VITAMIN AND MINERAL SUPPLEMENTATION IN ATHLETES, WITH SPECIAL REFERENCE TO THE ERGOGENIC EFFECTS AND POSSIBLE TOXIC SIDE-EFFECTS

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by

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DECLARATION

I, Lindsay Weight, declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other University.

I empower the University to reproduce for the purpose of research either the whole or any portion of the content in any manner whatsoever.

Part of this study was presented at the International Symposium on Research in Sport and Recreation held in Johannesburg on 25-27 June 1986.

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ABSTRACT.

The use of vitamin and mineral supplements as ergogenic aids is widespread amongst both athletes and the general population. Although there appear to be at least some theoretical reasons to suggest that supplementation may enhance athletic performance, there is limited scientific justification for this belief. There is also no evidence that the vitamin requirements of heavily training athletes eating a normal diet are increased. Furthermore, the possiblity that toxic side-effects may develop when high doses of vitamins and minerals are consumed, raises the question of the safety of this practice.

This study was designed to answer the following questions:-

(i) Does a commercially-available multi-vitamin and mineral supplement enhance the athletic performance of a group of trained marathon runners?

ii) Do athletes require additional vitamin and minerals in view of their increased energy expenditure?

(iii) Are there toxic side-effects associated with daily vitamin and mineral supplementation in these runners?

A nine-month cross-over, placebo-controlled study design was employed. The subjects were 30 competitive male athletes who had been running for at least three years and who were training more than 70 km per week. They were randomly assigned to two groups, so that 15 received placebo

("placebo group") and 15 the active agent ("active medication group") for the first 3 month period. This was followed by a 3 month 'wash-out' period during which both groups received no medication. During the last 3 month period the medications taken by the two groups were crossed over, so that the group who had initially received the placebo then ingested the active agent, and those who were initially on the active medication, received the placebo.

The following parameters were measured at 0, 3, 6 and 9 months:

i) Performance variables - maximal oxygen consumption, maximal heart rate, peak running speed and the lactate turnpoint were measured during a progressive treadmill running test to volitional exhaustion. Running time in a 15 km time-trial was also measured.

ii) Blood biochemical levels.

The serum levels of the following vitamins were measured:-
Riboflavin, nicotinic acid, pyridoxine, ascorbic acid, retinol and tocopherol.

The serum levels of the following minerals were measured:-
Zinc, magnesium, copper and iron.

iii) Haematological parameters.

The following haematological parameters were measured:-
Haemoglobin and haematocrit; serum iron, ferritin, folate, B_{12} and

red-cell folate levels; percentage saturation and total iron-binding capacity.

Throughout the trial the subjects were asked to report the occurrence of any side-effects they considered to have been due to the supplementation. In addition, they recorded in a standardized log-book on a daily basis, the following variables: distance run, waking pulse rate, mass, hours of sleep and perceived exertion during exercise. From these data, weekly averages were calculated.

During the initial phase of the study all subjects completed a 5 day diet record from which the average dietary intake of the macronutrients, vitamins and minerals was determined.

Statistical analysis at completion of the study determined that there were no significant differences between the two groups nor in the way they responded to the intervention; thus the data for the two groups for the appropriate sections of the study were pooled.

The study showed that three months of active supplementation:-

i) did not significantly alter the maximal oxygen consumption, maximal heart rate, peak treadmill running speed, lactate turnpoint or 15 km time-trial time;

ii) did not significantly alter any haematological parameters which have been shown to be related to physical fitness and work capacity; and

iii) significantly altered mean serum levels of riboflavin and pyridoxine. The mean serum vitamin and mineral levels were normal at the start of the trial, and remained so throughout the trial with the exception of serum pyridoxine levels which were abnormally elevated after the active medication phase.

iv) There were also no signs or symptoms of serious toxic side-effects apart from mild gastrointestinal disturbances.

iv) Dietary analysis showed that the athletes' mean daily intake of all vitamins and minerals was above the Recommended Daily Allowances for adult males.

I therefore conclude that although the mean blood levels of two vitamins and minerals increased significantly after multi-vitamin and mineral supplementation, this was not reflected in a measurable improvement in athletic performance in this group of athletes whose vitamin and mineral status was initially normal. The failure of all the blood vitamin and mineral levels to increase with supplementation may be the result of previously-documented interactions between the various components of the multi-vitamin and mineral supplement studied. There was no evidence of significant toxic side-effects from the chronic ingestion of this supplement.

ABBREVIATIONS.

b.p.m.	beats per minute
g	grams
mg	milligrams
µg	micrograms
ng	nanograms
pg	picograms
l	litre
dl	decilitre
ml	millilitre
mmol	millimole
µmol	microgram
hr	hour
I.U.	International Units
m	metre
km	kilometres
RDA	Recommended Daily Allowance
VO_2 max.	maximal oxygen uptake

In this thesis the vitamins have been referred to by their chemical and not their common name. Hence,

Thiamine	-	Vitamin B_1
Riboflavin	-	Vitamin B_2
Nicotinic acid	-	Vitamin B_3
Pyrodoxine	-	Vitamin B_6
Cyanocobalamin	-	Vitamin B_{12}
Folate	-	Folic acid
Ascorbic acid	-	Vitamin C
Retinol	-	Vitamin A
Tocopherol	-	Vitamin E

CHAPTER ONE

INTRODUCTION TO AND SCOPE OF THE THESIS.

In 1912 Funk coined the word 'vitamine' to describe certain "accessory factors" postulated to be present in the diet and essential to life. Subsequently it was shown that a dietary deficiency of these substances was related to certain disease states. However it was only in the mid-1930's that vitamins were first isolated and their molecular structure and biological role established, making possible their chemical manufacture. More recently, a 'vitamythology' (Percy 1978), based on the belief that vitamins are 'organically primitive substances' (Van der Beek 1985) with special properties (Jarvis 1983) has developed, so that it is widely believed by both the general population and athletes that the consumption of vitamins in addition to those supplied in the diet, is essential not only to maintain optimal health but also to enhance physical performance (Barnett and Conlee 1984; Costill 1982; Williams 1976). This belief has been strengthened by several authoritative scientific reports which have recommended the use by athletes of vitamin and mineral supplements, frequently in very large doses (Cureton 1954 and 1955; Klafs and Arnheim 1973; Sherman and Smith 1922; Williams 1976).

Thus as early as 1922, Sherman and Smith suggested that athletes should supplement their diets with an increased intake of vitamins A, B and C in order to 'elicit full stimulation of body secretions and to prevent nervousness'. They provided no data to support their suggestion. Other unsubstantiated claims for the use of vitamin and mineral supplements include those that work stress depletes critical body nutrients (Cureton 1969); that an additional vitamin intake is necessary to support the increased mitochondrial mass that develops with training (Buskirk and Haymes 1972); that large doses of ascorbic acid are necessary to

stimulate myocardial and skeletal muscle capillarization (Zauner and Updyke 1973) and to prevent fatigue in conditions of work stress (Baker 1967). More recent claims have been that supplementation with the B-group vitamins may be necessary when a high carbohydrate diet is eaten (Van der Beek 1985), and that women especially have an increased riboflavin requirement (Belko et al. 1983).

It has also been argued that because of their overall increased metabolic rate and possibly increased rate of elimination of vitamins and minerals during work stress, the vitamin requirements of athletes might be greater than those of non-athletes (Klafs and Arnheim 1973; Williams 1976; Yakovlev and Rogiskin 1975). The scientific evidence to support this is equivocal. In particular, there is no evidence that increased amounts of vitamins are lost in sweat as sweat is almost completely devoid of vitamins, although it does contain varying amounts of minerals (Consolazio et al. 1963; Costill 1977; Sarguet et al. 1944; Vellar 1968; Vellar and Hermansen 1971).

Intense endurance exercise such as long distance running has been associated with reduced plasma concentrations of certain mineral elements (Dressendorfer and Sockolov 1980; Hunding et al. 1981; Rose et al. 1970). These limited data suggest that strenous endurance training may increase dietary mineral requirements. However, little is known about the development of exercise-induced plasma mineral deficiencies and any special mineral requirements of endurance athletes (Dressendorfer et al. 1982).

The result of this 'vitamythology' has been that the use of vitamin and mineral supplements has now become widespread among athletes (Barnett and Conlee 1985; U.S. Senate Committee 1973; Van Huss 1974; Williams 1976) with one report concluding that 84% of Olympic athletes use vitamin supplements (Van Huss 1974). A further factor possibly contributing to the widespread use of vitamin and mineral supplements in these elite athletes is that such use is legal (despite the growing evidence that at very high doses, vitamins assume a pharmacological role), whereas the ingestion of other pharmacological agents is banned.

Despite the apparently widespread use of vitamin and mineral supplements among athletes, biochemical but not necessarily clinical evidence of vitamin and mineral deficiencies have been reported in different groups of athletes (Haralambie 1976 and 1981a and b; Van Dam 1978). Thus biochemical deficiencies of the B-complex vitamins, riboflavin, pyridoxine and nicotinic acid, have been reported in European athletes (Haralambie 1976 and 1981a and b; Van Dam 1978) and there is also considerable evidence of iron deficiency and even frank anaemia in athletes, most especially those involved in endurance sports (Banister and Hamilton 1985; Brotherhood et al. 1975; Colt and Hayman 1984; Clement et al. 1977; Clement and Asmundson 1982; Clement and Sawchuck 1984; de Wijn et al. 1981; Dickson et al. 1982; Dufaux et al. 1981; Ehn et al. 1980; Frederickson et al. 1983; Goforth et al. 1982; Hunding et al. 1981; Magnusson et al. 1985a and b; Matter et al. 1986; Nickerson and Tripp 1983; Pate 1983; Ross and Atwood 1984; Stewart et al. 1972; Stewart et al. 1984; Wishnitzer et al. 1983). In addition, hypozincaemia has been reported in German athletes (Haralambie 1981b) and there is also evidence that

strenuous exercise reduces the serum levels of copper and magnesium (Haralambie 1981a).

