zululand. The following blood parameters were measured: glucose, white and red blood cells (WBC and RBC), cholesterol, triglycerides, Lactate (LA), uric acid (UA) and C-reactive protein (CRP) using the analytical equipment Dimension Xpanda, Siemens, Germany according to the South African standards of good laboratory practice. All the participants were advised not to change their dietary habits and to refrain from physical exercise 24 hours before blood sampling.

The reference ranges were as follows: C-reactive protein (CRP range 0-8 mg/L), uric acid (UA, range 0.26-0.45 mmol/L) and lactate (LA range 0.63-2.4 mmol/L). The blood samples for biochemical parameters were taken before and after cricket training session. The training session was of 1hour and 30 min duration and consisted of bowling, batting and running with exercise intensity similar to their regular practicing sessions.

3.5 Physical and physiological parameters

The following physical and physiological parameters were measured: Grip strength (kg) as well as quadriceps and hamstring strength (Nm/kg), weight (kg), stature (cm), body mass index (BMI) in kg/m^2, lean body mass (LBM) in kg, fat body mass (FBM) in kg, fat percentage (%), heart rate (HR) in bpm and blood pressure (BP) in mmHg. The evaluation testing procedure as suggested by Ashton *et al*.(2004) was used for the measurement of grip strength. The IsoKnee α isokinetic dynamometer was used to determine the relative strength of the quadriceps (knee extension) and hamstring (knee flexion) muscles as suggested by Coetsee, (2007).

The estimates of daily energy requirements and metabolic rate (MR) were done using Cunningham equation (Thompson and Manore, 1996). The equation estimates metabolic rate at rest which is multiplied by an activity factor (within range 1.2 – 1.9) to establish mean daily energy requirements. This estimation has been shown to be the best energy requirement prediction equation for metabolic rate in athletic population (Watson et al., 2005).

3.6 Measurements

For the purposes of this study the following health related physical fitness components of physical activity were studied: Blood pressure (BP), Body composition, (fat percentage and BMI), hand and forearm muscles strength and finally, quadriceps and hamstring muscle strength.

Blood pressure (BP):-The subjects were allowed to sit quietly for at least 5 minutes in a chair with a back support, feet on the floor, arm supported at heart level. The cuff was wrapped firmly around the upper arm at the heart level and the cuff was aligned with the brachial artery. The stethoscope was placed over the brachial artery and the cuff pressure was quickly inflated to 180 mmHg. The pressure was released slowly at a rate 2-5mmHg per second. Systolic BP is the point at which 2 or more Korotkoff sounds are heard and diastolic BP is the point before the disappearance of the Korotkoff sounds (ACSM, 2010). Three measurements were taken for accuracy and the average was determined and recorded, for each subject The BP values obtained were compared against normative values.

Resting Heart rate (RHR):- was measured at rest by pressuring the radial artery on the left arm using the index and middle finger and counting the beats per minute using a stop watch for 60 seconds (Heyward et al., 2010).

Fat percentage: - A Lange skinfold calliper was used to determine the subject's body fat percentage. The 6 (six) skinfold sites that were measured were the calf, thigh, supraspinale, abdominal, subscapular and triceps as suggested by Yuhasz, (1974). A minimum of 2 (two) measurements were taken at each site.

Stature: - This measurement was recorded as the height from the floor to the top of the head. It was ensured that the floor surface was even and firm. The participants were measured barefoot. The participant stood straight up, heels together, arms hanging down sides (palms facing thighs). The heels, buttocks, upper back, and head were in contact with the wall. The participants were instructed to look ahead, take a deep breath, and stand as tall as possible (heels must not leave the ground).

The platform of the stadiometer was dropped until it made contact with the top of the head. The results were recorded to the nearest 0.1 cm (Ashton et al., 2004).

Mass:-The participants were weighed with as little clothes as possible, and without shoes. It was ensured that the scale measures 0 before the participant stepped on. The participant was asked to stand up straight and still, so that the body weight is evenly distributed between both feet, in the middle of the scale. The Results were recorded to the nearest 0.5 kg (Ashton et al., 2004).

Body mass index:- The procedure, as suggested by the ACSM (2000), was used to determine the subjects body mass index by dividing the body mass (kg) by the height in meters squared ($kg.m^{-2}$).

Grip strength:- A Takei Kiki Kogyo hand held grip dynamometer was used to measure the hand and forearm grip strength. The grip dynamometer and the testing procedure are both valid and reliable (Fischer et al., 2007).

A standard position for testing recommended by the ACSM (2000) requires that the participant sits in a straight back chair, with the feet flat on the floor. Shoulders adducted in neutral and the arms unsupported. Elbows are flexed at 90 degrees, and the forearm rotation was neutral. Wrist was at 0-30 degrees dorsiflexion and 0-15 degrees ulnar deviation (Ashton, et al., 2004). The handle of the dynamometer was adjusted when required - the base rested on first metacarpal (heel of palm), while the handle rested on middle of four fingers. When the participant were ready, they

squeezed the dynamometer with maximum isometric effort, which was maintained for about 5 seconds. No other body movement was allowed (Ashton et al., 2004).

The testing procedure consisted of three practice trials with a 15 second rest in-between each trial. This was done for both the left and the right hand. For standard procedure, the right hand was tested first. The participants were allowed a one minute rest before the testing began. The testing procedure consisted of three trials with a 15 second rest in-between each trial (Ashton et al., 2004). Both the right and left hand were tested.

Quadriceps and Hamstring muscle strength:- The IsoKnee α isokinetic dynamometer was used to determine the relative strength of the quadriceps (knee extension) and hamstring (knee flexion) musculature as suggested by Coetzee, (2007). The speed of rotation was set at 60 degrees for the measurement of peak muscular strength. A warm-up routine of two to three sets of 6 (six) repetitions interspersed by 30 seconds rest followed by 3 (three) maximum contractions was followed. The subjects were allowed to recover for a few minutes. The data was recorded with the subject performing maximal knee extension and knee flexion for duration of 10 seconds.

Basal metabolic rate (BMR) and energy expenditure:- The estimates of daily energy requirements and metabolic rate (MR) were done using Cunningham equation (Thompson and Manore, 1996). The equation estimates metabolic rate at rest which is multiply by an activity factor (within range 1.2 – 1.9) to establish mean daily energy requirements. This estimation has been shown to be the best energy

requirement prediction equation for metabolic rate in athletic population (Watson et al., 2005).

3.7 Genotyping

3.7.1 FTA cards preparation

Blood spots were collected on FTA® Classic cards according to the manufacturer's instructions (Whatman International, UK) in the pathology laboratory (Lancet Laboratories, Richards Bay) and were processed for genotyping in the University of Kwa-Zulu Natal Molecular Biology Unit. The samples were prepared by punching 1.2 discs from the cards and washing with FTA® purification reagent and TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) according to the manufacturer's instructions. PCR was then performed directly from the dried disc. The samples were run in triplicates for more accurate analysis.

3.7.2 ACE Gene Genotyping

The detection of the insertion (I) and deletion (D) alleles of the *ACE* gene was performed by a modified method of Alvarez and Coto (1998). The primer sequences were ACE F (forward): 5´- CTGGAGACCACTCCCATCCTTTCT -3´ and ACE R (reverse): 5´- GATGTGGCCATCACATTCGTCAGAT -3´.

PCR reactions were performed using the SensiMix™ dT kit according to the manufacturer's instructions (Quantace, UK). The final reaction mixtures contained 1× SensiMix (with a final Mg^{2+}concentration of 3 mM) and 200 mM of each of each

primer. 20 μl of the PCR mix was added to a single dried disc in a thin-walled 200 μl PCR tube.

All amplifications were performed in a Rotor-Gene 6000 (Corbett Research, Australia) using the following conditions: activation step 95^{o}C for 10 minutes followed by 40 cycles of 95^{o}C for 10 seconds, 60^{o}C for 20 seconds 72^{o}C for 20 seconds. 1 μl 6× loading buffer was added to 5 μl of each PCR reaction which was then loaded and analysed in a 2% (w/v) agarose 1× TBE gel.

3.7.3 ACTN3 and TNF-α genes genotyping

The detection of R577X polymorphism in*ACTN3* gene was performed by a modified method of Mills et al., (2001). The primer sequences were:

ACTN3 (forward): 5 CTGTTGCCTGTGGTAAGTGGG -3′ and

ACTN3 (reverse): -5′-TGGTCACAGTATGCAGGAGGG –3′.

The TNF G308A polymorphism was detected by a modification of the method of Wilson et al., (1999) using the primers:

TNFA1 (forward):5′-AGGCAATAGGTTTTGAGGGCCAT-3′and

TNFA2 (reverse):5′- TCCTCCCTGCTCCGATTCCG - 3′.

PCR reactions were performed using the SensiMix™ dT kit according to the manufacturer's instructions (Quantace, UK). The final reaction mixtures contained 1× SensiMix (with a final Mg^{2+} concentration of 3 mM) and 200 nM of each of the appropriate primer pair. 20 μl of the appropriate mix was added to a single dried disc in a thin-walled 200 μl PCR tubes.

All amplifications were performed in a Rotor-Gene 6000 (Corbett Research, Australia) using the following conditions: activation step 95^{o}C for 10 minutes followed by 40 cycles of 95^{o}C for 10 seconds, 60^{o}C for 20 seconds 72^{o}C for 20 seconds. Restriction digests were performed in a total volume of 5 μl: 2.5 μl PCR product, 0.5 μl NEB3 buffer (10×), 0.125 μl enzyme (1.25 U), and 1.875 μl water. Digests were performed for 16 hours at 37^{o}C. 1 μl 6× loading buffer was added to each digest and the whole sample loaded to a gel.

ACTN3 amplicons were digested with *DdeI* and resolved in 2.5% (w/v) agarose 1× TBE gels. In the 291 base pairs (bp) length PCR product, a permanent site exist which was recognized by the restriction enzyme and was used as a control. The products of the reaction were two fragments, namely: the 250bp and 86bp. In R allele homozygote individuals the second site recognized by the *DdeI* enzyme is missing and as a result only these two products could be visualized. In X-allele homozygote individuals there was a second site hydrolysed by *DdeI* enzyme and three products could be resolved namely: 108 bp, 97 bp and 86 bp. In heterozygote individuals four fragments could be visualized namely: 205 bp, 108bp 97 bp and 86 bp.

TNF-α amplicons were digested with *NcoI* and resolved in 3% (w/v) agarose 1× TBE gels. The following procedure was followed: The product was digested by *NcoI*, giving rise to two fragments of 87 bp and 20 bp. If this is mutated to an A *NcoI* site is abolished, the product is not digested and a single 107 bp band is seen. If there was a G at this position the following patterns were visible: homozygous G-87 bp and 20

bp fragments, homozygous A- 107 bp fragment and heterozygous G/A- 107 bp, 87 bp and 20 bp fragments.

3.8 Statistical analysis

An unpaired Student *t*-test was used to analyse the statistical difference in the blood biomarkers and physical characteristics between the cricket players and the control group. The results are presented as mean ± SEM. Statistical significance was accepted at $p<0.05$. Statistical analysis for the genotype associations is done using GenStatDiscovery Edition 3. The distribution of some variables is skewed; hence these variables are transformed for the Analysis of Variance (Unbalanced design). For the association tests, CRP levels are categorised as <3 mg/L (low) and >3 mg/l (high) according to Pearson *et al*. (2003). Other variables are categorised according to their median (M). After ANOVA the association is examined using Chi^2 maximum likelihood test and Fisher's exact test.

3.9 Ethics

The study was approved by the University of Zululand Ethics committee and all experimental protocols were conducted as per the Helsinski Declaration for Ethical Treatment of Human Subjects. The subject's participation was obtained through the written consent form signed by the subjects themselves. (See Appendix B and C for details).

CHAPTER FOUR

RESULTS

The ACE genotype and allele frequencies are presented in Table 4.1.

It was observed that ACE genotyping had displayed a complete absence of II genotype as shown in Fig. 4.1.

Table 4.1: ACE genotype and allele frequency (%) in cricket players and controls.

Group	Genotype frequency in % and number in brackets)					Allele frequency in %	
	DD		ID		II	D	I
Cricket players (n=14)	50.0	(7)	50.0	(7)	Null	75.0 (10.5)	25.0 (3.5) [a]
Controls (n=17)	82.4	(14)	16.7	(3)	Null	91.2 (15.5)	8.8 (1.5) [a]
Total (31)	**67.7**	**(21)**	**32.3**	**(10)**	**Null**	**83.8 (26)**	**16.2 (5.0)[a]**

[a]p= 0.004 Fisher's test - two tailed based on percentage

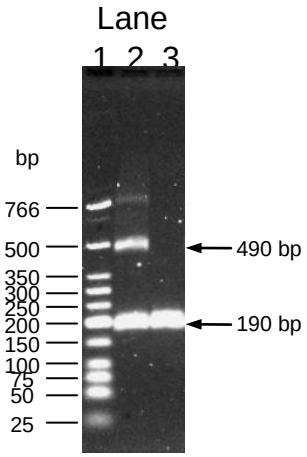


Figure 4.1: Analysis of ACE ID and DD genotypes. Amplified fragments were resolved in 2% (w/v) agarose, 1 ×TBE gels. Lane 1 - Low molecular weight DNA ladder (New England Biolabs); Lane 2 - ID genotype; Lane 3 - DD genotype. II genotype is absent.

For the whole group 67.7% DD and 32.3% ID genotypes were observed (Fig 4.2). In cricketers DD and ID genotypes were 50 % each compared to controls who showed 82.4% DD and 16.7% ID respectively The total frequency of 83.8% D allele for the cohort was significantly higher (p=0.004) compared to 16.2% I allele. (Table 4.1)

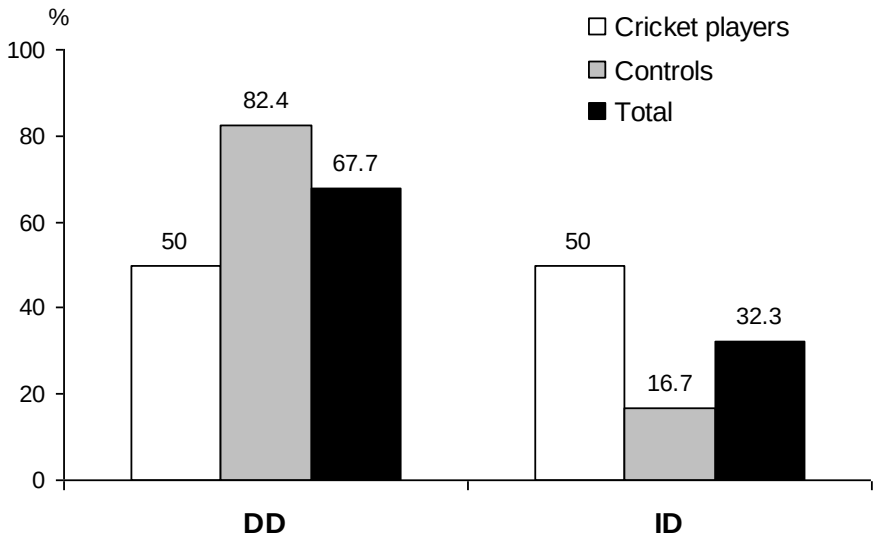


Figure 4.2: ACE DD and ID genotype frequencies (%) in cricketers, controls and the entire experimental group.

It was also found that in cricketers 25% ACE I allele frequency was higher (p=0.004) than 8.8% in controls, and 75% D allele frequency was lower (p=0.004) compared to 91.2% respectively (Table 4.1).

The biomarkers blood levels and the association tests with the allele frequency of ACE gene are shown in Table 4.2. C-reactive protein (in controls (5.81 mg/L) is much higher (p<0.001) than in cricketers (1.81 mg/L). In Ciba Laboratories of the European Union the CRP reference range are set up at 0-5 mg/L. It has to be mentioned that in South Africa the reference range of 0 -8 mg/L was accepted by

Lancet Laboratory, It was found that only one cricket player had CRP level above 6.0 mg/L. CRP levels above 8 mg/L were observed in four controls. The higher levels of CRP were discussed with the clinical pathologists. It was noted that no other changes were found in haematological markers of subclinical/clinical inflammation such as leucocytes and full blood count parameters with only exception of slight conversion changes of neutrophils and eosinophils. Therefore, these CRP levels were considered in the gene association tests where CRP levels were catergorised as above as < 3,0 mg/L and >3.0 mg/L according to Pearson's et al., (2003).

The results in cricketers have shown that 79% D allele frequency was associated ($p<0.001$) with lower CRP levels (< 3.0 mg/L). Uric acid (<0.30 mmol/L) was associated ($p=0.001$) with 43% D allele frequency.

Table 4.2: C-reactive protein (CRP), uric acid (UA) and lactate (LA) blood levels at rest in cricket players and control group (mean ± SEM) and ACE association tests.

Biomarkers	Cricket players	Control group
C-reactive protein (mg/L)	1.81 ± 0.37 [a,]	5.81 ± 0.51** NS
Uric acid (mmol/L)	0.31 ± 0.008 [b]	0.29 ± 0.007* NS
Lactate (mmol/L)	1.55 ± 0.08 NS	1.95 ±. 0.11** NS

Student's *t*-test

*p<0.05 control group vs cricket players

**p<0.001 control group vs cricket players

Association tests

[a]p=0.001 CRP <3 mg/L in cricketers was found to be in association with the ACE D allele frequency (79%), I allele (21%).

[b]p=0.001 UA <0.30 mmol/L in cricketers was in association with the ACE D allele frequency (43%), I allele (57%).

NS means there was no significant association tests.

