Comparing Male and Female 10km Runners with regards to both Performance and Training

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Declaration

I, the undersigned, hereby declare that the work contained in this assignment is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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Bowen, R.L.

Abstract

The objective of this study was to compare training and physiological variables in performance-matched 10 km female and male athletes in order to identify those factors allowing females to compensate for their lower haematocrit and higher % body fat, both of which are disadvantageous to performance. Eight well-trained competitive female runners and eight well-trained competitive male runners participated in the study. They were matched by performance in a controlled 10 km time trial in the field (TT_{10}). Training was monitored in each athlete for seven consecutive days using heart rate monitoring and training diaries. Each athlete gave a muscle biopsy for histological and biochemical analysis. Four maximal tests, two flat and two gradient (8%), were completed by each athlete in order to determine VO_{2max}, maximum heart rate and peak treadmill speed (PTS) under each condition. Each athlete also completed two submaximal tests (one flat and one gradient) and a ten minute race pace test, in which the pace was determined by their TT10 performance. These allowed fractional utilization of VO_{2max}, HR_{max} and PTS to be determined, as well as economy. Training data revealed a much greater training volume, both distance run and duration of training, in female athletes ($p < 0.05$ for distance; $p < 0.01$ for duration). VO_{2max} expressed per kg body mass was significantly higher in males ($p < 0.05$), however, when expressed per FFM, no difference was found between genders. Female athletes had a significantly greater percent composition of type I fibres and males had significantly more type IIX fibres, possible explanation of gender difference in PTS and contributing factor to equal TT_{10}. The relationships between training, performance and biochemical variables in either gender were very different.

Die vergelyking van vroue en mans 10km hardlopers met betrekking tot beide prestasie en oefening

Bowen, R.L.

Die doel van die studie was om die oefening en fisiologiese veranderlikes in 10 km vroue en mans atlete, wat afgepaar was volgens prestasie, te vergelyk om die faktore te indentisifeer wat vroue toelaat om te kompenseer vir hulle lae haematokrit en hoë persent liggaamsmassa, wat albei nadelig is tot prestasie. Agt mededingende vroue hardlopers en agt mededingende mans hardlopers het aan die studie deelgeneem. Hulle was gepaar volgens prestasie in 'n gekontroleerde padwedloop (TT_{10}). Oefening was gemonitor vir elke atleet vir sewe opeenvolgende dae deur gebruik te maak van harttempo monitors en oefeningsdagboeke. Elke atlleet het 'n spierbiopsie gehad wat histologies en biochemies geanaliseer was. Vier maksimale toetse, twee met 'n gradiënt van 0° en twee met 'n gradiënt van 5° (8%) was deur elke atleet voltooi om VO_{2maks}, maksimale harttempo en piek trapmeulspoed (PTS), vir beide situasies te bepaal. Hulle het ook almal twee submaksimale toetse gedoen (een teen 0° gradiënt en een teen 5° gradiënt) sowel as 'n tien minuut wedlooppas toets. Fraksionele benutting van VO_{2maks}, HR_{maks} en PTS was hiervan bepaal asook die ekonomie van elke atleet. Oefeningshoeveelheid ten opsigte van afstand en duur van oefening was baie hoër in vroue atlete ($p < 0.05$ vir afstand; $p < 0.01$ vir duur). VO_{2maks} uitgedruk per kg liggaamsmassa was aansienlik hoër in mans atlete ($p < 0.05$), maar uitgedruk per vetvrye massa (FFM) was daar geen verskil tussen geslagte. Vroue atlete het 'n merkwaardige hoër persentasie tipe I spiervesels gehad terwyl mans atlete 'n merkwaardige hoer persentasie tipe IIX spiervesels gehad het. Dit mag dalk 'n mootlike verklaring vir die geslagsverskil in PTS en 'n bydraende faktor tot gelyke TT_{10} wees. Verskillende verhoudings tussen oefening, prestasie en biochemiese veranderlikes was in die twee geslagte gesien.

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1. INTRODUCTION

Exercise scientists have long been fascinated with the effects of running on the human body and in 1973, the relationship between metabolic measurements and distance running performance was the focus of the study by Costill *et al* (1973). In this study oxygen consumption, heart rates and blood lactate accumulation in distance trained runners during both submaximal and maximal treadmill running were measured and each of the athletes competed in a 10-mile road race several days after the laboratory tests. The sixteen highly trained athletes competing in this study were selected based on their performance in a ten-mile race. A strong correlation between VO_{2max} and performance was found, as well as between % $VO_{2max,}$ % HR_{max} utilized during the race and distance running performance. The faster runners accumulated less blood lactate at all running speeds above 70% VO_{2max} than did the slower runners at similar speeds and relative percentages of their aerobic capacities. Thus, the study concluded that performance in distance running is dependent on the economical utilization of aerobic capacity and the ability to employ a large fraction of that capacity with minimal accumulation of lactate. This study set off a rush of running research and forms the background on which many subsequent studies are based, including those that I will review and my own study.

Much of the recent research has focused on the injuries acquired due to the sport (Egermann *et al*, 2002; Johnston *et al*, 2003; Taunton *et al*, 2003) and on the athletes participating in track events (Hill, 1999; Jensen *et al*, 1999; Yanagiya *et al*, 2001; Di Prampero *et al*, 2003; Bundle *et al*, 2003) or marathon running (Billat *et al*, 2003; Kratz *et al*, 2002; Billat *et al*, 2001; Hausswirth and Lehenaff, 2001; Roi *et al*, 1999). Very little research has focused specifically only on athletes specializing in the 10 km distance despite the fact that this is one of the most common distances run by athletes. To my knowledge, only three studies have been published in which 10 km was the focal running distance (Bale *et al*, 1986; Bentley *et al*, 2002; Vleck and Garbutt, 1998). However, two of these studies are actually on triathletes (Bentley *et al*, 2002; Vleck and Garbutt, 1998). Thus only one study, by Bales *et al* (1986), investigated training and performance in 10 km runners and in this one study the focus was only on male athletes. No studies on 10 km athletes thus far have focused on the performance of athletes comparing the male and female participants in this event.

It is generally presumed that male 10 km runners will be better than their female counterparts and for the elite runners this is the case (Billat *et al*, 2003; Billat *et al*, 2001) but if one turns the focus to well-trained competitive runners, there are female athletes whose performances match those of many males. It is well known that men have higher hematocrits and lower percentage body fat than women, yet these women runners still complete the races in the same times as the males and thus, they must in some way compensate for these variables.

The aim of this study is to determine how these well-trained competitive female 10 km athletes compensate for their gender-inherent disadvantages, in order to perform as they do. For this study we matched female and male athletes by their performance in a controlled 10 km time trial and studied various physiological variables in an attempt to discover the factors that allow the women runners to compete at the level they do.

Due to the minimal scientific information regarding 10 km running, this literature review will attempt to apply the information available regarding performance and training in other endurance running distances to our chosen distance. Wherever possible the information available on women runners will be presented, but because of the scarcity of studies with women subjects, I include the relevant literature even if only on men. The literature review will end with a specific section focusing on gender issues (pp 14 to 23)

2. LITERATURE REVIEW

2.1. ASSESSMENT OF RUNNERS

In a study by Christensen and Ruhling (1983), which focused on women marathon runners, the authors concluded that distance runners are generally lean and have high aerobic capacities when compared to sedentary women and other women athletes. They highlighted the fact that, although body composition seemed to be quite similar for all the women tested, the elite females had the highest VO_{2max}, thus suggesting that for trained women distance runners, successful performance is associated with a greater aerobic capacity rather than body composition. It was also stated that it is only when one has a homogeneous group, with regards to VO_{2max}, that factors such as running efficiency and fractional use of VO_{2max}, become important determinants of success, otherwise, when there is a great disparity in the VO_{2max} values of athletes, performance appears to be predictable.

2.1.1. Maximal oxygen consumption (VO_{2max}) testing

Maximal oxygen consumption (VO_{2max}) is a measure of the highest rate at which an individual can consume oxygen during exercise. It is known that oxygen uptake increases with running speed, and a high VO_{2max} has long been used as an indication of great endurance potential in athletes (Shephard, 1984).

Treadmill running until exhaustion has generally been found to produce the highest VO_{2max} values in runners (Scott, 1994; Hill and Rowell, 1996), with several authors suggesting that gradient treadmill running produces higher results than flat treadmill running (Kang *et al*, 2001; Helgerud, 1994). The highest oxygen consumption attained during a test is termed the athlete's VO_{2max}. This value is not, however cast in stone and the athlete may achieve a different value if tested again under different conditions or using a different protocol. This has led to some authors to prefer using the term VO_{2peak} (Millet *et al*, 2003; Billat *et al*, 2002; Demarie *et al*, 2000). There is an intra-individual day-to-day variation of 4-7% in VO_{2max} (Gazeau *et al*, 1997; Figueroa-Colon *et al*, 2000). As the gas analysers used in most laboratories these days, when calibrated correctly, are very accurate, the largest part of this variation can be ascribed to the daily variability in the level of physical capacity and mental state of the athlete. This unreliability of the VO_{2max} is the reason that the test is often repeated, to ensure the values achieved are more representative.

In order to be certain that an athlete is truly fatigued at the end of a VO_{2max} test, two of the following three criteria should be met. One indication of fatigue is a plateau in the VO_2. This classic "plateau" concept cannot be applied to all athletes, as modern studies have shown that only about 50% of individuals show a plateau in VO_2 at their maximum (Noakes, 1990; Noakes, 1998). Thus, there are two other criteria that serve as a good indication of fatigue, which are more applicable to all athletes. The first of these is a heart rate, at the end of the test, close to or greater than the predicted maximum and the second criteria, is a respiratory exchange ratio greater than 1.10 (Dressendorfer, 1991; Shephard, 1975; James and Doust, 1998). Any combination of two of the above three criteria indicates that the results achieved were valid indications of the maximal aerobic capacity of the athlete.

Just as VO_{2max} values vary between individuals, so too do they vary between genders, with females generally having lower VO_{2max} values than males. These lower

values are partly explained by the higher percentage body fat content and smaller muscle mass seen in females, as will be explained below in section 4.2.

As stated above, VO_{2max} may be a reasonable predictor of endurance performance in a heterogeneous group of athletes whose abilities are notably different, however, in a group of athletes with similar abilities, the usefulness of VO_{2max} as a predictor of performance is limited (Christensen and Ruhling, 1983). A number of studies have indicated that in such cases, the most valuable variable for predicting performance is the peak treadmill speed that an athlete can maintain during a VO_{2max} test.

2.1.2. Peak treadmill speed

Scrimgeour *et al* (1986) indicated that a relationship exists between the peak treadmill speed achieved during a continuous, flat, incremental treadmill test and race performance over distances between 10 and 90 km ($r = 0.72$). Noakes *et al* (1990) confirmed this relationship through their tests on 20 specialist marathon runners and 23 specialist ultra-marathon runners. They also found that peak treadmill speed was the best laboratory-measured predictor of running performance at any distance from 10 to 90 km ($r = -0.80$ to 0.92).

Of more relevance to the current study are the results of Scott and Houmard (1994) who again confirmed these findings after correlating performance in a self-paced 5 km time trial in a consistent laboratory setting to peak running speed ($r^2 = 0.94$, $p < 0.001$) and VO_{2max} ($r^2 = 0.13$, $p < 0.21$). They also examined whether a gender difference existed in the relationship between peak running velocity and distance running performance. Fourteen male athletes and nine female athletes, all of who were competitive athletes who had maintained consistent weekly training volumes for the three months prior to the study, participated in the study. The researchers found that the association was similar in both genders; thus, peak treadmill speed could be used in both well-trained males ($r^2 = 0.83$, $p < 0.001$) and females ($r^2 = 0.80$, $p < 0.001$) as a predictor of performance. The physiological determinants of peak treadmill velocity are not known. VO_{2max} cannot be the primary determinant otherwise it would be an equivalent predictor of performance. A criticism of this study is that they did not specifically discuss whether it is correct to do correlations with both male and female subjects in one group, as they did initially, or whether it is preferable to assess the relationships within each group as they subsequently did do. To my knowledge this is still an open question.

2.1.3. Field testing

Laboratory facilities are not always available for the determination of the factors affecting performance, such as maximal aerobic speed and VO_{2max}, and thus, field tests, in which these factors can be accurately predicted have been analysed (Berthoin *et al*, 1994). Multi-stage track tests and shuttle tests have been compared to VO_{2max} tests on a treadmill and it was found that VO_{2max} could be accurately estimated using the track test. The estimated VO_{2max} using the track test were not significantly different from the values measured during the treadmill test, but were higher than those estimated by the shuttle test. The means of the maximal aerobic speed observed in the track test and in the treadmill test were also not significantly different, while that of the shuttle test was again lower than the other two. Two important conclusions can be made. Firstly, as the authors concluded the multi-stage track test was a better field test for the estimation of performance. Secondly, it can be presumed that testing on a treadmill does not limit the maximal aerobic speed that can be achieved.

The physiological determinants of peak treadmill speed are not known. VO_{2max} cannot be the primary determinant otherwise it would be an equivalent predictor of performance. Other variables to consider are the fraction of VO_{2max} that can be sustained for the race distance and the mechanical efficiency, or aerobic energy cost of running (Berthoin *et al*, 1994), as well as the percentage composition of fast-twitch muscle fibres.

2.1.4. Physiology influencing economy

Running economy has traditionally been measured as the oxygen cost of running at a given velocity and is a reflection of the amount of fuel required for a particular running task. It is usually expressed as absolute VO_2 (Nindle *et al*, 1998) or VO_2 relative to body mass (Pate *et al*, 1992; Berthoin *et al*, 1994; Billat *et al*, 1994; Billat *et al*, 2001) or both. This factor has been accepted as the physiological criterion for 'efficient' performance and has been identified as a critical element of overall distance running performance (Anderson, 1996).

A link exists between running mechanics and energy cost of running, but research to date has not established a clear mechanical profile of an economic runner. Through training, it appears as though individuals are able to integrate and accommodate their unique combination of dimensions and mechanical characteristics in order to arrive at a running motion which is most economical for them.

Information in the literature suggests that biomechanical factors are likely to contribute to better economy in any runner. A variety of anthropometric dimensions could influence biomechanical effectiveness. These include: average or slightly smaller than average height for men and slightly greater than average height for women; low percentage body fat; and smaller than average feet. Stride length, which is freely chosen over considerable running time, may also be related to running economy. Martin and Morgan (1992) reviewed the results of the many studies in which the influence of structural and biomechanical factors on economy were the focus. They concluded that although relationships have been observed between economy and individual descriptors of body structural and gait mechanics, these relationships have generally been weak and inconsistent from study to study.

Pate *et al* (1992) examined the influence of heart rate and ventilation, body weight, VO_{2max} and age on economy, in a large, very diverse group of habitual runners. The group was composed of 119 male subjects and 69 female athletes, whose ages ranged from 20 to 60 years and weekly running mileage ranging from <10 miles to >70 miles. Economy was assessed by running on a flat treadmill at 6 mph. The findings of the study indicate that a better economy is associated with lower VO_{2max} (expressed as ml/kg/min), lower submaximal exercise minute ventilation and heart rate, younger age and higher body mass.

The association of relative economy with body weight indicated that heavier runners were more economical than lighter runners. This inverse relationship is thought to be due to differences in mass distribution between lighter and heavier runners. Lighter athletes, when compared to their heavier counterparts, have been shown to possess a greater percentage of their body mass in the extremities (Williams and Cavanagh, 1987) and thus would have to perform a relatively greater amount of work in the movement of these limbs.

Pate *et al* (1992) explained the lower VO_{2max} in the more economical runners, which at first may seem paradoxical, by suggesting that as VO_2 values in this study were expressed by dividing by body weight, in order to allow comparisons across individuals. However, most of the energy cost of running is associated with movements of the limbs, which constitute only a fraction of the total body weight. Persons who have a greater fraction of their body weight in their limbs, may have a higher VO_{2max} due to greater active muscle mass and these athletes would have a higher VO_2 at submaximal running speeds due to the increased energy cost of

moving the relatively heavier limbs. Another possible explanation of this phenomenon is that the observed relationship between VO_{2max} and $VO_{2submax}$ was a result of the test protocol employed during this study. As the submaximal speed chosen was such that it would be submaximal for all athletes tested, it may have been such that the athletes with the higher VO_{2max} values were in fact less comfortable with running at this slow pace and thus less economical.

The final variable that was associated with economy was age and it was found that younger athletes were more economical than older athletes, but the authors gave no explanation for this.

Although debated, the influence of height on running economy has usually indicated that the taller athletes are less economical. As height increases, it costs more to run at a particular pace, even when the cost is expressed per kilogram of body weight. (Bourdin *et al*, 1993). This can be explained due to the fact that bone mass increases exponentially, not linearly, as a function of height, which means that taller runners have both absolutely and relatively heavier bones, compared to shorter runners. It costs energy to carry the bones, and thus economy decreases.

Female runners have been shown to have a significantly lower cost of running, for a given mass, than male runners ($P < 0.05$), thus suggesting that women decrease their cost of running as a response to running training more efficiently than do men (Bourdin *et al*, 1993).

Maldonado *et al* (2002) investigated the influence of body dimensions on running economy in athletes specializing in different competition events. They assessed the influence of body mass and height on the energy cost of running in 38 highly trained male runners, specialized in either marathon, long middle-distance (5 000m to 10 000m) or short middle-distance (800m to 1 500m). Long- middle distance runners were found to have significantly higher mean energy cost of running and VO_{2max} than the other runners. The energy cost of running correlated negatively with height ($r = -0.86$, $p < 0.001$) and mass ($r = -0.77$, $p < 0.01$), but only in the short-middle distance group. They concluded that highly trained distance runners show counterbalancing profiles of running economy and VO_{2max}, with the more economical athletes having a lower VO_{2max}, and that anthropometric characteristics related with good performance are different in long and middle distance events.

Daniels and Daniels (1992) also investigated economy in short-distance (800- and 1500-metre), middle distance (3K, 5K, and 10K), and long-distance (marathon) runners and there findings contradicted popular beliefs that marathon runners tend to be the individuals who develop the most efficient running style. The short-distance competitors in this study almost always had the best economy at speeds of marathon race pace and faster.

2.1.5. Racing performance

In a competition against a handful of closely matched opponents the smallest change in a physiological variable, if sustained for the duration of the race, can have a substantial effect on the athlete's chances of winning.

VO_2 at average racing speed expressed relative to VO_{2max} is referred to as the fractional utilization of VO_{2max}. Marathon runners use approximately 75-90% of their VO_{2max} during competition (Costill *et al*, 1971).

The fractional utilisation of aerobic capacity in both elite and non-elite male and female marathon runners was investigated by Maughan and Leiper (1983). 28 marathon runners, of variable running ability, competed in the study (19 male subjects and 10 female subjects). Each subject completed both a maximal test and a submaximal test, at marathon racing pace, so as to determine their oxygen uptake at race pace. For all athletes, linear relationships were found to exist between marathon performance and aerobic capacity (males: r = 0.88 and females: r = 0.63). Similarly, the fraction of VO_{2max} sustained throughout the race was significantly correlated with performance for both male (r = 0.74) and female (r = 0.73) runners. The fastest runners were running at a speed requiring approximately 75% of VO_{2max}, while for the slowest runners, the work load corresponded to approximately 60% of VO_{2max}.

Scrimgeour *et al* (1986) further investigated this relationship, taking into account the effect of training on fractional utilisation. They investigated this relationship in 30 male athletes, who were divided into three groups of training according to training volume. The runners with higher training volume showed faster running speeds. Each athlete completed both a maximal and submaximal treadmill test in order to determine VO_{2max} and $\%VO_{2max}$ sustained during competition. Contrary to the findings in the study by Maughan and Leiper (1983), these two variables were found to be indistinguishable between the three groups, although the range of performances in the former study was much greater. The faster running speed of the more trained

runners, running at the same $\%VO_{2max}$ during competition, was due to their superior running economy (19.9%). Thus, the authors concluded that all of the group differences in running performance could be explained by the differences in running economy. They also suggested that the main effect of large volumes of training might be to increase running economy.

2.2. SKELETAL MUSCLE OF 10 KM RUNNERS

Skeletal muscle is a dynamic tissue that can adapt in many different ways to an exercise stimulus, such as by metabolic adaptations, muscle hypertrophy and other morphological adaptations that can change the contractile properties of the muscle (Ross and Leveritt, 2001).

2.2.1. Muscle fiber type composition

Prior to 1975, the physiological requirements for success in elite distance runners were mostly limited to measurements of metabolic and circulatory responses during exercise (Costill, 1970). After this, studies became much more invasive and histochemical and biochemical observations were made on muscle, to determine the effect of muscle composition on performance (Costill, 1975).

Skeletal muscle is composed of different types of fibers, differing from each other with respect to morphometric, contractile and metabolic properties (Pette and Starron, 1990). Brooke and Kaiser (1970) devised a method enabling the fibers to be categorized as slow twitch (type I), Fast twitch oxidative-glycolytic (type IIA) and fast twitch glycolytic (type IIX) based on different pH sensitivities of their myosin ATP-ase activity. Type IIX fibers exhibit the highest power outputs, with type IIA fibers exhibiting intermediate power outputs and type I fibers the lowest power outputs (Bottinelli *et al*, 1999). This property is confirmed by both ATPase activity that influences the velocity of contraction and cross-sectional area that influences force production.

Further characteristics of the three fiber types are summarized below:

2.2.1.1. Type I fibers

These fibres, also called slow oxidative fibres, contain large concentrations of myoglobin, many mitochondria and many blood capillaries. Type I fibres are red, split ATP at a slow rate, have a slow contraction velocity, are very resistant to fatigue and

have a high capacity to generate ATP by oxidative metabolic processes (Saltin and Gollnick, 1983).

2.2.1.2. Type IIA fibers

These fibres, also called fast twitch or fast-oxidative-glycolytic fibres, contain high concentrations of myoglobin, many mitochondria and many blood capillaries. Type II A fibres are red, have a very high capacity for generating ATP by oxidative metabolic processes, split ATP at a very rapid rate, have a fast contraction velocity and are resistant to fatigue (Saltin and Gollnick, 1983).

2.2.1.3. Type IIX fibers

These fibres, also called fast twitch or fast glycolytic fibres, contain a low content of myoglobin, relatively few mitochondria, relatively few blood capillaries and large concentrations of glycogen. Type II X fibres are white, geared to generate ATP by anaerobic metabolic processes, not able to supply skeletal muscle fibres continuously with sufficient ATP, fatigue easily, split ATP at a fast rate and have a fast contraction velocity (Saltin and Gollnick, 1983).