Whereas frank clinical vitamin deficiencies most notably those of the B-complex have been clearly demonstrated to limit work capacity (Berryman et al. 1947; Brozek et al. 1946; Brozek and Guetzkow 1957; Consolazio 1983; Keys et al. 1945; Van der Beek 1985; Wilson et al. 1949), no such direct effect on athletic performance has been shown for a sub-clinical deficiency state (Crandon et al. 1940; Hornig et al. 1981; Matter et al. 1986). In addition, while there is fairly comprehensive knowledge of the role of vitamins and minerals in normal physiological processes, many uncertainties exist regarding their role during exercise (Costill 1976; Haralambie 1975 and 1981a; Lehninger 1975; McGilvery and Goldstein 1979).

Consequently many researchers have concluded that vitamin and mineral supplementation will not markedly improve performance unless there is clinical evidence of a vitamin or mineral deficiency, in which case supplementation will return performance to normal levels but will not further enhance it (Barnett and Conlee 1985; McArdle, Katch and Katch 1986; Nelson 1975; Van der Beek 1985). Thus Van der Beek (1985) states that an adequate vitamin intake will maintain physical performance but that vitamin supplementation cannot lift the athlete's performance above levels determined by his physiological limitations. Thus while there may be an indication for the use of vitamin and mineral supplements in athletes if a clinical deficiency state exists, there is no apparent justification for the blanket recommendation that all athletes should

take multi-vitamin and mineral supplements (Harper 1974; Klafs and Arnheim 1973; Nelson 1975). This conclusion is supported by the recommendations of the National Research Council, Food and Nutrition Board of the United States of America who do not advocate the routine use of vitamin supplements, nor do they recommend an increased vitamin allowance for the physically active (National Research Council, Food and Nutrition Board Recommended Daily Allowances 1980).

Another argument against the widespread use of supplementary vitamins by athletes is the risk of vitamin toxicity. While it has been recognized for many years that excessive consumption of fat-soluble vitamins can have toxic effects, only more recently has it been demonstrated that this may also be true even for the water-soluble vitamins (Alhadeff et al. 1984; Marks 1984). In addition it is now clear that in very large doses, vitamins no longer act in their normal metabolic role but assume a pharmacological action (Marks 1984). While there is a considerable margin of safety for most vitamins with few serious side-effects even when they are ingested in doses far in excess of the Recommended Daily Allowances (RDA) for short periods, more serious complications such as allergic reactions (U.S. Senate Committee 1973; Marks 1984; Mosher 1970; Parsons 1960), 'rebound scurvy' (Hornig and Mosner 1981), anorexia, weakness, gastro-intestinal disturbances (Hornig and Mosner 1981), liver and kidney failure (Taylor 1972; Pardue 1961), calcification of the soft tissues (Linden 1974) and sensory neuropathy (Schaumberg et al. 1983) have been reported in persons ingesting large doses of supplemental vitamin and minerals for prolonged periods (Barness 1977; Fumig and Essig 1982; Hornig 1981; Korner and Weber 1972).

Finally, while many previous studies have investigated the effects of vitamin and mineral supplementation on athletic performance, many have been inadequately controlled and it has thus been difficult to assess the confounding effects of time and changes in fitness (Van der Beek 1985). There has also been little consideration given to the actual blood vitamin and mineral levels of the subjects and the effects on these levels of such supplementation. In addition, while there are reports of hypervitaminosis in athletes (Fumig and Essig 1982), there is no evidence to date on the possible side-effects of relatively prolonged chronic supplementation specifically in competitive athletes.

This study was therefore designed to address three major questions regarding chronic vitamin and mineral supplementation in athletes, specifically: (i) Does vitamin and mineral supplementation enhance athletic performance ? (ii) Do athletes require an increased intake of vitamins and minerals in view of their increased daily energy expenditure and (iii) what are the toxic side-effects, if any, of chronic vitamin and mineral supplementation ?

A nine-month cross-over, placebo-controlled study design was employed. The subjects were 30 competitive male athletes who were randomly assigned to two groups, so that 15 received placebo ("placebo group") and 15 the active agent ("active medication group") for the first 3 month period. This was followed by a 3 month 'wash-out' period during which both groups received no medication. During the final 3 month period the medications taken by the two groups were crossed over, so that the group who had initially received the placebo then ingested the active agent,

and those who were initially on the active medication, received the placebo.

At 0, 3, 6 and 9 months the subjects underwent a maximal laboratory treadmill test during which variables related to running performance were measured, and they also competed in a 15 km time-trial. Serum vitamin and mineral levels and haematological parameters were also measured on each of these four occassions. A 5-day diet record was completed by all subjects at the beginning of the trial. Throughout the trial the subjects kept detailed records of training distance with ratings of perceived effort and the occurence of any side-effects which they considered to be due to the supplementation.

This thesis is the result of this study.

CHAPTER TWO

THE EFFECT OF VITAMIN AND MINERAL STATUS ON ATHLETIC PERFORMANCE:

A REVIEW OF THE LITERATURE RELATED TO THE EFFECTS OF VITAMIN DEPLETION AND SUPPLEMENTATION ON ATHLETIC PERFORMANCE, AND OF THE ROLE OF THE SELECTED MINERALS IN HUMAN EXERCISE PHYSIOLOGY.

2.1 INTRODUCTION

Despite the apparently widespread use of vitamin and mineral supplements by both the general and the athletic population (Barnett and Conlee 1984; Van Dam 1978; Van den Berg 1978; van Huss 1974; Williams 1976), there are reports of mild to moderate vitamin and mineral deficiencies in these groups (Belko et al. 1983; Brotherhood et al. 1975; Ehn et al. 1982; Clement et al. 1977; Ehn et al. 1982; Haralambie 1981; Van Dam 1978; Van den Berg 1978) but the functional significance of these marginal biochemically-determined deficiencies is currently unknown.

Since the early 'forties there have been many studies on the effects of the vitamins, taken both individually and in multi-vitamin complexes, on both physical and psychological performance. These studies indicate that the capacity for physical and mental performance deteriorates in persons with clinically-apparent vitamin deficiencies, especially deficiencies of the water-soluble vitamins (Berryman et al. 1947; Brozek et al. 1946; Brozek and Guetzkow 1957; Buzina et al. 1982; Consolazio 1956 and 1983; Keys et al. 1945; Van der Beek et al. 1984; Wilson et al. 1949). In particular, physical work capacity is impaired by vitamin deficiencies which cause muscular weakness and inco-ordination (Consolazio 1983). With regard to the effects of vitamin supplementation on physical performance, the majority of studies do not support the widespread belief in the ergogenic effect of such supplementation (Barnett and Conlee 1984; Haralambie 1975; Keys and Henschel 1941 and 1942; Keys et al. 1945; Shepherd et al. 1974; Van den Beek 1985).

The following is a review of the laboratory and field studies of the effects on physical performance of either vitamin deficiency or vitamin supplementation with selected vitamins or with multi-vitamin complexes. Currently there are few studies of the effects of mineral deficiencies and mineral supplementation on physical performance. For this reason I have included a literature review on the physiological and metabolic aspects of selected minerals and trace elements including copper, zinc, magnesium and iron as they relate to physical work capacity.

2.2 THE VITAMIN B COMPLEX

The B group vitamins are theoretically those most likely to be of benefit to athletic performance and several authorities have advocated that athletes should supplement with these vitamins (Williams 1976). As the B-group vitamins are water-soluble and not stored in any appreciable amount in the body, a constant dietary supply must be provided. There are two reasons for believing that vitamin B supplementation will benefit athletic performance:-

(i) The B-complex vitamin co-factors, nicotinamide adenine dinucleotide (NAD), flavin adenine dinucleotide (FAD), riboflavin 5'-phosphate (FMN) and thiamine pyrophosphate (TPP) are required in the catabolism of carbohydrates, proteins and fats (McGilvery and Goldstein 1981). As physical conditioning increases the number of mitochondria in the muscle cells of trained limbs (Barnard and Peter 1971), it is postulated that higher levels of vitamin co-factors may be necessary to support the increased mass of mitochondrial enzymes. The three principal B-group vitamins involved in these processes are thiamine, riboflavin and nicotinic acid. These were the first to be studied to determine their effect on physical performance.

(ii) A deficiency of the B-complex vitamins is clearly associated with a decrease in physical performance (Barborka et al. 1943; Berryman et al. 1947; Brozek et al. 1946; Brozek and Guetzkow 1957; Consolazio 1983; Keys et al. 1945; Van der Beek et al. 1984; Wilson et al. 1949).

The well-controlled and complex early studies of Keys and Henschel (1942) and Keys et al. (1945) indicated that supplementation with thiamine, nicotinic acid, ascorbic acid or with thiamine, riboflavin, nicotinic acid, pyridoxine, ascorbic acid and calcium pentothenate did not influence any physiological or biochemical parameters measured during exercise, including oxygen consumption and blood lactate concentration. Previously Keys and Henschel (1941) had found that supplementation with thiamine, riboflavin and ascorbic acid did not affect either muscular resistance to fatigue or recovery from exertion.

Egana et al. (1942) reported a decreased time to exhaustion during treadmill testing and increased subjective ratings of fatigue in subjects eating a vitamin B deficient diet; the statistical analysis was suspect however. Other work supporting the view that vitamin B-deficiency causes a decrease in endurance capacity was that of Berryman et al. (1947), who also found that recovery to full performance capacity was slow on re-supplementation following vitamin depletion. There was also no indication that supplementation in nutritionally-replete subjects provided an additional ergogenic effect. Foltz et al. (1942) concluded that vitamin B-complex supplementation did not facilitate rapid post-exercise recovery. Similarly Friedman and Ivy (1947) found that subjects whose vitamin intake was below the RDA remained in good health without any deterioration in a wide range of physical performance tasks. Frankau (1943) studied the effects of nicotinamide alone and in combination with thiamine, riboflavin, retinol, cholecalciferol and tocopherol on physical endurance and co-ordination. He concluded, without statistical analysis, that both regimes significantly increased work efficiency.

Early and Carlson (1969) concluded that vitamin supplementation decreased fatigue, possibly because sweating induced a loss of vitamins during exercise in a hot environment. They therefore suggested that one or more of the B-complex vitamins, possibly thiamin or pantothenic acid may be necessary during training.