The physical characteristics and the ACE association tests are presented in Table 4.3. BMI, LBM and FBM were higher (p<0.001) in cricket players. High ACE D allele frequency (91-94%) were associated with BMI and FBM in cricketers (p=0.001) and in controls (p=0.029), where LBM has shown an association with 71% D allele (p<0.041) and with 94% D allele (p<0.029) respectively.

In this study, health related physical fitness components of physical activity were studied. BMI, LBM and FM were found to be significantly higher in cricketers than in the control group (Table 4.3).

Table 4.3: Physical characteristics of cricket players and control group (mean±SEM) and association tests with allele frequencies of ACE gene.

Physical characteristics	Cricket players	Control group
Weight (kg)	68.68 ± 2.54	61.00 ± 1.61 **
Stature (cm)	175.08 ± 1.25	170.58 ± 0.33 **
Body Mass Index-BMI (kg/m^2)	22.40 ± 0.81 [a,]	20.79 ± 0.36 **, [C]
Lean Body Mass-LBM (kg)	61.81 ± 2.01 [b]	55.41 ± 1.49 **,[C]
Fat Mass-FM (kg)	6.87 ± 0.54 [a,]	5.59 ± 0.22 **,[C]
Fat %	9.84 ± 0.39 NS	9.13 ± 0.32 * NS

Student's *t*-test

*p< 0.05 control group vs cricket players

**p<0.001 control group vs cricket players

Association tests for BMI, LBM and FM

[a]p=0.001 BMI below 22.4 kg/m^2, and FM below 6.9 kg – ACE D allele frequency (91%), I allele frequency (9%) in cricketers

The results for the physical tests (grip strength, quadriceps strength and hamstring strength) and the association tests with the allele frequencies of the ACE gene are shown in Table 4.4. No differences in grip strength and the strength of the quadriceps (knee extension) and hamstring (knee flexion) muscles between the groups were observed but higher quadriceps strength (knee extension) L >3.73 Nm/kg and R >3.63 Nm/kg was associated with D allele frequency of 86% (p=0.010) and 79% (p=0.014). Higher hamstring flexion (knee flexion) L >2.04 Nm/kg and R >2.0 Nm/kg was associated (p=0.014) with D allele frequency of 78% and 83%.

Table 4.4:Grip strength (kg), quadriceps strength (knee extension in Nm/kg) and hamstring strength (knee flexion in Nm/kg) of cricket players and control group (mean±SEM) and the association tests with the allele frequencies of the ACE gene. (L – left, R – right).

Physical characteristics	Cricket players	Control group
Grip strength – L (kg)	44.00 ± 2.12 NS	42.57 ± 1.97 NS
Grip strength – R (kg)	45.79 ± 2.25 NS	45.11 ± 1.84 NS
Knee extension – L (Nm/kg)	3.73 ± 0.11 [a,]	3.71 ± 0.13 [e,]
Knee extension – R (Nm/kg)	3.63 ± 0.08 [b]	3.59 ± 0.12 [e]
Knee flexion –L (Nm/kg)	2.04 ± 0.08 [c]	2.08 ± 0.88 [f]
Knee flexion –R (Nm/kg)	2.00 ± 0.02 [d]	2.00 ± 0.08 [f]

Association tests

NS – no significant association tests for grip strength (L) and (R) per group of cricketers and controls.

p=0.037 for the whole cohort grip strength (L) and (R) above 44.3 kg was in association with the ACE D allele frequency (86%) and I allele frequency (14%).

[a]p=0.010 Knee extension (L) above 3.7 Nm/kg was associated with the ACE D allele frequency (86%) and I allele frequency (14%) in cricketers.

[b]p=0.014 Knee extension (R) above 3.6 Nm/kg was in association with the ACE D allele frequency (79%)and I allele frequency (21%) in cricketers

[c]p=0.014 Knee flexion (L) above 2.0 Nm/kg was found to be in association with the ACE D allele frequency (78%) and I allele frequency (22%) in cricketers.

[d]p=0.014 Knee flexion R above 2.1 (Nm/kg) was in association with the ACE D allele frequency (83%) and I allele frequency (175) in cricketers.

[e]p=0.001 Knee extension (L) above 3.7 Nm/kg and (R) above 3.6 N/kg was associated with the ACE D allele frequency (83%) and I allele frequency (17%) in controls.

[f]p= 0.001 Knee flexion (L) and (R) above 2.0 Nm/kgwas found to be in association with the D allele frequency 87% and I allele frequency (13%) in controls.

Despite the fact that no statistically significant differences in grip strength between the controls and the cricketers were established it is important to point out that for the whole cohort 86% D allele frequency was significantly associated (p=0.037) with higher grip strength L (>43.3 kg) and R (R>45. 5 kg) as shown in Table 4.5.

Table 4.5: ACE D and I allele frequency (%) association with left (L) and right (R) grip strength in the whole cohort.

Physical Test		Whole cohort		
		D (%)	I (%)	P
Grip Strength (L)	< M	92	8	-
	> M	81	19	-
TOTAL	> M	86	14	0.037
Grip Strength (R)	< M	92	8	-
	> M	81	19	-
TOTAL	> M	86	4	0.037

ACTN3 genotype and allele frequencies among cricket players and control group are presented in Table 4.6. No significant differences between the two groups were noted. For the whole cohort the results obtained for ACTN3 genotyping have shown

that the frequency of the RR genotype was 90.3% and RX genotype was 9.7%. The XX genotype was absent as shown in Figure 4.3

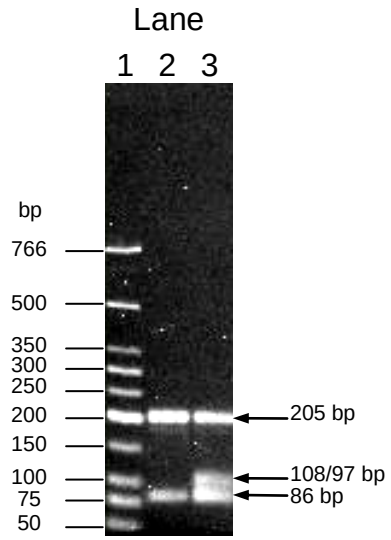


Figure 4.3: Analysis of ACTN3 RR and RX genotypes. Amplified fragments were digested with *Dde*I and resolved in 2% (w/v) agarose, 1 ×TBE gels. Lane 1- Low molecular weight DNA ladder (New England Biolabs); Lane 2 - RR genotype; Lane 3 - RX genotype. XX genotype is absent

Table 4.6:ACTN3 genotypes and allele frequency (%) in cricket players and controls.

Group	**Genotype frequency in % and numbers in brackets**					**Allele frequency in %**	
	RR		**RX**		**XX**	**R**	**X**
Cricket players (n=14)	85.7	(12)	14.3	(2)	Null	92.8	7.2
Controls (n=17)	94.1	(16)	5.9	(1)	Null	97.1	2.9
Total (31)	**90.3**	**(28)**	**9.7**	**(3)**	**Null**	**95.2**	**4.8**

The association tests of the allele frequency of ACTN3 gene with the blood levels of some biomarkers (CRP, UA and LA) that have been previously described are shown

in Table 4.7.This format of the table by repeating the biomarkers results has been chosen to clarify the association tests.

Table 4.7:C-reactive protein (CRP), uric acid (UA) and lactate (LA) blood levels at rest in cricket players and control group (mean ± SEM) and association tests with the allele frequencies of ACTN3 gene.

Biomarkers (mg/L)	Cricket players	Control group
C-reactive protein	1.81 ± 0.37[a,e]	5.81 ± 0.51**[b,e]
Uric acid	0.31 ± 0.008[c]	0.29 ± 0.007*NS
Lactate	1.55 ± 0.08[d]	1.95 ±. 0.11**NS

Student's t test:

*p<0.05 control group vs cricket players,**p<0.001 control group vs cricket players.

Association tests:

[a]p=0.0001, CRP < mg/L in cricket players. ACTN3 R allele frequency 100%,X allele(null)

[c]p=0.0001, UA<0.30 mmol/L in cricketers-ACTN3 R allele frequency (80%),X allele (20%),

[d]p=0.0001,LA<1.55 mmol/L in cricketers-ACTN3 R allele frequency(88%),X allele(12%),

*p=0.0001, CRP<mg/L in cricketers and control

TNF A allele frequency (40%),G allele (60%) in cricketers and A allele (18%),G allele (20%) in controls

N.S means no significant association test .

In this study the R allele at extremely high frequency (100%) was associated with lower CRP levels (<3.0 mg/L) in cricketers (p=0.0001) and lower lactate levels (p=0.0003) respectively as it is indicated in Table 4.7.

The association tests between the allele frequencies of the ACTN3 gene and the physical characteristics are presented in Table 4.8**.** The format of the table by repeating the physical characteristics results has been chosen again for the clarity of the ACTN3 gene association tests.

Table 4.8: Grip strength (kg), knee extension (Nm/kg) and knee flexion (Nm/kg) of cricket players and control group (mean±SEM) and the association tests with the allele frequencies of ACTN3 gene. (L – left, R – right).

Physical characteristics	Cricket players	Control group
Grip strength – L (kg)	44.00 ± 2.12 NS *	42.57 ± 1.97NS*
Grip strength – R (kg)	45.79 ± 2.25 NS	45.11 ± 1.84NS
Knee extension – L (Nm/kg)	3.73 ± 0.11 ' *	3.71 ± 0.13 ' *
Knee extension – R (Nm/kg)	3.63 ± 0.08 *	3.59 ± 0.12 *
Knee flexion –L (Nm/kg)	2.04 ± 0.08 *	2.08 ± 0.88 *
Knee flexion –R (Nm/kg)	2.00 ± 0.02 *	2.00 ± 0.08 *

Association tests

NS means no significant association tests for ACTN3 allele frequency and grip strength (L) and (R) per group of cricketers and controls.

*****p<0.001, Grip strength (L) and (R) above 44.3 kg with ACTN3 R allele frequency (86%) and X allele frequency (14%) in cricketers.

*p<0.014, Grip strength (L) and (R) above 44.3 kg with ACTN3 R allele frequency (100%) in controls.

*p<0.001, Knee extension (L) and (R) above 3.6 N/kg with ACTN3 R allele frequency (93%) and X allele frequency (7%) in cricketers

*p<0.014, Knee extension (L) and (R) above 3.6 N/kg with ACTN3 R allele frequency (100%) in controls.

*p<0.001, Knee flexion (L) and (R) above 2.0 N/kg with ACTN3 R allele frequency (89%) and X allele frequency (11%) in cricketers

*p<0.022, Knee flexion (L) and (R) above 2.0 N/kg with ACTN3 R allele frequency (100%) in controls.

The association tests between the allele frequencies of the ACTN3, TNF genes and the physical characteristics are presented in Table 4.9**.** The format of the table by repeating the physical characteristics results has been chosen for the clarity of the ACTN3 gene association tests.

Table 4.9: Physical characteristics of the cricket players and the control group (mean±SEM) and association tests with allele frequencies of ACTN3 and TNF genes.

Physical characteristics	**Control group**	**Cricket players**
Weight (kg)	68.68±2.54	61.00±1.61**
Stature (cm)	175.08 ± 1.25	170.58 ± 0.33 **
Body mass index (BMI)	22.40 ± 0.81a,[c]	20.79 ± 0.36 **[b,e]
Lean body mass (LBM	61.81 ± 2.01[d]	55.41 ± 1.49 **[b,e]
Fat%	9.84±0.39	9.13±0.32*
Fat body mass (FBM) (kg)	6.87 ± 0.54[a,c]	5.59 ±0.22**[b,e]

Student's t test:

*p<0.05 control group vs cricket players,**p<0.001 control group vs cricket players.

Association tests

BMI,LBM, and FBM: [a]p=0.0001 ,BMI below 22.4kg/m^2 and FBM below 6.9 kg was associated with the ACTN3 R allele frequency (88%), X allele (12%) in cricketers.

[b]p=0.0007, BMI below 22.4 kg/m^2, FBM below 6.9 kg and LBM below 55.4 kg was associated with ACTN3 R allele frequency (100%), X allele was(null).

[c]p=0.0202,BMI below 22.4 kg/m^2 and FBM below 6.9 kg was found to be in association with the TNF A allele frequency (9%),G allele (91%) in cricketers,

[e]p=0.0001, BMI below 22.4 kg/m^{2}LBM below 55.4 kg FBM below 6.9 kg was in association with the TNF A allele (16%),G allele (74%) in controls.

It is important to pinpoint that a strong association of R allele frequency of 100% was found with BMI and FBM in cricket players (p=0.0001) and controls (p=0.0007)..

The TNF G308A polymorphism was detected by a modified method of Wilson et al., (1999).After restriction digests were performed, fragments of different base pairs were observed. TNF genotype and allele frequencies are presented in Table 4.10 TNF genotyping displayed 42% GG, 45% GA and 13% AA frequency for the whole cohort but no differences between both groups were established.

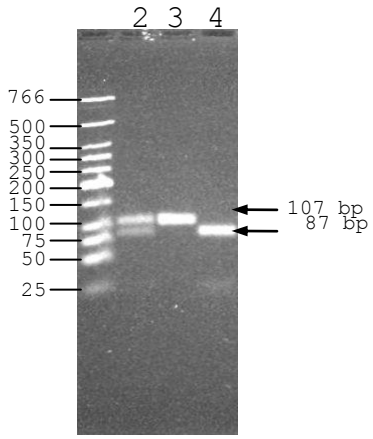


Fig. 4.4: Analysis of TNF-α GG and GA genotypes. Amplified fragments were resolved in 3% (w/v) agarose, 1 ×TBE gels. Lane 1 -, l ow molecular weight DNA ladder (New England Biolabs); Lane 2, GA genotype; Lane 3 - AA genotype; Lane 4 - GG genotype. A 20 bp fragment produced by digestion of the G allele is not clearly visible in this gel.

Lower CRP (<3.0 mg/L) was associated (p=0.0001) with the A allele frequency of

40% for the cricket players and 18% for the control group. Associations (p=0.0001) of the A allele frequencies from 9 to 22% with lower BMI, LBM and FBM were observed in (Table 4.10).

Table 4.10: TNF genotype and allele frequency (%) in the cricket players and the controls.

Group	**Genotype frequency in % and numbers in brackets**						**Allele frequency in %**	
	G G		**G A**		**AA**		**G**	**A**
Cricket players (n=14)	35.7	(5)	57.1	(8)	7.2	(1)	64	36
Controls (n=17)	47	(8)	35.3	(6)	17.7	(3)	65	35
Total (31)	**41.9**	**(13)**	**45.2**	**(14)**	**12.9**	**(4)**	**64.5**	**35.5**

Blood pressure was measured on both the control and the experimental groups to assess the health status of the participants. The blood pressure results are shown in Table 4.11: In the cricket players systolic blood pressure (SBP) was lower by 3.2 mmHg ($p<0.05$) and diastolic blood pressure (DBP) by 4.25 mmHg ($p<0.001$), where the values of systolic tension time (STT) were increased by 5.5% ($p<0.05$) compared to the controls. No differences in the heart rate and pulse pressure and no associations between blood pressure and allele frequencies of ACE gene were noted.

Table 4.11: Systolic and diastolic blood pressure (SBP & DBP mmHg), pulse pressure, heart rate (HR beats/min) and systolic tension time in the cricket players and the control group (mean ± SEM).

Parameters	Cricket players	Control group
Systolic Blood pressure (mmHg)	120.81 ± 2.03	123.75 ± 1.07*
Diastolic blood pressure (mmHg)	76.88 ± 1.4	81.13 ± 1.31**
Pulse pressure (mmHg)	43.00 ± 1.29	42.00 ± 2.01
Heart rate (beats/min)	60.75 ± 0.85	59.80 ± 1.11
Systolic tension time (SBP x HR)	7654.27 ± 147.59	7253.60 ± 124.48*

*p <0.05 , **p < 0.001

Estimates of daily energy requirements and metabolic rate (MR) were performed according to Thomson and Manore, (1996) guidelines. The cricketers have shown higher ($p<0.05$) basic metabolic rate and increased values ($p<0.001$) of metabolic rate by 10.3% and energy requirements by 14% (Table 4.12).

Table 4.12: Basic metabolic rate (BMR), metabolic rate (MR) and energy requirements (kilojoules) in the cricket players and the control group (mean ± SEM).

Parameters	Cricket players	Control group
Basic metabolic rate	1502.40 ± 25.91	1480.31 ± 13.34*
Metabolic rate	2246.99 ± 102.24	2035.40 ± 18.34**
Energy requirements (kj) per food intake)	9737.41 ± 76.79	8520.18 ± 76.79**

*p <0.05 , **p < 0.001

Blood parameters such as glucose, triglycerides and cholesterol were measured to assess the health status of the athletes and to observe the response to a one and half hour cricket training session in which bowling and batting were done. No

significant changes were observed in glucose, triglycerides and cholesterol among the cricketers before and after exercise (Table 4.13).

Table 4.13:Glucose, triglycerides and cholesterol blood levels in the cricketers before and after the training exercise session (mean±SEM).

Biomarkers (mmol/L)	Experimental group(before exercise)	Experimental group (after exercise)
Glucose	4.89±0.09	4.92±0.03
Triglycerides	0.84±0.05	0.77±0.04
Cholesterol	3.59±0.10	3.73±0.11

At rest no difference was noted in glucose, triglycerides and cholesterol blood levels between the controls and the cricketers as it is shown in Table 4.14.

Table 4.14: Glucose, Triglycerides and Cholesterol at rest in the control group and the cricket players (mean±SEM).

Biomarker	Control group	Experimental group
Glucose (mmol/L)	4.56±0.11	4.89±0.09
Triglycerides (mmol/L)	0.84±0.05	0.78±0.03
Cholesterol (mmol/L)	3.60±0.08	3.59±0.10

Full blood count (FBC) tests were performed to assess the levels of oxidative stress at rest in the cricket players compared to the control group. No statistical significance was noted. The results are tabulated in Table 4.15.