2.2.2. Cross-sectional areas of muscle fibers

The diameter and cross-sectional area of the muscle fibres is influenced by training. The effects of heavy resistance exercise over a period of months is an increase in muscle fiber hypertrophy: each individual muscle fiber becomes bigger, but the effects of distance running on muscle fiber cross-sectional area are less clear. Only three studies have focused on distance running and the cross-sectional area of fibers (Trappe *et al*, 1996; Sleivert *et al*, 1995; Costill *et al*, 1976).

Neuromuscular differences between volleyball players, middle distance runners and untrained controls were evaluated by Sleivert *et al* (1995). The runners partaking in the study were leaner than controls, while the volleyball players were taller, heavier and had larger thigh volumes than the other groups.

The volleyball players had higher absolute cycle ergometer power than both middle distance (26%) and control (15%) groups, although differences disappeared when expressed relative to body mass or thigh volume. The volleyball athletes were also stronger than both middle distance and control subjects for isokinetic leg extension

and plantar flexion. However, despite these indications otherwise, no differences in fiber cross-sectional area existed between groups.

Costill *et al* (1976) obtained biopsies from the gastrocnemius of 14 elite distance runners, 18 middle distance runners, and 19 untrained men. The elite runners' muscles were characterized by a high percentage (79%) of slow twitch (ST) fibers and, on average, the cross sectional area of their ST fibers was found to be 22% larger than the FT fibers ($P < 0.05$). No differences were found in the cross-sectional areas of the fiber types in middle-distance runners (800m – 5000m). This lack of difference is due to the fact that middle distance running places metabolic demands on both slow twitch and fast twitch fibres, thus both fiber types will undergo equal hypertrophy.

The muscle structure and performance capacity of Himalayan Sherpas was compared to that of Caucasian elite high-altitude climbers and the cross-sectional area was found to be similar in both groups (Kayser *et al*, 1991). In both cases, there was a reduction in fiber cross-sectional area and consequent increased capillary density. These changes were thought to facilitate O_2 transport due to the decreased diffusion distance from the capillary to the mitochondrion.

2.2.3. Oxidative capacity of muscles

In order to determine the oxidative capacity of muscle, which is the ability of the muscle to use aerobic energy systems, the activity of one of the key regulatory enzymes for the oxidative pathways can be measured (Ross and Leveritt, 2001). Citrate synthase (CS) and succinate dehydrogenase (SDH) are two such key enzymes. Citrate synthase is responsible for the condensation of oxaloacetate and acetyl coenzyme A to citrate in the Kreb's cycle. As the Kreb's cycle takes place within the mitochondria, citrate synthase activity is also an indicator of muscle mitochondrial content.

In a rat study by Delp and Duan (1996), muscle citrate synthase activity was measured to determine the relationship between fiber composition and muscle oxidative capacity. Citrate synthase activity, was most closely related to the population of type IIA fibers and was in the rank order of type IIA > I > IIX. This relationship in humans, and especially athletes has not been shown.

2.2.4. Anaerobic capacity of muscles

Glycolysis provides a rapid mechanism of energy production from carbohydrate stores. The pathway occurs independently of oxygen in the cytoplasm of the muscle fibres and is thus termed the anaerobic or non-oxidative pathway. Glycolysis is initiated at the onset of any intensity of exercise (Hultman *et al*, 1983) and contributes highly significantly to energy production during 10 seconds of maximal dynamic exercise (Jacobs *et al*, 1983). In fact, glycolysis may contribute up to 75% of the metabolic energy production during sprint exercise lasting about 10 seconds with additional major contributors being PCr breakdown (Ross and Leveritt, 2001). Thus, during sprint exercises the principal metabolic pathways used are the anaerobic.

2.2.4.1. Aerobic versus anaerobic contribution to 10 km races

The aerobic energy system requires the presence of oxygen, and although it is a slower energy system, the energy yield from this pathway is far greater than from the anaerobic pathways. The primary fuel sources for the aerobic energy system are fatty acids and glucose. In contrast glycolysis uses only carbohydrates. The relative contribution of each can be assessed by the respiratory exchange ratio (RER). The RER is the ratio of carbon dioxide produced to oxygen consumption (VCO_2/VO_2) and it indicates substrate utilization during steady state exercise in which the value of 1.0 represents 100% carbohydrate metabolism, and 0.7 represents 100% of fat metabolism.

The time course of anaerobic and aerobic energy expenditure in both sprint and endurance athletes was studied by Nummela and Rusko (1995). The athletes in this study (8 male sprint runners and six male endurance runners) completed a submaximal treadmill test to fatigue in order to determine the relative contributions of the energy systems. In both groups the running time was approximately 49 $\pm$ 5 minutes. The sprint group had a significantly higher peak blood lactate value after the run compared to the endurance group ($p < 0.05$), indicating a greater utilisation of the anaerobic pathway. The relative contribution of anaerobic energy yield decreased from 80% during the first 15 s to 60% within the first minute.

The relative contribution of aerobic energy yield was significantly higher ($p < 0.05$) in the endurance group (54-63%) than in the sprint group (43-47%) during the second half of the run, while in the first half the contribution was similar in both groups. Thus, during the second half of the exhaustive run, the sprinters relied more on the

anaerobic pathways and the endurance athletes on the aerobic pathways for energy production.

2.3. Influence of non-physiological factors on running performance

Performance in a race also depends on a variety of non-physiological, extraneous factors, such as different environmental conditions, terrain and familiarity with the race course. Hopkins and Hewson (2001) studied the variations from one competition to the next by looking at the performances of athletes in consecutive races. They used the official race times of athletes for consecutive races in a series within a competitive season and they concluded that in female runners, older runners and faster runners, there is less variation in their performance compared to male runners, younger runners and slower runners. They also speculated that differences in variability arose from differences in competitive experience and attitude toward competing.

As the above studies show, a relationship exists in distance runners between peak running velocity achieved during a continuous, horizontal, incremental test and 10-90km performances. However, the performance usually used when assessing the predictive power of physiological variables, is the best time achieved in a recent race, or a personal best ever (Scrimgeour *et al*, 1986; Powers *et al,* 1983). The use of these races may weaken the relationship between a given variable and distance running performance, as many factors like environmental conditions, terrain, familiarity with the race course and identity of the other competitors can influence race time independently of physiological performance ability. Scott and Houmard (1994) analyzed the relationships between peak running velocity and a self-paced 5km race time trial in a consistent laboratory setting to minimize these effects. Peak running velocity was strongly related to distance running performance (5km) in the athletes, but the relationship was evident both when performance was assessed with the controlled time trial (r^2 = 0.94) and with recent best field performance (r = 0.89). When performances of athletes need to be compared as a group however, such as in a subject-matching study as this, the individual athletes' performances in a recent race cannot be compared due to the varying factors contributing to the race. Thus, in such studies, the athletes all need to complete a standard race course, at the same time, so as to minimize these effects and achieve a true reflection of performance ability.

2.3.1. Reliability of testing

Reliability refers to the reproducibility of values of a test in repeated trials on the same individual (Hopkins, 2000). The more reliable a test, the greater the accuracy with which changes in variables can be seen.

There are many potential methods for testing endurance performance. In several studies (Millard-Stafford *et al*, 1992), competitive events or field trials have been used, but these assessments were performed in very uncontrolled conditions. While the laboratory protocols used make up for this element of control, the differences in research designs make it difficult to compare results among studies. Also, very few researchers have published reliability analyses of endurance performance protocols, reducing the confidence with which one can make any conclusions regarding the effects of experimental interventions.

Doyle and Martinez (1998) developed a highly reliable protocol for determining prolonged endurance performance, in which the initial segment of the test was sufficiently long and intense to deplete endogenous carbohydrate stores and this was followed by a time trial segment. The reliability of this protocol was found to be very high, especially when the first trial was considered to be a familiarization and thus, excluded from analysis. Although this protocol was found to be highly reliable, the total exercise duration approached two hours and thus, it was found to only be suitable for tests of endurance for athletes who compete in prolonged events. However, it was suggested that a part of the reliability of this protocol was due to the degree of control of the activity and due to the very controlled diet in the two days prior to the trials, both of which factors could be applied to a test of shorter duration.

2.4. GENDER ISSUES

Performance in long-distance running, as previously stated, is related to several physiological variables, such as VO_{2max}, the anaerobic threshold, running economy, and anaerobic capacity. Wright *et al* (2002), Marcell *et al* (2003), Von Tscharner and Goepfert (2003) and Billat *et al* (2002 and 2003) are recent studies that have delved into the gender differences in the relationships between long-distance performance and these factors, indicating that there is still substantial interest in this topic and that not all issues are resolved.

The gender differences observed in the relationships between physiological variables and running vary with respect to running distance. Thus the gender differences observed for marathon runners or middle-distance runners may not be applicable in the relationships between 10-km performance and these physiological variables. The gender differences in the specific relationships observed between 10km running and physiological variables thus need to be studied.

2.4.1. Reasons for better performance in men

Sparling *et al* (1998) studied the world rankings for the marathon (IAAF), 1500 m and 10 km track events in both women and men, from 1980 to 1996 in order to determine a) whether the gender difference in running events is declining or whether it has stabilized over the past 17 years and b) whether the relative decline in pace as distance increases is different between women and men.

In the 1500 m, the gender difference in world-best times (11.1 +/- 1.1%) was consistent from 1980 to 1996, and the slight rate of improvement in the event was similar for men and women. In the marathon, the gender difference in world-best times (11.2 +/- 0.9%) was essentially the same as for the 1500 m. In 1980, the marathon was a fairly new event for the women, having only just been sanctioned by the International Amateur Athletics Federation (IAAF). As a consequence, the depth of the field increased quickly from 1980 to 1984. Since the mid-1980s, the rate of improvement for women in 100th-ranked times has levelled off to equal that of men. The average improvements in relative pace for men and women from the 1500 m to the 10 km to the marathon were found to be remarkably similar, with no diminishing of the gender difference as race distance increased.

Thus based on worldwide indices of competitive distance running, the gender difference in distance running performance has plateaued in recent years. Thus, it seems likely that the current gender difference in performance will remain fairly constant because of biological differences between men and women that give men an advantage in distance running (Cureton and Sparling, 1980). The average male has less body fat, a greater aerobic capacity (VO_{2max}) and more favourable cardiovascular ratios, such as heart size, left ventricular mass and cardiac output (Speechly *et al*, 1996), thereby allowing for a greater work capacity in the average male athlete in comparison to the average female athlete. Another biological difference that accounts for a small but significant portion of the difference in distance running performance is the gender difference in hematocrit. This effect mediates

higher oxygen transport capacity and thus a higher maximum oxygen uptake in men compared with women.

2.4.2. The influence of percent body fat on performance

Many studies have investigated the extent to which differences between men and women in metabolic responses to exercise and distance running are due to the gender difference in % body fat (Cureton and Sparling, 1980; Sparling and Cureton, 1983; Ready, 1984). Female athletes have a higher % body fat that will always remain higher despite diet and training (Ready 1984) and this higher % body fat results in lower VO_2 values in female athletes (expressed per unit body mass).

In Cureton and Sparling's study (1980) the 10 male athletes participating in the study completed the tests under two conditions: (1) with normal body weight and (2) with external weight added to the trunk so that the total percent excess weight was equal to the % body fat of a matched female.

They found that by equating % excess weight, the mean gender difference in running tests was reduced. The study concluded that the greater gender-specific, essential body fat of women is one determinant of the gender differences in distance running performance. They also concluded that due to the greater body fatness, the average woman will utilize more oxygen per unit fat free weight to run at any given submaximal speed and will have a lower VO_{2max} expressed relative to body weight. As a result, females will maintain a speed on the 12-min run or other similar distance running event, which is slower than their male counterparts. They also concluded that due to the fact that this gender-specific, essential fat of women cannot be eliminated by diet or training, it provides part of a biological justification for separate distance running performance standards and expectations for men and women.

These findings were confirmed in a further study by Sparling and Cureton (1983), who compared the performances of 34 male and 34 female recreational runners in a 12-minute run performance test. The men differed significantly ($p < 0.05$) from women in % body fat (10.8 vs 19.8%), VO_{2max} (68.6 vs 65.1 ml/kg/min), and 12-minute run performance (3294 m vs 2747 m), but not in running economy. Multiple regression analysis of the data revealed that % body fat, VO_{2max} and running economy accounted for 74, 20, and 2% of the average gender difference in 12-min run performance, respectively. Thus, the average gender difference in 12-min run performance was found to be primarily due to differences in percent fat with a further

significant contribution due to difference in cardiorespiratory capacity. Their study also indicated that only 4% of the difference remained unexplained.

2.4.3. Scaling VO_2 to correct for differences in body size

Other factors that is different between groups of males and females are height and weight. Many studies have focused on various statistical modeling techniques in order to scale VO_2 between male and female groups. Much of the research in this field has been conducted on pre-pubertal children and adolescents, taking into account growth as well as differences in body fat (Eliakim *et al*, 1997; Armstrong *et al*, 1998; Eisenmann *et al*, 2001). However, as the athletes participating in this study are all mature, fully grown adults, the scaling methods used in these papers may not be correct for our study. However, several papers on an effective method of scaling gender differences in VO_2 in adults have also been published.

In 1996, Welsman *et al* studied the influence of different statistical modeling techniques on the interpretation of peak VO_2 data in groups of prepubertal, circumpubertal, and adult males (group 1M, N = 29; group 2M, N = 26; group 3M, N = 8) and females (group 1F, N = 33; group 2F, N = 34; group 3F, N = 16). A common mass exponent was identified (b = 0.8) and the contribution of stature was taken into account (0.44) to result in the authors using an allometric equation, which reduced the mass exponent to 0.71. Their results indicate that conventional ratio standards ($ml.kg^{-1}.min^{-1}$) do not adequately account for body size differences when investigating peak VO_2.

The relationship between oxygen consumption (VO_2) and both body surface area (BSA) and body size among 30 prepubertal children, 30 circumpubertal children, and 30 adults was the focus of the study by Rogers *et al* (1995) in order to determine which scaling model was most appropriate for making comparisons between these populations in both genders. Each of the subjects participating in the study completed a maximal treadmill test as well as a submaximal treadmill test and it was determined that the submaximal VO_2 -to-body size relationship provided the most appropriate model for comparisons. Analyses revealed a stronger linear relationship between submaximal VO_2 and body surface area than submaximal VO_2 and body mass. They concluded that, overall, a scaling factor of body surface area or body mass to the 0.75 power both provide a more appropriate method of comparison than a simple ratio standard of body weight.

Thus, it is clear that the scaling of VO_2 is vital when comparing male and female athletes. The most effective factor by which to scale these values however is still debated.

Neder *et al* (1999) completed a scaling study in cycling where body fat is less important. His subjects were non-athletic males (n = 34) and females (n = 37), aged between twenty and eighty years. Their peak VO_2 was compared to leg muscle mass and leg strength. The absolute values of all variables were higher in males and declined with age ($P < 0.01$). Allometric correction of peak VO_2 and leg strength by leg muscle mass slowed the age-related declines; the flattening effect however, was more evident in the females. In conclusion, they found that through using adequate scaling methods no gender differences are observed in peak VO_2 adjusted for leg strength. This study highlights that important body composition differences are not only % body fat but also fat free mass.

2.4.4. Muscle differences between male and female athletes

Muscle fibre composition and enzyme activities in elite female distance runners was compared to those of the males by Costill *et al* (1987). The female athletes were competing successfully in middle-distance (1500-3000 m) rather than long distance (10 km and marathon), which explained many of the differences observed between these "elite female runners" and the "elite male runners" studied by Costill in 1975. With the exception of differences in muscle fibre areas, the female runners show the same enzyme adaptations to endurance training that have been reported for male runners. The most notable finding of this study was the remarkable similarity of muscle fibre composition and enzyme activity for male and female runners when matched according to their preferred competitive distance.

In a comparative study by Esbjörnsson-Liljedahl *et al* (1999), the effect of a 30s sprint on the acute metabolic response in type I and type II fibres was analysed in twenty recreationally fit male subjects and nineteen recreationally fit female athletes. The athletes performed a 30s sprint on a cycle ergometer, before and after which a biopsy was taken. The analysis of the biopsies indicated that in women, there was a smaller reduction in glycogen content in type I fibers from the sprint. They concluded that this smaller sprint-exercise-induced reduction in glycogen content in women than in men might contribute to the smaller accumulation of blood lactate in women after sprint exercise.

2.4.5. Oxidative capacity and anaemia

Gregg *et al* (1989) demonstrated the interactions between oxidative capacity of the muscle, anaemia and endurance exercise. The rats in their study were evaluated under four conditions: untrained and endurance-trained with each group subdivided into anaemic and control animals.

The hemoglobin concentration and hematocrit in the anaemic rats were 38% and 41% lower than the levels in the control rats, respectively. The results of the study indicated that the anaemia significantly reduced endurance by 78% in untrained rats but only 39% in trained animals. Anaemic untrained and trained animals had similar VO_{2max}, both of which were lower than those of the control rats (both trained and untrained), but anaemic trained rats had higher muscle oxidative capacities and greater endurance.

In a further rat study by Davies *et al* (1982), the relationships between muscle oxidative capacity, anaemia and endurance were investigated in iron deficiency and during dietary iron repletion. The rats were made severely iron deficient by a diet containing 2 mg iron/kg, while control animals received the same diet but with 50 mg iron/kg. Blood hemoglobin in this instance was decreased to 3.6 +/- 0.5 g/dl compared to 13.7 +/- 0.6 in control animals.

This decreased iron content resulted in decreased mitochondrial enzyme specific activities and a 30% reduction in the mitochondrial content of muscle, the combination of which resulted in 60-85% decreases in muscle oxidative capacities. The endurance capacity was 90% lower in deficient animals than controls.
The iron-depleted rats were then given the iron sufficient diet and the course of dietary repletion was followed.

The hemoglobin levels increased substantially within 3 days, together with the VO_{2max} and VO_{2max} workload, while no significant improvements were seen in mitochondrial bioenergetic functions, mitochondrial content of muscle, muscle oxidative capacity, or endurance capacity until the 5th day. These findings led to the conclusion that VO_{2max} and VO_{2max} workload capacity were not limited by muscle oxidative capacity while the endurance capacity was not restricted by oxygen supply, but was primarily determined by the oxidative capacity of muscle.

The reduced work capacity due to anaemia in humans was assessed by Haas and Brownlie (2001). Work capacity in this study was assessed by aerobic capacity, endurance, energetic efficiency, voluntary activity and work productivity.

A strong causal effect of anaemia on aerobic capacity was seen, the mechanism for which was presumed to be the reduced oxygen transport associated with anemia and tissue iron deficiency through reduced cellular oxidative capacity.

Endurance capacity was also compromised in anaemic subjects, with energetic efficiency affected at all levels of iron deficiency.

The effect of an iron deficiency and subsequent iron supplementation in women was studied by Brownlie *et al* (2002). In this case the forty-one, untrained subjects were marginally iron-depleted and not anaemic. The subjects received either a placebo or a 50mg $FeSO_4$ supplement twice daily for six-weeks. The subjects trained five days a week for four weeks on a cycle ergometer, starting their training in the third week of the study.

The study revealed that the supplement resulted in improved serum ferritin and serum transferrin receptor (sTfR) concentrations and transferrin saturation without improvement in hemoglobin concentrations or hematocrit. The average VO_{2max} and maximal respiratory exchange ratio improved in both the placebo and iron groups after training, but the iron group experienced significantly greater improvements in VO_{2max}.

They thus concluded that, iron deficiency without anemia impairs aerobic adaptation among previously untrained women, but this impairment could be corrected with iron supplementation.

2.4.6. Matching male and female athletes

Several studies have focused on performance-matched female and male athletes (Helgerud, 1994; Speechly *et al*, 1996; Iwaoka *et al*, 1988), although none of these studies focused on runners specializing in the 10 km distance. Helgerud (1994) matched six female and six male marathon runners for performance (Mean time for men 199.4 minutes ± SEM 2.3 and for women 201.8 minutes ± SEM 1.8) and age (20-30 years). The assessment of these athletes indicated that while the men had higher VO_{2max} and anaerobic thresholds, women had higher weekly training distance,

superior running economy (a lower gross oxygen cost of running) as well as a higher fractional utilization of VO_{2max} during a race, a measure of resistance to fatigue.

There are several other ways to measure resistance to fatigue that are based on time to fatigue at a set workload. The minimal speed that elicits VO_{2max} is called the maximal aerobic running velocity ($V_{a\ max}$) and the maximal endurance at this velocity (t_{lim}) is a measure of resistance to fatigue and is thought to be an important predictor of middle-distance running performance (Yoshida *et al*, 1993; Scherrer and Monod, 1960). Thus, Billat *et al* (1996) investigated the gender effect on t_{lim} at $V_{a\ max}$ and anaerobic capacity in middle-distance runners (800 m to 3000 m). Fourteen female and fifteen male elite runners, who had similar race performances, participated in the study. No significant differences between male and female athletes with respect to t_{lim} at $v_{a\ max}$ or running economy at a set speed (14 km/h) or for oxygen cost of running at a relative speed (75% $v_{a\ max}$) was found.

Over longer distances such as ultramarathons, the top women can place very high on the race results. Speechly *et al* (1996) indicated that when aerobic capacity, defined as VO_{2max}, running economy, and training level, was matched between female and male distance runners, the female athletes tended to outperform their male counterparts in events longer than 24.1km. They also noted that some female endurance-athletes outperformed male endurance-athletes with similar aerobic capacities by more than 30 min over 90 km. Speechly *et al* (1996) decided to test this hypothesis in a more controlled study. They chose to investigate whether females would outperform over 90 km those males who were able to perform as well as them at 42,2 km. The marathon times for the female and male athletes were well matched (194.8 ± 12.9 m/min and 192.6 ± 16.3 m/min, respectively). However, they found that the performance for 90 km was significantly better ($p < 0.05$) in the female group (171.0 ± 11.7 m/min and 155.2 ± 14.7 m/min, respectively). The performances achieved by the females were attributed to the higher average fraction of the VO_{2max} sustained by the female athletes over the distance of the race (73.4 ± 5.5% vs 66.6 ± 3.7% for 42.2 km and 59.8 ± 6.2% vs 50.2 ± 3.1% for 90 km, for female and male athletes respectively). The decline in the fraction of the VO_{2max} sustained as the distance increased was found to be significantly less in the females ($p<0.05$). The mechanisms by which female athletes sustain this higher fraction, however, still remains to be identified but may be related to metabolism.