There has been limited subsequent experimental work on the B-complex vitamins since these early studies. Recently however, the possible benefits of supplementation are being re-examined, with special emphasis on the B-group vitamins pyridoxine, cyanocobalamin and pantothenic acid which have seldom been investigated. However there is no conclusive evidence for an ergogenic effect of these vitamins (Gray and Titlow 1982; Nice et al. 1984; Girandola et al. 1980; Van der Beek et al. 1984).

Read and McGuffin (1983) investigated the effect of B-complex supplementation on the endurance capacity of male athletes and found that such supplementation does not generally enhance endurance capacity. Similar findings come from the earlier studies by Foltze et al. (1942), Hilsendrager and Karpovich (1964) and Keys and Henschel (1941 and 1942).

Thus it would appear that prolonged deficiency of the B-group vitamins causes impaired performance (Barborka et al. 1943; Berryman et al. 1947; Brozek et al. 1946; Brozek and Guetzkow 1957; Consolazio 1983; Keys et al. 1945; Van der Beek et al. 1984; Wilson et al. 1949), re-supplementation with these vitamins restores performance to normal (Berryman et al. 1947; van der Beek et al. 1984; Consolazio 1956). However there is

no ergogenic benefit from additional vitamin B-group supplementation if the dietary intake is adequate.

2.3 THIAMINE.

Thiamine plays an important role in energy metabolism and in maintaining the integrity of the nervous system. Specifically it has a key function in the oxidative decarboxylation of pyruvate to acetyl-CoA which provides fuel for the Krebs cycle and thus for the generation of adenosine triphosphate (ATP). A deficiency of thiamine could thus result in increased muscle lactate production during exercise and the premature onset of fatigue (Peters 1936). Keys et al. (1943) and Horwitt and Kriesler (1949) considered the increase in venous lactate after an oral glucose load as an early sign of thiamine defciency. In addition, Early and Carlson (1969) noted that thiamine deficiency could result in the production of insufficient amounts of succinate, a component of heme which would limit the oxygen-carrying capacity of the blood.

Because of its close relationship with energy metabolism, the RDA of thiamine is often expressed per 1000 kcal of food energy consumed (Sauberlich et al. 1979). The National Research Council, Food and Nutrition Board of the United States of America (1974) noted that the need for thiamine is related to energy expenditure and is influenced by carbohydrate intake, but as the increased thiamine needs of the physically active should be met by the larger quantites of food they consume, there should be no need to supplement the diet with thiamine.

Since the role of thiamine in energy metabolism has been appreciated for over forty years, it has been one of the most studied vitamins in relation to physical exercise. Even so the total number of relevent studies are limited, and many of the studies have used thiamine in conjunction with other B-complex vitamins.

Studies in the 1940's of the effect of diets deficient in thiamine and other B-complex vitamins have consistently shown a deterioration of physical performance during the period of vitamin deficiency (Archdeacon and Murlin 1944; Barboka et al. 1943; Egana et al. 1942; Johnson et al. 1942). A marked improvement in physical performance and muscular endurance was found when yeast extract was administered following the period of deficiency (Archdeacon and Murlin 1944; Barboka et al. 1943; Egana et al. 1942; Johnson et al. 1942). However, Keys et al. (1945) found that a borderline thiamine intake for 5 months had no significant effect on health or fitness, but that when a thiamine-deficient diet was instituted following this phase anorexia, nausea and vomiting developed and there was a marked deterioration in endurance performance on the treadmill. All symptoms regressed rapidly after re-supplementation. Other studies using similar protocols confirm these findings (Brozek et al. 1946; Brozek and Guetzkow 1957; Berryman et al. 1947; Wilson et al. 1949).

Although the early and poorly controlled work by McCormick (1940) suggested that increased thaimine intake might improve physical performance, no subsequent studies have found that thiamine supplementation has any effect on physical capacity including muscular strength or endurance

(Archdeacon and Murlin 1944; Fotze et al. 1941; Irvine and Prentice 1962; Karpovich and Millmann 1942). More recently Steel (1970) noted that a group of Australian Olympian medallists were found to have higher thiamine intakes than the less successful athletes, and he thus suggested that athletes pay particular attention to their thiamine intake. McNeill and Mooney (1983) report that in conjunction with a high carbohydrate diet, thiamine taken at 100 x RDA significantly improved the endurance capacity of trained rats.

In summary it appears that although thiamine plays an important role in energy metabolism and that dietary deficiency is associated with impaired physical performance, there is no conclusive evidence to support the contention that supplemental thiamine will enhance performance.

2.4 RIBOFLAVIN.

There are few studies investigating the effects of riboflavin taken alone on physical performance. Keys et al. (1944) found no change in exercising heart rates or maximal power during a 5 month period in subjects eating a diet low in riboflavin. Consolazio et al. (1971) also found no impairment in work capacity during a 56 day depletion study. Their data suggest that a riboflavin intake of less than 0,07 $mg.day^{-1}$ was sufficiently low to deplete body stores, as evidenced by the increased erythrocyte glutathione reductase activity coefficient (EGRAC) values (Tillotson and Baker 1972). Early in the study, urinary excretion of riboflavin fell to levels associated with severe deficiency.

Belko et al. (1983) studied the effects of a change in energy requirements caused by exercise or by inactivity, on the riboflavin requirement of young females. It was found that in order to achieve a 'biochemically normal' riboflavin status, defined as an EGRAC of 1.2, both moderately and very active young females required a higher riboflavin than the accepted RDA of 0,6 mg.1000 $kcal^{-1}$. Even during six-weeks of restricted activity, these subjects became riboflavin deficient when ingesting the RDA for riboflavin. This was corrected by increasing the riboflavin intake. When the subjects began a six-week running programme, the increased riboflavin intake had to be increased yet further in order to maintain a normal riboflavin status.

Belko et al. (1983) did not comment on the effect of the decreased riboflavin status on exercise performance and the question arises as to whether marginal vitamin deficiencies, as defined by red blood cell enzyme activities actually have any physiological significance (Thurnham 1981). Depletion of riboflavin and also other B-complex vitamins first effects the red blood cell enzymes, before clinical symptoms or signs of deficiency appear. Marginal riboflavin deficiency may impair the the red blood cell stability, reduce its survival time and increase erythropoesis (Thurnham 1981). On this basis, Belko et al. (1983) have recommended that it is important to increase riboflavin intake when there is evidence of a reduced EGRAC.

The reason for increased riboflavin needs in exercising persons is not clear. Tucker et al. (1960) observed an acute decrease in riboflavin excretion after both sudden severe and prolonged exercise and attributed

this to a decreased renal blood flow. A possible explanation for the long-term decrease in riboflavin excretion in exercising persons is that riboflavin needs increase as energy intake is increased to maintain body weight as the flavoproteins, of which riboflavin is an important component, play an essential role in energy utilization (Bro-Rasmusson 1958). Tucker et al. (1960) suggested that riboflavin is retained in exercising persons for incorporation into new muscle tissue. If true, this would mean that riboflavin requirements are probably increased with exercise, but in relation to changes in lean body mass, rather than in energy expenditure.

However, Belko et al. (1983) found that the increased riboflavin requirement with exercise did not appear to be related to small changes in lean body mass. They propose rather that riboflavin requirements may be increased during periods of negative energy balance, that is during weight-loss, when there is clearly an inadequate energy, and therefore riboflavin intake.

2.5 NICOTINIC ACID.

The major function of nicotinic acid is to serve as a component of two important coenzymes concerned with glycolysis, lipid synthesis and tissue respiration. According to White, Handler and Smith (1973), no serious impairment of oxidative capacity has been demonstrated in tissues of nicotinic acid-deficient animals. An increase in blood nicotinic acid levels after short exhaustive exercise has been observed, but the

reason for this remains unclear.

An early report by Frankau (1943) suggested that nicotinic acid enhanced anaerobic capacity and increased efficiency in tests involving coordination and physical effort. However in a later and better controlled study, Hilsendrager and Karpovich (1964) studied the acute effects of either nicotinic acid, or nicotinic acid in combination with glycine, and found the treatment to have no significant effect on endurance capacity. Although nicotinic acid is a potent vasodilator they did not find any change in systolic blood pressure after administration of 75 mg of nicotinic acid.

Studies on the acute effects of nicotinic acid supplementation both at rest (Carlson and Oro 1962) and during exercise (Bergstom et al. 1969; Jenkins 1965) have shown a decrease of plasma free fatty acids levels due to impaired free fatty acid mobilization from adipose tissue. Depressed free fatty acid levels during endurance exercise would theoretically decrease the time to exhaustion, as muscle glycogen will be used at a faster rate (Noakes 1986).

A study by Bergstrom et al. (1967) on the effect of nicotinic acid on muscular endurance capacity showed that the ability to perform either short-term near maximal work or prolonged sub-maximal work was unchanged after the pre-exercise administration of nicotinic acid. Blood lactate levels were significantly increased and the muscle glycogen levels significantly lower. In addition, the subjects perceived the work to be heavier and more fatiguing after the administration of nicotinic acid.

This suggests that although a greater amount of glycogen was used during exercise the subjects did not perceive the exercise to be any easier. Pernow and Saltin (1971) found that the administration of nicotinic acid to glycogen-depleted subjects decreased the work capacity and increased the respiratory quotient during exercise, indicating increased carbohydrate combustion. Thus both these studies suggest that the administration of nicotinic acid prior to endurance exercise is likely to be detrimental.

2.6 PYRIDOXINE

Pyridoxine is a co-enzyme of glycogen phosphorylase (Cori and Illingworth 1956) and may therefore be important in short-duration, high intensity "anaerobic" exercise when glycolysis is maximal (Danforth and Lyon 1964; Haltman and Bergstrom 1973). Supplementation with pyridoxine may enhance glycogenolytic capacity (de Vos 1982; Hatcher et al. 1982) and should be considered during the carbohydrate-loading phase prior to competition (Noakes 1986).

There are few studies of the effects of pyridoxine on athletic performance. Richardson and Chenman (1981) reported an increased time to fatigue in gastrocnemius muscle isolated from rats receiving pyridoxine supplementation, and they speculated that pyridoxine increased muscle contractile efficiency. Marconi et al. (1982) concluded that an -ketoglutarate-pyridoxine complex stimulates aerobic metabolism after finding an increase in the maximal oxygen consumption and a decrease in the peak

lactate concentration after administration of the complex.