Table 4.15: Full blood count (10^9/L) parameters at rest - comparison between control group and cricketers' group (mean±SEM).

	White blood cells	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Control group at rest	6.22±0.23	3.01±0.24	2.19±0.09	0.52±0.02	0.38±0.04	0.02±0.01
Cricketers group at rest	5.94±0.25	2.87±0.22	2.26±0.05	0.47±0.02	0.46±0.19	0.12±0.01

A comparison was made to assess if any changes occur in full blood count parameters and especially in white blood cells as markers of exercise-induced oxidative stress in response to cricket training session in the cricketers group. The results are presented in Table 4.16**.** White blood cells and neutrophils were significantly higher ($p<0.05$), after the training exercise session, but within the reference ranges. No statistically significant differences in lymphocytes, monocytes, eosinophils and basophils were observed.

Table 4.16: **Full blood count (10^9/L) parameters - comparison in the cricketers' group before and after the training exercise session (mean±SEM).**

	White blood cells	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Cricketers group before exercise	5.94±0.25	2.87±0.22	2.26±0.05	0.46±0.02	0.38±0.04	0.01±0.01
Cricketers group after exercise	6.51±0.22*	3.41±0.19*	2.38±0.06*	0.49±0.02	0.33±0.02	0.02±0.01

***** $p< 0.05$ before vs after exercise training session

CHAPTER 5

DISCUSSION

The ACTN3 gene presents a polymorphism of R577X, which results in the conversion of the codon for arginine at position 577 to a premature stop codon (X). This conversion yields two versions of a functional R-allele and a null X-allele and a complete deficiency of α-actinin -3 in humans (Druzhevskaya et al., 2008)

In our study the finding of the complete absence of XX genotype was unexpected. XX null homozygosis was found in Nigerian athletes (Yang et al., 2007). Very low frequencies of XX genotype (1 to 2%) were observed in Kenyan, Jamaican and American sprinters (Scott et al., 2010)**.** Low frequencies (11%) were reported in Ethiopian athletes (Yang et al., 2005). European studies reported 6 to 14% XX allele genotype in Russians power athletes (Druzhevskaya et al., 2008), 19% in Swedish healthy volunteers (Norman et al., 2009) and 20% in Bulgarian athletes (Andonov et al., 2008). In Spanish athletes of European origin, Australian Caucasians and Asians (Japanese and Javanese) XX genotype frequencies from 18 to 25% were established (Yang et al., 2007).

Recently published data on the possible relationship between XX genotype and endurance performance remains controversial (Lucia et al., 2010; Paparini et al., 2007, MacArthur and North, 2007). The RR genotype of ACTN3 gene is considered an advantage for power/sprint activities in track and field athletes (Wessner et al., 2009), this advantage is likely related to the fact that α-actinin is the predominant fast fiber isoform in both mouse and human (Mills et al., 2001) and may present a greater capacity for the absorption or transmission of force at the Z- line during contraction

(Yang et al., 2003). The frequency of RR genotype found in elite Greek track and field athletes was 47.97% suggesting strong association with power performance (Papadimitriou et al., 2008).

Furthermore, male athletes with ACTN3 577RR genotype showed better results in long-distance rowing than carriers of RX or XX genotype (Ahmetov et al., 2010). In this study, the percent distribution of RR genotype (85.7%) in cricket players is much higher compared to 47.9% found in 60top level professional soccer players (Santiago et al., 2008). This finding shows that young cricketers of Zulu origin tend to display power/sprint genotype. This genotype is a potential basis for cricket-specific power and sprint development and trainability that are required for the successful performance in this sport.

Maximal rapid force production is a very important characteristic in fast bowling and batting. Even fielders stationed in deeper positions have to throw the ball with great power (Koley and Yadev, 2009). The ability of cricketers to cope with repeated muscle contractions may require substantial muscle strength to reduce the extent of muscle damage (Noakes and Durant, 2000). If the XX genotype enhances endurance performance as the R allele appears to enhance sprint ability, then the R and the X alleles may be maintained in the population because they both confer selective advantages under different environmental conditions and are thus kept at high population frequencies by balancing selection (Yang et al., 2003).

Data on the stature, BMI and LBM in cricket players is very similar to those observed by Christie and King (2008) in 25 male South African cricketers (age19.0± 1.1 years)

recruited from a provincial cricket academy and Rhodes University. The results of this study also support the findings of higher stature and LBM in102 Indian cricketers (age 18.3 ± 2.2 years) recruited from district and state level cricket teams of Amtrisar, Punjab (Koley and Yadev, 2009).

The percentage of body fat in the cricket players of the University of Zululand is lower compared to the cricket players in the above-mentioned studies. The difference may be due to the diet and effects of the training programme yet it should be noted that although the ranges of body fat percentage for athletes are related to successful performance, athletic performance cannot be accurately predicted only on the basis of body composition For example a heavier person has an advantage over a lighter person when activity demands that an inertia of another body or an external object be overcome (Jacqueline and Berning, 2006). In this study, particularly persons who are lighter with regards to body weight have an advantage when the goal is to propel the body for example the bats man.

For high level of sporting performance in cricket higher stature (177 ± 7.5 cm), BMI (23.3 ± 1.9 kg/m^2) and body fat of 13% have been suggested as an “ideal” body composition (Christie and King, 2008). In general, it is considered that height and LBM help cricketers to generate more force in the game (Koley and Yadev, 2009). This finding carry practical application in linking extensively used sports anthropometry with genotyping and talent selection.

The association of high R allele frequency with lower CRP, UA and LA biomarkers in cricket players is an important outcome of the study. UA and LA at rest are important

parameters for assessing overtraining and fatigue (Djarova et al., 2009, 2010). It is known that excessive free radical production during strenuous exercise could affect gene expression and regulation and act as secondary messengers in intracellular signaling cascades (Valko et al., 2007), having an impact on the circulating CRP, UA and LA levels (Djarova et al., 2009,2010).

In cricket, repeated eccentric muscle contractions occur during fast bowling, but also in repeated decelerations that occur when turning during batting and fielding may contribute to increased free radical production and exercise-induced oxidative stress inducing muscle damage (Noakes and Durant, 2000). On the other hand, low and physiological levels of free radicals are required for normal force production and adaptations in skeletal muscles (Power and Jackson, 2008). Further studies on large cohorts to confirm the association of R allele with above-mentioned blood markers, including markers for exercise-induced muscle damage are needed.

Significantly lower CRP levels were found in cricket players submitted to 5 to 6 training sessions weekly compared to controls. This outcome is in agreement with reduced levels of CRP observed by Lakka et al., (2005, 2006) in response to the exercise programme. It also supports previous results of the CRP- decreasing effect of strenuous training in swimmers and middle and long distance runners (Djarova et al., 2009, 2010). Changes in circulating CRP levels are not only markers of the immune status and sub clinical inflammation of individuals, but also indicators of the activation of signaling pathways including expression of TNF gene and cytokines production (Petersen and Petersen, 2005). Additional chemical pathology tests for

parasitic infections, tuberculosis, HIV test should be recommended for participants with higher CRP levels for future studies.

Investigating the TNF G-308A polymorphism association with CRP levels, Lakka et al., (2006) established genotype frequencies of 73.7% GG and 22.8% GA in 232 African Americans males. In this study, TNF genotyping has shown more balanced distribution of 41.9% GG and 45.2% GA frequencies. In Black Americans, 3.5% AA frequency was found to be lower compared to 12.9% AA frequency in Zulu South Africans, findings of 64.8% G allele and 35.5% A allele frequencies associated with CRP levels below and above3 mg/L are closer to the results of 75.6% G allele and 24.4% A allele frequencies reported by Lakka et al., (2006).

Several studies have suggested that the insertion allele or II genotype of the ACE gene is associated with elite endurance performance (Montgomery et al., *2005).* The ACE insertion allele or I allele has been reported to be over-represented in elite athletes. A complete absence of ACE II genotype was established for the first time in Zulu South Africans. A skewed distribution of the genotype for the whole cohort was observed (67.7% DD and 32.3% ID). A low frequency of II genotype was reported in African Americans, Kenyans, Jamaicans (Scott et al., 2005), Nigerians (Woods, 2009) and Xhosa South Africans (Payne et al., 2007). The ACE distribution in Caucasian Europeans (Woods, 2009) was found to be in ratio 1:2:1 (for example 26% DD, 50%ID, and 24% II in British males). The cricketers showed 25% of the I allele frequency as compared to controls that showed 8.8%.

The II genotype is linked with low ACE activity (Montgomery et al., 1998) and this leads to the reduction of bradykinin degradation. The reduction of bradykinin degradation may favorably alter substrate metabolism in II subjects with improvements in the efficiency of skeletal muscle. Cricketers of Zulu origin with ID genotype may have an advantage to succeed both in endurance and power/sprint events.

Collins et al., (2004) observed genotype frequencies of 24.3% DD, 54%ID and 21.6% II compared to 32.5% DD, 50.6% ID and 16.95% II in controls during ironman triathlons in a mixed group of South African born athletes, pointing out that significantly higher 51.5 % ACE I allele frequency was found in the fastest South African finishers. The frequency of I allele was higher in Lithuanian elite athletes than in controls (Gineviciene et al., 2010). On the other hand, a large study of East African distance runners did not find any association between ACE genotypes and elite endurance athletic status (Scott et al., 2010). In this study no overrepresentation of DD and ID genotypes was displayed in the Zulu cricketers.

The findings of high D allele frequency in Zulu South Africans are in line with the trend reported in Afro-Caribbean people (Berley et al., 1996), Nigerians (Woods, 2009) and elite Taekwondo athletes of Turkish. Wiliams et al., (2005) also reported a high level in D allele in Caucasian men who completed an 8 week program of Azerbaijan origin (Gunay *et al*., 2010). The excess of D allele was represented more in athletes participating in power-oriented and short-distance/high intensity exercise (Myerson *et al*., 1999, Woods *et al*., 2001, Nazarov et al., 2001, Cerit et al., 2006, Charbonneau et al., 2008).

The observation of the high D allele in the cohort possibly means that the conversion of angiotensin I to angiotensin II is higher in the presence of this allele, thus effecting the breakdown of bradykinin that influences various factors such as blood flow, substrate utilization and cell growth (Siushi et al., 2001) which are relevant to athletic performance.

After performing ACE genotyping, association tests were conducted using test results of biomarkers. It was found that the high frequency of power linked ACE D allele is also associated with lower CRP and uric acid levels in cricketers. It is worth mentioning that this is in concordance with the findings of strong association in the same cohort between these biomarkers and another power-related R allele of the ACTN3 gene (Djarova et al., 2011).

It seems that C-reactive protein might be involved via cytokines into triggering metabolic signalling pathways in the exercising muscles that could be under genetic control by both genes. It is important to emphasise that associations of the same trend as the above mentioned between high ACE D allele and high ACTN3 R allele frequencies, and BMI, LBM and FBM were also found. ACE I/D polymorphism associations with BMI and body fat have been reported (Thompson et al., 2007) suggesting that it may affect adherence to exercise training In sports events like cricket requiring short power/sprint bursts, considering the fact that both ACE D and R ACTN3 alleles have shown similarity in the association tests is a finding of interest that needs further studies.

When comparing cricketers to control subjects beneficial changes such as lower heart rate, lower BP and higher SST, higher metabolic rate and energy requirements were established. The I/D polymorphism may play a role in enhanced performance but this is not mediated by differences in the heart rate/VO_{2max} relationship to training (Woods, 2009). Despite the lack of ACE gene association the heart rate, lower blood pressure even only by 2 mm Hg based on three subsequent measurements and higher systolic tension time at rest indicate better cardiac efficiency in cricketers.

Interactions between ACE genotypes and alleles and serum ACE activity have been observed. The D allele has been related to higher circulating/tissue ACE levels and enhanced performance, but no associations between I and D alleles and BP have been reported (Bloem et al., 1996, Ostrander et al., 2009). Significantly higher estimated energy requirements were noted in cricketers. This corresponds to the findings of Noakes and Durandt (2000) that the energy demands of different cricket activities varied from 760 kJ/hours in fielding to 1064 kJ/hour in bowling and 1368 kJ/hours in batting. Strength parameters are related to cricketers' performance. Strength, flexibility and speed parameters are among many factors contributing to the success in cricket (Nunes and Coetsee, 2007).

The high D allele frequency association with grip strength that was established in the whole cohort in this study is in accordance with the findings that the D allele is related to the power/sprint output (Ruiz et al., 2010). Associations between the higher values of quadriceps/hamstring strength in cricketers and the frequency of the power/sprint linked D allele has been observed for the first time. In batting and especially in fast bowling the trunk must flex, extend and rotate within a short period.

The knee circumvents through flexion, rotation and extension. The bowling arm circumvents through extension, abduction, external rotation, thrusting flexion and internal rotation. (Stuelcken et al., 2007). The average running sprint during the wickets was found to be 18.7 km/hour which reflects high intensity work bouts (Christie and King, 2008).

One and half hour cricket training which consisted of bowling and batting was done by the experimental group and no significant changes were observed in glucose, triglycerides and cholesterol among the experimental group before and after exercise and also when comparing the control group to the experimental group. This might be an indication that the cricket players recover well after a short exercise period of one hour 30 minutes. The training period was short compared to the real game which takes about 10 to 12 hours per game.

Exercise intensity, duration and frequency have to be carefully considered to be able to effect changes in the blood biochemical markers of the experimental subjects. Our findings are similar to the observations made by Koch (2010) who studied handball players and noted no changes in blood lipids after a training period. Some studies have shown that there is no significant link in blood lipid levels with severity principles of exercise (Koch, 2010). Some authors argue that energy demands for a single endurance exercise can transiently alter lipid metabolism causing quantifiable changes in circulating lipids concentration which persist for hours or days after exercise regardless of intensity and duration (Crouse et al., 1997). This finding is worth of further investigation. It appears that, in our study based on the full blood count tests, the exposure to one low to medium intensity training does not precipitate

high levels of oxidative stress. The lack of significant changes in the full blood count parameters at rest when comparing cricketers to controls suggests the regular training sessions that there are no signs of accumulated oxidative stress that might result in fatigue and overtraining . It seems that the exercise intensity of these athletes does not exceed their recovery capacity. Good recovery in sports prevent underperformance (Kellmann, 2002)

When comparing the experimental group with the control group, no changes in white blood cells, eosinophils, basinophils, lymphocytes monocytes and erythrocytes were noted, this may also indicate good recovery of the athletes from oxidative stress due to exercise training. The short period of exercise also ensures that these cells are not exposed to molecular modifications. No changes in blood cells in response to exercise signifies good repair mechanisms, less production of free radicals that may damage various tissues and blood cells when produced in excess as it happens during prolonged strenuous exercise.

The interpretation of association studies has always been controversial especially when the limitation is a small sample. Genetic studies need large population samples, but it is difficult to reconcile this premise with the scarce number of world-class champions or a given ethnicity and sport event (Ruiz et al., 2010). When detailed phenotyping is properly applied it may improve the power to detect a biological effect in a small cohort as compared to a large cohort that is studied in less detail (Collins, 2009). The analysis of a single sport discipline and association with I/D ACE gene polymorphism has been done in groups of athletes from 25 elite climbers, 37 swimmers and 27 up to 291 runners (Woods, 2009).

The unique demands of cricket may require specific physical characteristics and genetic traits may play a substantial role. The perspective is to consider individual genetic endowment and develop training programmes that allow it to be optimized (Ostrander et al., 2009).This study in the future might provide some insight in talent identification and nurturing of young South African cricketers of various ethnicity.

CHAPTER 6

CONCLUSION

This study demonstrates for the first time a complete absence of ACTN3 XX genotype and ACE II genotype, providing evidence on the genotype distribution of previously unexamined cohort of Zulu South African athletes. Our findings show strong associations of two power/sprint linked alleles such as ACTN3 R allele and ACE D allele with low CRP, UA and LA and can contribute to the search of meaningful relationships between genetic traits and oxidative and inflammatory markers. The association of TNF G308A polymorphism with CRP inflammatory marker also supports this approach. The limitation of the present study is the small sample size but the homogeneity of the cohort is very important. Although the study involved limited number of participants it is concluded that Zulu cricket players display high frequency of ACTN3 RR genotype and a balanced ACE DD and ID genotypes distribution in conjunction with higher ACTN3 R and ACE D allele frequencies associated with physical characteristics and tests. In addition to these outcomes, beneficial differences in blood pressure and systolic tension time in cricketers compared to controls might be considered potentially advantageous parameters that could be used in monitoring cricket training and performance.

6.1 Suggestions for further work

.

The impact of genetics in sports has proved to have multiple influences, the effect of these studies on exercise performance must be combined with effective training programmes. An athlete's genetic profile can determine whether their main competitive advantage is in endurance, speed or power. Therefore, the results of this work can be presented to sports clubs to provide them with an understanding with regards to genetic suitability for specific positions and roles of each individual in the team and to gain insight of athlete's development in various sports or physical activities.

- In this study, high ACE D allele and high ACTN3 R allele frequencies were found to be in association with BMI, LBM and FBM. The finding of this similarity in association tests is of interest and is worth studying further.

- It would also be profitable to study the occurrence of the polymorphism of ACE, ACTN3 and TNF genes and their genotype/allele frequencies in conjunction with biomarkers and physical tests in population studies using large cohorts and whether they have a major effect on health or not.

References

Ahmetov, I.I., Druzhevskaya, A.M., Astratenkova, I.V., Popov, D.V., Vinogradova, O.L. and Rogozkin, V.A. (2010). The ACTN3 R577X polymorphism in Russian endurance athletes. British Journal of Sports Medicine, 44: 649-652.