Iwaoka et al (1988) examined the possible gender differences in lactate threshold (LT) and its relation to running performance. In their study ten male and eight female college distance runners, who were matched as closely as possible on the basis of maximal aerobic power relative to lean body mass (VO_{2max}/LBM), performed an incremental running test on the level treadmill. LT, which was determined from an inflection point in blood lactate, was found to be significantly higher in males than in females (49.2 vs 45.5 ml/kg LBM/min, $P < 0.05$). However, when LT was expressed as $\%VO_{2max}$, no significant difference was observed suggesting that there are no significant gender differences in LT when compared in relative terms.

Despite the factors discussed above that place male runners at an advantage, females may have inherent advantages as well, or they may try to compensate for their inherent disadvantages. Possible factors, which could be in the females' favour, are:

1) lower body mass
2) a higher training volume
3) higher training intensity
4) higher fractional utilization during races
5) increased peripheral adaptations in muscle citrate synthase activity
6) a more economical running style

In summary, due to the fact that we matched the runners in this study for performance, we hypothesized that several physiological factors should be the same between genders, such as VO_{2max} per fat free mass (FFM) and peak treadmill speed (PTS)

Known differences between the genders are hematocrit, which is higher in males, and percentage body fat, which is higher in females (Cureton and Sparling, 1980; Ready, 1984). Also VO_{2max} expressed per kilogram body mass is expected to be lower in females, due to the above two factors.

Factors between which there could be differences between the genders are height, fat free mass, gradient VO_{2max} and gradient peak treadmill speed.

Of these known and expected factors, four would put females at a disadvantage when considering running performance, which leaves the question of which factors put the male athletes at a disadvantage or how do female athletes compensate to still perform equally to the male athletes.

2.5. TRAINING

Training regimes have a large influence on the success of an athlete, although the type of training and quantity which is recommended for optimal performance varies considerably, depending on the competitive event of interest.

2.5.1. The effects on performance and physiology

Several studies have been conducted in which the training of athletes has been evaluated, to determine its effects on performance (Hewson and Hopkins, 1996; Billat *et al*, 2003; Billat *et al*, 2002; Berry and Moritani, 1985). Most of the information regarding the effects of training on performance again focuses on performance and training in the marathon athlete but provides a good background for application to the 10 km athlete.

Billat *et al* (2002) studied the effect of pre-competition training on the physiological factors of performance in elite marathon runners. Five male athletes and four female athletes participated in the study and the testing consisted of a flat 10 km run at the speed of their personal best marathon performance and, after a 6 minute rest, an all-out 1 km run. These two performance tests were repeated both before and after the training. It was found that VO_{2peak} increased after the 8 weeks of pre-competitive training (66.3 ± 9.2 vs 69.9 ± 9.4 ml/kg/min, $p<0.01$). The oxygen cost of running at marathon velocity was not changed after training, thus it was concluded the running economy was not changed by the training, rather that the fractional utilization of VO_{2max} decreased significantly (94.6 ± 6.2% VO_{2peak} vs 90.3 ± 9.5% VO_{2peak}, $p<0.05$).

Billat *et al* (2003) then focused their attention on the training practices of some of the top athletes in the world, the Kenyans. Training diaries were used to analyze the daily sessions. The training runs were classified by intensity, duration and distance. There is a great diversity of training methods in Kenya, as elsewhere, and thus two groups of athletes were defined according to the type of training program they followed. Those runners who trained at speeds greater than or equal to that velocity corresponding to 50% of the velocity at VO_{2max} made up one group of athletes doing

high speed training (HST), the others formed the low speed training group (LST). Those Kenyan athletes specializing in the 10 km distance mainly fell into the HST group.

Between the genders within the HST group, the women (n = 6) had significantly lower VO_{2max}, velocity at VO_{2max} and the fraction of VO_{2max} at lactate threshold running velocity than their HST male counterparts (n = 6). In this study, lactate threshold running velocity was defined as the speed at which an increase in lactate concentration corresponding to 1 mMol/L occurs between 3.5 and 5 mMol/L. The energy cost of running, when scaled to eliminate body composition differences, was not significantly different between genders (546 $\pm$ 39 ml/kg$^{0.75}$ vs 579 $\pm$ 20 ml/kg$^{0.75}$, for women and men, respectively, p = 0.09).

2.5.2. Training effects on muscle

Endurance training favourably modifies the capillarisation of skeletal muscle (Andersen and Henriksson, 1977). Some research findings actually suggest that specific training (or inactivity) may actually induce an actual conversion of type I to type II fibers (or vice versa). It appears that some transformation is possible in muscle fiber type with chronic and specific types of physical activities.

Bottinelli *et al* (1999) showed that the maximum unloaded shortening velocity of type IIX fibres is approximately ten times faster than type I fibres. Thus, a higher percentage of type IIX fibres, would be advantageous to sprinters and indeed, sprint performance has been strongly correlated with the percentage of type II fibres.

Adaptations of the contractile apparatus to a variety of training types has been reviewed by Pette (1998), with endurance training appearing to induce a shift toward type I fibres (IIX$\rightarrow$IIA$\rightarrow$I)

Enhancement of the oxidative capacity of fast-twitch fibres with endurance training brings them to a level at which they are almost as well equipped for oxidative metabolism as the slow-twitch fibres of untrained subjects. Endurance-trained men and women show some conversion of the type IIX fibre to the more aerobic type IIA fibre. This is accompanied by the well-documented increase in mitochondrial size and number and a corresponding increase in the total quantity of the enzymes of the Kreb's cycle and electron transport. Only the specifically trained muscles (muscle fibres) adapt to exercise.

The muscle adaptations to sprint training are clearly dependent on the duration of sprinting, the recovery between repetitions and the frequency of training bouts (Ross and Leveritt, 2001). All the above variables have profound effects on the metabolic, structural and performance adaptations of the muscles. Sprint runners have been shown to have a larger percent of type II fibers than other athletes and this percentage can be further increased by sprint training; however, the changes in fiber type are very closely related to the frequency of training.

2.5.3. The effect of training on cross-sectional area of muscle fibres

Sprint training alone doesn't appear to increase muscle fiber size significantly during short-term (6 to 7 weeks) training periods often despite performance improvements (Ross and Leveritt, 2001). However, significant increases in both type I and type II fibre area have been observed after sprint training ranging in duration from 8 weeks to 8 months (Sleivert *et al*, 1995).

The cross-sectional area of muscle fibers has great variance among athletes (Costill *et al*, 1975). However, elite distance runners' slow twitch fibers were 29% larger than the fast twitch fibers. Both fiber types in trained individuals have a larger area than those in untrained individuals. Saltin (1973) proposed that endurance training results in selective hypertrophy of the slow twitch and fast twitch fibers, respectively. As a result of the relatively larger slow twitch fibers, in distance runners, the cross-sectional area of their muscle was composed of 82.9% ST fibers. Slow twitch fibers are said to be responsible for developing a large fraction of the tension needed during distance running, thus this high % of slow twitch fibers would be a decided advantage.

2.5.4. Quantification of training

The question of training intensity is an important one, when comparing men and women. Do the female athletes train at higher intensities to compensate for low hematocrit? Do they spend a proportionally greater time or a greater absolute time per week in higher heart rate zones? One needs to take various factors into consideration and the following questions arise:

i) Is the proportion of time spent in each heart rate zone for the entire week's training important for racing performance?

ii) Or is the total amount of time spent in each heart rate zone, thus focusing on the absolute time, in any particular zone important?

iii) Is it better to focus on individual sessions and attempt to match the heart rate profile for each session with a characteristic type of training session?

2.5.4.1. Training specificity

One of the major organizing principles in the training of competitive athletes is the principle of specificity, which states that a training program should stress the physiological systems that are critical for the optimal performance in the given sport (Hewson and Hopkins, 1996; Foster *et al*, 1995). Thus, athletes specializing in different events should have training programs focusing on their different needs. The training practices of 353 coached distance runners (119 female and 234 male) were evaluated by Hewson and Hopkins (1996). Their primary goal was to determine the relationships between specificity, training and performance. The athletes specialized in distances from 800 m to the marathon. They completed six-month retrospective questionnaires reporting typical weekly durations of interval training and strength training, and typical weekly durations and estimated paces of moderate and hard continuous running for build-up, pre-competition, competition and post-competition phases of the season. Hewson and Hopkins (1996) found, on analysis of the six-month training logs, that training programs showed some evidence for specificity, especially for runners preparing for longer events, who had a significant correlation between performance and seasonal mean weekly duration of moderate continuous running. However, no other significant relationships were found between training specificity and performance, allowing the conclusion to be made that the training of better athletes is not, as one would expect it to be, characterized strongly by greater specificity.

2.5.4.2. The use of heart rate to monitor training

The intensity at which an athlete trains is critical to his or her performance. Exercise intensities that are too low may not result in the desired training effect, while a program with too much high intensity training may cause overtraining (Kuipers and Keizer, 1988). Thus, a method to observe training intensity is important for athletes and their coaches and heart rate monitoring is often the most practical tool to use. The questions of a) whether there are other means of assessing training intensity and b) whether heart rate can serve as an indication of training intensities were the focus of the article by Gilman (1996).

The intensity of training is often based purely on the athlete's perception of the intensity, the training distance per week or the type of training. However, these methods of intensity assessment are very subjective and thus may not always provide an accurate assessment of the metabolic stress experienced by the athlete. In Gilman's study (1996), the athlete's maximum heart rate was determined during laboratory testing and three training zones were defined in this matter – easy, moderate and hard. The training sessions were then recorded and the heart rates during each session categorized into an intensity profile. This served as a useful tool for scientists, athletes and coaches to study training patterns of both successful and overtrained athletes and in the development of individualized programs.

HR monitoring of training intensity was also found to provide more accurate information than self-reports of training intensity (Gilman and Wells, 1993). Six women runners of varying running ability participated in the study, which consisted of three phases. The first was a graded exercise test to determine the heart rates corresponding to ventilatory threshold and 4mM of blood lactate (OBLA), the second involved the monitoring of two weeks of training using heart rate monitors and the third was an 8 km race, in which heart rate was again monitored. From the training data it was determined that more than 70% of the race was performed at higher intensities than the heart rate at OBLA (173 bpm). 8 km race intensity corresponded very closely with that at OBLA, but from the two weeks training monitoring it was determined that very little training time was spent at that running intensity.

Potteiger and Weber (1994) confirmed the fact that heart rate is a more valid marker of exercise intensity than the perception of intensity by the athlete.

The phenomenon of cardiac drift, which is an increased heart rate during exercise over time, is a factor that may limit the use of heart rate monitoring in training (Jeukendrup and Van Diemen, 1998). Heart rates drift upwards by as much as 20 beats per minute during exercise lasting 20-60 minutes, despite unchanged work rates and steady lactate concentrations. Cardiac drift is further increased by both exercising in a hot environment and dehydration. Thus, the relationship between heart rate and intensity is susceptible to fluctuation and the heart rates recorded during training may not simply be a reflection of muscular work. A further factor that influences heart rate is altitude. When an athlete exercises at a certain work rate in hypoxic conditions, the heart rate recorded will be elevated compared to the same work rate at sea level and normoxic conditions. Thus, the relationship between heart

rate and workload may sometimes be dissociated. However, in warm environments or at altitude, heart rate can still be used as an indicator of whole-body stress levels since, should they train at a particular speed, which in a thermoneutral environment would lead to a positive training effect, in a hot environment, this same speed may be a factor that could contribute to overtraining.

2.6. SUMMARY AND AIMS

The performance of 10 km is affected by numerous factors, and due to its intermediate distance successful competitive athletes at this distance need to optimise the balance between endurance and speed.

Female athletes have a lower hematocrit and VO_{2max} and higher percent body fat than their male counterparts. However, some well-trained female athletes, specializing in the 10 km event are capable of performing equally to the well-trained men competing over the same distance, despite these genetic disadvantages.
Very few comparative studies of the various physiological and training variables between the genders specializing in 10 km have been done and although the information available from marathon running and track running has some relevance, it cannot be directly applied to 10 km distance due to its part-speed, part-endurance nature.

We hypothesize that the female athletes compensate for their inherent disadvantages by having a higher training volume than the performance-matched males, a more optimal muscle fibre composition for 10 km running, by sustaining a higher fractional utilization of their VO_{2max} throughout the race and by being more economical than the male athletes. Due to the fact that the male and female athletes perform equally, we also hypothesize that the peak treadmill speeds of the female athletes are equal to those of the male athletes. The females are also hypothesized to spend a greater percentage of time training and racing at intensities closer to their heart rate maximum.

3. METHODS

3.1. SUBJECTS

Eight well-trained competitive female runners and eight well-trained competitive male runners, who were matched for race performance, participated in the study. All subjects were between 19 and 29 years old and all specialized in the 10 km event. All athletes were either part of a training group or were actively involved in a training program. All female athletes recruited had to comply with the inclusion criteria of a sub-48 minute 10 km performance. Several of the male athletes were initially recruited for a larger study on sub-elite 10 km runners and after a controlled 10 km time trial, any of them having a race time of >37 min, were excluded from that study and could be considered as participants in this study. Those males not recruited in this manner were recruited in the same manner as the female athletes, with special attention given to their 10 km personal best time so as to match the female runners as closely as possible. Prior to the start of the study, all subjects received both verbal and written explanations of the purpose, protocol and potential risks of the study, after which they signed consent forms to participate in the study (see appendix 1 for introductory explanation and consent forms). The study and consent forms had been approved by the Sub-Committee C (Medical School) Ethics Committee of the University of Stellenbosch. All sixteen subjects agreed to have a muscle biopsy, performed by a general practitioner experienced in this technique and agreed to complete all tests.

3.2. TESTING

Each athlete completed a questionnaire regarding his or her training and racing. The questionnaire served to provide a brief history of athletic involvement in each of the participants and provided their race history for the past year (see appendix 2).
The study was divided into three stages:

Stage 1: 7-day training monitoring
Stage 2: 10 km time trial (TT_{10})
Stage 3: Laboratory testing

3.2.1. Stage 1: 7-day training monitoring

The first stage of the study was to monitor the normal training of each participant for 7 consecutive days. This was done using both a training diary (see appendix 3) and heart rate monitoring. The study was done during the winter season with all subjects focused on road running.

3.2.1.1. Training diaries

Each athlete completed a seven day training diary recording all training sessions for that week, both running and non-running. The type of session, duration of session and distance covered (if appropriate) were noted. The athletes were instructed to train as usual with respect to quantity and type of training, leaving those days in which they did not train blank in the diary. The training was then quantified by adding up the distance and duration of each training session and categorising the various sessions.

3.2.1.2. Heart rate monitoring

On receiving the training diary, each athlete also received a downloadable heart rate monitor (Polar S610/S710, Polar electro, Kempele, Finland) and transmitter belt. During each training session recorded in the training diary, the heart rate monitor was worn and the athlete's heart rates for the session were recorded, at either 5 s or 15 s intervals, depending on the personalised settings of the watch, which was not standardised for this aspect. These heart rate files were stored by the receiver and downloaded via the infrared down-loader into the analysis software (Polar Precision Performance) on completion of the week.

3.2.2. Stage 2: 10 km time trial (TT_{10})

In order to match athletes for performance, each completed a 10 km time trial over the same route. The athletes' personal best times (PB) in the previous 12 months could not be used for matching as each athlete's personal best was in a different race, with different routes and training may have changed. The route was measured twice with a measuring wheel and each kilometre along the route was marked. A route of mixed terrain that included several steep hills was chosen. Heart rate monitors were worn for the duration of the race to record athletes' racing heart rates. Water was available for the athletes at 3 km and 7 km, to simulate race conditions as closely as possible. Time trials were held on four occasions to accommodate the time course of subject recruitment. Time trails were done in combination with another study so that the number of athletes racing against each other ranged from 6 to 38.

3.2.3. Stage 3: Laboratory tests

Each subject visited the laboratory on eight separate occasions, the first time for a muscle biopsy, anthropometry assessment and familiarisation, and the following seven for their exercise testing. The testing was performed over a period of 3 to 4 weeks for each athlete, with at least one day off between each test. The biopsy was performed the day after the completion of their 7-day training monitoring for each athlete and TT_{10} was within two weeks of their biopsy. The maximal and submaximal tests were completed by all athletes within the two weeks after the TT_{10}. The following tests were performed by each athlete:

VO_{2max} test: flat (2 separate tests)
VO_{2max} test: gradient (2 separate tests)
Submaximal test: flat
Submaximal test: gradient
10-minute race-pace test

3.2.3.1. Muscle biopsy and anthropometry

On the subjects' first visit to the exercise laboratory, they were familiarized with all equipment relevant to the project. They completed a short treadmill run in which they were instructed on the correct manner to get onto and off the treadmill. Hereafter they were assessed anthropometrically. The subjects were weighed (HD308 Scale, Tanita Corporation, South Africa) wearing lightweight running vest and lightweight running shorts. This body mass (in kg with 2 decimal place accuracy) was used to calculate body fat (Bale *et al*, 1985). Height was measured using a fixed wall-measure and anthropometric measurements were taken for each subject according to the procedure described by Behnke and Wilmore (1974). A standardised measuring tape was used to determine circumferences and a standardised calliper for taking skinfold measurements. All anthropometrical measurements were performed by an experienced anthropometrist (Level II registered, International society for anthropometrists and kinanthropometrists).

Skinfold measurements were taken at the following sites on each athlete:

Biceps: A vertical fold halfway between the shoulder and elbow joints, on the anterior midline of the upper arm
Triceps: A vertical fold halfway between the shoulder and elbow joints, on the posterior midline of the upper arm

Subscapular: A diagonal fold at the back, just below the inferior angle of the scapula, at a 45° angle

Mid-axilla: A vertical fold halfway at the level of the nipple, on the midaxillary line.

Iliac crest: A horizontal fold just above the iliac crest, in line with the level at which the midaxillary line intercepts the iliac crest.

Supraspinal: A diagonal fold just below the iliac crest, at the position where the anterior axillary line would come down, at about 30°.

Abdomen: A vertical fold 2.5 cm to the right of the umbilicus.

Front thigh: A vertical fold in the middle of the front thigh, halfway between hip and knee joint.

Medial calf: A vertical fold in the middle of the medial head of the gastrocnemius.

The circumferences measured were as follows:

Relaxed arm: Halfway between the shoulder and elbow joints, connecting the anterior and posterior midlines of the upper arm

Waist: The narrowest part of the waist, approximately four centimetres above the umbilicus

Gluteus: The widest part of the gluteus maximus and the hips.

Thigh: The middle of the thigh, halfway between the hip and knee joint

Calf: The medial head of the gastrocnemius.

Three measurements were taken at each site and the average was used to calculate % body fat according to the formulas recommended by Forsyth and Sinning (1973) for the male athletes and by Jackson *et al* (1980) for the female athletes.

Following the anthropometry, each athlete had a muscle biopsy. The procedure has been used since 1868 (Duchenne) for muscle pathology assessments and was re-popularised by Bergstrom (1962) and Edwards (1971) for Sport Science assessments. Prior to the procedure, the position of the biopsy was marked on the upper left thigh. The procedure was performed by a medical doctor. The samples of muscle were extracted using a trephine biopsy needle.

After sterilizing the area around the biopsy site (Betadine antiseptic solution, Adcock Ingram, Bryanston, South Africa) the athletes were given a local anaesthetic injection (Xylotox® L, Adcock Ingram LTD, Bryanston, South Africa), above the site to be biopsied. A small incision was then made through the skin, fat and fascia with a surgical blade (carbon steel surgical blade, Premier, UK). The biopsy needle was then introduced into the muscle, to a depth of ~ 5 centimetres.

Once the needle was in the leg, suction was applied using a 50 ml syringe and a small portion of muscle pulled into the window of the biopsy needle's barrel. The blade was then passed through the barrel, cutting off the small piece (20 to 40 mg wet weight, containing approximately 100 to 700 muscle fibres). The sample remained inside the barrel as it was extracted from the muscle. This piece was quickly dissected into smaller pieces and one of these pieces was oriented and mounted directly on a labelled cork disc and frozen in pentane cooled by liquid nitrogen to near-freezing point. The other samples were then frozen in liquid nitrogen and all were placed in the -80ºC freezer for later analysis.

The athlete's wound was cleaned using Friar's Balsam (Allied Drug Company, Durban, South Africa) and sealed using Steri-Strips (3M Health Care, Borken, Germany). It was then covered with a sterilized dressing (Tegaderm, 3M Health Care, Borken, Germany). A pressure bandage was wrapped around the thigh to inhibit bleeding. The athlete was instructed to remove the bandage after several hours and not to exercise for at least the following two days, and thereafter, at a low intensity for the next few days.

3.2.3.2.VO_{2max} test: flat

The maximal oxygen consumption test (VO_{2max}) was completed twice by each athlete. The VO_{2max} for each athlete was determined from whichever of the two tests provided the Peak treadmill speed (PTS). The test was a continuous, incremental protocol to subjective exhaustion on a treadmill (Technogym Runrace, Gambettola, Italy) with a 0% gradient. From this, each athlete's VO_{2max}, PTS and maximum heart rate (HR_{max}) were determined. After a 10 minute warm-up of self-selected intensity, the test commenced at 12 km/h for the female athletes and 14 km/h for the male athletes. The velocity of the treadmill was increased by 0.5 km/h every 30 seconds until exhaustion. PTS was calculated according to the time spent at the highest level.

Equation: Speed of last completed workload (WL) + (time at highest WL)*0.5
30 seconds

An on-line gas analyser system (Jaeger Oxycon Version 4.5, Hoechberg, Germany) was used to perform respiratory gas analyses continuously throughout both tests. The analyser was calibrated with gases of known composition prior to each exercise test. A mask was worn by the subjects during each test. The mask was fitted with a ventilometer that was calibrated simultaneously with the gas analyser system.

The system calculated oxygen consumption (VO_2), carbon dioxide production (VCO_2), minute ventilation (VE) and the respiratory exchange ratio (RER) at 10-s intervals. Heart rate (HR) was monitored using a transmitter belt (Polar) and a receiver, which was attached to the system. HR was averaged every 10 s and the highest 10-s average was recorded as the maximum heart rate (HR_{max}) achieved during the flat VO_{2max} test, which was used as the maximum heart rate for any further calculations.

3.2.3.3. VO_{2max} test: gradient

The gradient VO_{2max} test was also completed twice by each athlete. The same protocol as in the flat test was used but the gradient of the treadmill was 8% (5°) and the starting speed was 10 km/h for the female athletes and 12 km/h for the males. The computerised metabolic system was again used to determine the VO_2, VE, RER and HR.

3.2.3.4. Submaximal tests

Prior to the submaximal tests (both flat and gradient), an indwelling cannula (Jelco™ IV Catheter (22G), Johnson and Johnson Medical, Brussels, Belgium) was inserted in the subject's left forearm vein whilst the subject was supine on a bed. A 3-way stopcock (Brittan Healthcare, Isando, South Africa) was attached to the canula to allow for multiple sampling. The canula and stopcock were fastened to the arm using strips of tape (Transpore, 3M Health Care, Borken, Germany). A 3 ml resting sample was then drawn for lactate and hematocrit analysis and was put on ice. The athlete then warmed-up for 10 minutes at self-selected intensity prior to the commencement of the test.