The pyridoxine requirement appears to increase with the consumption of high protein diets, but as the main source of pyridoxine is meat and to a lesser extent, dairy products such a diet should cover dietary needs. Primary pyridoxine deficiency appears to be rare and is usually associated with the use of certain drugs, in particular oral contraceptives, and alcohol abuse. Wirth and Lohman (1982 and 1984) have however shown that the detrimental effect of oral contraceptive agents on static muscle endurance is not related to pyridoxine deficiency.

2.7 CYANOCOBALAMIN.

Cyanocobalamin has a general role in protein, fat and carbohydate metabolism. The physiological roles of cyanocobalamin and folate are interrelated in ways that are not clearly understood (Van der Beek 1985). As there is no cyanocobalamin in plant foods all strict vegetarians risk the development of cyanocobalamin deficiency leading to pernicious anaemia over a period of time (Noakes 1986).

No studies of the effects of cyanocobalamin deficiency on exercise performance have been reported, but there are two studies on the effect of cyanocobalamin supplementation on physical performance. Both reported no significant change in performance that could be attributed to supplementation (Montoye et al. 1955; Tin-May-Tan et al. 1978). Several studies of the effects of cyanocobalamin supplementation in combination

with the other B-complex vitamins have also failed to reveal any ergogenic effect (Early and Carlson 1969; Haralambie 1975; Read and McGuffin 1983).

2.8 FOLATE.

Folate deficiency with or without anaemia is possibly the most common vitamin deficiency in America and Eastern Europe, especially during pregnancy. Previous work in this department (Matter et al. 1986) has shown that the endurance capacity of female marathon runners with low serum folate levels but without macrocytic anaemia did not improve with supplementation that corrected the biochemical evidence of deficiency.

Currently there are no other studies of the effects of folate supplementation or deficiency on physical performance although there are several studies on the combined effects of folate and several of the lesser B-complex vitamins, for example pantothenic acid (Cogswell et al. 1946; Egana et al. 1942; Keys et al. 1944 and 1945).

2.9 ASCORBIC ACID.

Little is known of the mechanisms of action of ascorbic acid and there is controversy about the dietary requirements in both health and disease (Schaffer 1970; Williams 1976). Many claims have been made for the benefits of megadoses of ascorbic acid including prevention of the common

cold (Pauling 1970), the treatment of various cancers, the lowering of blood cholesterol levels (Schaffer 1970) and curing diabetes (Lamb 1974; Stone 1967). Baker (1967) claimed that there is an increased need for ascorbic acid in all forms of stress including physical exertion especially in the heat. Indeed, it is reported that athletes use large doses of ascorbic acid to prevent fatigue and enhance performance (Hanley 1980; Van Huss 1966). Cureton (1969) indicated that supplementation with ascorbic acid was effective in decreasing the oxygen debt while Horstman (1972) noted a shift in the oxygen dissociation curve following supplementation with ascorbic acid and decreased physical work capacity in subjects with ascorbic acid deficiency. Hettinger (1961) noted that ascorbic acid deficiency adversely affected the adaptive response of muscle to strength training.

There is evidence that ascorbic acid is involved in many of the metabolic processes important in physical exertion (Baker et al. 1966; Boddy et al. 1974). Ascorbic acid has been associated with the synthesis of catecholamines (Howald et al. 1975) and increases in serum cortisol levels (Boddy et al. 1974). Blood levels of both these hormones increase in response to stress. Several studies of experimental scurvy have suggested that physical exertion accelerates the depletion of the ascorbic acid pool (Baker et al. 1971; Hodges et al. 1971; Norris 1983). In addition, Boddy et al. (1974) found a decrease in leucocyte ascorbic acid levels following a two hour soccer practice, and von Huss (1966) found that the ascorbic acid content of the adrenal gland was decreased following exhaustive physical exercise. In contrast, Garry and Appenz-

eller (1983) reported increased blood ascorbic acid levels in athletes after an extremely stressful 46km run. The explanation for this is not clear, but they conclude that ascorbic acid supplementation is not required in endurance athletes.

A number of early reports suggested that ascorbic acid increased work efficiency and capacity and resistance to fatigue (Jokl and Suzman 1940;. Williams 1976). However most of these studies were not properly designed. More recent studies have also reported increased physical work capacity and mechanical efficiency with ascorbic acid supplementation. Interestingly the German, Russian and Central European studies have generally indicated a beneficial effect of ascorbic acid supplementation on physical performance whereas the studies from Western countries have not reported similar findings (Williams 1976). Possibly the central European athletes were initially deficient in ascorbic acid; this deficiency was corrected with supplementation.

Thus both Gey et al. (1970) and Keren and Epstein (1980) found no significant effect on performance of prolonged ascorbic acid supplementation. Bailey et al. (1967 and 1970) also reported that daily supplementation with 2 mg ascorbic acid was of no benefit in meeting the additional stress of maximal exercise in healthy subjects, regardless of their state of training or smoking habits.

Margaria et al. (1964) and von Huss (1974) studied the acute effect of ascorbic acid supplementation and found that neither running time to exhaustion nor the maximal oxygen uptake were altered by

supplementation. Howald et al. (1975) found that ascorbic acid decreased blood glucose and increased plasma free fatty acid levels. They concluded that ascorbic acid may exhibit a glycogen-sparing effect.

Other claims for ascorbic acid supplementation are related to the known effects of ascorbic acid deficiency on wound healing. In a review of the topic, Schwartz (1970) reported that a certain minimum dietary intake or minimum body ascorbic acid pool is required for adequate wound healing. Stanton (1952) did not find that the administration of ascorbic acid had a significant effect on muscle soreness. Although there may be some rationale for ascorbic acid supplementation in the treatment of athletic injuries, data supporting this postulate are not yet available.

One should be careful in interpreting the results of previous studies. Ascorbic acid is one of the most heat-labile vitamins and dietary intakes can vary widely. Unless the pre-study vitamin status of the tested subjects is known, the incorrect conclusions may be drawn. In some studies, only the acute effects of ascorbic acid were studied with little attention being paid to the possible need to build up the ascorbic acid pool. Other studies may have been of too short a duration. Nevertheless, those studies which have used substantial supplementation over prolonged periods of time (Gey et al. 1970; Williams 1976) do not support the belief that ascorbic acid supplementation is necessary in athletes.

2.10 RETINOL.

There are no studies on the effects of retinol supplementation on physical performance. However retinol supplementation would not appear to be justified on either theoretical of practical grounds (Williams 1976; Van der Beek 1985).

The sole study of the effects of retinol deficiency upon maximal endurance performance was conducted by Wald et al. (1942). Plasma retinol levels did not change during the 6 months of retinol deficiecny, nor did endurance capacity.

2.11 TOCOPHEROL.

Tocopherol has been one of the vitamins most widely used by athletes. There are several proposed physiological reasons why tocopherol should have an ergogenic effect. Williams (1976) quotes several reports suggesting that tocopherol facilitates oxygen utilization, reduces the tissue oxygen requirements, decreases lactic acid accumulation and increases free fatty acid mobilizaton and utilization during aerobic exercise. Greater resistance to hypoxia has been reported in animals receiving tocopherol (Williams 1976).

Cureton and Pohndorf (1955) conducted a number of poorly-designed studies and found that wheatgerm oil and tocopherol apparantly improved endurance capacity even though the supplementary dose of tocopherol

was lower than the RDA of 30 $mg.day^{-1}$.

More recent reports from better controlled studies have however concluded that tocopherol fails to influence performance during either aerobic of "anaerobic" exercise. Sharman (1971) studied the effects of tocopherol supplementation in schoolboy swimmers, Shepherd et al. (1974) in college middle-distance swimmers, Watt et al. (1974) in ice hockey players and Lawrence et al. (1975) in both well-trained and less well-trained swimmers. All failed to show an effect. Studies performed at altitude did however report decreased post-exercise blood lactate levels and an increase in maximal oxygen uptake after tocopherol supplementation (Haymes 1983). In other studies Dillard (1978) found that supplementation with α-tocopherol significantly reduced pentane production, suggesting decreased lipid oxidation, both at rest and during exercise. Helgheim et al. (1979) reported that tocopherol supplementation had no significant effect on the increase in the serum enzymes creatinine kinase and lactate dehydrogenase following exercise.

From these studies we can conclude that the earlier reports of the beneficial effects of tocopherol supplementation probably resulted from the studies being poorly-controlled; more recent studies have failed to show a significant effect of tocopherol supplementation on athletic performance.

2.12 MULTIVITAMINS

There are numerous studies of the ergogenic effects of the individual vitamins but comparitively few, apart from those on the B-complex vitamins discussed previously, which have used a multi-vitamin or mineral preparation (Haymes 1983; Williams 1981).

Barboroka et al. (1943) found that two months on a reduced dietary intake of the thiamine, riboflavin, ascorbic acid, retinol, phosphorus, calcium and iron adversely affected physical work capacity and that 4 to 6 weeks of supplementation restored physical work capacity to normal. Keys and Henschel (1942) conducted a comprehensive series of experiments on American soldiers who ingested large doses of supplemental thiamine, riboflavin, pyridoxine, ascorbic acid and calcium pantothenate for 4 to 6 weeks. They concluded that supplementation did not enhance performance during either short-term "anaerobic" or more prolonged endurance-type exercise. Buzina et al. (1982) reported that maximal oxygen uptake was related to the serum iron, ascorbic acid and ribcflavin status, and that two weeks of supplementation with these vitamins and minerals normalized any relative deficiencies and increased maximal oxygen consumption. Haralambie et al. (1975) investigated the effect of a polyvitamin-granulate product on sub-maximal endurance capacity. They found a decrease in sub-maximal heart rate after supplementation. Consolazio (1956) reported a significant improvement in performance efficiency in vitamin-deficient Chinese Nationalist troops after they had consumed a diet enriched with thiamine, riblofavin, nicotinic acid, retinol and iron.