Alvarez, R.,Terrados, N., Ortolano, R., Iglesias-Cubero, G., Reguero, J.R., Batalia, A., Cortina, R., Fernandez-Garcia, B., Rodriguez, C., Braga, S., Alvarez, V. and Coto, E. (2000). Genetic variation in the rennin-angiotensin system and athletic performance. European Journal of Applied Physiology, 82:117-120.

Alvarez, V., Coto, E. (1998). Angiotensin-converting enzyme and angiotensin II receptor 1 polymorphisms: association with early coronary disease. Cardiovascular Research, 40: 375-379.

American College of Sports Medicine. (2010). Guidelines for exercise testing and prescription. 6th Ed. Philadelphia, PA: Williams & Wilkins.

American College of Sports Medicine Annual Meeting, Nashville, USA (2005). Medical Science, Sports Exercise, 37: 472 (personal communication).

Amir, O., Amir, R., Yamin, R., Attias, E., Enion, N., Sagiv, M. and Meckel, Y. (2007). The ACE deletion allele associated with Israeli elite endurance athletes. Exercise. Physiology, 92:881-886.

Andonov, S., Saraeva, R., Andonova, S., Kaneva, R., Gigova, V., Stefanov, L., Kremenski, I. and Atanassov, P. (2008). Polymorphism of ACTN3, ACE and AMPD1 genes and physical performance in Bulgarian sub-elite athletes. European Journal of Human Genetics, (2): 288-289.

Ashton, L.A. and Myers, S. (2004). Serial grip strength testing-Its role in assessment of wrist and hand disability. The International Journal of Surgery, 5:1528-1642.

Beggs, H. and North, K.N. (1994). Deficiency of a skeletal muscle isoform of α-actinin (α-actinin-3) in merosin-positive congenital muscular dystrophy. International Journal of Biochemistry, 6(4):134-143.

Bertelli, M., Alushi, B. and Veiscteinas, A. (2008). Physical exercise to prevent multifactorial diseases: a warning written in our genes? Sport Science and Health, 4: 33-35.

Blake, B. and Volpie, R. (2004). Encyclopaedia of endocrine disease, 135-138.

Bloem, L.J., Manatunga, A.K. and Prat, J.H. (1996). Racial differences in the relationship of an angiotensin I–converting enzyme gene polymorphism to serum angiotensin I–converting enzyme activity. Hypertension, 27: 62-66.

Bray, M.S., Hagbero, J.M., Perusse, L., Rankinen, T., Roth, S.M., Wolfarth, B. and Bouchard, C. (2008). The human gene map for performance and health-related

fitness phenotypes: The 2006-2007 update. Journal of Medicine and Science in Sports and Exercise, 41: 34-72.

Brooks, G.A. (1986). The lactate shuttle during exercise and recovery. Medicine and Science in Sports and Exercise 18, 360-368.

Calo, C.M. and Vona, G. (2008). Gene polymorphisms in athlete's performance. Journal of Anthropological Sciences, 86: 113-131.

Cerit, M., Colakoglu, M., Erdogan, M., Berdeli, A. and Cam, F.S. (2006). Relationship between ace genotype and short duration aerobic performance development. European Journal of Applied Physiology, 98:461-465.

Charbonneau, D.E., Hanson, E.D., Ludlow, A.T., Delmonico, M.J., Hurley, B.F. and Roth, S.M. (2008). ACE genotype and the muscle hypertrophic and strength responses to strength training. Medical Science and Sports Exercise, 40: 677-683.

Christie, C.J. and King, G.M. (2008). Heart rate and perceived strain during batting in a warm and cold environment. International Journal of Fitness, 4(1): 33-38.

Christos, P., Chrysohoou, P., Skoumas, J. and Masoura, C. (2003). The emerging anti-inflammatory role of HDL-cholesterol, illustrated in cardiovascular disease free population: the ATTICA study. International Journal of Cardiology, 122: 29-33.

Clarkson, P.M., Devaney, J.M., Gordish-Dressman, H., Thompson, P.D., Hubal, M.J., Urso, M., Price, T.B., Angelopoulos, T.J., Gordon, P.M., Moyna, N.M., Pescatello, L.S., Visch, P.S., Zoeller, R.F., Seip, R.I and Hoffman, E.P. (2005). ACTN3 genotype is associated with increases in muscle strength in responses to resistance training in women. Journal of Applied Physiology, 99: 154-163.

Coates, D., Isaac, R.E., Cotton, J., Siviter, R., Williams, T.A., Shirras, A., Corvol, P., and Dive, V. (2000). Functional conservation of the active sites of human and Drosophila angiotensin I converting enzyme. Biochemistry, 39(30):8963– 8969.

Coetsee, M.F (2008). Isoknee α Instruction Manual, Isokinetic Equipment cc Publisher, South Africa, 1-163.

Collins, M. (2009). Genetics and Sports. Karger Publishers, Basel, Switzerland, 9.

Collins, M., Xenophontos, S.L., Cariolou, M.A., Mokone, G.G., Hudson, D.E., Anastasiades, L. and Noakes, T.D. (2004). The ACE gene and endurance performance during the South African Ironman Triathlons. Medical Science and Sports Exercise, 36: 1314-1320.

Cooper, C., Vollaard, N.B.J., Choueiri, T. and Wilson, M.T. (2002). Exercise: free radicals and oxidative stress. Biochemical Society Transactions, 30(2): 231-244.

Corvol, P., Williams, T. A., and Soubrier, F. (1995). Peptidyl dipeptidase a-angiotensin-I converting-enzyme. In Proteolytic Enzymes: Aspartic and Metallo Peptidases, 283–305.

Costerousse, O., Allegrini, J., Lopez, M. and Alhenc-Gelas, F. (1993). Journal of Biochemistry, 290: 33–40.

Couillard, C. (2001). Arteriosclerosis, Thrombosis, and Vascular Biology, effects of endurance exercise training on plasma HDL cholesterol levels depend on levels of triglycerides: evidence from men of the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) Family Study.

Clarkson, P.M., Devaney, J.M., Gordish-Dressman, H., Thompson, P.D., Hubal, M.J., Urso, M., Price, T.B., Angelopoulus, T.J., Gordon, P.M., Moyna, N.M., Pescatello, L.S., Visich, P.S., Zoeller, R.F., Seip, R.L. and Hoffman, E.P. (2005). ACTN3 genotype is associated with increases in muscle strength in response to resistance training women. Journal of Applied Physiology, 99: 154-163.

Crouse, F.S., O'brien, B.C., Grandjean, Robert P.W., Lowe, C., Rohack, J.J., Green, J.S. and Tolson, H. (1997). Training intensity, blood lipids, and apolipoproteinsin men with high cholesterol. Journal of Applied physiology, 83(6):2019-2028.

Cullinane, E., Siconolfi S., Saritelli, A. and Thompson, P.D. (1982). Acute decrease in serum triglycerides with exercise: Is there a threshold for an exercise effect. Metabolism, 31(8):844-847.

Davids, K. and Baker, J. (2007). Genes environment and sports performance. Sports Medicine, 37: 901-980.

Davis, B. (2000). Training for physical fitness. Physical education and the study of sport. 121-122.

Deurenberg, P., Weststrate, J.A. and Seidell, J.C. (1991). BMI to body fat percentage formula, Body mass index as a measure of body fatness: age- and sex-specific prediction formulas. British Journal of Nutrition, 65 (2):105-14.

Djarova, T.**,** Andreeva, L., Stefanova, D., Mateev, G., Tzvetkov, S. and Bonov, P. (2010). Dark chocolate supplementation impact on blood oxidative biomarkers in track athletes submitted to maximal exercise tests*.* Proceedings of the 5th International Congress "Sport, Stress, Adaptation-Olympic Sport and Sport for All", Journal of Sport and Science, Special issue 1: 330-336.

Djarova, T., Andreeva, L., Stefanova, D., Mateev, G., Basson, A., Tzvetkov, S. and Kamenov, L. (2009). Influence of dark chocolate supplementation on uric acid, liver enzymes, and lactate and glucose changes induced by submaximal exercise in athletes. South African Journal for Research in Sports, Physical Education and Recreation, 31:1-8.

Djarova, T., Andreeva, L., Stefanova, D., Mateev, G., Basson, A., Tzvetkov, S. and Kamenov, L. (2009). The effects of dark chocolate supplementation on C-reactive protein, immunoglobulins, leucocytes and platelets in response to maximal exercise

in swimmers. Proceedings of the 6th European Congress of Sports Medicine Supplement 11, European Journal of Sports Medicine, (14): 329-335.

Djarova, T., Watson, G., Basson, A., Grace, J., Cloete, J. and Ramakoaba, A. (2011). ACTN3 and TNF gene polymorphism association with C-reactive protein uric acid, lactate and physical characteristics in young African cricket players. African Journal of Biochemistry Research, 4:22-27.

Druzhevskaya, A.M., Ahmetov, I.I., Astratenkova, V. and Rogozkin, V. (2008). Association of the ACTN3 R577X polymorphism with power athlete status in Russians. European Journal of Applied Physiology, 103: 631-634.

Eleanor, N.W. and Rolfes, S.R. (2003). Understanding nutrition, 311.

Fielding, R.A., Manfredi, T.J., Ding, W., Fiatarone, M. A., Evans, W.J. and . Cannon, J.G. (1993). Acute phase response in exercise: Neutrophil and IL-1 beta accumulation in skeletal muscle. American Journal of Physiology, 265 (1): 166-172.

Fischer, M.B. and Birren, J.E. (2007). Standardization of a test of hand strength. Journal of Applied Physiology, 30 (4):380-387.

Folland, J., Leach, B., Little, T., Howker, K., Myerson, S. and Montgomery, H. (2000). Angiotensin converting enzyme genotype affects the response of human skeletal muscle to functional overload. Exp Physiology, 85:75-579.

Franks, D. and Howley, T.E. (1998). Fitness leader's handbook, 1-4.

Gayagay, G., Bing, Y., Brett, H., Boston, T., Hahn, A., David, S.C. and Trent, R. J.(1998). Elite endurance athletes and the ACE I allele – the role of genes in athletic performance. Human Genetics , 103(1):48-50.

Gineviciene, V., Pranckeviciene, E., Millasius, K. and Kuchinskas, V. (2010). Relating fitness phenotypes to genotypes in Lithuanian elite athletes. Actamedica Lithuanica, 17: 1-10.

Goodwin, M.L. (2010). Blood Glucose Regulation during Prolonged, Submaximal, Continuous Exercise: A Guide for Clinicians. Journal of Diabetes Science and Technology, (3): 694–705.

Gomez-Gallego, L.A., Santiago, F., Gonzalez-Freire, C., Muniesa, C. A., Fernadez Del Valle, M., Perez, M., Foster, C. and Lucia, A. (2008). Endurance performance: genes or gene combinations? International Journal of Sports Medicine, 30: 66-72.

Gunay, M., Ulkuer, M.K., Celenk, C., Bezci, S., Gokdemiir, K., Gevat, C. and Kesici, T. (2010). Angiotensin-converting enzyme polymorphism in elite Taekwondo athletes of Turkish and Azzerbaijan Taekwondo teams.Ovidia University Annals, Series Physical Education and Sports Science, Movement and Health, 10: 165-168.

Hagberg, J., Farrell, M., McCole, R.E., Wilund, S.D. and Moore, H.E. (1998). VO_{2max} is associated with ACE genotype in postmenopausal women. Journal of Applied physiology, 85: 1842-1846.

Halle, M., Berg, A., Von Stein, T., Baumstark, M.W., König, D. and Keul, J. (1996). Lipoprotein in endurance athletes, power athletes, and sedentary controls. Journal of Medicine and Science in Sports and Exercise, 28(8): 962-966.

Haung, W., Xie, C., Zhou, H., Yang, T. and Sun, M. (2003). Association of the angiotensin-converting enzyme gene polymorphism with chronic heart failure in Chinese Han patients. The European Journal of heart failure, 23-27.

Hawley, J.A., Muburg, K.H., Noakes, T.D and Dennis, S.C. (1997). Training to improve fatigue resistance and enhance endurance performance. Journal of Sports Science, 15:325-333.

Hayward, V.H. (2010). Advanced fitness assessment and exercise prescription, 233.

Heck, A.L., Barroso, C.S., Callie, M.E. and Bray, M.S. (2004). Gene Nutrition Interaction in Human Performance and Exercise Response. Nutrition, 20:598-602.

Helge, W.J. (2002). Long-term fat diet adaptation effects on performance, training capacity, and fat utilization. Medicine and Science in Sports and Exercise, 34 (9): 1499-1504.

Hellsten, Y., Svensson, M. and Sjodin, B. (2001). Allatonin formation and urate and glutathione exchange in human muscle during submaximal exercise. Free Radical Biology and Medicine, 31: 1313-1322.

Heunks, L.M. and Dekhuizen, P.N. (2000). Respiratory muscle function and free radicals: from cell to COPD, Thorax, 55: 704-716.

Hollozy, J.O., Kohrt, W.K. and Hansen, P.A. (1998). The regulation of carbohydrate and fat metabolism during and after exercise. Bioscience, 3:1011-1027.

Howley, E.T. and Franks, B.D. (1998). Fitness leader's handbook, 1-4.

Horowitz, J.F and Klein,S.(2000). Lipid metabolism during endurance exercise. American Journal of clinical nutrition, 72: 558–563.

Hruskovicova, D., Selingerova M., Bohus, B., Timkanicova, B. and Kovacs, L (2006). The angiotensin converting enzyme I/D polymorphism in long distance runners. Journal of Sports Medicine and Fitness, 46(3):509-513.

Hurmuz, K. The comparison of some blood lipid levels before and after handball match in players. (2010). World Applied Science Journal, 10(6): 727-731.

Huttley, G. and Wilson, A. (2000). Testing for concordant equilibria between population samples. Genetics, 156:2127–2135.

Jackson., M.J. (2005). Reactive oxygen species and redox-regulation of skeletal muscle adaptations to exercise. Philosophical transactions of the Royal Society, Biological Sciences, 360:2285-229.

Jacqueline, R. and Berning, S.N.S. (2006). Nutrition for Sports and exercise, 4th Edition: 65.

Jamil, K., Syed, R. and Rao, H. (2009). Implications of I/D (rs4340) polymorphism in CAD among South Indian population. International Journal of Medicine and Medical Sciences, 1(5):151-15.

Jonatan, R., Arteta ,D., Buxens, A., Artieda, M., Gómez-Gallego, F., Santiago, C., Yvert, T. and Morán, M. (2010). Can we identify a power-oriented polygenic profile? Journal of Applied Physiology, 108: 561-566.

Jones, A. and Woods, D.R. (2003). Skeletal muscle RAS and exercise performance, International Journal of Biochemistry and Cell Biology, 35: 855-866.

Jones, A., Montgomery, H.R., and Woods, D.R. (2002). Human performance: A role for the ACE genotype? Exercise and Sport Science Review, 30(4): 184-190.

Kaplan, N.L., Hudson, R.R. and Langley, C.H. (1989). The “hitchhiking effect”: Revisited genetics, 123:887–899.

Katz, A. and Sahlin, K. (1990). Role of oxygen in regulation of glycolysis and lactate production in human skeletal muscle. Exercise and sports science reviews. Baltimore, Williams & Wilkins, 1-28.

Kellman, M. (2002). Under recovery and overtraining: different concepts-similar impacts: Kellman M (ed): Enhancing recovery-preventing underperformance in atheletes, Champaign, Human Kinetics, 3-24.

Kisner, C. and Lynn, A. (2002). Therapeutic Exercises, 5: 692-93.

Koening, W., Sund, M., Frohlich, M., Fischer, H.G., Lowel, H., Doring, A., Hutchingson, W.L. and Pepys, M.B. (1999). C-reactive protein, a sensitive marker of inflammation, predicts future risk of coronary disease in initially healthy middle-aged men: results from the MONICA (Monitoring trends and Determinants in cardiovascular Disease). Circulation, 99:237-242.

Koley, S. and Yadav, M.K. (2009). An association of hand grip strength with some anthropometric variables in Indian cricket players. Facta Universitatis, Physical Education and Sport, 7: 113-123.

Lakka ,T. A., Lakka, H. M., Rankinen, T., Leon,. A. S., Rao, D. C., Skinner, J.S, Wilmore, J. H. and Bouchard, C. (2005). Effect of exercise training on plasma levels of C-reactive protein in healthy adults: The Heritage Family Study. European Heart Journal, 34:338-343.

Lakka, H. M., Lakka, T. A., Rankinen, T., Rice, T., Rao, D. C., Leon, A. S., Skinner, J. S. and Bouchard, C. (2006). The TNF-α G308A polymorphism is associated with C-reactive protein levels: The HERITAGE Family Study. Vascular Pharmacology, 44: 377-383.

Langford, B.R., Davis, D., Blaufox, A., Oberman, S. and Hawkins, W. (1991). Effect of drug and diet treatment of mild hypertension on diastolic blood pressure, American Hearts Association Journals of Hypertension, 17:210-217.

Lawrence, R.D. (1946). Lipodystrophy and hepatomegaly with diabetes, lipaemia, and other metabolic disturbances: a case throwing new light on the action of insulin. Lancet, 724–731, 773–775.

Li Li, J.T. (1994). Oxidative stress during exercise: Implication of antioxidant nutrients. Free Radical Biology and Medicine, 18(6):1079-1086.

Libby, P., Ridker, P.M. and Maseri, A. (2002). Inflammation and atherosclerosis. Circulation, 105:1135-1143.

Lidinger, M.I., Kowalchuk, J.M., and Heigenhauser, G.J. (2005). Applying physiochemical principles to skeletal muscle acid-base status. American Journal of Regulatory, Intergrated and Competitive Physiology, 289: 891-894.