The submaximal, discontinuous, incremental protocol consisted of 5 minute intervals at 64%, 72%, 80% and 88% of their individual PTS, determined from the best of the two flat VO_{2max} tests, separated by a minute rest, during which a 3 ml blood sample was drawn. Each sample was placed with the resting sample on ice until the end of the test. The test was performed at a 0% gradient and the athlete was encouraged to complete all workloads. The gas analyzer system performed respiratory gas analyses continuously throughout the test.

On completion of the test, five further 3 ml blood samples were drawn from the athlete at 3 min, 6 min, 12 min, 15 min and 18 min post-exercise for lactate analysis. Once all samples were collected, the lactate samples were centrifuged at 4°C at 3000 rpm (LC 1-K, Sarstedt, Nümbrecht), and plasma was obtained and frozen for later analysis, while the hematocrit was determined in triplicate (Micro-haematocrit centrifuge, Hawksley, England).

The same protocol used for the flat submaximal test was used for the gradient test, but the treadmill speeds were determined from the peak speed achieved during the better of the two gradient VO_{2max} tests. The test was, as in the case of the gradient VO_{2max} tests, performed at 8% gradient.

The blood samples drawn were again centrifuged and the plasma frozen for later analysis.

3.2.3.5. 10-minute race-pace test

After a thorough warm-up of self-determined intensity, the athletes ran at their 10 km race pace, determined from their TT_{10}, for 10 minutes on the treadmill at 0% gradient. Heart rates, oxygen consumption and respiratory exchange ratios were measured by the gas analyser for the duration of the test. Fractional utilisation of maximum VO_2 was calculated using the VO_{2max} of the 2nd flat test.

3.3. BIOCHEMICAL ANALYSES

3.3.1. Muscle biopsy

The sample previously frozen in liquid nitrogen was freeze-dried overnight (Alpha 1-4, LCD-1, Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and used to determine the citrate synthase activity of the muscle. The sample

previously mounted and frozen in chilled pentane was used for fibre typing and determination of the cross-sectional area of the muscle fibres.

3.3.2. Citrate synthase activity

Each sample was dissected, at -20°C, to remove any visible remaining connective tissue or blood and to obtain a sample of approximately 1 mg. The sample was then weighed using an electronic microbalance (Sartorius, Germany) to determine the exact mass in mg to three decimals. Homogenizing buffer (see appendix 4) was then added to each sample at a ratio of 1:90 for dry weight and the sample was vortexed and put onto ice. Each sample was then sonicated while held semi-immersed in a beaker of ice and water for six second intervals (VirSonic 300, The Virtis Company, Inc., Gardiner, New York, USA), with two second rest intervals, until all visible traces of muscle had disappeared, whereafter the sample was placed back on ice.

The citrate synthase activity assay was performed according to the method described by Srere (1969). The absorbance was read every 30 seconds for 5 minutes at a wavelength of 412 nm, yielding a linear curve (FLX800 Microplate Fluorescence Reader, Bio-Tek Instruments, Inc., Winooski, USA) and the citrate synthase activity determined as µmol/min/g dry-weight (for details of method see appendix 4). Following this procedure, any remaining connective tissue in the muscle homogenate was removed, dried overnight and weighed and the results gained for the assay were corrected accordingly. To determine the activity as µmol/min/g of protein a sample of the muscle homogenate was also assayed for protein content using the method of MM Bradford (1976) (EL800 Universal Microplate Reader, Bio-Tek Instruments, inc., Winooski, USA) the absorbance was read at 595 nm (for details of method see appendix 5).

3.3.3. Fibre typing

In order to determine the fibre type composition for each athlete, the samples were analysed using the mATPase staining protocol previously described by Padykula and Herman (1955) and Wattenburg and Long (1960).

The muscle samples were sectioned, at -20°C, using a cryostat (Leica GM1100, Leica Instruments, Nussloch, Germany). The cork block was attached to the chuck with tissue freezing medium (Jung, Leica Instruments, Nussloch, Germany) and left for about 10 minutes to adjust to the temperature of the cryostat. Sections were 10 µm each and were placed on a slide, previously coated with poly-L-lysine.

Three slides were prepared per athlete, one for each pre-incubation solution (pH4.3, pH4.6 and pH10.3). A 10μm slice was placed in the upper corner of each slide and the completed slides stored overnight in copelin jars at 4°C. In order to be able to compare the various different pH slides for each athlete, when cutting the muscle, the consecutive muscle slices were put onto the three slides. The solutions for the following day's staining were prepared.

The following day the pHs of the three solutions were checked prior to starting using a pH meter (Microprocessor pH/mV/°C meter, Hanna Instruments 8417, Singapore).

Once the staining procedure was completed (for details see appendix 6), the slides were allowed to dry and fixed with glycerine gelatine.

The slides were then viewed at 4x and 10x magnifications (Nikon Eclipse E400, Japan). Two areas per sample were matched per slide and these areas were photographed at the 10x magnification. The three different fibre types stained different colours due to the staining protocol, enabling the fibres to be categorized as slow twitch (Type I), fast twitch oxidative-glycolytic (Type IIA) or fast twitch glycolytic (Type IIX) and the photographs of the fibres were used to count the different types of fibres present in each sample. At least 200 fibres were counted per slide. The photographs were analysed by four assessors each to ensure accuracy and the co-efficient of variance between the assessors was found to be 16.3 %.

The cross sectional areas of the fibres were determined using high performance imaging software (Simple PCI Version 4.0, C.Imaging Systems, Cranberry Townships, USA). A general 'macro' was designed in which the fibres from which the average cross-sectional area should be calculated were marked. The program then blocked out the fibres that did not conform to certain specifically set requirements (the fibres that had not stained clearly or those whose borders were not clear) and the average fibre cross-sectional area was calculated. This process was followed both for Type I and Type II fibres, with no distinction made between Type IIA and Type IIX fibres.

3.3.4. Lactate samples

The plasma lactate concentrations of each athlete's samples were supposed to be determined using an electroenzymatic technique with an automatic analyser (YSI

1500 Sport Lactate Analyser, YSI Inc., Yellow Springs, Ohio). This analyser is usually calibrated prior to each test using standard solutions of known lactate concentrations. Unfortunately, due to moving buildings, the cable was lost and the new cable has not arrived yet from overseas.

3.4. DATA ANALYSIS

3.4.1. Training diary analysis

Seven consecutive days of typical training were analyzed from the training diaries completed by each athlete for that week and the heart rate files recorded for the sessions. Training sessions were classified according to their intensity and terrain: long slow run, high intensity training (HIT) (track, high intensity road training) or hills, and non-running sessions. The number of sessions, distance completed, and duration of running training were calculated.

3.4.2. Heart rate data analysis

The heart rate data was downloaded from the watches using the infrared down loader into the software program (Polar Precision Performance, Polar, Finland) and percentage of time in each HR zone was calculated for each session, along with the percentage of weekly training time in each HR zone.

3.4.3. Statistical analysis

The statistical data analysis was performed using the program Statistica (Version 6, Statsoft Inc, Tulsa, OK, USA). The differences between genders with respect to physiological and training data were analysed using non-parametric Mann-Whitney U-Tests. Differences within the gender groups were analysed using parametric paired t-tests. Correlations between the variables for each of the groups were determined using the non-parametric Spearman rank order correlations. Results are presented as mean ± standard deviation. Statistical significance was set at either $p < 0.05$, $p < 0.01$ or $p < 0.005$.

4. RESULTS

SECTION 1: SUBJECT CHARACTERISTICS AND LABORATORY TESTS

4.1. Subject characteristics. Significant differences between the female and male athletes were only found for body mass ($p < 0.01$) and fat free mass ($p < 0.005$) (Table 1), both of which were significantly higher in the male athletes. Both height and hematocrit tended to be higher in male than female athletes ($p = 0.058$ and 0.059 respectively). Percentage body fat tended to be higher in the female athletes ($p = 0.058$). There were no differences in the ages and 10 km personal best times of the athletes of opposite gender. The small sample number for each gender may explain the lack of significance, therefore trends between groups for several of the variables will be presented.

Table 1: Subject characteristics

	Females		Males	
	Mean ± SD	Range[¤]	Mean ± SD	Range[¤]
Age (yr)	22.2 ± 3.3	19 - 29	21.9 ± 1.1	20 - 23
Height (cm)	170 ± 9[c]	159 - 188	179 ± 6	169 - 184
Body mass (kg)	57.8 ± 9.9[b]	43.5 - 69.5	71.8 ± 8.5	54 - 83.5
% Body fat	19.0 ± 4.6[c]	9.6 - 23.4	14.2 ± 4.3	9.8 - 21.6
Fat free mass (FFM) (kg)	46.5 ± 6.5[a]	36.3 - 53.2	61.5 ± 6.8	47.8 - 68.1
Resting hematocrit	31.64 ± 2.17[c]	26.8 - 34.3	36.81 ± 1.87	34.5 - 39.5
10 km PB (min)	40.72 ± 2.33	37.18 - 43.00	39.43 ± 3.17	34.00 - 44.63

Abbreviations: PB, personal best

[¤] Minimum to maximum

[a] $p < 0.005$; Mann-Whitney U-test

[b] $p < 0.01$; Mann-Whitney U-test

[c] trend: $p < 0.06$

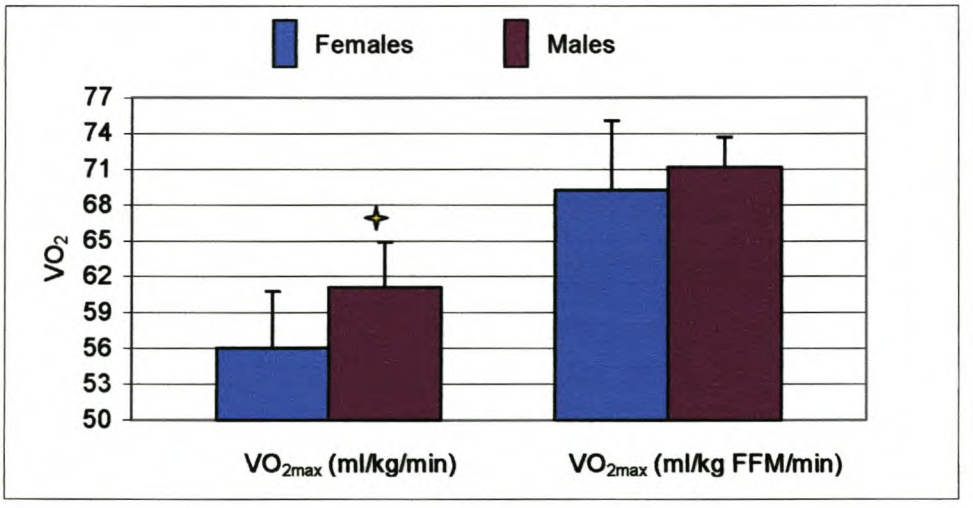
4.2. TT_{10} times. The individual results achieved for the controlled 10 km time trial (TT_{10}) are recorded in Table 2. The race times are not significantly different between the male and female athletes ($p > 0.05$) and within each group a paired t-test showed that TT_{10} versus 10 km PB times were not significantly different ($p > 0.05$). Each female athlete was matched to a male athlete as closely as possible. The matched athletes are indicated by corresponding numbers (1 - 8) for the fastest to slowest in each group and for all comparative aspects of the study, these matches were used.

Table 2: Individual results for TT_{10}, matching female and male athletes

Females		Males	
Subject	Time (min)	Time (min)	Subject
1	38.92	38.87	1
2	39.15	39.55	2
3	40.77	39.82	3
4	41.98	41.85	4
5	42.87	42	5
6	43.78	43.46	6
7	44.93	43.53	7
8	47.75	43.7	8
Mean	42.52	41.99	Mean
SD	± 3.00	± 1.74	SD

4.3. Athlete responses to treadmill tests.

4.3.1. Athlete responses to maximal tests. As each athlete completed two of each of the flat and gradient tests, the highest results achieved in either of the tests was used. Figure 1 indicates VO_{2max} from the flat test. Although significant differences were seen between the female and male athletes for flat VO_{2max}, once this value was corrected for body fat and expressed per kg of fat free mass, there was no longer a difference between the genders.



✦ significantly different from female athletes at $p < 0.05$

Figure 1: Maximal oxygen consumption relative to body mass and FFM

Within the gender groups, paired t-tests were used to determine whether a significant difference existed between the VO_{2max} (ml/kg/min) of the flat test and that of the gradient test. No difference was found in either gender ($p > 0.05$)

Table 3 presents the remaining results of the female and male athletes for both the flat and gradient tests. The data indicate that the difference between the genders for peak treadmill speeds (flat and gradient) was at a more significant level than the differences in VO_{2max}.

Table 3: Summary of results from flat and gradient VO_{2max} tests

	Females		Males	
	Mean ± SD	Range	Mean ± SD	Range
Flat PTS (km/h)	17.85 ± 0.87[a]	16.5 - 19.0	19.69 ± 0.87	18.6 - 20.9
Flat RER_{max}	1.17 ± 0.03	1.12 – 1.20	1.19 ± 0.02	1.15 – 1.23
Gradient PTS (km/h)	12.83 ± 0.67[a]	12 – 14	14.82 ± 0.48	14 - 15.5
Gradient RER_{max}	1.23 ± 0.05	1.16 – 1.31	1.26 ± 0.06	1.17 – 1.36
Gradient VO_{2max} (ml/kg/min)	55.1 ± 4.7[b]	47.6 - 60.75	61.7 ± 2.5	58.2 - 64.7
Gradient VO_{2max} (ml/kg FFM/min)	68.1 ± 6.5	59.8 – 77.9	72.0 ± 3.0	66.5 – 76.3
Flat heart rate maximum (bpm)	189 ± 1[b]	187 – 190	196 ± 5	188 - 206
Gradient heart rate maximum (bpm)	186 ± 2[c]	184 – 189	193 ± 6	184 - 203

PTS, peak treadmill speed

[¤] Minimum to maximum

[a] Significantly different from male athletes, $p < 0.005$

[b] Significantly different from male athletes, $p < 0.01$

[c] Significantly different from male athletes, $p < 0.05$

4.3.2. Athlete responses to economy tests. The results from the submaximal test were used to determine both the female and the male runners' economy at 14 km/h (using the data from the flat submaximal test) and 9.5 km/h (using the data from the gradient submaximal test). The results are summarized in Table 4. No significant difference between genders was found for any of the variables ($p > 0.05$).

Table 4: Economy of athletes as determined from submaximal tests (14 km/h from flat test, and 9.5 km/h from gradient test)

	Females		Males	
	Mean ± SD	Range	Mean ± SD	Range
VO_2 at 14 km/h (ml/kg/min)	52.58 ± 4.02	47.0 - 60.3	54.49 ± 4.26	49.2 - 61.8
VO_2 at 9.5 km/h (ml/kg/min)	52.23 ± 4.83	40.8 - 56.3	55.32 ± 3.17	50.3 - 60.73

Statistical analysis: not significant ($p > 0.05$)

The relationships between both VO_2 at 14 km/h and 9.5 km/h and TT_{10} performance in male runners were found to be non-significant (VO_2 at 14 km/h: $r = -0.02$; VO_2 at 9.5 km/h: $r = -0.05$). The associations in female athletes between these variables were also non-

significant (VO_2 at 14 km/h: r = -0.07), although there was a trend in females towards a relationship between TT_{10} performance and VO_2 at 9.5 km/h (r = -0.60, p < 0.12).

4.3.3. 10-minute race-pace results. The first four minutes of the race-pace test were not analysed, as the athletes had not reached steady state by this stage. As the subjects were matched for performance, there was no significant difference between the average race-pace of the female and male athletes (p < 0.05). The oxygen consumption, heart rate and respiratory exchange ratios for the third, fourth and fifth fifths of the test were determined and there was no significant difference between any of the variables over any of the times between the genders (p > 0.05). As there is indeed a significant difference between the maximal oxygen consumption obtained by the genders in their maximal tests, the lack of significance in the 10-minute race-pace test would suggest that the female athletes race at a higher percent of their VO_{2max}, however, after averaging the oxygen consumption of the 10 km race-pace test to achieve a 'race' VO_2 and determining the fractional utilization for both males and females, it was found that there is no significant difference (p > 0.05). Table 5 is the race-pace test summary.

Table 5: 10-minute race-pace test summary

		Females		Males	
		Mean ± SD	Range[¤]	Mean ± SD	Range[¤]
	Race pace (km/h)	14.18 ± 0.96	12.6 - 15.4	14.45 ± 0.69	13.7-15.4
Min 5 & 6	VO_2 (ml/kg/min)	51.88 ± 6.65	37.7 - 59.9	54.60 ± 4.06	45.85 - 60.25
	Heart rate (bpm)	179 ± 5.83	172 - 187	185 ± 7.36	174 - 194
	RER	1.017 ± 0.041	0.978 – 1.057	1.018 ± 0.020	0.979 - 1.038
Min 7 & 8	VO_2 (ml/kg/min)	51.3 ± 8.83	32.6 - 59.2	55.17 ± 3.81	47.40 - 60.10
	Heart rate (bpm)	182 ± 5.36	175 - 190	186 ± 7.39	173 - 197
	RER	1.01 ± 0.03	0.975 - 1.057	1.006 ± 0.018	0.967 - 1.02
Min 9 & 10	VO_2 (ml/kg/min)	50.61 ± 9.53	32.0 - 59.6	55.20 ± 4.62	46.25 - 60.95
	Heart rate (bpm)	184 ± 5.15	178 - 193	189 ± 7.49	175 – 199
	RER	1.01 ± 0.03	0.96 - 1.041	1.002 ± 0.025	0.965 - 1.015
	"Race" VO_2 (ml/min/kg)	51.27 ± 8.27	34.1 - 59.6	54.99 ± 4.09	46.5 - 60.2
	Fractional utilization of VO_{2max} (%)	91.05 ± 9.80	71.06 - 102.79	90.42 ± 5.76	80.76 - 97.49
	"Race" HR (bpm)	182 ± 5	176 - 190	187 ± 7	174 – 197
	Fractional utilization of HR_{max} (%)	96.23 ± 3.19	92.4 - 101.4	95.28 ± 2.82	88.9 - 98.2
	Race pace (km/h)	14.2 ± 0.96	12.6 – 15.4	14.5 ± 0.69	13.7 – 15.4
	Fractional utilization of PTS (%)	79.4 ± 2.7[a]	75.4 – 82.4	73.4 ± 1.6	70.8 – 76.3

[¤] Minimum to maximum

[a] Significantly different from male athletes, p < 0.005

The VO_2, HR and RER variables attained by the female athletes at each stage of the submaximal test showed no significant differences to the corresponding variables in the

male athletes. The fractional utilization of flat PTS, however, was significantly different between genders ($p < 0.005$). Thus, when racing, the female athletes are running at a much higher relative intensity than their male counterparts.

4.3.4. Relationships between subject characteristics, field test and laboratory performance. TT_{10} performance was correlated with the subject characteristics in Table 1 and performance variables in Table 3 for both the female athletes and the males. As described in more detail below, several of the relationships seen between the various variables and TT_{10} performance were similar between the genders, whereas others were very different with male athletes showing more significant correlations than females.

The only strong correlation for the female runners, which was also strong for the males, was between flat peak treadmill speed and TT_{10} ($r = -0.78$, $p < 0.05$ and $r = -0.92$, $p < 0.005$ for females and males respectively). The flat VO_{2max} for both genders showed no significant association with TT_{10}, although for the female athletes a trend was identified (females: $r = -0.60$, $p = 0.12$; males: $r = -0.26$).

The male athletes also showed a good correlation between personal best 10 km time and TT_{10} performance ($r = 0.88$; $p < 0.005$), which was not the case for the women athletes ($r = 0.50$). Good correlations also occurred in male athletes between gradient PTS and TT_{10} ($r = -0.77$, $p < 0.05$) and between gradient VO_{2max} and TT_{10} ($r = -0.81$, $p < 0.01$), with no association being present in either case for the female runners (gradient PTS: $r = -0.56$; gradient VO_{2max}: $r = -0.24$, respectively). Percentage body fat in male athletes was also associated with TT_{10} ($r = 0.74$, $p < 0.05$), which was again not the case in female athletes ($r = 0.47$).

Further trends were identified in the female runners between fat free mass and TT_{10} ($r = 0.67$, $p = 0.07$) and hematocrit (Hct) and TT_{10} ($r = -0.67$, $p = 0.07$); these trends were not identified in the male athletes (FFM: $r = -0.14$; Hct: $r = 0.05$).

The relationships between fractional utilization of HR_{max} and TT_{10} in the male and female athletes were non-significant, with that for the female athletes' showing a tendency towards a negative correlation ($r = -0.50$ for males and $r = -0.60$, $p < 0.12$ for females). The fractional utilization for maximum heart rate during the submaximal testing showed no association, in either the male or the female athletes, with TT_{10} performance.

SECTION 2: TRAINING ANALYSIS

4.4. Weekly training summary. Training diaries, in which the training on seven consecutive days was reported, were kept by each athlete. The mean training distance per week, as well as the number of high intensity sessions and hill training sessions per week of the monitored training are reported in Table 6. Any running sessions not classified as HIT sessions or hill training were long, slow distance runs. The training sessions other than running were gym, cycling, basketball or swimming. There were significant differences both in the distance run per week ($p < 0.05$) as well as in the duration of training per week ($p < 0.01$), with the female athletes training more with respect to both variables. The female athletes also showed a trend of having a greater number of running sessions per week ($p = 0.057$). However, the average speed per kilometre of training was similar in both groups.

Table 6: Summary of weekly training reduced from the 7-day training diaries

	Females		Males	
	Mean ± SD	Range[¤]	Mean ± SD	Range[¤]
Running sessions/wk	6 ± 1[c]	5 - 7	5 ± 2	4 - 8
Distance (km/wk)	69 ± 16[b]	45 - 87	43 ± 20	18.2 - 75
Duration/week (min/wk)	368 ± 90[a]	210 - 464	219.0 ± 86.4	95 - 357
Ave. speed per kilometres (min/km)	5.35 ± 0.37	4.66 - 5.78	5.47 ± 0.95	4.35 - 6.56
Ave. speed of training (km/h)	11.26 ± 0.82	10.38 – 12.86	11.69 ± 1.43	9.14 – 13.79
HIT sessions/wk	2 ± 1	0 - 3	1 ± 1	0 - 2
Hill training sessions/wk	1 ± 1	0 - 3	0 ± 1	0 - 1
HIT sessions + hill sessions	3 ± 2	0 - 5	1 ± 1	0 - 3
Exercise sessions/wk other than running	1 ± 1	0 - 3	1 ± 2	0 - 4

HIT, high intensity training

[¤] Minimum to maximum

[a] Significantly different from male athletes, $p < 0.01$

[b] Significantly different from male athletes, $p < 0.05$

[c] trend: $p < 0.06$

4.5. Relationships between training and TT_{10} performance or VO_{2max}

4.5.1. Training and TT_{10}. TT_{10} performance had no significant correlation with any training variable in either gender (Table 7). However, the combination of HIT and hill sessions per week showed a trend to relate to performance in female runners ($r = -0.63$, $p = 0.09$), which was not the case for the male runners ($r = -0.25$).