More recently, a comprehensive supplement containing vitamins and iron was used to evaluate the effect of such supplementation on maximum oxygen consumption (Van der Beek 1985). Four months of supplementation failed to produce an effect other than that due to training. In a further study, Van der Beek et al. (1984) examined the physiological effect of a restricted intake of thiamine, riboflavin, nicotinic acid and ascorbic acid and found that reduced serum levels of the restricted vitamins could be detected after 4 weeks on the deficient diet. On re-supplementation with twice the RDA of these vitamins, serum vitamin levels returned rapidly to normal. The marginally-deficient vitamin status was associated with a 16% and 24% decrease in maximum oxygen consumption and anaerobic threshold respectively; these values had returned to control values after 2 weeks of supplementation. They attributed the decrease in maximum oxygen consumption to the low ascorbic acid levels.

Barnett and Conlee (1985) reported that 4 weeks of supplementation with a commercially-available multi-vitamin preparation had no effect on the rate of muscle glycogen utilization, or on blood glucose and free fatty acid levels and maximal and submaximal oxygen consumption during a progressive treadmill test to exhaustion.

Thus although there appears to be some theoretical evidence to support the claim that multivitamin supplementation may be beneficial in some circumstances, there is little scientific evidence to support this. The majority of reports which do not substantiate the claims for an ergogenic effect of multi-vitamin supplementation in athletes eating an adequate diet.

2.13 COPPER

Copper is an essential trace element that is present in several oxidative enzymes (Klevay et al. 1980; McGilvery and Goldstein 1981). Plasma copper levels are homeostatically controlled and remain very constant in the healthy adult (Kubota et al. 1968). Plasma levels are unchanged either post-prandially or during fasting (Cartwright and Wintrobe 1964). It has been reported that compared to non-athletes, athletes in training have significantly elevated resting copper levels, compared to non-athletes, and it appears that regular training has an influence on the mechanisms regulating plasma copper levels (Haralambie 1975; Kubota et al. 1978). These elevated serum copper levels may also be related to the role of copper in the function of enzymes such as cytochrome oxidase (Holloszy et al. 1973), and its role in the mobiization of iron (Osaki et al. 1966). There is no published evidence of copper deficiency in athletes (Noakes 1986).

Increased serum levels of copper and ceruloplasmin which is a copper-binding protein, have been reported after acute exercise in trained and sedentary rats and also in untrained men (Haralambie 1975). More the 95% of copper is bound to ceruloplasmin, which is increased by various non-specific stressors, including physical trauma (Mason 1979). However, after prolonged athletic events lasting several hours, serum copper and ceruloplasmin levels are unchanged (Anderson et al. 1984; Haralambie 1972). A moderate amount of copper is indeed lost in sweat during exercise (Costill 1976).

Copper is intimately associated with maintaining iron in its ferric state, and thus plays an important role in iron absorption and mobilization (Milne and Omaye 1980; O'Dell 1984). Therefore, a complex relationship exists between copper, ascorbic acid and iron, as ascorbic acid decreases the intestinal absorption of copper, and excess ascorbic acid exaccerbates the copper deficiency (Finley and Cerklewski 1983; Milne and Omaye 1980). Serum iron levels tend to be low in copper deficiency, with the development of hypochromic microcytic anaemia even though the liver iron stores are increased (O'Dell 1984; Underwood 1977). High levels of dietary zinc also induce copper deficiency, possibly by induction of metallothionine synthesis (O'Dell 1984). Even a normal intake of dietary zinc aggravates copper deficiency.

2.14 MAGNESIUM.

There appears to be no documented research on the effects of magnesium supplementation on athletic performance, nor any published reports of magnesium toxicity (Williams 1976).

Lukaski et al. (1983) reported a significant inverse relationship between magnesium and maximal oxygen consumption which suggests a metabolic role for magnesium in exercise other than its role as an enzyme co-factor. Yakovlev (1977) found increased muscle magnesium levels in trained animals. In sedentary subjects, serum magnesium levels remain remarkably constant whereas during exercise, serum magnesium levels fall and are significantly decreased after prolonged endurance exercise

Haralambie 1981; Rose et al. 1970). It is thought that during exercise magnesium is redistributed to the red blood corpuscles and muscle cells (Costill et al. 1976) as the trivial magnesium losses in sweat cannot explain the decreased plasma levels during exercise (Rose et al. 1970).

It has been suggested that the low serum magnesium levels found in trained athletes at rest may be due to an inadequate dietary intake and associated with increased sweat loss during prolonged exercise (Haralambie 1981b). However, as stated, the amount of magnesium lost in sweat although variable is generally low, and the body magnesium balance is not greatly affected by either sweat loss or fluctuations in dietary intake as efficient intestinal and renal conservation mechanisms exist (Haralambie 1975). Provided renal function is normal, supplementing a normal dietary intake with oral magnesium causes increased excretion of the mineral without significant changes in normal serum magnesium levels (Heaton 1969).

Muscle magnesium stores are more labile and may become depleted before serum and erythrocyte levels decrease (Limm 1972). There is also doubt concerning the reliability of plasma and erythrocyte magnesium measurements as indicators of tissue magnesium levels (Shils 1969). Consequently Haralambie (1981) states that conclusive evidence of magnesium deficiency in athletes must be obtained either by muscle biopsy or by analysis of the response to dietary loading with magnesium salts.

2.15 ZINC.

There are few data on the zinc status, zinc losses or zinc requirements of athletes. (Haralambie 1981b; Lukaski et al. 1983). Short duration exercise induces a sharp rise in serum zinc levels whereas endurance exercise causes only a slight rise in blood levels with return to pre-exercise levels within 24 hours (Hetland et al. 1975). An increase in serum activity of the zinc-containing enzymes, lactate dehydrogenase and glutumate dehydrogenase (Dixon and Wells 1979) as well as α_2-macroglobin, a zinc-transporting protein (Parisi 1970), have been found after prolonged exercise (Haralambie 1969). Halstead and Smith (1970) reported low plasma zinc concentrations after various temporary stress conditions. Zinc losses in sweat in excess of 1 mg day^{-1} can occur when environmental temperatures are high (Prasad et al. 1983) and this could conceivably contribute to the low serum zinc levels found in athletes (Haralambie 1981).

Dressendorfer and Sockolov (1980) studied the relationship between weekly training distance and serum zinc and copper levels in trained runners. They found that the athletes had significantly decreased average serum zinc concentrations compared to sedentary controls and that the serum zinc levels were inversely related to the weekly training distance. They attributed these findings to the high carbohydrate diet (low in zinc) that is followed by endurance athletes. Greger and Snedeker (1980) have found a direct relationship between dietary zinc requirements and protein intake. Lindeman et al. (1972) describe significant changes in zinc metabolism after acute tissue injury in man. As zinc is essential for protein synthesis and tissue repair, the hypermetabolic

state with increased protein synthesis induced by exercise may increase the zinc requirements of trained athletes.

Animal experiments show a strong correlation between changes in plasma and skeletal muscle zinc concentrations (Wang and Pierson 1975). Oh and Whanger (1978) showed that plasma zinc levels decrease in rats after acute endurance swimming, and this was related to increased synthesis of hepatic metallothionein - a zinc-binding protein. Recent animal studies have shown the importance of zinc for muscle performance (Isaacson and Sandow 1978) and resistance to fatigue (Richardson and Drake 1979). Similar findings have been made in humans (Krotkwiewski et al. 1982).

2.16 IRON.

See Chapter 3.2

2.17 CONCLUSION.

A restricted intake (35-45% of the RDA) of some B-complex vitamins, particularly thiamine, either individually or in combination, over a period of weeks may lead to decreased endurance capacity (Berryman et al. 1947; Brozek et al. 1946; Brozek and Guetzkow 1957; Consolazio 1983; Keys et al. 1945; Van der Beek et al. 198 ; Wilson et al. 1949). Impaired psychomotor and psychological functioning has not been consistently found under these conditions (Van der Beek 1985). Studies of the effects of ascorbic acid or retinol deficiency have not demonstrated any decrease in endurance capacity (Crandon et al. 1940; Wald et al. 1942), although in a few epidemiological surveys a biochemical ascorbic acid deficiency was shown to be associated with reduced aerobic capacity (Hodges et al. 1971 and 1979).

There have been numerous claims for the ergogenic effect of vitamin supplementation but these are difficult to support on scientific grounds. The available evidence from the better-controlled experimental studies indicates that vitamin supplementation does not enhance the performance of athletes eating an adequate diet. However it is possible that supplementation with the vitamin B-complex may be appropriate for athletes involved in sports with a high energy expenditure if these athetes are eating diets with a low vitamin content (Van der Beek 1985).

Although prolonged endurance exercise such as long distance running has been associated with reduced plasma concentrations of certain mineral

elements (Dressendorfer and Sockolov 1980; Hunding et al. 1981; Rose et al. 1970) there is little evidence to suggest that athletes eating a well-balanced isocaloric diet, without mineral supplementation will develop any mineral deficiencies even when engaged in repeated strenuous exercise (Dressendorfer et al. 1982).

CHAPTER THREE.

HAEMATOLOGICAL PARAMETERS AND ATHLETIC PERFORMANCE.

3.1 INTRODUCTION.

The haematological status and most particularly the iron status of athletes has received much attention (Andersen and Barkve 1970; Banister and Hamilton 1985; Berry et al. 1949; Clement et al. 1977; Clement and Asmundson 1982; Clement and Sawchuck 1984; Colt and Hayman 1984; de Wijn et al. 1971; Dickson et al. 1982; Dufaux et al. 1981; Ehn et al. 1980; Finch et al. 1976 and 1979; Frederickson et al. 1980; Gardner et al. 1975 and 1977; Goforth et al. 1982; Hunding et al. 1981; Magnusson et al. 1985; Nickerson and Tripp 1983; Pate et al. 1979; Pate 1983; Puhl and Runyan 1980; Ross and Atwood 1984; Stewart et al. 1972; Stewart et al. 1984; Wishnitzer et al. 1980; Yoshimura 1970 and 1980). This can be attributed largely to the fundamental role that iron plays in the formation and physiological function of haemoglobin, myoglobin and the cytochtomes, all of which are involved in oxygen transport and energy production and thus play a vital metabolic role, especially in athletes.