Lucia, A., Moran, M., Zihong, H. and Ruitz, R. (2010). Elite Athletes: Are the Genes the Champions? International Journal of Sports Physiology and Performance, 5: 93-102.

Lucki, N.C. and Nicolay, C.W. (2007). Phenotypic plasticity and functional asymmetry in response to grip forces exerted by intercollegiate tennis players. The American Journal of Human Biology, 19: 566-577.

MacArthur, D.G and North, K.N. (2004): A gene for speed? The evolution and function of alpha-actinin3. Bioassays, 26: 786– 795.

MacArthur, D. G. and North, K. N. (2007). ACTN3: A genetic influence on muscle function and athletic performance. Exercise and Sports Review, 35(1): 30-34.

Manore, M., Meyer, L.N. and Thompson, J. (1995). Sport Nutrition for Health and Performance, 155.

Margonis, K., Fatouros, I.G., Jamurtas, A.Z., Nikolaidis, M.G., Douroudos, I., Chatzinikolaou, A., Mitrakou, A., Mastorakos,G., Papassotiriou, I., Taxildaris, K., and Kouretas, D. (2007). Oxidative stress biomarkers responses to physical overtraining: Implications for diagnosis. Free Radical Biology and Medicine, 43(6):901-910.

Martens, R. (2004). Successful Coaching Human Kinetics Campaign

Martin, H., Berg, A., Von Stein, T., Baumstark, M.W., Konig, D. and Keul, J. Lipoprotein (a) in endurance athletes, power athletes and sedentary controls. (1996). Journal of Medicine and Science in Sports and Exercise, 8: 962-966.

Mattei, M.G., Hubbert, G.C., Alhenc-Gelas, F., Roeckel, N., Corvol, P. and Soubrier, F. (1989). Angiotensin–I converting enzyme gene is on chromosome 17. Cytogenetics and Cell genetics, 51: 1041.

Mattusch, B., Dufaux, O., Heine, I., Mertens, H.and Rost, R. (2000). Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. International Journal of Sports Medicine, 21: 21-24.

Maughan, R.J. (2008). Sports Nutrition, 89.

Melrose, D.K., Spaniol, F.J., Bohling, M.E., and Bonnete, R. A. (2007). Physiological and performance characteristics of adolescent club volleyball players. Journal of Strength and Conditioning Research, 21(2): 481-487.

Milander, L., Stein, D.J. and Collins, M. (2009). The interleukin-6, serotonin transporter, and monoamine oxidase. A genes and endurance performance during the South African Ironman Triathlon. Applied Physiology, Nutrition and Metabolism, 34: 858-865.

Mills, M., Yang, N., Weinberger, R., Van der Woude, D.L., Beggs, A.H., Easteal, S. and North, K. (2001). Differential expression of the actin-binding proteins, α-actinin-2

and -3, in different species: implication for the evolution of functional redundancy. Human Molecular Genetics, 10: 1335-1346.

Montgomery, H and Payne, K.(2003). The Renin- Angiotensin system and physical performance. Biochemical Society Transactions, 31(6): 222-242.

Montgomery, H., Clarkson, P., Barnard , M., Bell, J., Brynes, A., Dollery, C., Hajnal, J., Hemingway, H., Mercer, D., Jarman, P., Marshall, R., Prasad, K., Rayson, M., Saeed, N., Talmud, P., Jubb, M., World, M. and Humphries, S. (1999). Angiotensin-converting-enzyme gene insertion/deletion polymorphism and response to physical training Journal of Sports Science and Medicine, 353(9152): 541-545.

Moran, C., Yang, N., Bailey, M.E.S., Tsiokanos, A., Jamurtas, A., MacArthur, D.G., North, K., Pitsiladis, Y.P. and Wilson, R.H. (2007) .Association analysis of the ACTN3 R577X polymorphism and complex quantitative body composition and performance phenotypes in adolescent Greeks. European Journal of Human Genetics , 15:88–93.

Mougios, V. (2006). Exercise Biochemistry, 170.

Murakami, S., Kurihara, S., Koikawa, N., Nakamura, A., Aoki, K., Yosigi, H., Sawaki, K. and Ohtani, M. (2009). Effects of oral supplementation with cystine and theanine on the immune function in athletes in endurance exercise: randomised, double-blind, placebo-controlled trial. Bioscience, Biotechnology and Biochemistry, 73: 817- 821.

Myerson, S., Hemingway, H., Budget, R., Martin, J., Humphries, S. and Montgomery, H. (1999). Human angiotensin I-converting enzyme gene and endurance performance. Journal of Applied Physiology, 87: 1313-1316.

Nazarov, I.B., Woods, D.R., Montgomery, V.A., Shneider, O.V., Kasakov, V.T., Tomilin, N.V. andRogozkin, V.A. (2001). The angiotensin converting enzyme I/D polymorphism in Russian athletes. European Journal of Human Genetics, 9:797-801.

Noakes, T.D. and Durandt, J.J. (2000). Physiological requirements of cricket. Journal of Sports Sciences, 18: 919-929.

Norman, B., Esbjornsson, M., Rundqvist, H., Ostrelund, T., Von Walden, F. and Tesch, P.A. (2009). Strength, power, fiber types, and mRNA expression in trained men and women with different ACTN3 R577X genotypes. Journal of Applied Physiology, 106: 959-965.

North, K.N., Yang, N., Wattanasirichaigoon, D., Mills, M., Easteal, S. and Beggs A.H. (1999). A common nonsense mutation results in α-actinin-3 deficiency in the general population. Nature and Genetics, (21): 353–354.

Nunes, T and Coetsee, B. (2007). The contribution of isokinetic strength parameters to the performance of cricket batsmen. Isokinetic and Exercise Science, 15: 233-244.

Oh, S.D. (2007). The distribution of I/D polymorphism in the ACE gene among Korean male elite athletes. Journal of Sports Medicine and Physical Fitness, 47: 250 - 254.

Ostrander, E.A., Hudson, H.J. and Ostrander, G.K. (2009). Genetics of athletic performance. Annual Revision. Genomics and Human Genetics, 10: 407-429.

Papadimitriou, I.D., Papadopoulos, C., Kouvatsi, A. and Triantaphylidis, C. (2008). The ACTN3 gene in elite Greek track and field athletes. International Journal of Sports Medicine, 29: 352-355.

Paparini, A., Ripani, M., Giordano, G.D., Santoni, D., Pigozzi, F. and Romano-Spica, V. (2007). ACTN3 genotyping by real-time PCR in the Italian population and athletes.Medicine and Science in Sports and Exercise, 39: 810-815.

Payne, J.R., Dhamrait, S.S., Gohlke, P., Cooper, J., Scott, R.A., Pitsiladis, Y.P., Humphries, S.E., Rayner, B. and Montgomery, H.E. (2007). The impact of ACE genotype on serum ACE activity in a Black South African male population. Annuals of Human Genetics, 71: 1-7.

Peake, J.M., Suzuki, K. and Coombes, J.S. (2007). The influence of antioxidant supplementation on markers of inflammation and the relationship to oxidative stress after exercise. The Journal of Nutritional Biochemistry ,18 (6): 357-371.

Pearson, T.A., Mensah, G.A., Alexandra, R.W., Anderson, J.L., Cannon, R.O., Criqui, M., Fadl, Y.Y., Fortmann ,S.P., Hong, Y., Myers, G.I. et al. (2003). Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centres for disease

control and prevention and the American Heart Association. Circulation, 107:499-511.

Pescatello, L.S, Kostek, M.A., Gordish-Dressman, H., Thompson, P.D., Seip, R.I., Price, T.B., Angelopoulos, T.J., Clarkson, P.M., Gordon, P.M., Moyna, N.M., Visich, P.S., Zoeller, R.F., Devaney, J.M. and Hoffman, E.P. (2006). ACE ID genotype and the muscle strength and size response to unilateral training. Medicine and Science and Sports Exercise, 38: 1074-1081.

Petersen, A.M. and Petersen, B.K. (2005). The anti-inflammatory effects of exercise. Journal of Applied Physiology, 98: 1154-1162.

Pieruzzi, F., Abassi, A., Keiser, H.R. (1995). Expression of rennin-angiotensin system compounds in heart, kidneys and lungs of rats with experimental heart failure. Circulation, 92:3105-3112.

Pitzavos, C., Chrysohoou, D.B., Panagiotakos, J., Skoumas, A., Zeimbekis, P. and Kokkinos, P. (2003). Association of leisure-time physical activity on inflammation markers (C-reactive protein, white cell blood count, serum amyloid A and fibrinogen) in healthy subjects. American Journal of Cardiology, 1(3): 368-370.

Power, S.K., and Jackson, M.J. (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiological Reviews , 88: 1243-1276.

Rader, D.J. (2007). Effect of insulin resistance, dyslipidemia, and intra-abdominal adiposity on the development of cardiovascular disease and diabetes mellitus. American Journal of Medicine, 120(3): 12-8.

Rankinen, T., Perusse, L., Gagnon, J., Changon, Y.C., Keon, A.C., Skinner, J.S., Wilmore, J.H., Rao, D.C. and Bouchard, C. (2000). Angiotensin-converting enzyme ID polymorphism and fitness phenotype in the HERITAGE family study. Journal of Applied Physiology, 88:1029-1035.

Rankinen, T., Pérusse, R., Rauramaa, M. A., Wolfarth, B., and Bouchard, C. (2004). The Human Gene Map for Performance and Health-Related Fitness Phenotypes: The 2003 Update. Medicine and Science in Sports Exercise, 36(9): 1451-1469.

Richter, E.A., Garetto, L.P., Goodman, M.N and Neil, B. (1982). Muscle Glucose Metabolism following Exercise in the ratincreased sensitivity to insulin. Ruderman journal of clinical investigations, 69(4): 785–793.

Rigat, B., Hubert C., Alhenc-Gelas, F., Cambien, F., Corvol, F. and Soubrier, F. (1990). An insertion(I)/deletion (D) polymorphism in the Angiotensin I-Converting Enzyme gene accounting for half the variance of serum enzyme levels. Journal of Clinical Investigation, 86(4):1343–1346.

Robergs, R.A., Ghiasvand, F. and Parke, D. (2004).Biochemistry of exercise-induced metabolic acidosis. American Journal of Physiology, 287: 502-516.

Rodriguez, N.R., Vislocky, L.M., Gaine, P.C. (2007). Dietary protein, endurance exercise and human skeletal-muscle protein turnover. Current Opinion in Clinical Nutrition and Metabolic Care, 10: 40-45.

Romero-Corral. A., Montori, V.M., Somers, V.K., Korinek, J., Thomas, R.J, Allison T.G. and Mookadam, F. (2006). Association of bodyweight with total mortality and with cardiovascular events in coronary artery disease: a systematic review of cohort studies. Lancet, 368(3695): 666–678.

Ross, C.H. and Rősbald, B. (2002). Norms of grip strength in children aged 14-16 years. Acta Paediatrica, 91(6) : 617-625.

Ruiz, J.R., Arieta, D., Buxens, A., Arieda, M., Gomez-Galego, F., Santiago, C., Yvert, T., Moran, M. and Lucia, A. (2010). Can we identify a power-oriented polygenic profile? Journal of Applied Physiology, 108: 561-566.

Sahlin, K., Katz, A. and Bobergs, S. (1990). Tricarboxylic acid cycle intermediates in human muscle during prolonged exercise. American Journal of physiology, 25(5): 834-841.

Santiago, C., Gonzalez-Freire, M., Serratosa, L., Morate, F.J., Meyer, T., Gomez-Galego, F. and Lucia, A. (2008). ACTN3 genotype in professional soccer players. British Journal of Sports Medicine, 42: 71-73.

Scanavini, D., Bernardi, F., Castoldi, E., et al (2002). Increased frequency of the homozygous II Ace genotype in Italian Olympic endurance athletes. European Journal ofHuman Genetics, 10: 576-577.

Schneider, A.J. and Rupert, J.L. (2009). Constructing winners: The science and ethics of genetically manipulating athletes. Journal of the Physiology of Sport, 36: 182-206.

Scott, R., Irving, R., Irwin, L., Morrisson, E., Charlton, V., Austin, K., Tladi, D., Deason, M., Headly,S., Kolkhirst, F., Yang, N. and North, B. (2010). ACTN3 and ACE Genotypes in Elite Jamaican and US Sprinters. Medicine and Science in Sports and Exercise, 42(1):107-112.

Scott, R.A., Moran, C., Wilson, R.H., Onywera, V., Boit, M.K., Goodwin, W.H., Gohike, P., Montgomery, H., Pitsiladis, Y.P. (2005). No association between angiotensin converting enzyme (ACE) gene variation and endurance athlete status in Kenyans. Compiled Biochemistry and Physiology. A Molecular Integration in Physiology, 141:169-175.

Sentűrk, G.F., Kuru, O., Aktenin, M.R., Yalein, O, Bor-Kűcűkatay, M., Yesilkaya, A. and Baskurt, O.K. (2001). Exercise induced oxidative stress affects erythrocytes in sedentary rats but not exercise-trained rats. Journal of applied physiology, 91(5):1999-2004.

Sipahi, T., Budak, M., Sen, S., Ay, A. and Seneq, S. (2006). Association between the Ace gene insertion (I)/deletion (D) polymorphism and primary hypertension in Turkish patients of Trakya region. Biotechnology and Biotechnology, 20: 104-108.

Shils, M.E. and Shike,M. (2006). Modern nutrition in health and disease. 144.

Speakman, J.R. and Selman, C. (2003). Physical activity and resting metabolic rate. Champaign (IL): Human Kinetics, 62(3): 621-634.

Stainsby, W.N. and Brooks, G.A. (1990). Control of lactate metabolism in contracting muscles and during exercise. Exercise and sports science reviews. Baltimore, Williams & Wilkins, 29-63.

Steensberg, A., van Hall G., Osada. T., Schjerling, P., Pedersen, B., Saltin, B., and Febbraio, M. (2002). Muscle glycogen content and glucose uptake during exercise in humans: influence of prior exercise and dietary manipulation. Journal of Physiology, 541(1): 273–281.

Stuelcken, M.,Pyne, D. and Sinclair, P. (2007). Anthropometric characteristics of elite cricket fast bowlers. Journal of Sports Sciences, 25: 1587-1597.

Suzuki, K., Yamada, M., Kurakake, S., Okumura, N., Yamaya, K., Liu, Q., Kudoh,S., Kowatari, K., Nakaji, S., and Sugawara, K. Circulating cytokines and hormones with immunosuppressive but neutrophil-priming potentials rise after endurance exercise in humans.European Journal of Applied Physiology, 81(4): 281-287.

Tan, B., Aziz, A. R., Teh, K.C. and Lee, H.C. (2001). Grip strength measurement in competitive ten-pin bowlers. Journal of Sports Medicine and Physical Fitness, 41: 68-72.

Taylor, R.R., Mamotte, C.D.S., Fallon, K. and van Bockxmeer, F.M. (1999). Elite athletes and the gene for angiotensin-converting enzyme. Journal of Applied Physiology, 87:1035-1037.

Thayer, J. F., Merritt, M.M., Sollers , J.J. III, Zonderman, A.B., Evans, M.K., Yie, S. and Abernethy, D.R. (2003). Effect of the Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism DD Genotype on High-Frequency Heart Rate Variability in African Americans American Journal of Cardiology, 92: 1487-1490.

Thiriet P., Gozal, D., Wouassi, D., Oumarou, T., Gelas, H., and Lacour, J.L. (1993). The effect of various recovery modalities on subsequent performance, in consecutive supramaximal exercise. Journal of Sports Medicine and Physical Fitness 33, 118-129.

Thompson, J. and Manore, M.M. (1996). Predicted and measured resting metabolic rate of male and female endurance athletes. Journal of the American Diet Association 96: 30-34.

Thompson, W.R., Gordon, N.F., Pescatello, L.S. (2000). Guidelines for exercise testing and prescription. American College of Sports Medicine. 6th Ed. Philadelphia, PA: Williams & Wilkins.

Thomson, J., Raitt, J., Hutchings, I., Bjargo, E., Loset, A., Grocott, M., Montgomery, H. and Caudwell, E. (2007). Angiotensin-converting enzyme genotype and successful ascent to extreme high altitude. Extreme Everest Research Group. High Altitude Medicine and Biology, 8: 278-283.

Tsianos, G.I., Evangelou, E., Boot, A., Zillikens, M.C., van Meurs, J.N.J., Uitterlinden, A.G.and Ioannidis, J.P.A. (2009). Associations of polymorphism of eight muscle or metabolism-related genes with performance of Mount Olympus marathon runners. Journal of Applied Physiology, 108: 569-574.

Tuan, T.C., Hsu, T.G., Fong, M.C., Hsu, C.F., Tsai, K.C.C., Lee, C.Y. and Kong, C.W. (2008). Deleterious effects of short-term, high-intensity exercise on immune function: evidence from leukocyte mitochondrial alterations and apoptosis. British Journal of Sports Medicine, 42:11-15.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Masur, M. and Telser, J. (2007). Free radicals and antioxidants in normal physiological function and human disease. International Journal of Biochemistry and Cell Biology, 39: 44-84.

Van Berlo, J. and Pinto, Y. (2003). Polymorphisms in the RAS and cardiac function. International Journal of Biochemistry and Cell Biology, 35**:**932-943..

Vescovo, G., Zennaro, R., Sandri, M., Carraro, U., Leprotti, C., Ceconi, C., Battista, G.A. and Libera, L.D.(1998). Apoptosis of Skeletal Muscle Myofibers and Interstitial

Cells in experimental heart failure. Journal of Molecular and Cellular Cardiology, 30(11):134-150.