Table 7: Correlation coefficients for training variables in relation to TT_{10}

	r-values (p-values)	
	Females	Males
Sessions/week	-0.48	-0.46
Distance (km/wk)	-0.02	-0.33
Duration (min/wk)	0.05	-0.24
Ave Speed (min/km)	0.05	0.19
HIT (sess/wk)	-0.42	-0.19
Hill (sess/wk)	-0.56	-0.17
HIT + hill (sess/wk)	-0.63 (p=0.09)	-0.25
Other (sess/wk)	-0.16	-0.45

4.5.2. Training and VO_{2max} variables. In both male and female athletes, several good correlations occurred between training variables and those of the VO_{2max} tests, however, in each gender the good relationships were seen between different variables.

In the female athletes a strong association was seen between gradient PTS and the number of hill sessions per training week ($r = 0.88$, $p < 0.005$) and between gradient PTS and the combined number of hill and HIT sessions ($r = 0.77$, $p < 0.05$); these associations were not present in the male athletes (hill sessions: $r = 0.34$; HIT + hill: $r = 0.11$). However, gradient PTS was significantly related to the number of running sessions per week for the male athletes ($r = 0.75$, $p < 0.05$), an association that was not present in the female runners ($r = 0.26$).

Further significant correlations were seen in the female athletes between HR_{max} and the number of sessions per week, the distance run in the week and the duration of training (Sessions/wk: $r = 0.81$, $p < 0.01$; distance: $r = 0.82$, $p < 0.01$; duration: $r = 0.82$, $p < 0.01$), none of which were significant correlations in the males, although there was a trend

between the duration of training and maximum heart rate in the males (sessions/wk: r = -0.41; distance: r = -0.57; duration: r = -0.63, p = 0.09).

In the male athletes significant associations were also seen between flat VO_{2max} and average training speed (r = 0.74, p < 0.05), and HR_{max} and number of sessions per week of training other than running (r = -0.92, p < 0.001), all of which were non-significant correlations for the female runners (r = 0.17, r = 0.26 and r = -0.5, respectively)

4.6. Indications of individual differences in heart rate data from weekly monitoring. The heart rates for each athlete were analyzed, per session and over the whole week, into heart rate (HR) zones, taking the maximum heart rate reached in the better of the two VO_{2max} (Flat) tests as the athlete's maximum heart rate.

Heart rate data for the two genders for the week are presented in Table 8, first with HR divided into 3 zones and then expanded into 10 zones. In each gender the data is presented expressed relative to total time for the week (percentage of time) as well as the absolute time.

Table 8: Mean time, relative and absolute, spent in each HR zone during training week by each gender

	Percent of Time		Absolute Time	
HR Zones	Females	Males	Females	Males
Easy	36.9 ± 12.6	36.7 ± 14.1	140.6 ± 60.0[a]	80.9 ± 43.4
Moderate	33.0 ± 11.3	32.6 ± 10.4	128.0 ± 50.7[a]	76.5 ± 37.4
Hard	30.1 ± 14.1	30.7 ± 17.7	107.5 ± 41.0	77.8 ± 58.2
<60%	15.4 ± 8.79	8.53 ± 8.26	56.1 ± 33.4[a]	21.9 ± 25.3
60-69%	8.42 ± 4.77	11.6 ± 4.34	32.3 ± 20.8	26.0 ± 13.4
70-74%	13.1 ± 10.6	16.5 ± 12.5	52.2 ± 42.7	33.0 ± 21.9
75-79%	16.0 ± 9.01	18.2 ± 13.9	62.7 ± 37.3	39.1 ± 28.3
80-84%	16.9 ± 6.44	14.4 ± 6.86	65.4 ± 28.1	37.3 ± 28.4
85-89%	13.9 ± 5.63	17.7 ± 12.4	51.7 ± 23.1	46.0 ± 37.7
90-94%	8.78 ± 6.97	10.1 ± 6.81	30.6 ± 22.2	26.3 ± 20.7
95-100%	6.78 ± 6.21	1.58 ± 1.21	22.8 ± 16.7[a]	4.31 ± 3.44
100% +	0.31 ± 0.61[a]	1.28 ± 3.60	2.4 ± 3.23[a]	1.25 ± 3.46

[a] Significantly different from male athletes, p < 0.05

Individual variation in the data will be presented below in 4 different ways:

i) Percentage of *weekly* training time in each of 10 HR zones

ii) Percentage of *weekly* training time in each of 3 HR zones

iii) Absolute time of *weekly* training in each of 10 HR zones

iv) Absolute time of *weekly* training in each of 3 HR zones

Figure 2a and 2b represent the relative time each athlete spent in each zone over the week. The performance-matched male and female athletes, matched for TT_{10}, are indicated by corresponding numbers and colours, with athletes ranked from best to worst TT_{10} (1-8).

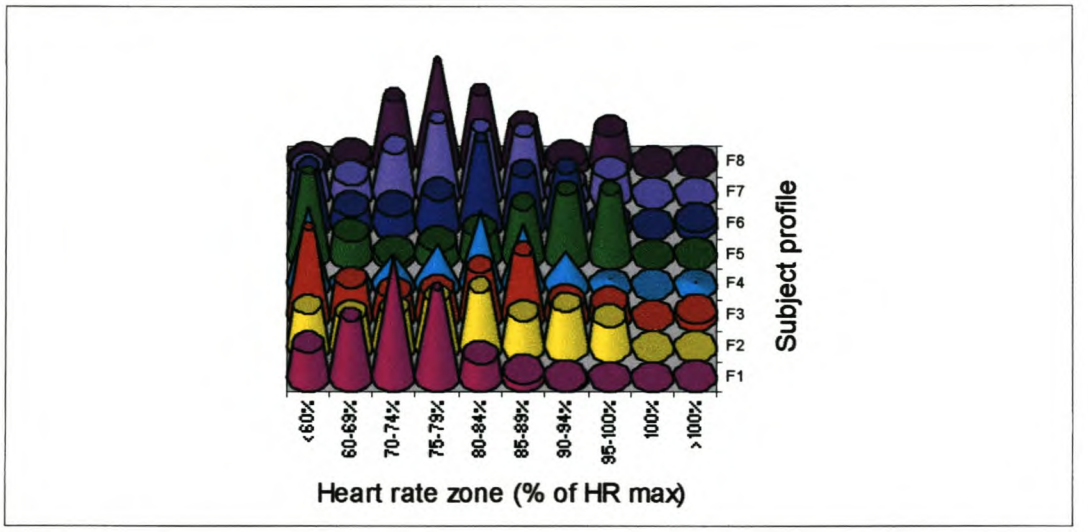


Figure 2a: Percentage of female athletes' weekly training time spent in each heart rate (HR) zone.

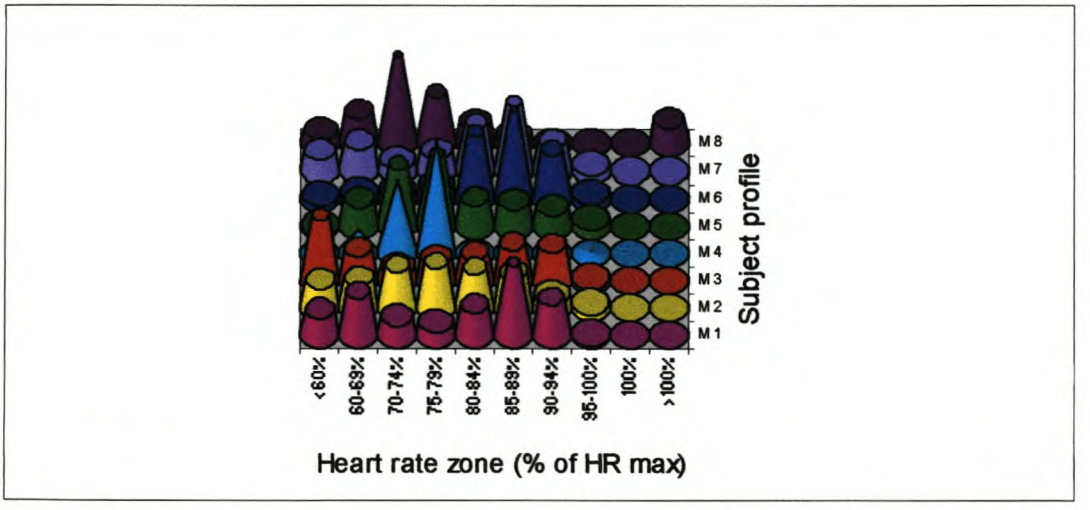


Figure 2b: Percentage of male athletes' weekly training time spent in each heart rate (HR) zone.

Heart rates from training sessions can also be separated into the more commonly used heart rate zones which describe the intensity of the session: easy, moderate and hard. The divisions for these three zones are as follows: <74% is EASY, 75-84% is MODERATE and >85% is HARD. Figure 2c shows the relative time spent by each athlete in these zones during the seven consecutive days of training.

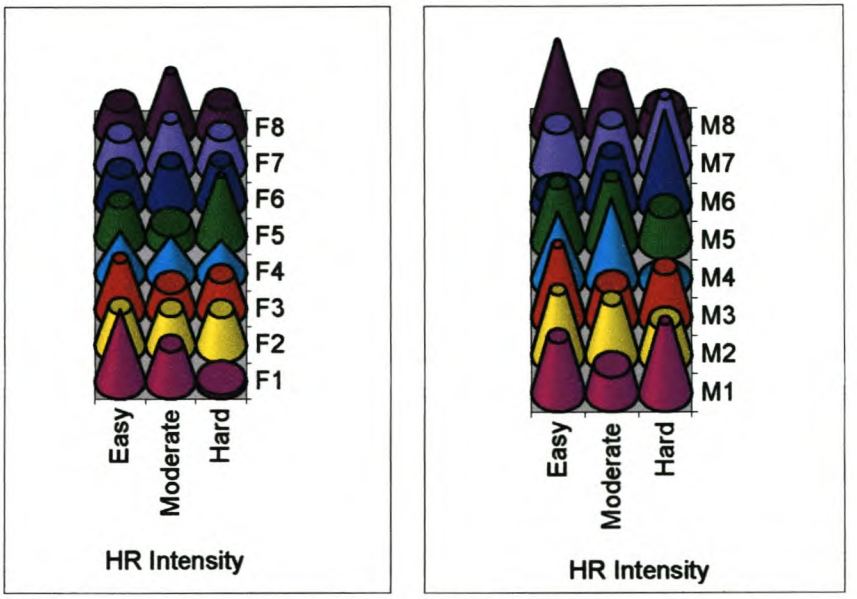


Figure 2c: Percentage of athletes' weekly training spent in HR intensity zones

Figure 3a summarizes the absolute time spent by each athlete in the three HR intensity zones during the seven consecutive days of training monitoring.

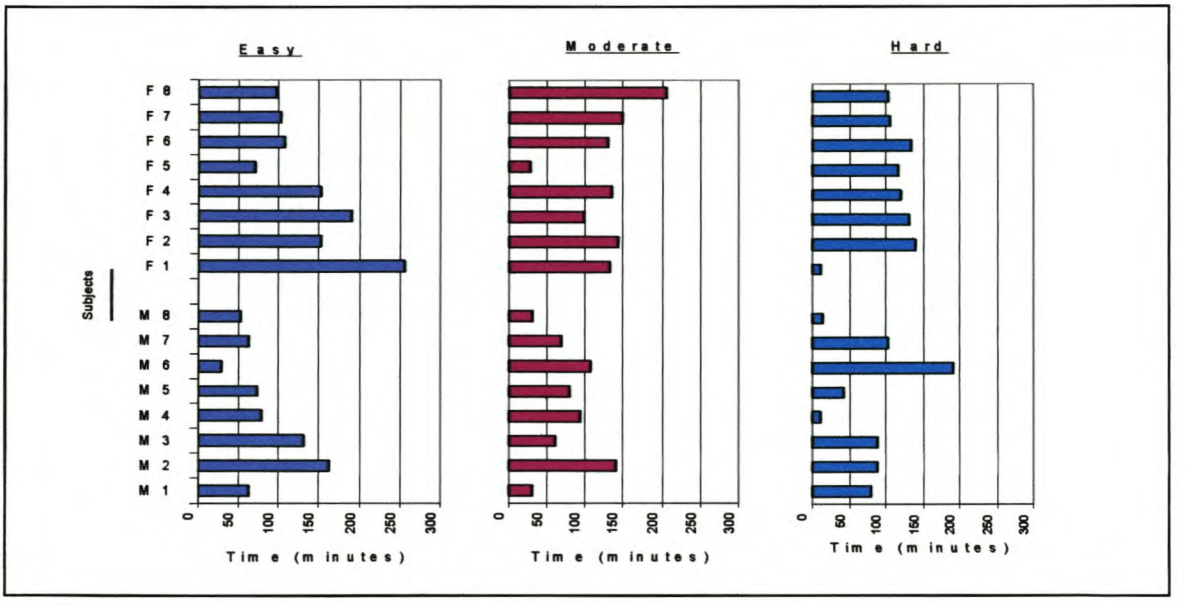


Figure 3a: Absolute time spent by each of the athletes in the easy, moderate and hard HR Zones for 7 consecutive training days

Figure 3b and 3c represent the same data but in absolute time, illustrating the total time spent training as well as the absolute time spent at various heart rate intensities during this time. In these figures, the performance-matched male and female athletes are also indicated by corresponding numbers (1-8).

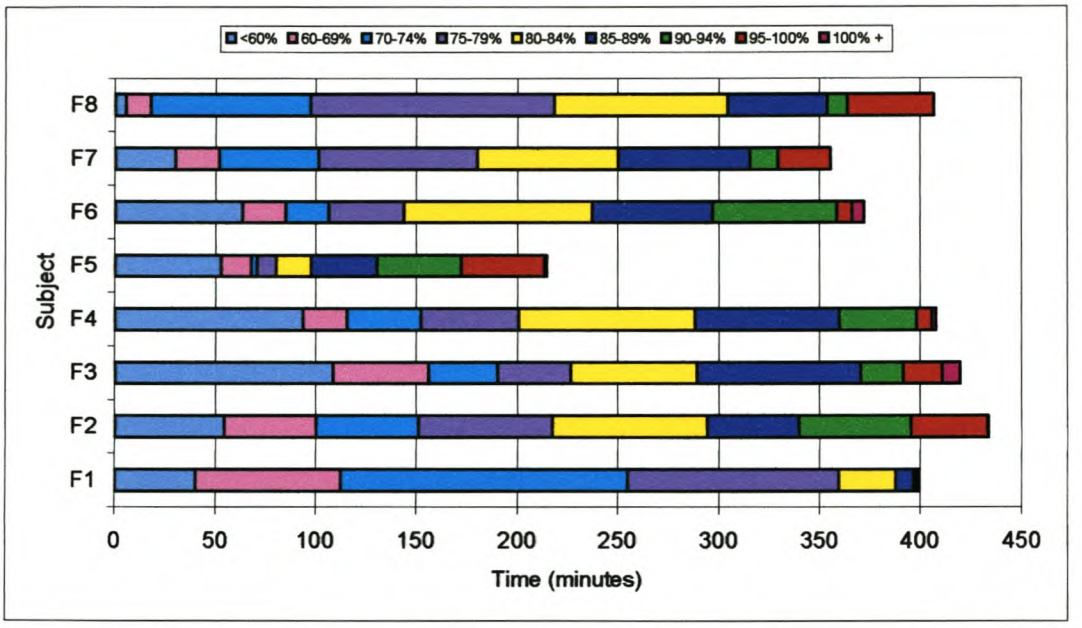


Figure 3b: Absolute time spent in each HR Zone over the weeks training by each of the female athletes

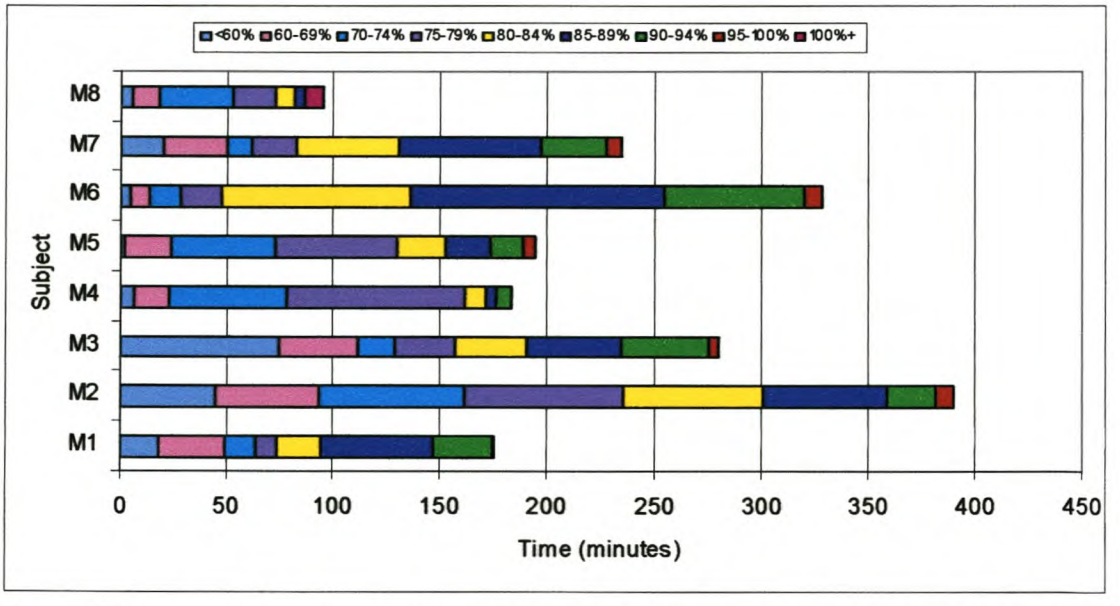


Figure 3c: Absolute time spent by each of the male athletes in each HR Zone for 7 consecutive training days

4.6.1. Relationships between the individual differences in time spent in various heart zones and performance

Better performance in the TT_{10} (i.e. lower race time) was related to more absolute training time spent at 69-69% HR_{max} zone in men ($r = -0.71$, $p < 0.05$) and women runners ($r = -0.81$, $p < 0.05$), as well as the corresponding EASY zone in women runners ($r = -0.70$, $p < 0.05$). Neither VO_{2max} nor PTS correlated with the absolute time spent in any HR zone. However, economy (VO_2 at 14 km/h, expressed as ml/kg/min) was associated with training intensity in the following ways: male runners who spent more time in the 90-94 % HR_{max} zone were less economical (higher VO_2) ($r = 0.71$, $p < 0.05$); in contrast, more time spent by women runners in the moderate intensity zones was associated with poorer economy (MOD HR zone: 0.79; 70-74 % HR max zone: $r = 0.76$ and 75-79 % HR max zone: $r = 0.79$, all $p < 0.05$). Fractional utilization of VO_{2max} at race pace was associated with the amount of training in the EASY zone for both men ($r = 0.71$, $p < 0.05$) and women runners ($r = 0.81$, $p < 0.05$).

When training time in each zone was expressed relative to each subject's total training time, thus reflecting the way in which the programmes were structured, there was no pattern in the data for men with only one significant correlation (VO_{2max} vs. relative time spent at 75-79 % HR_{max} zone: $r = -0.71$, $p < 0.05$). However, interesting patterns emerged from the women runners' data: more time spent in the 60-69% HR_{max} zone was associated with faster TT_{10} ($r = -0.79$, $p < 0.05$), higher PTS ($r = 0.71$, $p < 0.05$), higher VO_{2max} ($r = 0.79$, $p < 0.05$), higher fractional utilization of VO_{2max} at race pace ($r = 0.71$, $p < 0.05$), but lower economy in the gradient test ($r = 0.83$, $p < 0.05$). Time spent in the middle zones was positively related to VO_{2max} ($r = 0.78$ and 0.74, $p < 0.05$, for zones 70-74 and 75-79 % HR_{max} respectively), but negatively associated with fractional utilization of VO_{2max} at race pace ($r = -0.74$, $p < 0.05$). More time spent between 80 and 84% of HR_{max} was associated with a better economy running at 8% gradient ($r = -0.83$, $p < 0.05$).

SECTION 3: TT_{10} HEART RATES

4.7. Heart rates during TT_{10}. The percentages of time spent in each HR zone during the TT_{10} are recorded in Figure 4a for the female athletes and Figure 4b for the males. Due to a technical error with one of the heart rate monitors used on the day of TT_{10}, the heart rate file for F4 was blank and thus is not included in Figure 4a. It is clear that both male and female athletes spent the greater part of running time very close to their maximum heart rate, indicating that they indeed treated TT_{10} as a race.