These studies have provided considerable evidence for sub-optimal serum iron and blood haemoglobin levels in athletes, most especially for endurance athletes (References as above). However the explanation for this so-called 'sports anaemia' (Yoshimura 1970) remains controversial (Dill et al. 1974; Dufaux et al. 1981; Holmgren et al. 1960; Lindemann et al. 1978; Magnusson et al. 1985; Maron et al. 1977; Paulev et al. 1983; Steenkamp et al. 1986; Vellar 1968; Vellar and Hermansen 1971). These issues as well as the conflicting findings on the effects of iron supplementation in athletes will be addressed in this chapter. In addition, a brief outline of the various haematological parameters measured in

this study and their significance in assessing the haematological status of athletes is included.

3.2 IRON

Iron metabolism is largely concerned with the synthesis and breakdown of haemoglobin, the oxygen-carrying protein of the red blood cells (Worwood 1977). Theoretically, low iron levels will lead to low haemoglobin levels and a consequent decrease in the oxygen-carrying capacity of the blood which will thus limit the maximal oxygen uptake (VO_2 max.). It is known that alpha glycerolphosphate oxidase is an iron-containing enzyme (Finch et al. 1976) as are the cytochromes. Thus iron deficiency could lead to reduced levels of one or more of these enzymes and thereby cause impaired aerobic metabolism at the tissue level.

3.2.1 Iron deficiency in athletes.

There is good evidence for impaired iron status in athletes and most especially endurance runners with studies reporting that as many as 50% of endurance athletes are iron deficient (de Wijn et al. 1971; Ehn et al. 1980; Haymes et al. 1972; Hunding et al. 1981). More recent reports have indicated low serum ferritin levels and deficient bone marrow iron stores in long distance runners (Ehn et al. 1980; Magnusson et al. 1985a and b). Kilbom (1971) found serum iron levels of sedentary females significantly reduced following 6 weeks of training and Frederickson et al. (1980) reported decreased serum iron levels and percentage iron saturation in runners following 10 weeks of training. Ericsson (1970)

reported an inverse relationship between improvement in physical work capacity and changes in the bone marrow iron content among subjects who were not receiving iron supplements. However, little change was observed in a group of females undergoing a 10 week physical training programme (Puhl and Runyan 1980a) and also no significant change in iron saturation in female athletes during a 10 week competitive season (Puhl and Runyan 1980b).

3.2.2 The effect of iron deficiency and iron repletion on athletic performance.

Iron deficiency anaemia of even a mild nature has been shown to lower physical work capacity (Edgerton et al. 1972; Gardner et al. 1977), probably through the combined effects on maximal oxygen uptake and lactate production (Banister and Hamilton 1985; Davies et al. 1973 and 1982; Finch et al. 1979; Matter et al. 1986; Perkkio et al. 1985; Viteri and Torun 1974), to increase the heart rate at sub-maximal work loads (Davies et al. 1973; Gardner et al. 1977) and to prolong the post-exercise recovery time (Andersen and Barkve 1970). Experiments with iron-deficient rats whose haemoglobin levels had been restored acutely by transfusion suggest that tissue iron deficiency reduces aerobic metabolism and endurance performance and elevates blood lactate levels (Finch et al. 1979). Iron supplementation increased performance to normal levels within 4 days in anaemic rats (Edgerton et al. 1972; Finch et al. 1976). Finch et al. (1976 and 1979) suggested that restoration of alpha glycerolphosphate oxidase activity was responsible for the improvement in performance since myoglobin and cytochrome iron levels were remained

depressed after therapy. Anaemic persons given iron supplements have lower heart rates at submaximal work loads compared to subjects given placebos (Gardner et al. 1975; Ohira et al. 1979).

There is some evidence that iron supplementation may be beneficial during training in subjects who are iron deficient but not anaemic. Plowman and McSwegin (1980) reported a significant increase in haemoglobin levels and an almost significant increase in serum iron levels in females who received iron supplements during training. The serum ferritin levels and percentage saturation increased to normal when the diet of seven iron-deficient athletes was supplemented for a two week period. Maximal lactate levels were reduced following exercise, but no significant changes were observed in maximal oxygen intake or performance time following iron therapy (Nilson et al. 1981).

However, there is little evidence that supplementing with iron the diet of athletes whose blood haemoglobin levels are normal, will improve performance. Thus Brotherhood et al. (1975) have argued against iron supplementation in athletes unless iron deficiency has been confirmed. Vellar and Hermansen (1971) found little difference in maximal oxygen uptake, haemoglobin concentration or blood iron levels between non-anaemic groups receiving either iron supplementaton or a placebo. Several studies have reported no significant improvement in iron status or haemoglobin levels amongst athletes in training who received iron supplements compared to those receiving placebo therapy (Cooter and Mowbray 1978; Pate et al. 1979; Weswig and Winkler 1974). A study done in this department found that although the serum ferritin and folate levels of

iron- and folate-deficient female marathon runners returned to normal with supplementation, there were no changes in any performance variables including the lactate turnpoint (Matter et al. 1986). It has also been suggested that supplemental oral iron intake during heavy training may be of little value, as transferrin saturation is also very high at this time, thereby impeding enhanced dietary iron absorption (Banister and Hamilton 1985).

3.2.3. Aetiology of iron-deficiency in athletes.

Iron deficiency is one of the major nutritional deficiency states in the world (Fielding et al. 1965; Haymes 1983; Hallberg 1968; McFarlane et al. 1967). Even in the Western World, iron deficiency is widespread due mainly to the decreased iron content of the western diet in which a larger proportion of food energy is derived from fats and sugars (Hallberg 1981). However, nutritional inadequacies are not sufficient to explain the apparently high prevalence of iron deficiency in endurance athletes, and it would seem paradoxical that healthy athletes with a high energy intake and thus presumably an adequate dietary iron intake should become iron deficient (Hallberg and Magnusson 1985). It has therefore been proposed that the athletes most likely to become iron deficient are those training high weekly mileages and eating an iron-poor diet, for example vegetarians (Noakes 1986).

Currently four main hypotheses have been advanced to explain the cause(s) of this so-called 'sports anaemia':-

i) Haemolytic anaemia.

Serum haemoglobin levels increase and haptoglobin levels decrease after exercise due to erythrocyte destruction, probably due to mechanical trauma in the feet. This condition is therefore analagous to march hemoglobinuria (Dressendorfer et al. 1982; Dufaux et al. 1981; Lindeman et al. 1978; Poortmans and Haralambie 1979). However careful observations show the increased rate of haemolysis to be only slight if at all (Steenkamp et al. 1986), and even a moderately increased red cell destruction rate is easily compensated for by increased red cell production, thus maintaining the haemoglobin status (Hallberg and Magnusson 1985).

ii) Increased iron losses in sweat and urine.

The limited capacity the body has for iron excretion was originally observed by McCance and Widdowson (1937) and has since been confirmed by various workers (Vellar 1968; Vellar and Hermansen 1971). The rate of iron loss depends on the iron saturation of the body, and as long as blood haptoglobin levels remain above 300 $mg.l^{-1}$ there is no notable urinary iron loss. However, in rare cases, considerable amounts of haemoglobin are lost via the kidney, after the 'protection barrier' constituted by haptoglobin is exceeded (Haralambie 1981; Refsum and Stromme 1974). Sweat contains ±0,4 mg $iron.l^{-1}$ (Vellar 1968). Since athletes may lose as much as 5-8 litres of sweat per day during heavy training in hot environments, a significant amount of dietary iron (2-3 $mg.day^{-1}$) may be lost via this route.

iii) Haemodilutional anaemia.

The anaemia may be a favourable adaptation to the increased oxygen

demands as a lower haematocrit reduces the peripheral vascular resistance and may in this way lead to improved oxygen delivery to the tissues (Murray et al. 1963; Thorling and Erslew 1968). There are however many observations which make this hypotheses unlikely (Clement et al. 1977; Ekblom et al. 1972; Hallberg and Magnusson 1985).

iv) Inadequate dietary iron absorption.

There are two major forms of dietary iron: heme iron, which is derived from haemoglobin and myoglobin, and non-heme iron which is derived from cereals, fruits and vegetables. Heme iron forms a relatively minor part of total iron intake so that even in diets with a high meat content, heme iron usually accounts for only 10 - 15% of the total daily iron intake. However the proportion of heme iron that is absorbed is greater than that of non-heme iron, which account for 25% and 10-12% respectively of the total iron absorbed from the daily diet. While meal composition and the iron status of the individual can markedy influence the absorption of non-heme iron (ascorbic acid and meat increase absorption while phytates and tannin decrease absorption), heme iron aborption is not so affected (Brodan et al. 1968; Elwood et al. 1968; Hallberg et al. 1979).

A complex relationship exists between iron, ascorbic acid, copper and zinc (Heth et al. 1966; Milne and OMaye 1980; Monson et al. 1968). As discussed above, ascorbic acid increases the absorption of non-heme iron and its incorportion into ferritin, while copper is important in maintaining iron in its ferric state and also for iron absorption and mobilization (Milne and Omaye 1980). However, ascorbic acid exacerbates low

serum copper levels as does zinc (Milne and Omaye 1980). Thus in combination, iron, copper and ascorbic acid form a complex relationship. With the variable absorption of each of these compounds in levels above the RDA, it is possible that optimal absorption of all or one may be affected. Monson and Cook (1976) have also reported that calcium and phosphorus salts combine to inhibit markedly iron absorption, possibly through the formation of an insoluble complex.

Iron overload (haemochromatosis - Powell et al. 1980; Woodliff and Herman 1973), a condition in which body iron stores are pathologically increased, is a rare condition usually of genetic origin, but which may also be caused by excess intake of oral iron supplements or by a very high concentration of iron in the diet as found in South African blacks who brew a beer rich in ascorbic acid in iron pots (Bothwell et al. 1979; Green et al. 1968). The dangers of iron overload have been emphasized in a debate regarding the usefulness of iron fortification of staple foods (Bothwell et al. 1979; Crosby 1978).

3.3 HAEMOGLOBIN.

Both male and female endurance athletes have been reported to have lower haemoglobin and haematocrit levels than non-athletes, although their total amount of circulating haemoglobin is higher (Berry et al. 1949; Clement et al. 1977; Clement and Asmundson 1982; de Wijn et al. 1971; Holmgren et al. 1960; Stewart et al. 1972). There has been speculation that this could be caused by a haemodilution effect of plasma volume

expansion, but the studies that have examined blood volume in athletes have produced equivocal results. Several studies have reported no significant change in blood volume or haemoglobin content in athletes (Colt and Hayman 1984; Cook et al. 1969; Glass et al. 1969: Moore and Buskirk 1974), whereas Brotherhood et al. (1975) and Dill et al. (1974) reported increases of 20 and 21% in the blood volume of athletes. Brotherhood et al. (1975) also reported that total body haemoglobin was on average 20% higher in athletes.