Wagner, H., Thaller, S., Dahse, R. Sust, and M, (2006). Biomechanical muscle properties and angiotensin-converting enzyme gene polymorphism: a model-based study. European Journal of Applied Physiology, 98: 507-515.

Wang, P., Fedoruk, M.N. and Rupert, J.L. (2008). Keeping pace with ACE. Sports Medicine, 38:1065-1079.

Watson, T.A., Callister, R., Taylor, R.D., Sibbritt, D.W., MacDonald-Wicks, L.K. and Garg, M.L. (2005). Antioxidant restriction and oxidative stress in short-duration exhaustive exercise. Medicine and Science in Sports Exercise, 37: 63-71.

Waugh, M. and Halligan, S. (2008). Blood Pressure Monitoring. 7(6):309-312.

Webster's new world medical dictionary, third edition, 2008.

Weltman, A. (1995). The blood lactate responses to exercise. Human Kinetics, Champaign IL, 17-18.

Werner, W.K. and Hoeger, S.A. (2010). Lifetime physical and wellness: A personalised program. 214.

Wessner, B., Tschan, H. and Bachi, N. (2009). Genes associated with athletic performance. Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology, 1:153: 74-75.

Williams, A.G. and Folland, J.P. (2008). Similarity of polygenic profiles limits the potential for elite human physical performance. Journal of Physiology, 1:586, 113-121.

Wilson, A.G., di Guovine, F.S., Blakmore, A.J. and Duff, G.W. (1992). Single base polymorphism in the human tumour necrosis factor -α (TNF-α) gene detectable by *NcoI* restriction of PCR product. Human Molecular Genetics, 1(5): 353.

Wilson, R.F. (1992). Critical care manual: applied physiology and principles of therapy, 32.

Wolfarth, B., Rivera, M.A., Oppert, J.M., Bowlay, M.R. and Dionne, F.T. (2000). A polymorphism of the alpha 2 -α adrenoreceptor gene and endurance athlete status. Medicine and Science in Sports and Exercise, 32: 1709-1712.

Woltfarth, B., Bray, M., Hagberg, J.M, Perusse, L.R., Rainer, R., Miguela, R., Stephen, M., Rankinen, T. and Bouchard, C. (2005). Medicine and Science in Sports and Exercise. 37(6): 881-903.

Woods, D.R. (2009). Angiotensin-converting enzyme, rennin-angiotensin system and human performance. Collins M (Ed) Genetics and Sports. Medicine and Sport Science. Basel, Karger, 54: 72-87.

Woods, D.R., Brull, D. and Montgomery, H.E. (2000). Endurance and ACE I/D polymorphism. Science Programme, 84: 317-336).

Woods, D.R., World, M., Rayson, M.P., Williams, A.G., Jubb, M., Jamshidi,.Y, Hayward, M., Mary, D.A.S.G., Humphries, S.F. and Montgomery, H.F. (2002). Endurance enhancement related to the human angiotensin -converting enzyme I-D polymorphism is not due to differences in the cardiorespiratory response to training. European Journal of Applied Physiology, 86: 240-244.

Yang, N., MacArthur, D, G., Wolde, B., Onywera, V.O., Boit, M.K., Wilson, R.H., Scott, R.A., Pitsiladis, Y.P. and North, K. (2005). ACTN3 genotype is not associated with elite endurance athlete status in Ethiopians and Kenyans. Medicine and Science in Sports and Exercise, 37: 472-473.

Yang, N., MacArthur, D. G., Gulbin, J.P., Hahn, A.G., Beggs, A.H., Eastel, S. and North, K. (2003), ACTN3 genotype is associated with human elite athletic performance, American Journal of Human Genetics, 73: 627-631.

Yang, N., MacArthur, D.G., Wolde, B., Onywera, V.O., Boit, M.K., Lau, S.Y.M., Wilson, R.H., Scott, R.A., Pitsiladis, Y.P. and North K. (2007). The ACTN3 R577X

polymorphism in East and West African Athletes. Medicine and Science in Sports and Exercise, 39: 1985-1988.

Yudkin, J.S., Stehouwer, C.D., Emeis, J.J., Coppack, S.W. (1999). C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arteriosclerosis, Thrombosis, and Vascular Biology, 19: 972–978.

Yuhasz, M.S. (1974). Physical Fitness Manual, London Ontario, University of Western Ontario.

Zhang, B., Tanaka, H., Shono, N., Miura, S., Kkiyomaga, A., Shindo, M. and Saku, K. (2003). The I allele of the angiotensin-converting enzyme gene is associated with an increased percentage of slow twitch type I fibres in human skeletal muscle. Clinical Genetics, 63: 139-144.

Appendix A

Details of Methods on PCR protocol and reagents preparation

A.1 GENOTYPING

A 1.1 FTA cards preparation

Three disks from each sample were punched out and put into a 1,5 µl tube, the following procedure was used to wash the disks:

a) 500 ml of FTA reagent were put into each tube and incubated for 5 minutes at room temperature using a mixer the tubes were shaken moderately in between washes
b) All spent FTA purification reagent was discarded using a pipette
   The above steps were repeated 2-3 times for a total of 3 washes
c) 200 µl of TE^{-1} Buffer (10mM Tris-HCl, 0,1 mM EDTA,pH 8.0) Solution was added and incubated for 5 minutes at room temperature
d) All spent TE Buffer was removed and discarded
e) Steps c-d were repeated three times for a total of 3 washes
f) The discs were allowed to dry at room temperature using air dryer and were left over night

The washed and dry disc was put for PCR analysis.

A.2 ACE gene primer preparation

The stock solution was prepared using the following protocol:

The tubes were marked 1-32, 32 being the Non Template Strand, 25 (µl) of the stock solution was dispensed into each tube .The tubes were ready and loaded into the Rotor Gene for amplification and PCR analysis.

Table A.1: ACE Genotyping preparation

Content	Volume(µl)	X by number of samples(µl) X 33
Water	10.0	330
2x Master Mix	12.5	412.5
SYBR	0.5	16.5
Forward Primer	1.0	33.0
Reverse Primer	1.0	33.0

Table A.2: Programme for amplification of I/D Polymorphism in ACE gene

Step	Temperature	Time
1.Initial denaturation	94 ºC	5 min
2. Denaturation	94 ºC	40 sec
3.Hybridization	61ºC	40 sec
4.Elongation	72 ºC	40 sec
5.Final Elongation	72 ºC	5min

After completion the PCR reaction ,the obtained products were tested on 1.2 % agarose and were visualised with Ethidium Bromide under UV light. For the insertion of the gene a product with the length of 490 base pairs was visible on agarose gel and for the deletion a product of 190 bp was visible. In heterozygote individuals with the two alleles both products were visualised -490 and 190 bp

A.3: PCR-RLFP (Restriction Fragment Polymorphism) method for detection of R577X polymorphism in ACTN3 gene.

Systemic approach

i) PCR amplification of the fragments containing the polymorphic sites that have to be identified, followed by:
ii) RLFP-analysis

Though the detection of R577X polymorphism in ACTN3 gene was done by Mills *et al.,* 2001 method. Standard optimisation steps were conducted in order to adapt the method to our laboratory and obtain optimal gain and purity of the products. The programme for amplification of the required DNA fragments after the optimisation steps is presented on table A.3. and A.4.

Table A.3 Programme for amplification of R577X polymorphism in ACTN3 gene

Steps	Temperature	Time
1.Initial denaturation	95 ºC	5min
2.Denaturation	95 ºC	30sec
3.Hybridization	65 ºC	40sec
4.Elongation	72 ºC	40sec
5.Final Elongation	72 ºC	5min

Steps 2, 3 and 4 were repeated 35 cycles

Table A.4. ACTN3 Genotype preparation

Content	Volume(µl)	X by number of samples(µl) X 33
Water	8.0	264
2x Master Mix	10.00	330
SYBR	0.4	13.2
Forward Primer	0.8	26.4
Reverse Primer	0.8	26.4

20(µl) of the stock solution was put into each tube

The amplified product with 291 bp length was tested in 1.2% agarose. After the PCR reaction, the products were submitted to endonuclease treatment with the restriction enzyme DdeI (RFLP-analysis) for identifying the alleles.

A.4 Restriction enzyme reaction:

Water :	4. 5µl
Buffer 3 :	1.2 µl
PCR Product :	6.0 µl
Ddel :	0.3 µl (10U/ µl) 12.0 µl

A.5 TNF G308A polymorphism detection

All amplifications were performed in a Rotor-Gene 6000 (Corbett Research, Australia) using the following conditions: activation step 95^{o}C for 10 minutes followed by 40 cycles of 95^{o}C for 10 seconds, 90^{o}C for 20 seconds 72^{o}C for 20 seconds. Restriction digests were performed in a total volume of 5 µl: 2.5 µl PCR product, 0.5 µl NEB3 buffer (10×), 0.125 µl enzyme (1.25 U), and 1.875 µl water. Digests were performed for 16 hrs at 37^{o}C. 1 µl 6× loading buffer was added to each digest and the whole sample loaded to a gel. *ACTN3* amplicons were digested with *Dde*I and resolved in 2.5% (w/v) agarose 1× TBE gels. TNF-α amplicons were digested with *Nco*I and resolved in 3% (w/v) agarose 1× TBE gels.

Appendix B

Consent form

PARTICIPANT INFORMATION LEAFLET AND INFORMED CONSENT FORM

INTRODUCTION

You are invited to volunteer for a research study. This information leaflet is to help you to decide if you would like to participate. Before you agree to take part in this study, you should fully understand what is involved. If you have any questions, which are not fully explained in this leaflet, do not hesitate the study doctor. You should not agree to take part unless you fully understand the procedures involved.

By signing this document, you indicate that you understand the information and that you give your consent to the medical procedures to be performed and to participate in the research study. Please read this document carefully.

NATURE AND PURPOSE OF THE STUDY

- To assess genetic and biochemical markers associated with physical performance in cricket players.
- To establish the polymorphism in ACE, ACTN3. and TNF genes.
- To assess the changes in red and white blood cells ,haemoglobin, C-reactive protein, uric acid, lactate, glucose, triglyceride, cholesterol in cricket players and control experimental subjects
- To evaluate the physical characteristics (height, weight, BMI, FBM, isometric grip strength, isokinetic strength of quadriceps and hamstring muscles.

WHAT IS THE DURATION OF THE STUDY AND WHAT PROCEDURES ARE INVOLVED?

During three periods of 4 weeks each, volunteers are going to be examined. Your blood pressure, heart rate, weight, handgrip strength, isokinetic strength of quadriceps and hamstring muscles.

WHAT IS THE DURATION OF THE STUDY AND WHAT PROCEDURES ARE INVOLVED?

During three periods of 4 weeks each, volunteers are being examined. Your blood pressure, heart rate, weight, handgrip strength and quadriceps ad hamstring strength will be measured. Blood tests will be taken and sent to the laboratory for measurement of the biochemical markers and assessment of the gene polymorphism.

At the time of the study, researchers will review your past and preset medical history and you will have a routine medical examination. You will have to answer to few questions and to fill a questionnaire.

HAS THE STUDY RECEIVED ETHICS COMMITTEE APPROVAL?

This study protocol was submitted to Ethics and Biosafety Committee of the University of Zululand

WHAT ARE MY RIGHTS AS A PARTICIPANT IN THIS STUDY?

Your participation in this study is entirely voluntary and you can refuse to participate or stop at any time without stating any reasons. Your withdrawal will out affect your access to other medical care.

POTENTIAL BENEFITS

During this study, you will receive a very high standard of medical advice and assessment of your physical performance by the researchers who are very experienced in this field. Lastly, the information obtained from your participation, may benefit other athletes.

CONFIDENTIALITY

Any information uncovered regarding your test results or state of health as a result of your participation will be held in strict confidence.

INFORMED CONSENT TO PARTICIPATE

I have read the above information.
The content ad meaning of this information has been explained to me by the study doctor.
I have read ad understood the written information in this form.
I may, at any stage, without prejudice, withdraw my consent and participation in the study.
I have had an opportunity to ask questions about this study and I am giving permission to take part in the study.

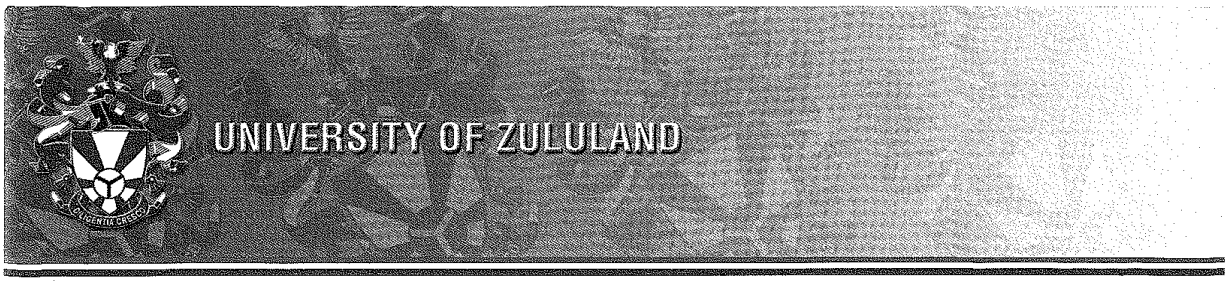
Participant's Name Participant's Signature Date

I, Prof T. Djarova-Daniels, herewith confirm, that the above participant has been informed fully about the meaning of this study.

Researcher's Name Researcher's Signature Date

Appendix C

Ethical Approval



Ethics Committee
Faculty of Science and Agriculture
University of Zululand
C/O Mr L Vivier
Department of Zoology
University of Zululand
Private Bag 1001
KwaDlangezwa
3886
Tel: 035 – 902 6741
Email: hjerling@pan.uzulu.ac.za

20 September 2010

To whom it may concern

ETHICS EVALUATION OF RESEARCH PROJECT PROPOSAL

This letter serves to confirm that **A Ramakoaba** (Student No **20053333**), registered for a Masters Degree in the Department of Biochemistry and Microbiology at the University of Zululand, in accordance with appropriate rules submitted a research project proposal to the Ethics Committee of the Faculty of Science and Agriculture at the University of Zululand. The research project will investigate: **Angiotensin converting enzyme (ACE), actinin (ACTN3), tumour necrosis factor (TNF) gene polymorphism associated with biomarkers and physical characteristics in cricket players**. Based on the research protocol stipulated, this committee could find no reason from an ethical standpoint to reject the proposed research.

Yours sincerely

Dr H.L. Jerling
Ethics Committee
Faculty of Science and Agriculture
University of Zululand

Appendix F

List of Research outputs

Publications

Djarova, T., Watson, G., Basson, A., Grace, J., Cloete, J. and **Ramakoaba, A.** (2011). ACTN3 and TNF gene polymorphism association with C-reactive protein uric acid, lactate and physical characteristics in young African cricket players. African Journal of Biochemistry Research, 4:22-27.

Trayana Djarova, **Abigail Ramakoaba**, Albert Basson, Sam Mugandani, Jeane Grace, Jaque Cloete, and Gregory Watson. (2011). Angiotensin Converting Enzyme genotypes relationship with blood pressure , C-reactive protein and selected physical tests in Zulu South African cricketers.African Journal of Biochemistry 5(7).xxx.xxx

International Conference Proceedings

Djarova, T., Watson, G., Basson, A., Grace, J., Cloete, J. and **Ramakoaba, A.** . ACTN3 and TNF gene polymorphism association with C-reactive protein uric acid, lactate and physical characteristics in young African cricket players. 26th International Council for physical Activity and Fitness Research Conference,Ithala game reserve, South Africa, 16-20 August 2010.

Djarova, T., **Ramakoaba, A.,** S. Mugandani**,** Basson, A., Grace, J., Cloete and Watson, G., ACE I/D polymorphism, blood pressure, C-reactive protein and physical

tests in Zulu South African cricketers. 16th Annual Congress of European College of Sport Science (ECSS), 6-10 July 2011, Liverpool, UK.

Local Conference Proceedings

Ramakoaba, A., Mungandani, S., Watson, G., Cloete, J., Grace, J., Basson, A., and Djarova, T. ACE gene polymorphism, blood pressure and physical tests in cricket players of Zulu origin. 5th Annual Science Symposium, University of Zululand, South Africa, 25 November 2010.