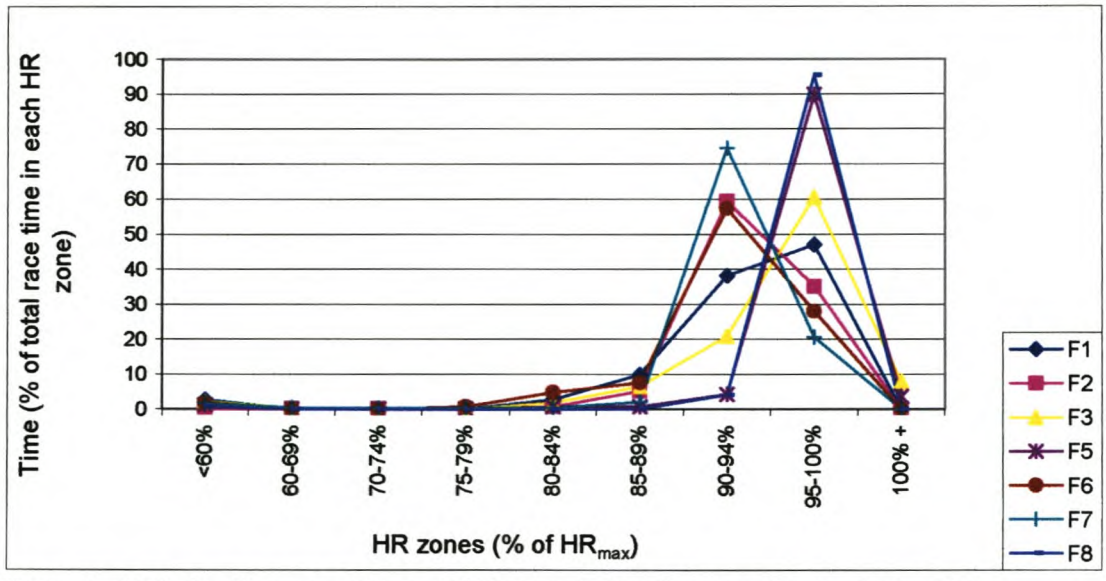


Figure 4a: Relative time spent in each HR zone by the female athletes during TT_{10}

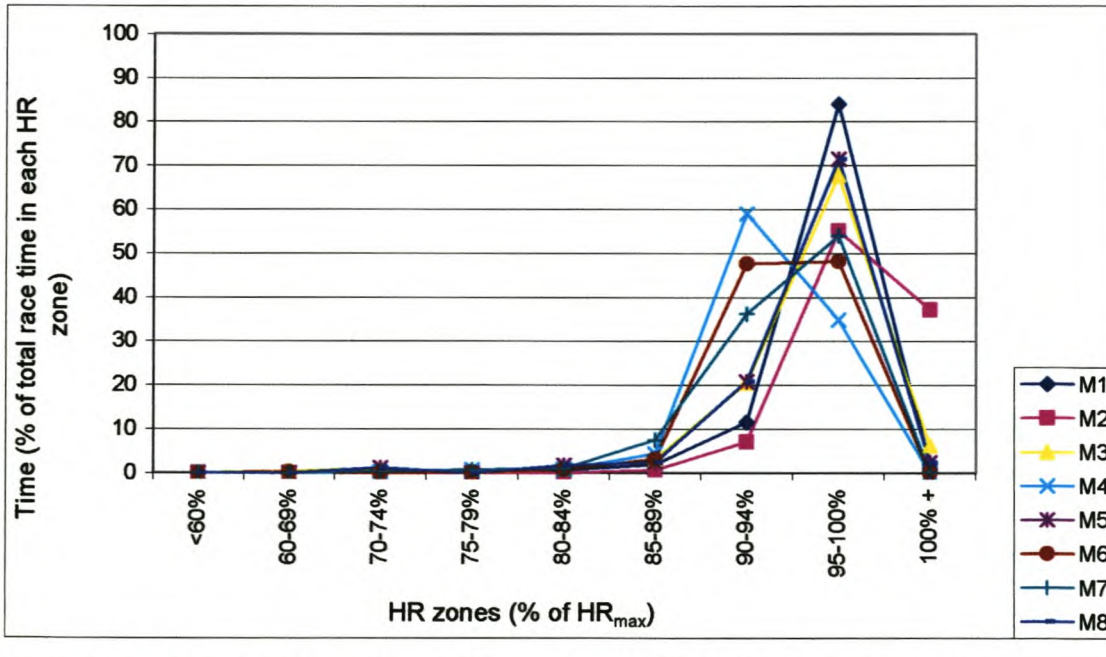


Figure 4b: Relative time spent in each HR zone by the male athletes during TT_{10}

The mean data for each group showed that the female and male athletes matched very well, with a Mann-Whitney U-Test showing no significant difference between the profiles of the two groups ($p > 0.05$), as can be seen in Figure 5.

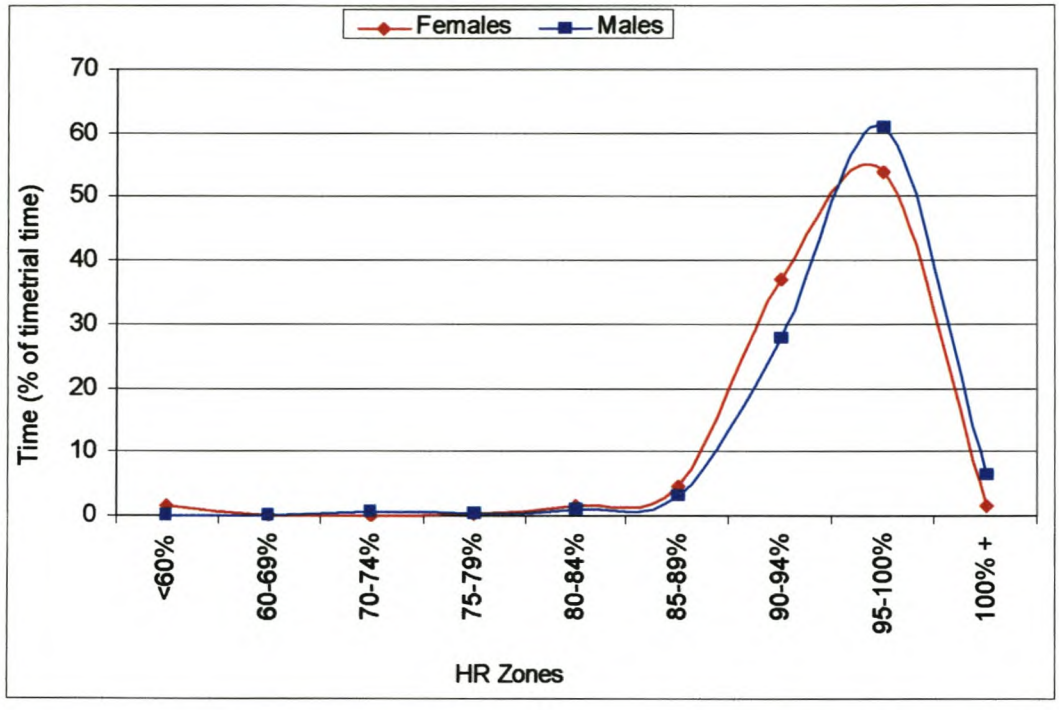


Figure 5: Average relative time spent by each group of athletes in each HR zone during TT_{10}

SECTION 4: LABORATORY TESTS

4.8. Muscle analysis

4.8.1 Summary of oxidative capacity, fibre composition and fibre cross-sectional area of muscle samples.

Figure 6 shows the results from the mATPase staining, indicating the different fibres at pH10.3, pH4.3 and pH4.6 respectively, for two subjects (M1 and M8). The slides at pH10.3 and pH4.3 are almost mirror image of each other, with the type I fibres being white at pH10.3 and black at pH4.3 and type II fibres being black at pH10.3 and black at pH4.3. The pH4.6 sample serves to identify the type IIA and type IIX fibres.

Figure 6: Example of the mATPase staining results for pH 10.3, 4.3 and 4.6 respectively at 10x magnification in two of the athletes (M1, top and M8, bottom)

The average fibre type composition present in the males and females is summarized in Table 9, along with the cross-sectional areas of the fibers and the muscle citrate synthase activities. The citrate synthase activity is expressed per gram of protein in the homogenate. Citrate synthase activity is one of the key enzymes in the aerobic energy system, and its activity is an indication of the oxidative capacity of the muscle.

Table 9: Mean percentage fiber type compositions, cross-sectional areas and citrate synthase activities of the two genders

	Females		Males	
	Mean ± SD	Range[¤]	Mean ± SD	Range[¤]
Type I	63.1 ± 13.1[a]	43.3 - 80.8	49.9 ± 11.3	34.2 - 63.8
Type IIA	35.5 ± 13.7	18.0 - 56.7	37.3 ± 9.6	29.0 - 55.6
Type IIX	1.45 ± 0.99[a]	0.00 - 2.90	12.8 ± 10.3	0.00 - 33.2
XSA (μm^2):TI	4,934 ± 856	4,018 - 6,217	6,494 ± 2617	3,323 - 10,273
Diameter (µm):TI	78.5 ± 6.77	71.1 - 87.8	88.8 ± 18.4	64.8 - 114.1
XSA (μm^2): TII	4,376 ± 981	3,154 - 5,805	5,808 ± 1709	3,477 - 8,274
Diameter (µm):TII	73.8 ± 8.34	63.2 - 85.9	82.2 ± 16.9	66.2 - 100.8
CS (µmol/min/g prot)	97.7 ± 30.1	33.9 - 155.9	84.6 ± 39.2	52.1 - 143.6

Abbreviations: XSA – Cross-sectional area
CS – Citrate synthase activity
TI – Type I fibres
[¤] Minimum to maximum
[a] $p < 0.05$
TII – Type IIA and IIX fibres

Muscle fiber composition of the male athletes was significantly different from that of the female athletes (Table 9). The greatest difference is seen in the type IIX fibers, which make up a significantly larger percentage of the male athletes' muscle ($p < 0.05$). This finding has implications for oxidative capacity analysis of homogenate (see 4.8.2.3). Significant differences are also seen between the percentage of type I fibers in the female and male athletes ($p < 0.05$), with the female athletes having considerably more.

The citrate synthase (CS) activities of the muscle samples varied considerably between individual athletes and there were consequently no differences seen between the genders ($p > 0.05$).

The range in values measured for fibre cross-sectional area was also large, although much more so within the male athletes than the females. The difference in fibre cross-sectional areas between the genders, both for type I fibres and type II fibres, were also not significant, although both fibre types tended to be larger in the male athletes (Type I XSA: 32% greater in males, type II: 33% greater in males).

4.8.2. Relationships with oxidative capacity of muscle samples

4.8.2.1. Oxidative capacity and TT_{10} performance. Oxidative capacity had no relationship with TT_{10} performance in female athletes ($r = 0.26$) or male athletes ($r = -0.33$). For both genders, there were no relationships between the oxidative capacity of the muscle samples and their performance in the VO_{2max} tests, except for one relationship seen in the male runners between training volume and oxidative capacity ($r = 0.81$, $p < 0.05$), which was not seen in the female runners ($r = -0.05$).

4.8.2.2. Oxidative capacity and weekly training. The female athletes showed no correlation between CS activity and either distance trained ($r = -0.05$; see Figure 7a) or duration of weekly training ($r = 0.12$). The male athletes, however, showed good correlations between the two training variables and CS activity ($r = 0.81$, $p < 0.05$ for distance trained, see Figure 7a, and 0.83, $p < 0.01$ for duration). In male athletes a further significant correlation was found between number of hill training sessions and CS activity ($r = 0.85$, $p < 0.01$). This relationship in female athletes was non-significant ($r = -0.32$). In the female athletes there was a trend towards an association between CS activity and

average training speed for the monitored week ($r = 0.62$, $p = 0.10$, see Figure 7b); this trend was not seen in the male athletes ($r = -0.23$, see Figure 7b).

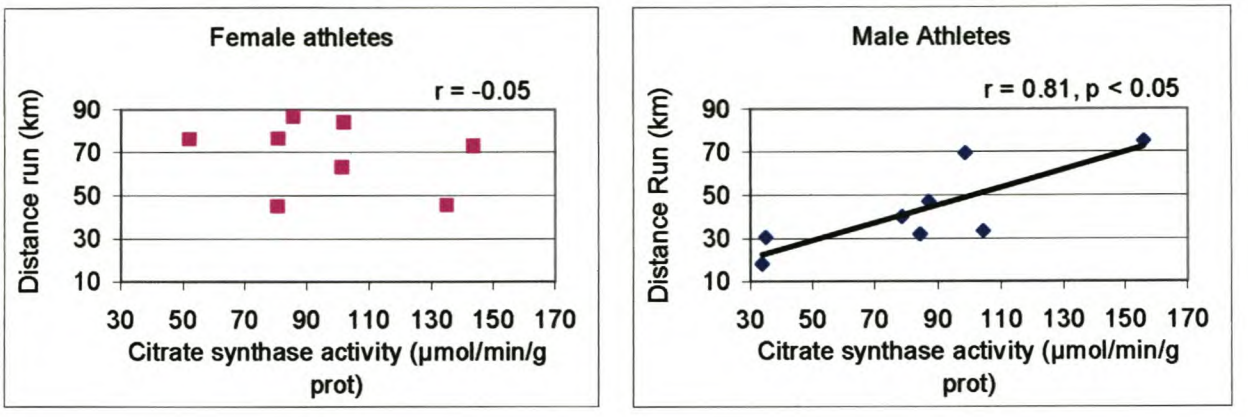


Figure 7a: Relationship between citrate synthase activity and distance run in monitored training week for each of the genders

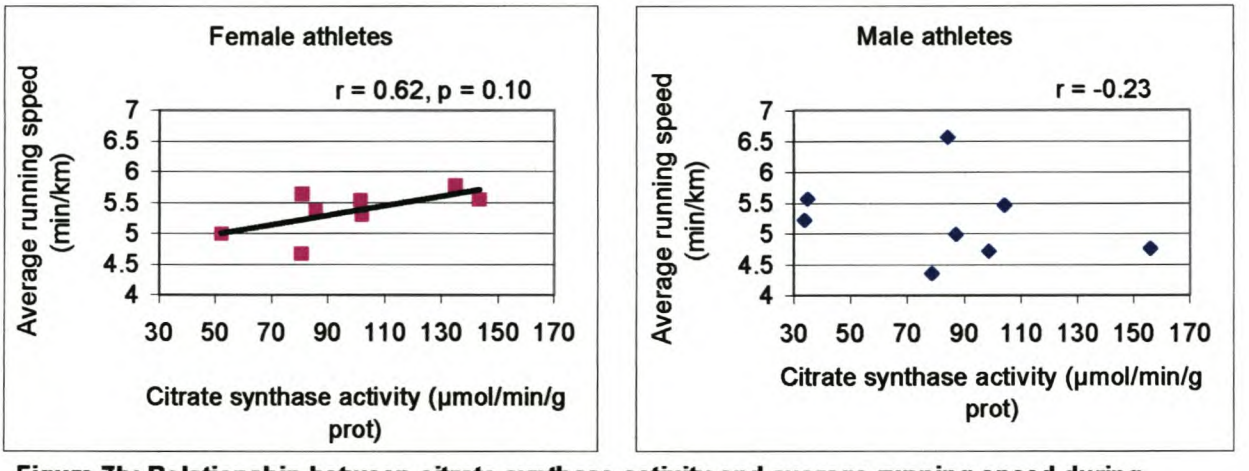


Figure 7b: Relationship between citrate synthase activity and average running speed during monitored training week for each of the genders

The higher the absolute weekly training time spent by male athletes in the EASY HR zone, the higher their citrate synthase activity ($r = 0.83$, $p < 0.01$). In female athletes, the highest citrate synthase activity was seen for those athletes spending the greatest absolute training time in the 85 – 89 % HR_{max} zone ($r = 0.86$, $p < 0.01$). For the female athletes, the there was a trend towards the same relationship with respect to the relative time spent in the 85 – 89 % HR_{max} zone and citrate synthase activity ($r = 0.64$, $p = 0.09$). The relative time in male athletes spent in the 100%+ HR_{max} zone showed a trend towards a negative relationship with citrate synthase ($r = -0.65$, $p = 0.08$).

4.8.2.3. Oxidative capacity, fibre composition and cross-sectional area of fibre types. The relationships between oxidative capacity and fibre type and cross-sectional area are summarized in Table 10. The relationship between all fibre types in both male and female athletes and CS activity were found to be weak. With respect to fibre cross-sectional area, both fibre types in the male athletes had a good relationship with oxidative capacity ($r = 0.76$, $p < 0.05$ for both type I and type II). The relationships between cross-sectional areas of both type I and type II fibres in the female athletes with oxidative capacity indicated a trend toward a positive relationship (type I XSA: $r = 0.60$, $p < 0.12$; type II XSA: $r = 0.64$, $p < 0.09$). These trends together with the relationships in the males indicate that an increased cross-sectional area is associated with an increased oxidative capacity.

Table 10: Relationships between citrate synthase activity and muscle fibre type composition and cross-sectional area

	r-values (p-values)	
	Females	Males
Type I composition	0.05	0.33
Type IIA composition	-0.05	0.10
Type IIX composition	-0.12	-0.38
Type I XSA	0.60 ($p = 0.12$)	0.76 ($p < 0.05$)
Type II XSA	0.64 ($p = 0.09$)	0.76 ($p < 0.05$)

Abbreviations: XSA – Cross-sectional Area

4.8.3 Relationships between muscle histology and both performance and training variables

4.8.3.1. Fibre histology and performance. TT_{10} performance was not correlated with fibre types for male athletes or female athletes. In the case of both fibre types, for both genders, the relationships between the cross-sectional area of the fibres and the athletes' performance in TT_{10} were not significant.

The female runners had no significant correlations between their fibre type compositions and VO_{2max} results. The correlations for the male athletes were not much better with only two having significance: the type IIA composition of the male athletes was associated with their weight ($r = -0.90$, $p < 0.005$) and their FFM ($r = -0.74$, $p < 0.05$). The cross-sectional area of both type I and type II fibres in the male and female athletes were not related to

any of the variables of the VO_{2max} tests, although in the male athletes the cross-sectional area of the type II fibres tended towards an association with flat VO_{2max} ($r = 0.62$, $p < 0.10$).

4.8.3.2. Fibre histology and training variables. The distances run by the athletes during the monitored training week were found to have no significant associations with fibre types. The relationship between type IIX fibres and duration of training in male athletes indicated a slight trend toward a negative association ($r = -0.50$, $p < 0.21$).

Type I and type IIA fibre composition in female athletes correlated significantly, but oppositely, with HIT (Type I: $r = -0.79$, $p < 0.05$; type IIA: $r = 0.79$, $p < 0.05$), while in the male runners fibre types I and IIA tended to correlate, although not significantly, with the average running speed during training (Type I: $r = 0.64$, $p = 0.09$; type IIA: $r = -0.62$, $p = 0.10$).

Type IIX fibre composition showed strong relationships with the number of running sessions in female athletes ($r = 0.91$; $p < 0.01$) and number of combined hill and HIT sessions in the male runners ($r = -0.82$; $p < 0.05$).

Relationships between the time spent training (both relative and absolute) in various heart rate zones (both the narrow and broad range), and fibre histology were investigated and several strong correlations were found with fibre type composition. When times spent in the various training HR zones were expressed relative to total time, a positive correlation was found in both men and women between time spent <60% HR_{max} and % type IIX fibres ($r = 0.71$ and $r = 0.76$, respectively, both $p < 0.05$). In men, the % type I fibres was significantly correlated with relatively more time spent in the MODERATE HR_{max} zone ($r = 0.76$, $p < 0.05$) and specifically the 75-79 % HR_{max} zone ($r = 0.74$, $p < 0.05$), as well as absolute time spent in the 70 – 75 % HR_{max} zone ($r = 0.74$, $p < 0.05$). In women athletes, the % type I fibres correlated with time spent in the MODERATE % HR_{max} zone when this time was expressed in absolute terms (MOD zone: $r = 0.78$, $p < 0.05$; 70-74 % HR max zone: $r = 0.79$, $p < 0.05$; 75-79 % HR_{max}: $r = 0.88$, $p < 0.01$). More slow twitch fibres in the female runners were also associated with less absolute time spent in the 100%+ HR_{max} zone ($r = -0.78$, $p < 0.05$). This relationship was also true for the male athletes, with those athletes having a greater percentage of slow twitch fibres spending less absolute and relative time spent in the +100% HR_{max} zone ($r = -0.74$ and $r = -0.73$ respectively, both $p < 0.05$).

The type IIA fibres in males showed a positive correlation with the relative time spent at 80-84% ($r = 0.88$, $p < 0.005$), thus higher intensity, and negative correlations with the time spent in the more moderate zones: 70-74% zone and 75-79% zone ($r = -0.90$, $p < 0.005$ and $r = -0.95$, $p < 0.005$, respectively), which contributed to the negative correlation seen between the % type IIA fibres and time spent in the easy HR zone ($r = -0.76$, $p < 0.05$). When looking at the absolute time spent per week in the various HR zones, the findings are complimentary with % type IIA correlating positively with the time spent at 85-90 % HR_{max}, 90-95 % HR_{max} and the HARD HR zone ($r = 0.86$, 0.88 and 0.86 respectively, $p < 0.01$). In contrast, in both men and women athletes, the % of type IIA fibres was negatively correlated with the absolute time spent at more moderate HR zones: 75 – 79 % HR_{max} (men: $r = -0.71$, $p < 0.05$; and women: $r = -0.88$, $p < 0.01$). This negative correlation still held for the women for the zone 70-74 % HR_{max} ($r = -0.79$, $p < 0.05$).

The relationship between % fibre IIX and the relative time spent in the +100% HR_{max} zone for both genders was positive ($r = 0.71$ for men and $r = 0.76$ for women, both $p < 0.05$), but in absolute terms only for the men ($r = -0.73$, $p < 0.05$).

However, no relationships between cross-sectional area of fibre types and the time spent training in each heart rate zone were found in either gender for the relative time spent in each zone, and only one was seen in absolute time correlations, between the time spent by male athletes at 85-89% of HR_{max} and the cross-sectional area of type II fibres ($r = 0.76$, $p < 0.05$).

5. DISCUSSION

In this study, eight well-trained competitive female 10 km runners were matched to eight well-trained competitive male 10 km runners according to performance in a controlled 10 km time trial (TT_{10}). The aim of the study was to identify those factors in the female athletes allowing them to perform equally when compared to the men, despite their inherent disadvantage due to lower hematocrit and higher percentage body fat.

Due to the small number of subjects in each gender group, type II statistical errors could emerge when analysing the data. Thus, several trends towards significance were reported in the results, especially in those variables in which differences between genders, and relationships with performance variables, were expected and not found, such as haemotocrit and % body fat. This study should therefore be considered a pilot study and more subjects will have to be tested prior to publication of results.

The main findings of this study were that the women runners did tend to have lower haematocrits and higher percentage body fat than their male counterparts (both $p < 0.06$), the combination resulting in significantly lower VO_{2max} ($p < 0.05$). The variables that clearly compensated for this were: lower body mass, higher training volume (both $p < 0.01$) and higher % type I fibres ($p < 0.05$). Also, there was a range in TT_{10} time of ~9 min for women runners, so that various parameters could be related to performance to further inform about the factors positively influencing their performance.

5.1. Subject Characteristics

The athletes participating in this study all fell into the same age bracket, with no difference in ages between the two groups. The similarity in ages made comparisons easier due to the fact that both muscle mass and oxygen consumption decrease with age (Saltin, 2001).

The running background of the athletes varied considerably, with some of the athletes starting as track athletes in school and moving on to 10 km running later, and others participating in other sports in school and only starting road running after school. All the athletes, however, had been training for and competing in the 10 km distance for at least three years.