Although total haemoglobin has been found to be significantly related to to maximal oxygen intake, both total haemoglobin and maximal oxygen intake increase with body size, and when this is taken into account the strength of the relationship is reduced (Vellar and Hermansen 1971). Runyan and Puhl (1980b) also found no significant relationship between total haemoglobin and distance running performance.

3.4 HAEMATOCRIT.

Significant reductions in the erythrocyte count have been observed during the early phase of training, which is usually followed by an increase back to normal levels (Pugh and Runyan 1980a; Yoshimura 1970 and 1980). Decreased haematocrit values are reported in novice runners at the beginning of training (Ross and Atwood 1974).

3.5 TOTAL IRON BINDING CAPACITY (TIBC).

The TIBC has a well-defined negative correlation with iron stores, in particular bone marrow heme iron, with increased levels when body iron stores are depleted and decreased levels in iron overload (Weinfeld 1964). Although serum iron concentration rises rapidly after oral iron ingestion, TIBC levels remain constant. However the wide range of values in normal individuals and such variables as the increased rate of haemolysis and mild infection which may alter the levels significantly (Magnusson et al. 1985) limits the usefulness of the TIBC as an isolated parameter of iron status (Hallberg et al. 1968).

3.6 PERCENTAGE SATURATION (TRANSFERRIN SATURATION).

The percentage saturation (also termed transferrin saturation) is a measure of the amount of iron bound to transferrin, that is the ratio of serum iron : total iron binding capacity. It is considered the most relative index of iron supply to erythroid bone marrow and to non-erythroid body tissues. Physiological changes in transferrin saturation occur slowly and are limited in degree, with day-to-day variations of about 8% (Statland 1976). A value below 16% indicates iron-limited erythropoeisis, and is associated with a low sideroblast count, and is therefore widely used as an indicator of iron-deficiency (McFarlane et al. 1967).

The inherent variability of serum iron levels and the wide range of

transferrin levels in normal individuals (Statland et al. 1976; Wieppl et al. 1973) limits the usefulness of either variable when considered separately, although the percentage saturation of transferrin is a more useful diagnostic indicator than either value alone. The factors that control the plasma levels of serum iron and transferrin although interrelated, operate independently and thus a change in the TIBC : serum iron ratio (that is, percentage saturation) may result from a change in either component, but will not change if the values change proportionally. Therefore, for clinical purposes, more information is gained from considering serum iron, TIBC and percentage saturation together (Fielding et al. 1980).

3.7 SERUM FERRITIN.

Previous studies have reported a high incidence of low ferritin levels in male competitive long-distance runners with the incidence among female runners possibly being even higher that is as much as 80% (Clement and Asmundson 1982; Clement and Sawchuck 1984; Colt and Hayman 1984; Dickson et al. 1982; Dufaux et al. 1981; Magnusson et al. 1985; Matter et al. 1986; Nickerson and Tripp 1983; Pate 1983). Serum ferritin levels are regarded as a reliable index of iron stores (Jacobs and Worwood 1975; Lipsitchz et al. 1974) and the effects on physical performance of low serum ferritin levels when associated with iron-deficiency anaemia are well documented (Davies et al. 1973; Dickson et al. 1982; Finch et al. 1979; Schoene 1983). However the effects of supplementation are less well studied (Cooter and Mowbray 1978; Gardner et al. 1975; Matter

et al. 1986; Ohira et al. 1979; Weswig and Winkler 1974). Previous studies have shown that correction of iron-deficiency anaemia returns the maximal oxygen uptake rapidly to normal but the correction of the metabolic defect in muscle lags behind the increase in the maximal oxygen uptake. The effects of tissue iron deficiency alone, as evidenced by decreased serum ferritin levels in the absence of anaemia, and the effects of therapy on the performance of such athletes is not known, although current work in this department would suggest that neither increased or decreased iron and folate levels appear to influence maximal treadmill performance in female athletes who are not also anaemic (Matter et al. 1986).

Recently Magnusson et al. (1985b) compared whole body iron status in two groups of long distance runners, the one group considered to be iron-deficient because of low serum ferritin levels and low bone marrow haemosiderin content; the other with normal values for these parameters. The interesting finding was that there was no other evidence for iron-deficiency in the group with low serum ferritin levels. In particular, the bone marrow sideroblast counts, Desferal tests, MCV and red cell protoporphyrin values were all normal in this group. In addition, the dietary iron intake was high ($>18 mg.day^{-1}$) and the urinary iron excretion was not increased.

The authors concluded that the low serum ferritin levels in that group did not indicate iron deficiency. Rather they suggest that the reduced bone marrow haemosiderin and low serum ferritin levels reflect a shift in the catabolism of senescent red cells undergoing intravascular

haemolysis during exercise, from the reticulo-endothelial system to the hepatocytes. Thus they conclude that the major site of iron storage in some runners is in the liver rather than in the bone marrow. This would explain why these runners with low serum ferritin levels were not iron-deficient as serum ferritin levels reflect specifically bone marrow iron stores. It remains to be established why some but not all runners show this adaptation.

3.8 SERUM B_{12}, SERUM AND RED CELL FOLATE.

Red cell folate levels give a more accurate estimate of the whole body folate status as they correlate with the liver folate levels (Wu et al. 1976). Vitamin B_{12} (cyanocobalamin) deficiency is associated in the majority of cases with an increase in serum folate, but a decrease in red cell folate levels. Therefore serum folate, red cell folate and Vitamin B_{12} levels must be measured when assessing whole body folate status.

(Further information on the effects of cyanocobalamin and folate deficiencies and also of their supplementation on athetic performance can be found in Chapter 2, sections 2.6 and 2.7. The toxic side-effects associated with these vitamins is discussed in Chapter 4, sections 4.5 and 4.8).

CHAPTER FOUR.

VITAMIN TOXICITY.

4.1 INTRODUCTION.

The vitamins are by definition "naturally occuring constituents of food- ... essential for the life and well-being of animals and man" (Marks 1975). From this it follows that their ingestion in quantities equivalent to those found in a normal mixed diet must be beneficial rather than harmful and would not be associated with any adverse reactions.

As already discussed, unscientific claims that athletic performance will improve with nutritional supplementation abound so that many athletes are known to consume high doses of vitamins on the false assumption that "if a little is good, more is better" (Van der Beek 1985). At such high doses, toxic side-effects are possible. In addition, when taken in such large amounts, vitamins may no longer act in their conventional role but might assume a pharmacological action which could potentially either harm or improve athletic performance.

The levels of vitamins ingested by the majority of the population including athletes, whether in the diet or by oral supplementation do not normally exceed the RDA prescribed by the National Research Council, Food and Nutrition Board of the United States of America (1980) by more than a factor of 2, and hence are safe (Marks 1984). The risk of adverse reactions are highest when prolonged high doses are taken without professional advice. Cases of hypervitaminosis and allergic reactions have indeed been reported in athletes (Fumig and Esser 1982; United States Senate Committee 1973; Williams 1976).

The following is a review on the reported toxic side-effects of the vitamins and minerals which were investigated in this study.

4.2 THIAMINE

Hypersensitivity reactions to thiamine are found after injection of thiamine. In patients with a history of allergic reactions, these can occur at relatively low doses (Marks 1984). Very few such reactions have been reported after oral administration (Mills 1941). These reactions were however transient. Thus the safety factor for oral thiamine administration is considered to be very high (Marks 1984).

4.3 RIBOFLAVIN.

No adverse reactions have been reported for riboflavin as it appears that the gastrointestinal tract has a limited absorption capacity for this vitamin. In addition, rboflavin is also rapidly excreted. The magnitude of absorption is increased by the presence of food. In large doses riboflavin causes discolouration of the urine. Several metals and drugs including zinc, copper, iron, nicotinic acid, ascorbic acid and caffeine form chelates with riboflavin which alter its solubility. The clinical sigificance of this has yet to be determined (Levy and Jusko 1966).

4.4 NICOTINIC ACID.

Nicotinic acid induces the release of the vasodilator, histamine, which causes flushing. This is a common phenomenon with doses above 75mg.

However it is debatable whether this effect can be regarded as a true adverse reaction as it is not harmful and clears rapidly (Marks 1984). However individuals with peptic ulcer disease and asthma should use nicotinic acid with caution due to its vasodilatory action, that is the release of histamine (Ivey 1979; Wentzler 1979). Large doses have produced isolated reports of some toxic manifestations including cholestatic jaundice, cardiac arrhythmias, increased blood uric acid levels and dermatological problems (Alhadeff et al. 1984; Marks 1984; Winter and Boyer 1974).

4.5 PYRIDOXINE.

Pyridoxine has a rapid turnover value and shows large daily plasma fluctuations (Schaumberg et al. 1983). Until recently pyridoxine was considered to be completely safe but recent reports of sensory neuropathy after the administration of high doses of pyridoxine (Schaumberg et al. 1983) suggest that this is not the case.

4.6 CYANOCOBALAMIN.

Cyanocobalamin appears to be totally safe and is therefore an ideal placebo (Herbert et al. 1982).

4.7 FOLATE.

Unconfirmed reports descibe rare gastrointestinal disturbances with high

doses of folate (Marks 1984). However it appears that doses of even 80 $mg.day^{-1}$ (2000 x RDA) have no adverse side-effects (Marks 1984).

4.8 ASCORBIC ACID.

The desirable level of ascorbic acid intake is still widely disputed. Recently there have been claims that at high doses, ascorbic acid may prevent the common cold and may be of value in the treatment of terminal cancer (Pauling 1970).

Absorbed ascorbic acid readily equilibrates with the body pool of which 3-4% is turned over daily. Isotopic studies have shown that a daily intake of 60 mg will maintain serum ascorbic acid levels and the body pool at optimum levels (Baker et al. 1971). When the pool is saturated, any excess is metabolized or excreted (Marks 1984). Large daily intakes are therefore not beneficial, and may in the long-term pose the risk of 'rebound scurvy' once supplementation ceases. However this phenomenon has not been routinely observed even in persons taking very high doses of ascorbic acid suggesting that toxicity occurs rarely (Sauberlich et al. 1982).