Appendix D

Supplementary Data

Table D.1 Control group Blood parameters

Name & Surname & (No) from Genotyping	CRP rest	WBC	NEU	LY	MO	EO	BA	URIC ACID	CHOL	TRIG	GLU	LACT
Ndlovu Ntando (14)	1	5.35	0.89	3.28	0.37	0.78	0.03	0.39	3.25	0.66	3.9	0.97
Ntombela Mduduzi (15)	3.2	9.49	5.71	2.83	0.67	0.23	0.05	0.27	3.48	0.62	4	1.72
Mthinunye Ntokozo (16)	1.2	6.2	3.5	1.87	0.67	0.15	0.01	0.29	4.17	0.74	3.5	0.83
Zulu Sphelele (17)	4	7.07	**1.65**	1.53	0.34	**3.53**	0.02	0.31	2.63	0.5	**3.2**	1.18
Mkhize Senzo (18)	1.8	4.73	**1.49**	2.61	0.36	0.25	0.02	0.34	2.97	0.48	4.7	1.26
Shange Nhtlandla (19)	40.4	6.85	2.82	3.02	0.93	0.06	0.02	**0.21**	2.97	1.42	5.1	**2.93**
Mabuza Nhlandhla (20)	6.1	5.72	3.04	1.85	0.66	0.16	0.01	0.34	3.74	0.6	5	1.91
Ngomane Thokozane (21)	1.8	6.28	2.15	3.09	0.64	0.37	0.03	**0.21**	3.87	0.59	4.7	2.02
Kgantiloe Gabo (22)	2	8.43	6.08	1.5	0.38	0.34	0.03	0.38	4.33	0.99	4.8	**2.69**
Mhlongo Muzomuhie (23)	10	6.76	4.4	1.37	0.43	**0.53**	0.03	0.29	3.51	0.56	5	**2.97**
Mngadi Thabiso (24)	11.4	7.93	3.57	3.14	0.85	0.34	0.03	0.32	3.27	0.62	4.8	1.1
Mthembu Mbulisis (25)	3.8	4.65	2.08	2.03	0.39	0.13	0.02	**0.25**	3.88	**1.92**	4.6	1.53
Cele Mukelani (26)	3.3	5.13	**1.96**	2.08	0.37	**0.69**	0.03	0.29	3.61	0.44	3.6	1.06
Ngubane Sithembiso (27)	2.9	**3.09**	**1.29**	1.33	0.38	0.08	0.01	**0.21**	2.8	0.62	4.5	**2.89**
Nkosi Sibusiso (28)	1.7	4.21	2.13	1.58	0.36	0.11	0.03	0.29	3.87	0.61	**4.7**	2.03
Zikhali Lindokuhle (29)	2.9	8.91	6.35	1.9	0.64	0.01	0.01	0.29	4.23	0.92	5.4	**2.46**
Mchunu Thulani (30)	1.3	4.92	2.03	2.29	0.47	0.11	0.02	**0.24**	4.69	0.98	**6.1**	**3.58**

Table D.2: Experimental group blood parameters

Name & Surname Cricket & (No) from Genotyping	CRP Pre T	CRP Post	WBC Pre x10 9/L	WBC Post	NEU Pre x10 9/L	NEU Post	LY Pre T x10 9/L	LY Post	MO Pre x 10 9/L	MO Post	EO Pre x10 9/L	BA Pre x10 9/L
Sikhosana Themba (1)		6.1	4.26	**5.65**	**1.52**	**2.28**	2.09	**2.68**	0.44	**0.5**	0.2	0.01
Ndwandwe Musa (2)		1	4.97	**6.4**	**1.73**	**3.22**	1.94	**2.38**	0.45	**0.49**	**0.84**	0.01
Matjila Kitso (3)		3.2	4.64	**4.63**	**1.46**	**1.93**	2.6	**2.13**	0.37	**0.38**	0.2	0.01
Nyavo Thandanani (4)		1.3	5.79	**6.02**	2.05	**2.34**	2.57	**2.54**	0.65	**0.62**	0.49	0.03
Makhanya Mpilo (5)		1	3.43	**4.29**	**1.08**	**2.11**	1.85	**1.75**	0.3	**0.26**	0.3	0.01
Buthelezi Nkululeko (6)		1	5.67	**5.21**	2.32	**2.2**	2.83	**2.56**	0.44	**0.4**	0.07	0.01
Shezi Siyabonga (7)		1.1	5.96	**6.43**	3.48	**3.56**	1.62	**1.83**	0.55	**0.63**	0.25	0.06
Dlamini Sandile (8)		3.4	8.16	**8.52**	3.8	**4.13**	3.43	**3.32**	0.8	**0.9**	0.12	0.01
Gumede Mafika (9)		1	7.84	**7.91**	4.51	**4.72**	2.45	**2.43**	0.42	**0.4**	0.43	0.02
Cebekhulu Siyabonga (10)		1	6.98	**7.25**	4.63	**4.58**	1.57	**1.91**	0.64	**0.63**	0.11	0.02
Mnguni Siyanda (11)		1.5	5.18	**5.48**	**1.54**	**2.3**	2.6	**2.08**	0.33	**0.4**	**0.7**	0.01
Bekhva Siyabonga (12)		1	5.46	**6.28**	2.76	**2.81**	2.32	**2.91**	0.34	**0.51**	**0.8**	0.01
Ngema Sandile (13)		1.1	7.04	**9.1**	3.39	**5.66**	2.49	**2.41**	0.32	**0.29**	0.8	0.04
Jali Lionel (31)		1.6	7.78	**8**	5.95	**6.01**	1.31	**2.4**	0.48	**0.5**	0.03	0.01
CRP > 3.0 in RED												
LOW in BLUE												
HIGH in PINK												

Table D.2 Continued...

Eo Post	BA Pre	BA Post	UA Pre	UA Post	CHOL Pre	CHOL Post	TRI Pre	TRI Post	GLU Pre	GLU Post	LA Pre	LA Post
	x10 9/L		mmol/L		mmol/L		mmol/L		mmol/L		mmol/L	
0.2	0.01	**0.01**	**0.24**	**0.27**	4.61	**4.87**	0.98	**1.11**	4.9	**5.1**	1.09	**0.27**
0.28	0.01	**0.02**	**0.25**	**0.34**	3.13	**3.73**	1.32	**0.77**	5.9	**4.92**	0.96	**1.81**
0.18	0.01	**0.01**	0.29	**0.32**	2.98	**3.06**	0.37	**0.35**	4.9	**4.6**	2	**1.92**
0.51	0.03	**0.01**	0.33	**0.37**	3.07	**3.05**	0.68	**0.67**	4.5	**4.9**	0.94	**2.17**
0.16	0.01	**0.01**	0.37	**0.4**	3.6	**4.06**	0.97	**0.68**	4.3	**4.8**	1.76	**1.88**
0.04	0.01	**0.01**	**0.25**	**0.26**	3.6	**3.43**	0.51	**0.47**	4.4	**4.7**	1.69	**1.02**
0.3	0.06	**0.11**	0.37	**0.38**	4.52	**4.88**	1.35	**0.85**	6.9	**5.4**	2.21	**3.67**
0.15	0.01	**0.01**	**0.25**	**0.27**	3.55	**3.56**	1.11	**0.92**	4.9	**4.3**	1.15	**1.22**
0.35	0.02	**0.01**	0.26	**0.28**	2.59	**2.61**	0.78	**0.68**	4.9	**4.5**	1.07	**1.27**
0.11	0.02	**0.02**	0.31	**0.32**	4.46	**4.62**	1.01	**1.33**	4.7	**4.8**	**2.63**	**2.39**
0.68	0.01	**0.02**	0.33	**0.37**	3.94	**4**	0.42	**0.48**	4.5	**4.5**	2.12	**2.5**
0.7	0.01	**0.02**	0.4	**0.43**	3.92	**3.87**	0.97	**1.06**	4.7	**6.3**	1.54	**1.89**
0.7	0.04	**0.04**	0.35	**0.38**	2.72	**2.72**	0.56	**0.63**	4.5	**5.1**	1.17	**1.5**
0.32	0.01	**0.02**	0.34	**0.35**	3.57	**3.75**	0.78	**0.8**	4.4	**4.98**	1.37	**1.97**

Table D.3 (Control Group)BMR, Blood Pressure, HR and STT table.

Name & Surname (CONTR)	BMR(KJ)	Metabolic rate	Energy requirements	SBP mmHg	DBP mmHg	HR bpm	STT	Apnoeic Test(sec)
Ndlovu Ntando	1425.0	1959.38	8201.9	122	82	68	8296	54
Ntombela Mduduzi	1324.5	1821.19	7623.49	108	80	64	6858	35
Mthinunye Ntokozo	1408.9	1937.24	8109.28	110	60	68	74802	24
Zulu Sphelele	1327.2	1824.9	7639.03	110	65	66	7260	32
Mkhize Senzo	1369	1934	7502	110	60	60	6600	35
Shange Nhlanhla	1302	1934.2	8150.2	118	78	53	6354	28
Mabuza Nhlanhla	1512.1	2079.14	8703.27	128	70	60	7680	38
Ngomane Thokozane	1477.4	1990.18	8330.87	134	70	60	8040	50
Kgantiloe Gabo	1598.4	2197.8	9199.9	122	88	78	9516	70
Mhlongo Muzomuhie	1320	2153	8544	130	90	60	7800	32
Mngadi Thabiso	1649.4	2267.9	9493.5	122	70	60	7320	56
Mthembu Mbulisis	1503.9	2067.86	8656	140	80	64	8890	27
Cele Mukelani	1432	1998	8443	117	78	60	7020	92
Ngubane Sithembiso	1501.5	2064.56	8642.26	120	90	60	7200	64
Nkosi Sibusiso	1424.9	2148.03	8991.6	128	70	66	8448	81
Zikhali Lindokuhle	1302	2061	7205	130	90	58	7540	55
Mchunu Thulani	1558.4	2142.8	8968.76	122	70	60	7320	54

Table D.4 (Experimental Group) BMR, Blood Pressure, HR and STT table.

Name	BMR(KJ)	Metabolic Rate	Energy requirements	SBP Pre	SBP Post	DBP Pre	DBP Post	HR Pre	HR Post	STT Pre	STT post	Apnoeiic Test

Jali	1408.5	2183.18	9138.77	115	112	78	68	66	57	7590	6384	42
M. Gumede	1463.4	2268.27	9494.98	110	120	76	70	34	50	7260	9000	41
T.Skhosana	1779.8	2758.69	11547.87	122	126	70	78	57	60	6954	7560	90
Matjila	1539.08	2385.6	9986.01	115	112	78	68	66	57	7590	6384	42
S. Bekwa	1504.54	2332.04	9761.91	128	138	72	78	60	65	66	6144	55
Shezi S.	1576.34	2443.33	10227.77	128	138	72	78	56	60	6144	9108	55
S.Dlamini	1184.24	1835.57	7683.69	120	132	78	68	66	60	7920	7910	35
Nyawo	1449.76	2247.13	9406.5	118	122	78	70	60	57	7080	6954	49
M.Makhanya	1548.1	2399.56	10044.54	138	128	90	98	58	60	8004	8280	28
S.Cebekhulu	1561.56	2420.42	10131.87	120	118	78	68	60	48	9360	8024	37
S.Ndwandwe	1532.3	2375.07	9942.02	105	118	60	68	54	60	5670	7080	28
S. Ngema	1481.18	2295.83	9610.34	122	122	74	70	56	48	6832	12740	96
N. Buthelezi	1132.2	2193	9786.3	136	140	84	85	84	60	11424	8400	46
S. Mnguni	1112	2034	1103.4	120	118	90	88	60	54	7200	6372	35

Table D.5 (control group) Physical parameters

Name & Surname	Birth Date	Weight	Stature	BMI	Fat%	LBM	Fat Mass	Grip Strenght - L	Grip Strenght - R	Knee Ext (L)	Knee Ext (R)	Knee Flex (R)	Knee Flex (L)
		(kg)	(cm)	(kg/m^2)	(%)	(kg)	(kg)	(kg)	(kg)	(Nm/kg)	(Nm/kg)	(Nm/kg)	(Nm/kg)

T Mchunu	01/12/1987	71.5	178	22.6	10.15	64.2	7.26	53.5	55	4.7	4.2	2.8	2.9
M Mthembu	23/12/1986	68	169	23.8	10.36	60.96	7.04	56.5	58	4.3	4	2.2	2.4
S Ngubane	04/04/1986	67	173	22.4	10.25	60.12	6.87	43	40.5	4.6	3.8	1.9	1.8
S Nkosi	17/11/1989	61.5	174	20.3	7.73	57.01	4.49	50.5	54.5	4	3.8	1.9	1.9
G Kganticoe	26/08/1988	46.5	158	18.6	12.36	40.75	5.75	27.5	32.5	4.6	4.8	2.3	2.5
M Senzo	23/11/1989	58	168	20.5	7.62	53.58	4.41	46.5	47	3.6	3.3	2.2	2.1
S K Zulu	17/12/1988	50	159	19.8	7.73	46.35	3.65	42	40.5	2.6	2.9	1.3	1.5
T Mngadi	21/08/1989	80	178	25.2	10.15	71.88	8.12	52	55	3.1	3.3	2.3	2
N Mthimunye	31/10/1988	56	175	18.3	7.95	51.55	4.45	33	37.5	3.3	3.1	1.7	1.8
T Ngomane	02/09/1987	60.5	175	19.8	8.05	55.63	4.87	34.5	35	3.3	3.1	1.7	2.2
N Mabuza	04/04/1988	67.5	171	23.1	10.99	60.08	7.42	43.5	50	3.4	3.8	1.8	1.8
M Ntombela	23/10/1986	49.5	168	17.5	6.16	46.5	3.05	35	38.5	3.1	3.3	1.9	2.3
N Ndlovu	25/05/1989	57	176	18.4	9.21	51.75	5.25	36	42.5	3.5	3.3	1.8	1.8

Table D.6 (experimental group) Physical parameters

Name & Surname	Birth Date	Weight	Stature	BMI	Fat%	LBM	Fat Mass	Grip Strength - L	Grip Strength - R	Knee Ext (L)	Knee Ext (R)	Knee Flex (R)	Knee (R)
		(kg)	(cm)	(kg/m^2)	(%)	(kg)	(kg)	(kg)	(kg)	(Nm/kg)	(Nm/kg)	(Nm/kg)	(Nm/

L Jali	05/05/1986	57.5	172	19.4	7.21	53.35	4.15	51	52.5	3.9	3.9	2.1	
K Matjiba	31/10/1987	72.3	163	27.2	11.4	64.05	8.25	36.5	34	4.4	4.2	1.9	
M Gumede	22/10/1989	61	176	19.7	8.37	55.9	5.1	31	34.5	3.2	3.1	1.9	
S Bekwa	13/08/1988	65.4	178	20.5	8.68	59.72	5.68	48	48	4	4	2.1	
T Sikhosana	08/04/1987	94	181	28.7	12.57	82.18	11.81	61.5	64.5	2.9	3.4	1.9	
S Shezi	17/11/1984	75.4	175	24.6	12.57	65.92	9.48	47.5	44.5	4.2	3.7	2.5	
S Dlamini	19/05/1989	64.6	171	22.1	9.63	58.38	6.22	48	47.5	3.3	3.2	2.1	
N Thandanani	09/12/1990	58.6	176	18.8	10.78	52.28	6.32	23.5	35.5	3.5	4	1.8	
S Cebekhulu	12/08/1983	72.1	181	22	10.36	64.6	7.47	44.5	45	3.9	3.8	2.1	
M Ndwandwe	06/02/1990	67.5	177	21.5	8.16	62	5.5	42	49	3.8	3.6	1.5	
S Ngema	18/09/1984	65.3	176	21.1	8.89	59.49	5.81	42	37	3.5	3	1.8	
M Makhanya	27/09/1988	70.5	175	23	9.42	63.86	6.64	52.5	56.5	4.2	3.7	2.3	

APPENDIX E

Gel Electrophoresis of the ACE, ACTN3 and TNF genes

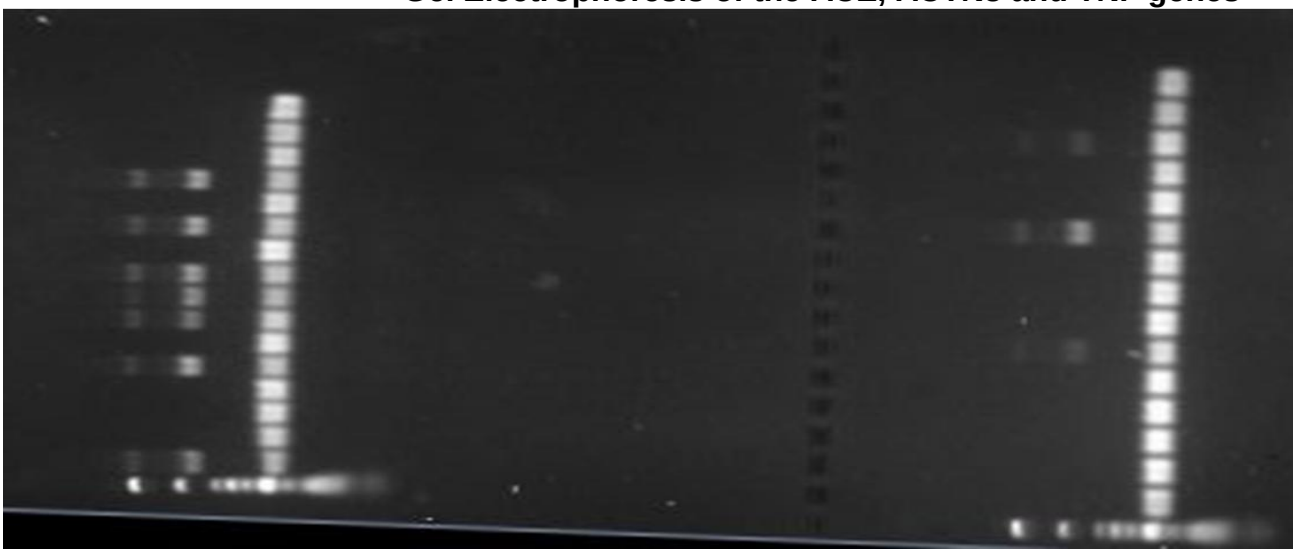


Figure E.1

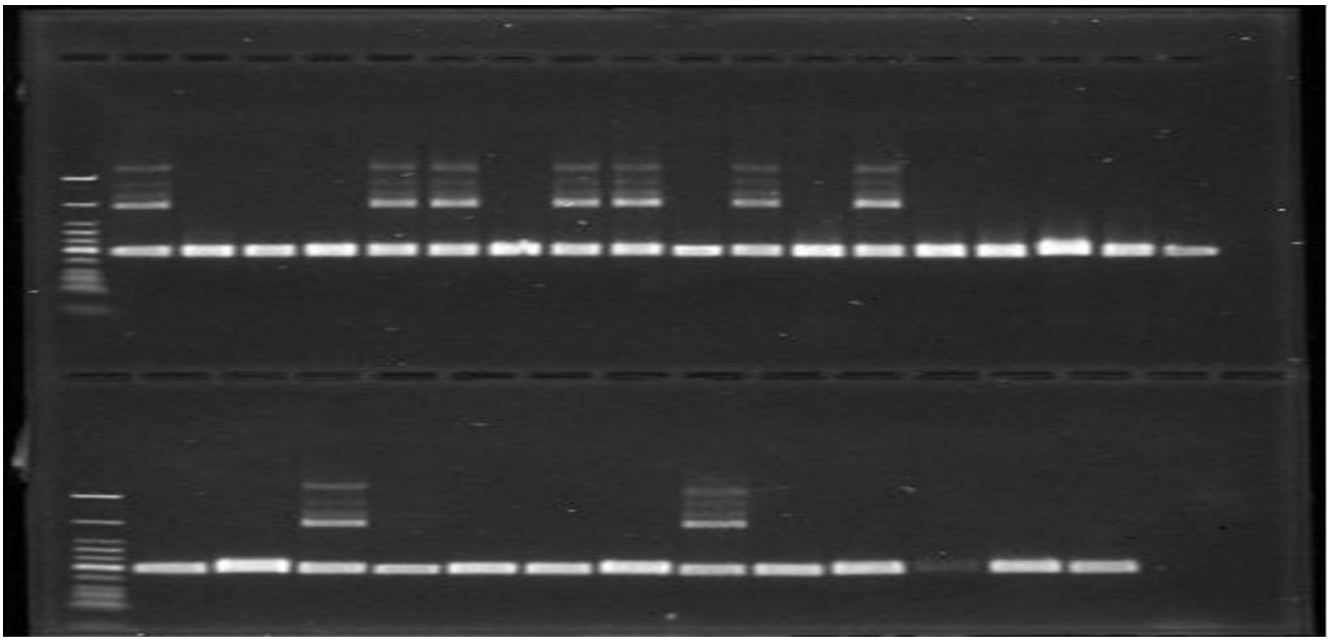


Figure E.2

Figure E.1 and E.2: Images of amplified fragments of the ACE I/D genotypes. Fragments were resolved in 2% (w/v) agarose for the analysis of ACE I/D genotypes. Figure E.1 - Control group and Figure E.2- Experimental group