The percent body fat in female runners was ~5% greater than that of the male runners. In the study in the literature most similar to ours, Sparling and Cureton (1983) compared men and women recreational runners with relatively good VO_{2max} (>60 ml/kg/min) with respect to performance in a 12-min time trial. Although this study used a much shorter performance test, it is possible to compare our subject cohorts for subject characteristics. Our athletes had a lower average VO_{2max} for the relative gender group that in Sparling and Cureton's study (males: 61.0 vs 68.6; females: 56.0 vs 65.1). The % body fat observed in the females in this study was very similar to those of the athletes in Sparling and Cureton's study (Current study: 19.01%, Sparling and Cureton, 19.8%) however, the % body mass recorded for the male athletes of this study was somewhat higher than those of the male athletes in Sparling et al's study and explains why, where they found a significant difference between the genders, the current study only showed a tendency. % Body fat affects the oxygen consumption of the athlete, when expressed per kilogram of body weight, resulting in significantly higher oxygen consumption in the male athletes. If however body mass is corrected the percent body fat, the VO_{2max} relative to fat free mass did not differ between the genders in the study. This would imply that % body fat probably has a greater influence on VO_{2max} than does haematocrit. Sparling and Cureton (1983) revealed that % body fat, in fact, accounted for 74% of the average gender difference seen for their 12-min test. The statistics performed to achieve this relative contribution to gender difference cannot be performed for our study due to the small subject number. However, the results of our study do suggest this.

While the smaller contribution of haematocrit may seem surprising, it is important to remember that lower haematocrit does not necessarily mean lower total red blood cell mass, due to runners' 'pseudo-anaemia' (Dang, 2001; Weight *et al*, 1992; Weight *et al*, 1991). This condition is due to an expansion in plasma volume due to training. Thus, while the athlete would appear to have a low haematocrit, this could largely be attributed to a dilutional effect. The normal range for haematocrit in males is 47% ± 5%, while for females it is 42% ± 5%. The average haematocrit for the female runners in the current study was 31.6% ± 2.17%, while that for the male participants was 36.81% ± 1.87%. These vales were somewhat lower than the normal ranges and may have indicated the prevalence of runner's anaemia within these athletes. Dang (2001) suggested that runner's anaemia is well tolerated by an avid runner and thus, may not be easily detected. Should the athletes have been suffering from this

complaint, it could explain why only a trend was seen in the difference in Hct between genders. However, as the women runners in the current study had a higher training volume, they may have had a greater plasma volume expansion than the male runners.

Christensen and Ruhling (1983) suggested that when a great disparity exists in the VO_{2max} between athletes, performance can be predicted by VO_{2max}. They evaluated the marathon performance of elite, experienced and novice marathon runners and concluded that the elite athletes had the greatest VO_{2max}. However in a homogeneous group of runners, the usefulness of this variable as a predictor of performance decreases. Within our group of women, VO_{2max} did not correlate significantly with performance, which is expected due to the relative homogeneity of the group. The same was found for the male athletes. In both gender groups, however, peak treadmill speed, both fat and gradient, did show correlations with performance. This finding would support Noakes (1990) and Scott and Houmard (1994) who suggested that PTS is the best laboratory-measured predictor of performance.

Certain variables cannot be matched between genders, and in this study, these invariable differences were clear.

Firstly, as expected, the male athletes were significantly taller than the female athletes ($p < 0.05$), and although this difference in height is not the primary factor resulting in gender differences in performance, it can play a contributory role. Taller athletes, thus the males, generally have a greater stride length, making it easier for them to run at faster speeds, but amongst male runners, taller runners have been found to be less economical (Maldonado, 2002; Bourdin, 1993). Thus, while being able to run faster, the taller athletes will consume more energy to do so. This may provide a partial explanation of the ability of the female athletes to perform equally to the male runners. However, the male runners were not less economical in the present study. While the male athletes may have the capacity to run faster, as illustrated by their significantly higher PTS ($p < 0.005$) due to their greater stride length, this speed was not translated into better 10 km performance. Therefore, the resistance to fatigue was lower in the men who could not maintain a high speed for the duration of 10 km and thus, they will race at slower speeds relative to PTS (see Table 5). Despite this difference ($p < 0.005$) in fractional utilisation of PTS in men, there was no difference in the fractional utilisation of VO_{2max}. This can probably be

explained by the finding that, although not significant, the men ran at a 7% higher absolute VO_2 at race pace.

The second predictable difference, was the greater body mass of the male runners compared to their female counterparts. Due to the differences in body mass seen between subjects, Berthoin et al (1994), Billat et al (2001) and Billat et al (1994) chose to express VO_{2max} relative to mass (ml/kg/min) so as to normalise a population for comparative purposes.

In contrast to height, higher body mass is associated with a better economy, even when expressed per kg (Pate *et al*, 1992). This type of within-group comparison is not relevant when comparing genders, as illustrated by our data of equal economy, despite marked differences in height and body mass.

5.2. 10 km time trial (TT_{10})

Each of the female athletes was matched, as closely as possible, by their performance in TT_{10}, to a male athlete. Thus, the average performance time for TT_{10}, between the two genders was not significantly different.

The first 5 km of TT_{10} route was predominantly uphill with the second 5 km being predominantly downhill. Thus the first 5 km of TT_{10} was quite slow and the second 5 km, faster. On the whole the route was much tougher than many of those in which the athletes achieved their PB's, hence the slower times for several of the athletes. However, it served its purpose to allow female and male athletes to be matched for their performance over the same course. The high percentage of HR_{max} at which the athletes raced during TT_{10} indicated that the athletes treated TT_{10} as they would a race and gave 100% effort, thus allowing us to match the athletes for performance. Costill et al (1971) studied performance in marathon runners and found that marathon runners race at approximately 75-90% of their VO_{2max}. The athletes in the current study raced at an average of 91% of their VO_{2max}, although the range within the athletes was very similar (71% to 102%). These high fractional utilizations of VO_{2max} also serve as an indicator that the athletes performed to their optimal capacity during TT_{10}.

The relationship between performance in TT_{10} and their PB for the male athletes was found to be significant, although the same was not true for the female athletes.

Perhaps, an explanation for this could be that the women found the course with the relatively long slow uphill for the first 5 km to be more difficult than did the men. The difference in gradient PTS between the two groups was 16%, whereas for the flat test it was 10%.

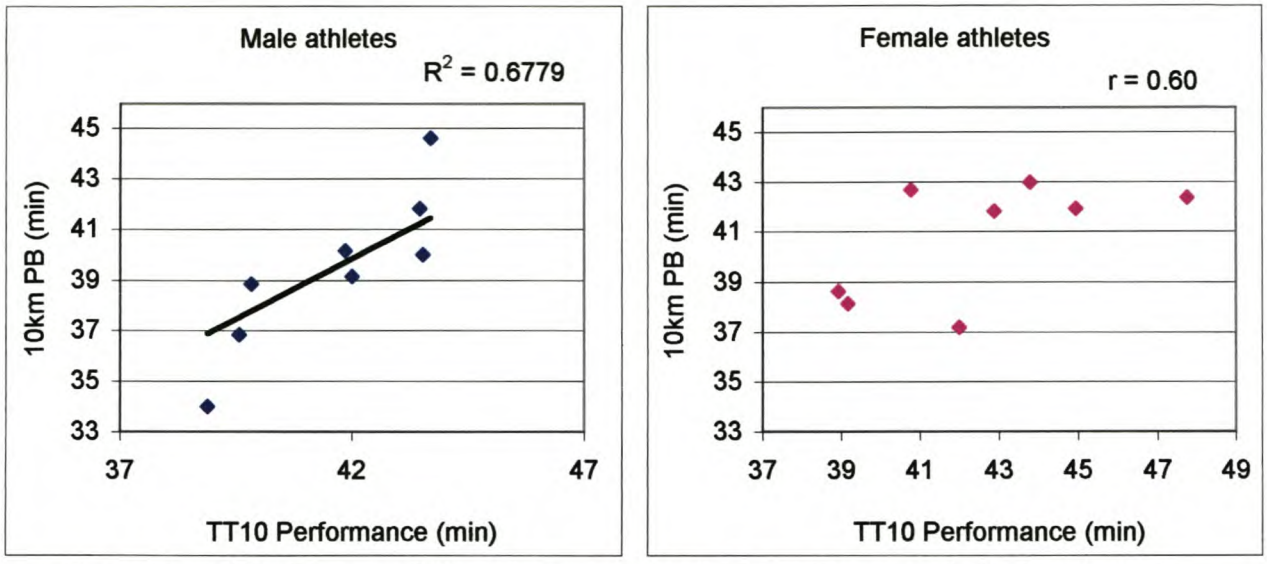


Figure 8a and 8b: The correlations between PB and TT_{10} performance, indicating lack of significant relationship for female athletes

Kang *et al* (2001) and Helgerud (1994) suggested that gradient treadmill running produces higher results with regards to VO_{2max} than flat treadmill running. However, the results achieved by the athletes in this study do not support this suggestion as the VO_{2max} achieved during the gradient test was not significantly different to that of the flat test.

5.3. Weekly Training

Each athlete was instructed to train as normal over the seven consecutive days in which their training and training heart rates were monitored and the athletes completed this seven day training analysis in different weeks, since quite a few of the athletes usually trained together. Athletes F1, F2, F3, F4, F6, M2, M3, M6 and M7 all had the same coach, but all these subjects did not necessarily attend all training sessions. The training volume, both numbers of sessions, distance and duration per week, was significantly greater in the female athletes, indicating that they either train more regularly with the coach, or do additional training to their coaching sessions.

After analysis of the training diaries, in which the training during seven consecutive days was recorded, it was found that the female runners on average train more often (a greater number of sessions per week), cover a greater distance and spend more time per week training than do the male athletes. This is opposite to the finding by Billat *et al* (2003) in their study on elite Kenyan runners, in which the females trained significantly less. However, Billet *et al* (2003) did not aim to match their subjects for performance, thus explaining this discrepancy.

The findings of the current study with regards to training volume are more comparable to those of Helgerud (1994) who, in his comparison of six male and six female marathon runners, found that the males had higher VO_{2max} values and anaerobic thresholds, but that the women had higher weekly training distance.

The higher training volume in female athletes could indicate that the female athletes took their running more seriously and thus, more committed to coaching sessions and possibly doing extra sessions too.

The higher volume of training in females, however, did not correlate with those variables expected to increase with training volume, such as citrate synthase activity and may indicate that the female athletes did extra training in their monitored week compared with what they normally did and thus, that the training records of the males could have been more representative of their normal training. This possibility is substantiated by the good correlation between training distance per week and citrate synthase activity in men (see Figure 7a), as would have been expected for both groups. However, the adaptation of muscle to training may not simply be related to training distance. In the women runners, the average training speed tended towards a correlation with citrate synthase activity ($r = 0.62$, $p = 0.10$, see figure 7b), which was not seen in the men.

Although the men trained less than the women, within their group the strongest relationship with performance was seen for the number of sessions completed, with the general tendency to be that the male athletes who trained more often, performed better. In the case of the male athletes, their training volumes were very low, in some cases (see Table 6) thus, simply by increasing the number of sessions per week, they would be able to improve their performance.

The average speed of training, averaged for the whole week, was similar in both genders ($p > 0.05$), despite the higher PTS of the men, and this is another factor that could explain the equivalent TT_{10} performance. The average speed for the female athletes was 14.2 ± 0.96 km/h, and that of the males was 14.5 ± 0.69 km/h. While non-significant differences were also recorded for the type of sessions completed in the week by the two genders, within the female group of runners a correlation was found between the number of high intensity sessions (track plus hill) and TT_{10} performance. Those female runners recording a higher number of HIT sessions per week had better performance. An explanation of the differences seen within the genders with respect to these training variables and their relation to performance could be that all the female athletes had a high volume of training, thus a further increase in the volume of training will not make too much difference, whereas speed sessions may be critically influencing their PTS and thus, their performance.

Therefore, we examined the intensity of training and racing in greater detail.

5.4. Heart rates during testing, racing and training

The maximum heart rate recorded by the athletes, which was taken as the maximum result achieved during the flat VO_{2max} test, was found to be significantly higher in male athletes. This could be explained by the fact that the male athletes have a higher fat free mass than females and the muscle compartment requires oxygen, thus, the heart has to supply the extra muscle with sufficient oxygen. However, if heart size and body size are linearly correlated, heart rate should not be affected. Thus a different explanation is required for the finding in the current study. Bradycardia at rest has been reported to result from training (Leicht *et al*, 2003; Melanson and Freedson, 2003). This could be due to left ventricular hypertrophy, which has been found to occur as an adaptation to high training volumes (D'Andrea *et al*, 2002). Left ventricular hypertrophy results in an increased stroke volume of the heart. As the required cardiac output remains constant, the heart rate can thus be lower ($Q = HR \times SV$). The female runners in this study had considerably higher training volumes than the male athletes and thus, their lower maximal heart rates could be the result of left ventricular hypertrophy.

One might argue that the females did not reach their true maximum heart rate during the VO_{2max} tests. This is not believed to be the case as for both the flat and the gradient VO_{2max}, the RER_{max} achieved by the female athletes was above 1.10, which

is commonly used as a criterion for maximal efforts (Doherty *et al*, 2003). The validity of the HR_{max} achieved during the flat VO_{2max} test is further strengthened by the fact that the profile of the $\%HR_{max}$ utilised during the race for the female athletes is very comparable to that of the male athletes, who achieved higher heart rates (Figure 9).

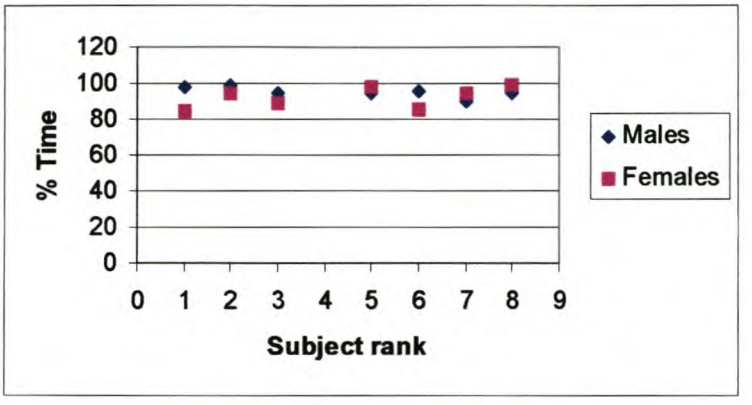


Figure 9: Comparison of amount of the relative time spent running at 90-100%+ HR_{max} during TT_{10} for performance matched individuals.

Both the female and male athletes spent more than 50% of TT_{10} time at heart rate intensities greater than 80% of their maximum (Figures 3a and 3b). There was no significant difference between the relative heart rate breakdown between the genders, although in absolute terms, as the female athletes had lower maximal heart rates, they also raced at lower absolute heart rates. As stated in the results section, the heart rate file of one of the female runners recorded during TT_{10} was not downloadable and thus, F4's HR file for the race was excluded from any relationships determined and figures in which race HR are presented. This further affected the likelihood of type II errors.

The relative percent of time spent in each heart rate zone by each athlete was found to be similar between the two genders. Thus, the division of the total time was similar. The time spent training at intensities less than 75% of HR_{max}, defined by us as EASY, was 36.6% in males and 36.9% in female athletes. 53% and 46.8%, in male and female athletes respectively, was spent between 75 and 90% of heart rate maximum and 13% of the relative weekly training time in males and 16.2% in females was spent training at intensities between 90 and 100% of their maximum. Therefore, the way the genders chose to structure their training was similar despite the differences in volume.

We therefore determined whether the structure of a training programme has a greater influence on muscle composition, or the absolute quantity.

5.5. Muscle Composition and Function

In both groups of athletes muscle samples were primarily composed of type I fibers. Since endurance training may induce a shift of fibers to type I (Pette, 1998), or persons with higher type I fibre percentages may select to participate in endurance exercise, this result was not surprising. However, of more interest is the fact that the female athletes had a significantly greater percentage of type I fibers ($p < 0.05$) than did the males. When Costill et al (1987) matched elite men and women runners for preferred racing distance but not for times over 10 km, they found remarkably similar fibre type between the groups. In the current study, the type IIA content of the samples of both genders was very similar, meaning that the male athletes had a significantly greater percentage of type IIX fibers than the female athletes ($p < 0.01$). This difference in fiber type composition may play a very important role in explaining some of the differences and similarities between the genders.

The higher percentage of type IIX fibers in the males may explain their higher peak treadmill speeds. Type IIX fibers are those with the highest contractile velocity, producing the greatest force per contraction (Bottinelli *et al*, 1999). Thus, a higher percentage of these fibers allows for greater speed but only over a short distance because these fibers are also very quick to fatigue. This may explain, in part, why the males are not faster than the females over 10 km. They may have been faster than the females over the shorter time of the VO_2 tests, but over 10 km, the slow-twitch type I fibers and the oxidative type IIA fibres are mainly recruited. Type IIX fibres are more easily converted to type IIA fibres, than are type IIA to type I (Pette, 1998), but they do need to be recruited consistently. In this study, the men had not converted as many type IIX fibres to type IIA as had the women over the previous months and years of training. This may have been related to the lower volume of training or a lack of high intensity training and IIX fibre recruitment in the men. A relationship was seen in the male athletes between the relative weekly training time spent at 60-69% HR_{max} and the % type IIX fibres. Contrary to what one would first think, the relationship suggested that the more time spent training at these low intensities, the greater the % type IIX fibres. However, this relationship indicates that the male athletes are training at intensities so low that their type IIX fibres are not being recruited and thus cannot transform to type IIA fibres. This may be a key factor in the performance

equality between the genders. As the females have a lower % type IIX composition, they will be outperformed in a short, speed test, such as the maximal test. This, along with the fact that the female runners had a higher percent of the type I fibers, may have provided more endurance, and may have allowed them to race at a higher relative intensity (fractional utilization of VO_{2max} and PTS) than the male athletes for TT_{10} ultimately allowing them to perform equally to the males. The results indicate that while the females did not race at a higher fractional utilization of VO_{2max}, their fractional utilization of PTS during TT_{10} was significantly greater.

However, fibre type alone is not the only characteristic that could be influenced by recruitment. Endurance training can cause muscle fibre hypertrophy (Saltin, 1983). This study therefore, also assessed fibre cross-sectional areas and related these to training intensity.

The average cross-sectional area of type I fibers and type II fibers was determined for each athlete, without differentiating between type IIA and type IIX fibers. Saltin (1973) suggested that endurance training results in a selective hypertrophy of type I fibers. The fact that the type I fibers were not significantly larger than the type II fibers, together with the fact that both genders had a relatively high percent of type IIA fibers, may indicate that, as in the case of middle distance runners (800-5000m) in the study by Costill *et al* (1975), both types of fibers are used for the 10 km distance and thus both types will undergo hypertrophy. A gender difference in fiber type cross-sectional area was not significant but this factor could be further investigated in a larger subject group.

As in the study by Costill *et al* (1975), a great inter-individual difference was found between the average cross-sectional areas of both fiber types in both genders. Individual differences in cross-sectional areas of both fiber types may influence muscle power, thus influencing hill running abilities and flat peak treadmill speed. Conversely, the greater cross-sectional area may adversely affect fatigue resistance. From the relationships found in this study, we propose that the slightly greater cross-sectional area of both fibre types in the male athletes can explain the greater gradient PTS achieved by the males. Also, because much of the TT_{10} route was uphill, and thus one would expect the males, with their superior climbing ability, to outperform the females, it can be deduced that the increased cross-sectional area did indeed reduce the fatigue resistance in the male athletes, thus causing their performance to be worse than expected.

Another factor that could be further investigated in relation to the intensity of training is citrate synthase activity. Our findings also showed a large range in the muscle oxidative capacity between the athletes. In the male athletes, those who spent a greater % of absolute weekly training time in the EASY heart rate zone had greater citrate synthase activity, while those spending a greater relative time at 100%+ HR_{max}, had lower citrate synthase activity. These relationships would be expected since citrate synthase is an oxidative enzyme involved in the aerobic metabolic pathways. Exercising at 100%+ HR_{max} would rely heavily on anaerobic metabolism, hence the relationships seen in the males. The relationships seen in the female athletes are less obvious. For both relative and absolute time, those athletes spending more time in the 85-89% HR_{max} zone had a higher citrate synthase activity.

5.6. Conclusions

This is the first study to focus on training and performance in competitive, sub-elite female 10 km runners. By matching the female runners for performance with male runners, we were able to dissect out the reasons that some women have endurance racing performance capacity similar to men, rather than evaluating why elite level men are better than elite level women runners (Billat *et al*, 2003).

The females, as predicted had a lower haematocrit and a higher % body fat compared to the male athletes, both of which effect performance negatively and thus put female athletes at an immediate disadvantage compared to the male athletes. The influence of % body mass has shown to account for more than 70 % of the difference in VO_{2max} seen between genders and the findings from the current study confirm the extent to which % body mass influences VO_{2max}. The VO_{2max} achieved by the male athletes was significantly greater than that of the females, when expressed as ml/kg/min. However, as soon as the % body mass was deducted to give a VO_{2max} relative to fat free mass, the difference between genders disappeared.

This study only touches on the surface of the physiological mechanisms by which female athletes compensate for their inherent performance disadvantages, however, the main conclusion of this study is that the main mechanism by which females compensate in order to perform equally is by having a higher training volume compared to the male athletes. Further advantages of the female athletes is their higher % fibre type I composition and their higher fractional utilization of PTS while racing. The latter two variables can be a result of the high training volumes.

Although it was predicted that the PTS of male and female athletes would not be significantly different due to their equal TT_{10} performance, the male athletes had a significantly greater PTS and while this PTS was a good predictor of performance within gender groups, it could not be applied across the genders. The fractional utilization of PTS during TT_{10} by the female athletes was, however, significantly higher than in the males, allowing them to run at speeds equal to the men.

The higher PTS in the males is due, in part, to their higher % type IIX fibre composition. Type IIX fibres can transform to the more oxidative type IIA fibres as a result of training. However, in order to transform, they need to be recruited and thus, the high percentage of type IIX fibres in the males indicates that their training is either insufficient in volume or of the wrong intensity, to recruit these type IIX. This will result in fewer endurance fibres (type I and type IIA) and thus, while the male athletes are able to run fast for short times, these high speeds cannot be maintained over long distances.

Type I fibres are related to a greater endurance capacity in athletes. The 10 km distance can be categorised into the lower end of endurance events because it is not an event that can be completed without some training. Thus, a higher endurance potential is desirable. The significantly higher % type I fibre composition seen in females can be a genetic factor or can be the results of training adaptation.

Further studies with a greater number of athletes could help clear up many of the tendencies reported. Even four more male and four more female athletes would be very beneficial.

Thus, our hypothesis that the female athletes compensate for their inherent disadvantages by having a higher training volume than the performance-matched males was found to be true. The females had a more optimal muscle fibre composition for 10 km running and could maintain a higher fractional utilization of the PTS throughout TT_{10}. They did not however, as predicted that they would, have a higher fractional utilization of their VO_{2max} throughout the race and were also found not to be more economical than the male athletes but equally economical. The females were also hypothesized to spend a greater percentage of time training and racing at intensities closer to their heart rate maximum, but this was not the case.

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7.1. APPENDIX 1: ATHLETE INFORMATION SHEET AND CONSENT FORM

University of Stellenbosch
Department of Physiological Sciences
Training Study 2003
Information sheet to athletes
Project: Comparing Male and Female 10km Runners with regards to both Performance and Training

Co-ordinator:	Ms. Robyn Bowen	Tel: (021) 808 4564	Cell: 084 660 7063
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Dr. Jannie Brink		
Prof. Kathy Myburgh		

You are hereby cordially invited to participate in a research study that will compare female and male 10 km athletes.

Background
It is well known that at the elite level, male runners will out-perform female athletes. However, should one turn one's focus to the well-trained competitive runners, there are many females who are capable of performing equally to their male counterparts. This study aims to determine the factors, whether they be physiological or training factors, that allow the females to compete with the males. 10 km athletes will be the focus of the study.

What will you gain from this study?
- You will be involved in scientifically backed training procedures
- You will gain valuable training advice and feedback on your progress throughout the testing period
- The opportunity to educate yourself about your own body
- And more ....

Prior to starting the study, please read through all information contained in this sheet and read and signed the consent form. Should you have *ANY* queries, please don't hesitate to contact me.

Layout of tests and training

Initial phase before physical testing
You will receive a heart rate monitor and a training diary and will be required to monitor your training activity for 7 consecutive days. Each training session, both running and non-running, must please be recorded, and if you have rest day, this should be left blank in the training diary. Please do not run any extra mileage in this monitored week as we would like to get an accurate record of your usual weekly training.

10km time trial
In order to match your performance to that of a member of the opposite gender, you will be required to run a controlled 10 km time trial. The route for this time trial will be clearly marked and water will be available along the route. Times will be taken and heart rate will be monitored during the timetrail.

Testing Phase
Your percentage body fat will be calculated for this study and will require that anthropometry is performed. Dr Theo Nell will measure 9 skinfold measurements and several circumferences. You will be required to wear running shorts and a short running top (for the females).
A muscle biopsy will be performed by Dr. Jannie Brink who is a qualified medical practitioner. All instruments and environment are sterilized. Local aesthetic will be administered to your quadriceps leg muscle, an incision made and a small piece (±150 mg) of muscle will be removed. After this, the doctor will thoroughly attend to the wound according to medical procedures. There is the slight possibility of infection, but the chances are very slim. Only one case has been reported, but this was because of negligence of the subject and not abiding to the instructions given to him by the doctor.

After five (5) consecutive days of rest, you will be asked to perform maximum running tests. You will first be familiarized with the treadmill, and when you feel completely comfortable, four (4) maximum running tests will be performed. These four tests will be spread out so that you have at least one (1) resting day in between. You will wear a mask that does not impair breathing and a heart rate monitor so that we can measure the amount of oxygen that you use and also your heart rate. The test takes approximately 15 minutes to complete.

After the maximum exercise tests, you will be asked to perform two (2) running economy tests. This involves running for 5 minutes at fractions of your maximum speed achieved in the above tests. During this, we will also insert a cannula in a venous forearm vain with a small tap. Roughly 3 ml of blood will be drawn each time (10 times = 30ml) during the economy test to see how much lactate you produce.

The final laboratory test will be a ten-minute race pace test. The pace will be calculated from your 10 km time trial performance and you will be required to run on the treadmill for 10 minutes at this pace, with your heart rate and oxygen consumption being measured throughout.

Things to remember:

- You are always free to withdraw from the study
- This experiment takes dedication and will take time. At some stage you will feel like quitting, but we would like to encourage you to continue. We therefore ask that before discontinuing the tests, that you consult one of the researchers so that we can assess in anything that is on your mind.
- Always feel free to contact us if you are experiencing problems of any kind. Our doors are open!
- You will not be responsible for any extra expenses on your behalf.
- All your data and personal communications will be held strictly confidential, but we will use the data collectively in scientific publications by taking your name out.
- The researchers are available 24 hours on their cell phones for any problems

Form 2 - Training Study 2003

CONSENT AND INDEMNITY FORM

The University of Stellenbosch
Department Physiological Sciences
University of Stellenbosch.

Project Title:

Comparing Male and Female 10km Runners with regards to both Performance and Training

Statement of understanding:

I have read and understand the explanation attached. I have had opportunity to ask the researcher questions and I understand that I am free to withdraw from the study at any time should I choose to do so.

I confirm that:

1. I was invited to participate in a research project by the Department Physiological Sciences at the University of Stellenbosch.
2. It was explained that:
   2.1. The aim of the study is to compare physiological variables in performance matched male and female 10 km runners.

   2.2. Informed consent is needed for the procedures below, which form part of the research study.

   2.3. The study is expected to be completed with a total of **16** athletes. Ideally, the project will be completed over a period of 2 months per athlete.

   2.5. I will need to undergo the tests tabulated below. Where blood samples are required, it will be drawn from a forearm vein by a qualified person. Also, where a muscle biopsy is needed, a qualified medical doctor will make an incision under local aesthetic and attend properly to the wound.

EXPLANATION OF PROCEDURE

	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7	Test 8	Test 9
Tests required	Muscle biopsy Performed by medical doctor	10 km time trial – field test	VO_2max and peak treadmill velocity – flat	VO_2max and peak treadmill velocity - flat	VO_2max and peak treadmill velocity – gradient 5°	VO_2max and peak treadmill velocity – gradient 5°	Running economy – flat	Running economy – gradient 5°	10-min race pace test - flat
Blood samples							30ml	30ml	
Running test		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

I also confirm:

3. I have been informed of any possible side effects, discomfort or detrimental effects by participating in this study. I have been informed of the possible side effects of blood drawing.
4. All the possible advantages of the study have been explained.
5. Information gathered in this study will be confidential. No names will be associated with individual results since every subject will be represented by a number only. The results will be used for a scientific assignment, publication or thesis, or all of these.

6. Results will be made available regarding the complete project as well as my own individual results once the project is completed.
7. I am free to withdraw from the study or any procedure at any time and the researcher may withdraw me from the study, without any disadvantage to me.
8. The information was presented in a language that I can understand and I had the opportunity to ask any questions and my questions were answered.
9. My participation was of my own choice without any pressure from the researcher / the department / the doctor and I am allowed to withdraw at any given time.
10. My participation will not involve any personal monetary costs.
11. This project has been evaluated and approved by Sub-committee C of the Research Committee of the University of Stellenbosch.

I, ...am .....years of age and hereby give consent to undergo all of the above procedures according to the explained protocol.

Signed at ..... on .....20 ....
(place) *(date)*

....
Signature of patient/participant *Signature of witness*

STATEMENT BY OR ON BEHALF OF INVESTIGATOR(S):

I, ..., declare that I explained the information given in this document to ..... *(name of the patient/participant)* and that he/she was encouraged and given ample time to ask me any questions in a language that he/she understands well.

Signed at ..... on .....20 ....
(place) *(date)*

....
Signature of investigator *Signature of witness*

7.2. APPENDIX 2: ATHLETE TRAINING HISTORY

Training Monitoring Questionnaire for Athlete Subject.
University of Stellenbosch

Please fill in this questionnaire as completely as possible with the help of an investigator.

FULL NAME:.....

Address:.....
.....
.....

Gender:.....

Age:..... Date of Birth:.....

Current Weight:..... Height:.....

Phone no.: (H)..... (cell)..... Fax:.....

Email:..... Date today: .....

Event specialization:.....

Best times/achievements for:

Year	Half-Marathon (Month)	10km (Month)	Other
2002			
2001			
2000			

1) What is your Personal best for your event and when was it if prior to 2000?
.....
.....

2) How long have you been training seriously?.....

3) Summary of racing and training history:.....
.....
.....
.....
.....
.....

.....
.....

4) Have you improved over 10km or other events in the last six months?(Y/N)
.....

5) If Yes, how much have you improved over 10km or other events in the last 6 months (if possible give examples):
.....
.....
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6) If no, has your performance declined? If so, by how much and why? E.g.: injury, Off-season.
.....
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.....

7) On a scale of one to five, how fit are you at the moment:
1: Very Unfit, battle with training
2: Unfit but training
3: Average Fitness
4: Fit and running well
5: Personal best, in top form

A) For Half-Marathon Competition?.....
B) For 10km Competition?.....

8) What are your goals for the following 12 months? (Give at least three goals or up to 6 different goals)
.....
.....
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9) For approximately how many hours do you train in a week?
a) On-season?.....
b) Off-season?.....

12) Approximate distance run in a normal week?
a) On-season?.....
b) Off-season?.....

13) How often do you usually train in a week?...

14) Injury History:

Year	Injury	Duration of reduced training	Duration of no training
2000			
2001			
2002			

15) Describe any problems you experience with training or racing. Use as much space as you require.

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7.3. APPENDIX 3: 7 DAY TRAINING LOG BOOK

7 DAY TRAINING LOG BOOK

ATHLETE'S NAME:_____

Operation of Polar S610 and S710 Heart Rate Monitor to record multiple sessions

NB: IF YOU HAVE PROBLEMS IN USING THE WATCH, PLEASE CONTACT THE RESEARCH STUDY COORDINATOR.

A. Recording a training session (Wear the heart rate transmitter around your chest, just below pectorals)

1. Press **RED BUTTON** function until the ***HEART*** indicator flashes.
2. Turn the sound off by pressing **SIGNAL** and holding it in for 1 second. (This improves the battery life).
3. Press **RED BUTTON again ONCE**. A long beep will follow and the timer will start. The watch records it automatically.
4. To end a training session, press **STOP button (bottom left).** Again a beep will follow. Then press **STOP BUTTON** again ONCE to go to the normal time screen. This last function is necessary for the watch to know that the training session is finished, otherwise it will record the next session in the same file.

B. Deleting a file

NB. THIS FUNCTION IS ONLY IF YOU HAVE MADE YOURSELF ACCUSTOMED TO THE WATCH. DO NOT DELETE ANY FILES THAT YOU RECORDED FOR TRAINING. RATHER BRING THE WATCH TO THE LAB AND THE COORDINATOR WILL CONSULT WITH YOU. THE FILE NUMBERS BELOW ARE ONLY EXAMPLES.

Deleting all information:

1. From the normal time mode, press **UP ARROW (top right)** once. The ***FILE*** function will appear. Press the **RED BUTTON** and it will give you the details of the last file recorded
2. Hold the **LIGHT BUTTON (top left)** and the word ***DEL FILE F3*** will appear.
3. Confirm deleting by pressing the **RED BUTTON.** The screen will display **"Are you SURE",** if you are, then press the **RED BUTTON** again. To cancel deleting, press **STOP BUTTON** until you return to the time screen.
4. After deleting, the screen will return to the next in line file being File 02.
5. Do this procedure for all files until memory is clean. To exit from the screen, press **STOP** until you return to the time screen.

Procedure adapted from Polar S610 and S710 manual by R. Bowen 2003

Your name:_____

Today's:_____

Your telephone number: _____

Coach's name: _____

Coach's telephone: _____

Please note when completing the forms:

1. You can write as much as you like – the more the better for us to relay back to you
2. Fill in ALL the days
3. YOU must fill in the forms – not the coach – you can ask for advice on training etc.
4. Always wear your heart rate monitor and the watch when doing any type of training or running – even when you run to work or to school.
5. Fill in the correct dates and times when logging data as this is correlated with the heart rate monitoring.
6. If you have problems, contact the supervising researcher as soon as possible (details below)
7. Make sure you understand the operation of the heart rate monitor and watch – see back page for operation.
8. Enjoy the 7-day training!

Supervising researcher: Robyn Bowen
Tel: 084 660 7063
Email: rlb@sun.ac.za

Training Log	Today's date	

Remember to wear HEART RATE MONITOR and WATCH

How many training sessions today in total

Road		Off-Road		Track		Strength		Other	

Training Session 1

Time of Session:		Duration of Session:	
Weather Conditions:			

Group Training/Alone:

Type of Session

Long Run		High Intensity		Competition		Other (Specify)	

Track		Road		Off-Road		Strength		(Make an X where appropriate)

Approximate Total Distance (km) of:	Warm-up		Training:		Warm-down	

Describe the following in detail | The warm up:

The route:

Breakdown of Session: (eg: Hard, fast run – intensity (min/km), did you rest, were there intervals, etc)

For High Intensity Training (HIT):

Hill		Fartlek		Track	
Speed of each HI interval (km/h):		Distance of each Interval			
Number of Intervals:		Activity during rest:			
Duration of each HI Interval:		Duration of each rest Interval:			

Describe how you felt during session (including HIT):

How did you feel before the session: (eg: Eager to train/ tired etc)

How did you feel during the session: (Strong/ not a good run etc)

How did you feel after the session:

Any change of plans during the session ie: route/ distance?

RPE of Session		Max. Heart Rate		Average Heart Rate	

Training Session 2

Time of Session:		Duration of Session:	
Weather Conditions:			

Group Training/Alone:

Type of Session

Long Run		High Intensity		Competition		Other (Specify)	

Track		Road		Off-Road		Strength		(Make an X where appropriate)

Approximate Total Distance (km) of:	Warm-up		Training:		Warm-down	

Describe the following in detail | The warm up:

Breakdown of Session: (eg: Hard, fast run – intensity (min/km), did you rest, were there intervals, etc)

For High Intensity Training (HIT):

Hill		Fartlek		Track	
Speed of each HI interval (km/h):		Distance of each Interval			
Number of Intervals:		Activity during rest:			
Duration of each HI Interval:		Duration of each rest Interval:			

Describe how you felt during session (including HIT):

How did you feel before the session: (eg: Eager to train/ tired etc)

How did you feel during the session: (Strong/ not a good run etc)

How did you feel after the session:

Any change of plans during the session ie: route/ distance?

RPE of Session		Max. Heart Rate		Average Heart Rate	

Training Session 3

Time of Session:		Duration of Session:	
Weather Conditions:			

Group Training/Alone:

Type of Session

Long Run		High Intensity		Competition		Other (Specify)	

Track		Road		Off-Road		Strength		(Make an X where appropriate)

Approximate Total Distance (km) of:	Warm-up		Training:		Warm-down	

Describe the following in detail | The warm up:

The route:

Breakdown of Session: (eg: Hard, fast run – intensity (min/km), did you rest, were there intervals, etc)

For High Intensity Training (HIT):

Hill		Fartlek		Track	
Speed of each HI interval (km/h):		Distance of each Interval			
Number of Intervals:		Activity during rest:			
Duration of each HI Interval:		Duration of each rest Interval:			

Describe how you felt during session (including HIT):

How did you feel before the session: (eg: Eager to train/ tired etc)

How did you feel during the session: (Strong/ not a good run etc)

How did you feel after the session:

7.4. APPENDIX 4: CITRATE SYNTHASE PROTOCOL

SPECTROPHOTOMETRIC ENZYME ASSAYS

Homogenising buffer

100 mM Phosphate buffer pH7,40

KH_2PO_4	0.572g
K_2HPO_4	2.752g
EDTA-$2H_20$	0.149g
MgCl2-$6H_20$	0.203g

Make up to 150 ml with dH_20. adjust pH to 7.40. Fill up to 200 ml. Store in opaque bottle in fridge.

Before first use

Add to	12.5 ml PO_4 Buffer	25.0 ml PO_4 Buffer
BSA (0.02%)	2.5 mg	5 mg

This can be stored for less than a week in the fridge

Homogenizing and sonnication of samples

- Dissect sample free of any remaining connective tissue or blood. This should preferably be done at -20°C.
- Weigh the sample
- Chop sample with fine scalpel and add homogenizing buffer:
  1:20 for wet weight (eg. 25 mg x 19 = 475 µl)
  1:90 for dry weight (eg. 8 mg x 89 = 712 µl)
- Vortex sample to ensure proper mixing
- Sonnicate sample on ice
- Pipette into eppindorfs and freeze

CITRATE SYNTHASE ASSAY (SPECTROPHOTOMETRICAL)[1]

A: 100 mM Tris buffer pH8.30

Tris-base	Mr(121.1)	1.850 g
Tris-HCl	Mr(157.6)	1.535 g

Fill ¾ and adjust pH. Fill up to 250 ml with 250 ml dH_2O. store in opaque bottle in fridge

B: 1 mM DTNB

DTNB	Mr(396.4)	10 mg

Fill up to 25 ml with Tris buffer

C: 4mM Acetyl-CoA

Must prepare fresh

Acetyl-CoA Mr(827.4) 5 mg
Fill up to 1.5 ml with dH_20

D: 10mM Oxaloacetate

Oxaloacetate Mr(132.1) 2.67 mg
Fill to 2 ml Tris buffer

Procedure:

- Add 10 µl sample, 50 µl C, 100 µl B and 795 µl A to a cuvette
- Add 50 µl D, invert cuvette, and read every 30 sec for 5 min at 412 nm
- Calculation: Gradient = Abs/min

$$E = 13\,600\ M^{-1}.cm^{-1}$$

$$\frac{\text{Abs} \times 100}{E \times [\text{Muscle}]\ g/l}$$

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1. **Srere, P.A.** Citrate Synthase. In Lowenstein, J.M., ed., Methods in Enzymology. New York and London, Academic Press. 1969, 3-11

7.5. APPENDIX 5: BRADFORD ASSAY PROTOCOL

BRADFORD ASSAY (ELISA PLATE READER) RANGE: 0.05 – 0.5 g/l

Description:

To determine the protein concentration of a unknown sample using the Coomassie Brilliant Blue G250 stain

Materials:

Coomassie G 250 powder

95% (v/v) EtOH

Distilled water

Phosphoric acid (H_3PO_4; 85% m/v)

Whatman no 1 Filter paper

BSA crystals

100 mM Phosphate Buffer pH 7.40

Method

Bradford reagent

- Weigh off 0.1g Coomassie Brilliant Blue G250 and add 25ml 95% EtOH.
- Add 50ml 85% (m/v) phosphoric acid (H_3PO_4) slowly and stir with glass rod.
- Add the above to 100ml dH_2O in a 500ml volumetric flask. Add dH_2O to mark.
- Filtrate through filter paper.
- Store at 4°C in brown bottle.

0.5mg/ml BSA Standard

- Weigh off 5mg BSA and add 10ml 100mM Phosphate buffer pH 7.40 and mix.
- Store at –87°C
- Standards must be performed in duplicate.

Standard BSA series (work very carefully – accuracy is important)

Tube no	BSA (µl)	Phosphate buffer	[BSA] (mg/ml)
0 (Blank)	0	10	0.0
1	2	8	0.1
2	4	6	0.2
3	6	4	0.3
4	8	2	0.4
5	10	0	0.5

Unknown sample preparation (muscle wet weight or dry)

- Mark eppindorfs or use a second ELISA plate.
- The dilution will be 1:29.
- Pipette 10µl of sample into the eppindorfs followed by 290µl 100mM Phosphate buffer pH 7.40 and mix well.

- Of the above, pipette 10µl in duplicate into the appropriate wells.
- Pipette for all samples and standards, 200µl Bradford reagent into each well.
- Incubate for 5 minutes at room temperature.
- Read absorption at 595nm.

Other tips

- When using the ELISA reader, make sure the machine is switched on 30 minutes before the time.
- The eppindorfs might be a better idea when enzymes have to be measured. Make sure about concentrations before proceeding.
- It is essential that the samples be very well mixed. Use the pipette. Make sure the tips are clean.
- The phosphate buffer can be substituted for dH_2O
- Calculation: $\frac{\text{Abs of sample}}{\text{Slope}} \times 30 = \text{Protein concentration in mg/ml}$

7.6. APPENDIX 6: mATPase STAINING PROTOCOL

Muscle Histochemistry

Cutting of Muscle

- Muscles must be at -20°C, therefore leave it in the cryostat for 10 minutes
- Before slide collection, orientate muscle and cut 20 μm slices. Check wether the section is orientated correctly under the microscope
- Mark previously made poly-L-lysine slides for each of the pre-incubation pHs
- Change the slice thickness to 10 μm and place on section in the each of the top two corners of each of the three slides, with consecutive slices being placed on the three different pH slides
- When finished cutting place slides in copelin jars, marked for the relative pre-incubation pH – they can be placed bacl-to-back – seal the jars and place overnight in the -20°C freezer. The slides must be stained the day following cutting.

MATPase Staining Solutions

- Prepare the solutions the day before staining, on the same day that the samples are cut

Solution 1

2.253 g Glycine
2.40 g $CaCl_2$
1.755 g NaCl
300 ml dH_2O
Mix with 270 ml 0.1 M NaOH (1.08 g NaOH to 270 ml)
Adjust pH with concentrated HCl or 5 M NaOH to 10.3

Solution 2 (a and b)

3.90 g Na-acetate
3.70 g KCl
500 ml dH_2O
Use glacial acetic acid to adjust pH to 4.30 or 4.60
PH 4.60 solution is VERY important and very sensitive, work extra carefully with it.

Solution 3

0.017 g ATP in 10 ml solution 1 (There are three cups of 10 ml, thus 30ml in total)

Adjust pH with HCl to 9.40

ATP: Sigma A-5394

Staining of samples

- Be very precise with timing
- Turn on waterbath to 37°C prior to start of staining so that it is at the correct temperature on commencement of the staining.
- Room temperature should be controlled to between 20 and 25°C
- For the duration of the staining procedure, the slides should remain in the copelin jars
- Pre-incubate the slides in:

10.3: Solution 1	9 min, shaking bath 37°C
4.3: Solution 2a	1 min, at room temperature
4.6: Solution 2b	1 min, at room temperature

- Rinse the slides well with dH_2O
- Incubate in solution 3 at 37°C for 30 minutes
- Rinse the slides well with dH_2O
- Incubate the slides in 1% $CaCl_2$ at room temperature for 3 minutes (NB: the solution must be at room temperature)
- Rinse the slides well with dH_2O
- Incubate the slides in 2% $CoCl_2$ at room temperature for 3 minutes (Wear gloves at this stage as the solution is very poisonous)
- Rinse the slides well with dH_2O
- Incubate in 1% NH_4S at room temperature for 1 minute (2.5ml 20% NH_4S solution, fill to 50 ml dH_2O). This step of the staining process needs to be performed in the fume cupboard
- Rinse the slides well with dH_2O
- Allow to dry well and mount with glycerine gelatine