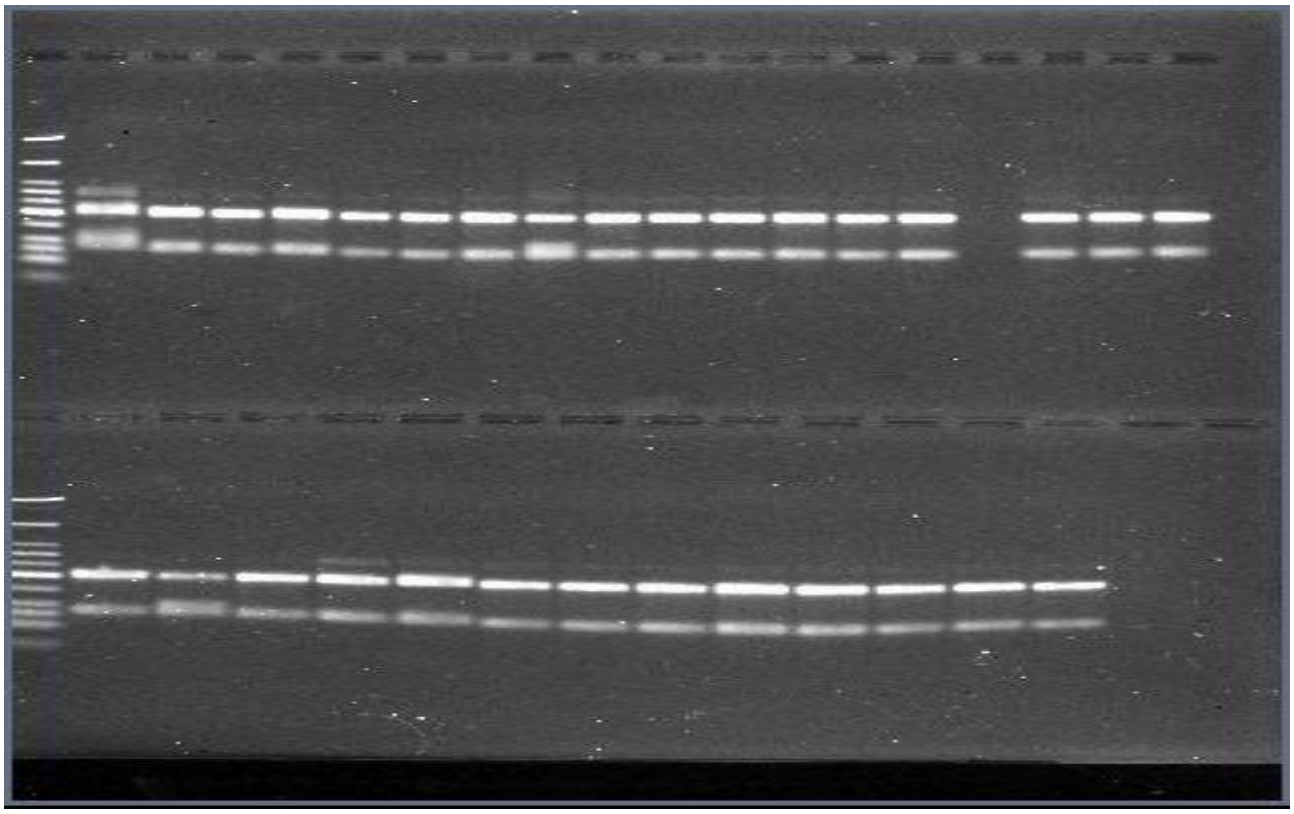


Figure E.3

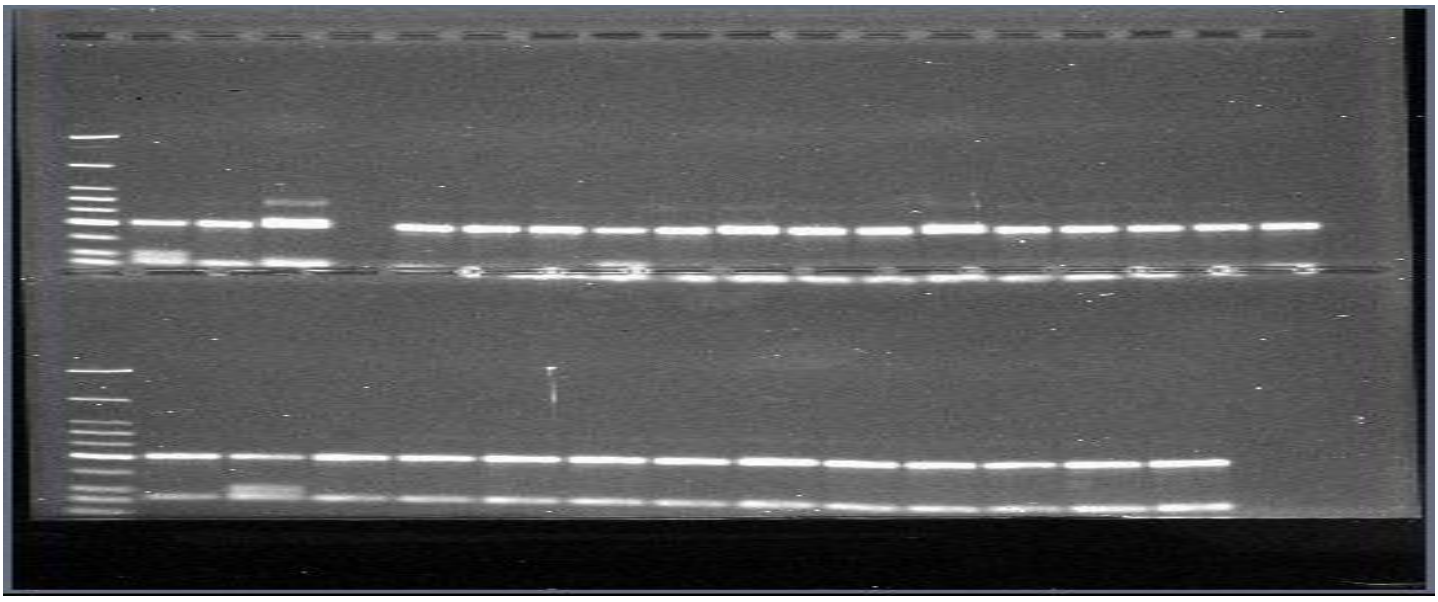


Figure E.4

Figure E.3 and E.4: Amplified fragments in the analysis of ACTN3 RR and RX genotypes which were digested with DdeI and resolved in 2% (w/v) agarose, 1x TBE gels. E.3 - control group, E.4 - Experimental group

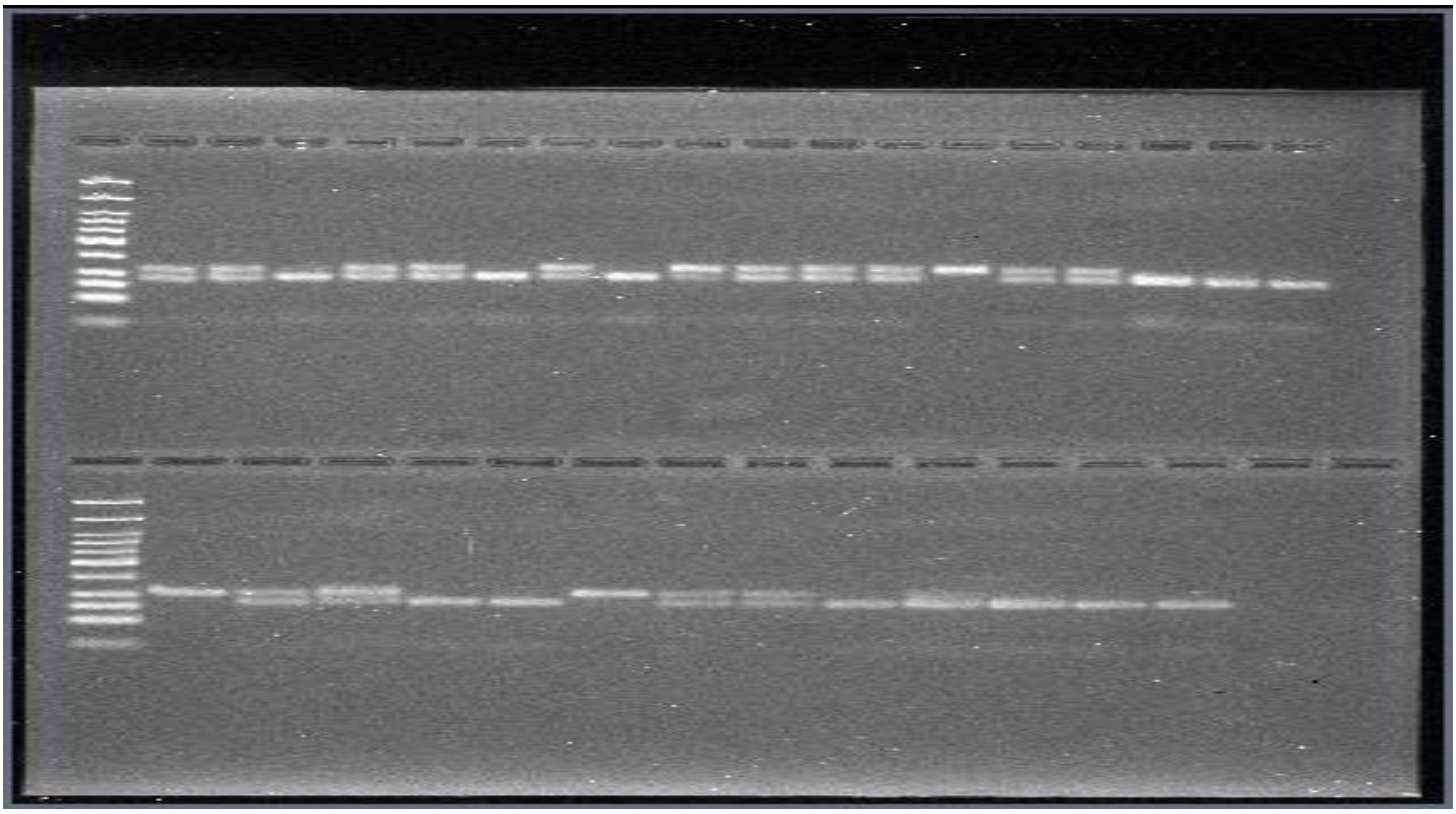


Figure E.5

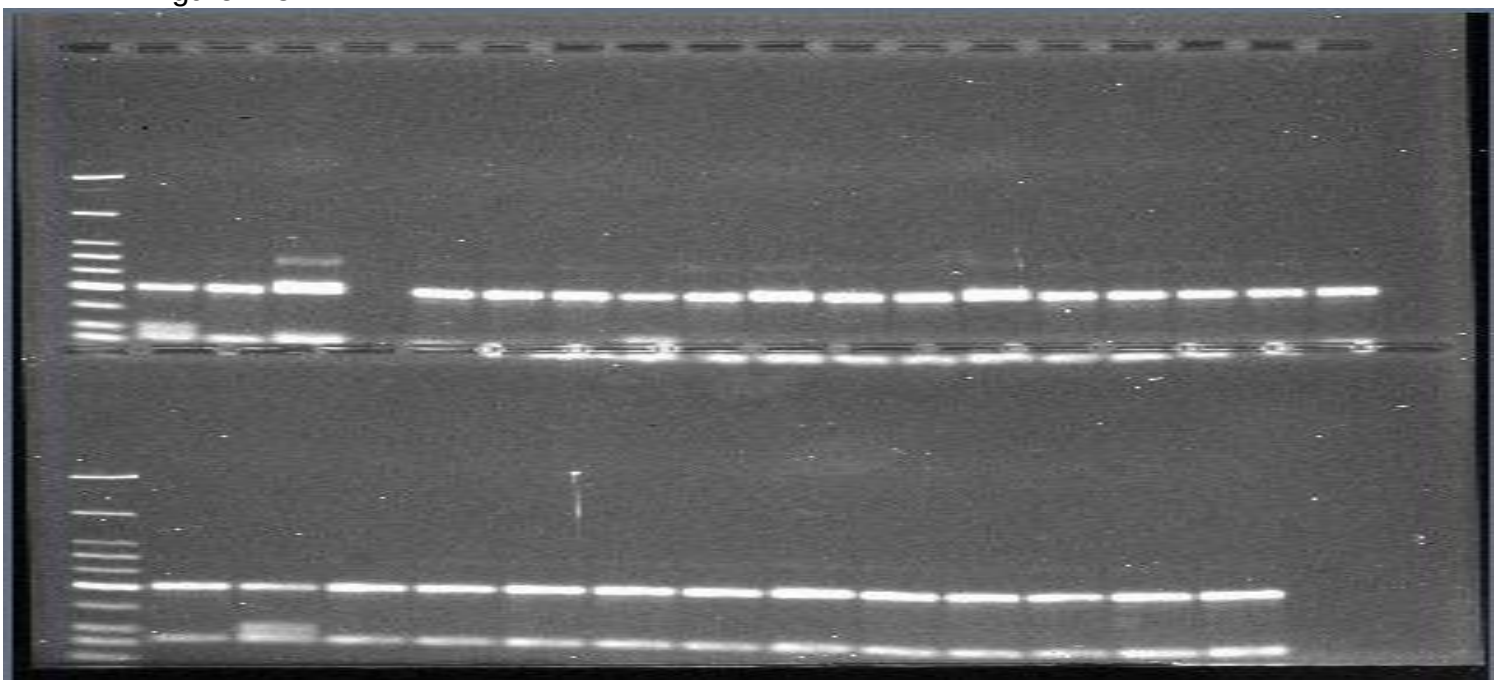


Figure E.6

Figure E.5 and Figure E.6: Images of the amplified fragments of the TNF-α GG and GA genotypes. Amplified fragments were resolved in 3% (w/v) agarose. E6-Contol,

Appendix F

List of Research outputs

Publications

Djarova, T., Watson, G., Basson, A., Grace, J., Cloete, J. and **Ramakoaba, A.** (2011). ACTN3 and TNF gene polymorphism association with C-reactive protein uric acid, lactate and physical characteristics in young African cricket players. African Journal of Biochemistry Research, 4:22-27.

Trayana Djarova, **Abigail Ramakoaba**, Albert Basson, Sam Mugandani, Jeane Grace, Jaque Cloete, and Gregory Watson. (2011). Angiotensin Converting Enzyme genotypes relationship with blood pressure , C-reactive protein and selected physical tests in Zulu South African cricketers.African Journal of Biochemistry 5(7).xxx.xxx

International Conference Proceedings

Djarova, T., Watson, G., Basson, A., Grace, J., Cloete, J. and **Ramakoaba, A.** . ACTN3 and TNF gene polymorphism association with C-reactive protein uric acid, lactate and physical characteristics in young African cricket players. 26th International Council for physical Activity and Fitness Research Conference,Ithala game reserve, South Africa, 16-20 August 2010.

Djarova, T., **Ramakoaba, A.,** S. Mugandani**,** Basson, A., Grace, J., Cloete and Watson, G., ACE I/D polymorphism, blood pressure, C-reactive protein and physical tests in Zulu South African cricketers. 16th Annual Congress of European College of Sport Science (ECSS), 6-10 July 2011, Liverpool, UK.

Local Conference Proceedings

Ramakoaba, A., Mungandani, S., Watson, G., Cloete, J., Grace, J., Basson, A., and Djarova, T. ACE gene polymorphism, blood pressure and physical tests in cricket players of Zulu origin. 5th Annual Science Symposium, University of Zululand, South Africa, 25 November 2010.