Ascorbic acid has been alleged to cause the formation of oxalate stones in the kidney as oxalate is the main metabolite of ascorbic acid (Herbert et al. 1978). However recent studies have shown no increased rate of stone formation in persons taking ascorbic acid despite high oxalate excretion rates (Barness 1977; Hornig 1981; Korner and Weber 1972).

4.9 TOCOPHEROL.

Tocopherol has not been shown to have any toxic side-effects (Marks 1984) and thus it has a very high safety ratio. As the intake of tocopherol increases above the RDA, tissue storage of the vitamin becomes less efficient. Several grams of tocopherol can be stored in adipose tissue from where it is only slowly eliminated. Although tocopherol is widely used in many pharmocological preparations, there is no scientific evidence to justify its widespread use.

4.10 RETINOL.

A review on the toxic effects of retinol by Korner and Vollm (1975) claims that retinol is much safer than had been thought, with reports of toxic side-effects occurring only after the ingestion of excessive amounts over a prolonged period (Bair 1951). On withdrawal of the vitamin the symptoms of toxicity regress within days.

Fumig and Esser (1982) report a case of hypervitaminosis in a 15 year old soccer player, who ingested megadoses of retinol daily while concurrently following a diet high in retinol. In diseased individuals (most notably those with liver disease), side-effects may occur at much lower doses. Because retinol is stored it is difficult to define the highest safe levels, and only the RDA for adult males is based on experimental evidence (Reitz et al. 1974; Rodriguez and Irwin 1972). However since it is stored in the body and is thus available during periods of short-term deficiency, there is little indication for daily supplementation.

CHAPTER FIVE.

IMPORTANT CONSIDERATIONS IN THE LABORATORY ASSESSMENT OF NUTRITIONAL STATUS AND PHYSICAL PERFORMANCE.

5.1 Introduction.

The vitamin status of an individual is defined as the total amount of a particular vitamin present in the body and which is able to catalyze optimally the normal biochemical processes for which that vitamin is essential (Van der Beek 1985). A marginal or sub-clinical deficiency state is one in which there is neither optimal vitamin status nor frank clinical vitamin deficiency. It is characterized by blood biochemical values which deviate from statistically-defined reference limits obtained from 'normal' or healthy populations and the absence of clinical signs and symptoms of vitamin deficiency.

5.2 The laboratory assessment of vitamin status.

Laboratory tests assume a correlation between the vitamin and mineral levels in either the tissues, blood or urine and functional vitamin status (Baker et al. 1980). Thus the usefulness of such tests in nutritional investigation depends on (i) the establishment of such a relationship, (ii) the range in which changes in vitamin and mineral concentrations in the tissues can be interpreted (Baker et al. 1980), and (iii) whether the technique reflects only the supply of vitamins and minerals to the body, or whether they indicate abnormal metabolism caused by their relative deficiencies (Baker and Frank 1968). At present, little is known about the functional and clinical consequences associated with the range of vitamin and mineral levels measured in other tissues.

In addition, many biological and technical factors affect the determination of the vitamin and mineral levels which also argues against the assumption of a direct realtionship between tissue vitamin and mineral status and their functional capacity (Solomons and Allen 1983). When the supply of vitamins or minerals becomes limited, homeostatic regulation of the circulating levels occurs either by depletion of the body stores before circulating levels are reduced (as with the fat-soluble vitamins, retinol and tocopherol), or by reducing the excretion rate (as with the water-soluble vitamins, pyridoxine and ascorbic acid). Thus body reserves may be significantly depleted before a measurable change in tissue levels occurs.

Thus, while the circulating vitamin and mineral levels can be easily measured, they do not necessarily reflect the body stores which are situated primarily in the liver, adipose tissue and muscle, and which cannot therefore be accessed except by invasive techniques, which although possible are not entirely practical.

However, several alternate methods for evaluating the functional dimension of vitamin status have been developed. These are based on the fact that vitamins, especially those that are water-soluble are found in co-enzymes involved in cellular metabolism. Thus by measuring the activity of a specific co-enzyme - enzyme complex, an indication of that vitamin's availability and also its functional concentration at the cellular level is obtained. Co-enzyme stimulation tests therefore make it possible to discriminate a marginal or sub-clinical deficiency state from both optimal vitamin status and serious deficiency (Brin 1980). This

measurement of coenzyme stimulation of vitamin dependent enzymes in the erythrocyte is a widely accepted approach to the assessment of the water-soluble vitamins thiamine, riboflavin, pyridoxine and nicotinic acid (Vuilleumier et al. 1983). In this study, the levels of thiamine, riboflavin, pyridoxine and nicotinic acid were assessed by such methods.

5.3 Important considerations in the investigation of the nutritional status of athletes

Most knowledge regarding the metabolic roles of vitamins and minerals comes from animal experiments and from data on isolated organs and tissues. The extrapolation of these data to intact humans during severe exercise stress may not be entirely justifiable. Furthermore the majority of humans studies consist mainly of empirical observations drawn under very variable conditions of exercise and subjects characteristics. It is therefore impossible to raw firm conclusions fron these studies.

There are several other important considerations in the field of nutritional research especially as it relates to vitamin , mineral and electrolyte studies:-

i) Assessment of an adequate determination procedure:

As discussed, each of the procedures which are used to assess vitamin and mineral status has associated difficulties and inaccuracies. At At present, plasma and red cell indices of most vitamins and minerals are regarded as a 'practical' index of vitamin and mineral status.

ii) Definition of the normal vitamin and mineral status of athletes as compared to control subjects:

While normal clinical values for vitamin and mineral status are compatible with common daily activities these may not always correspond to optimum values for top athletes.

iii) Assessment of the acute and chronic effects of exercise:

This is an important consideration, as exercise may cause a redistribution of body mineral stores. Work by Magnusson et al. (1985) on iron, Costill et al. (1976) on magnesium and Haralambie (1975) on copper, suggest that this may well be the case.

iv) Other factors:

Other factors that need to be considered are sex, age and diurnal and seasonal variations, all of which may alter the vitamin and mineral status significantly at any one time, and for which control must be allowed. This is especially true for serum iron levels which show a marked diurnal variation as well as sex-related differences (Statland et al. 1976 and Statland 1977).

Thus these factors must be recognized and consequently adequatelly controlled for in studies related to functional nutritional status.

CHAPTER SIX.

AN EXPERIMENTAL STUDY ON VITAMIN AND MINERAL SUPPLEMENTATION IN ATHLETES, WITH SPECIAL REFERENCE TO THE ERGOGENIC EFFECT AND THE POSSIBLE TOXIC SIDE-EFFECTS.

6.1 INTRODUCTON.

There have been comparatively few studies of the ergogenic effects of multi-vitamin and mineral supplementation on athletic performance, and few well-controlled studies on the individual effects of the various vitamins. This despite the widespread claims that such supplementation is essential for both optimal health and athletic performance. Further, the more recent appreciation that vitamin supplementation in very high doses may have toxic side-effects raises the question of the safety of this practice.

This study was designed to answer some of these questions by adequately controlling for any variables such as training distance, diet, performance potential or anthropometry that may have influenced the results. In particular, a double-blind placebo-controlled study design was employed with the athletes acting as their own controls, thereby reducing the compounding influences of these variables.

The subjects were competitive male athletes who had been performing at a consistent levels for two or more years and whose performances were thus not likely to improve dramatically as a result of training during the trial. All were eating a normal diet and were not taking any other medications.

The multi-vitamin and mineral supplement used was one which is widely available. The placebo tablets resembled the active medication in form and colour and consisted of the same inactive ingredients as found in

the active agent. These were taken daily for two 3 month periods, separated by a three month washout phase during which no medication was taken.

The performance variables measured in the laboratory were those shown to be related to actual running performance. In addition the subjects ran four time-trials, designed to reproduce the competitive situation.

An attempt was made to assess the subjective ratings of perceived effort during training by using a defined self-reporting rating scale. A psychological benefit of vitamin supplementation would have shown as reduced levels of perceived exertion during training.

The vitamins and minerals measured were those which are routinely analyzed by the two clinical departments who performed these analyses. The haematological measures used were those parameters which provide a comprehensive haematological profile.

The 5-day diet records were analyzed to determine the habitual vitamin, mineral and macronutrient intake of these athletes.

The detailed protocol is described in the following section.

6.2 METHODS AND MATERIALS.

A. SUBJECTS:

Thirty well-trained male volunteers who were between 20-45 years, had been running competitively for at least three years and trained at least 70 km per week were recruited from local Cape Town running clubs as subjects for the study. None had known food allergies, nor were any taking prescription medications. Those who had been taking any vitamin or mineral supplements were asked to stop for at least 6 weeks prior to the start of the trial. All subjects signed written consent forms after they had been informed by the investigator of all possible demands, risks and discomforts of the testing procedures associated with the study.

B. EXPERIMENTAL DESIGN.

A nine month double-blind cross-over, placebo-controlled study design was employed. The volunteers were randomly assigned to two groups, so that 15 received placebo ("placebo group") and 15 the active agent ("active medication group") for the first 3 month period. This was followed by a 3 month 'wash-out' period during which both groups received no medication. During the last 3 month period the medications taken by the two groups were crossed over, so that the group who had initially received the placebo then ingested the active agent, and those who were initially on the active medication, received the placebo.

C. SUPPLEMENTS.

Daily supplementation involved the ingestion of 7 tablets (3 capsules, 2 tabules and 2 tablets) with food, preferably breakfast. The vitamin and mineral content of the supplement are detailed in Tables 6.1 and 6.2. The placebo tablets were identical in external appearance to the tablets containing the active agents and contained the same inactive ingredients that were present in the active tablet.

TABLE 6.1: Contents of the test supplement for which the RDA* have been established.

	Contents of capsules	RDA	% RDA
Vitamin A(fish liver oil)	10 000i.u	5 000i.u	200
Vitamin D (fish liver oil)	400i.u	400i.u	100
Vitamin E (α-tocopherol)	500i.u.	15i.u.	3 333
Vitamin B_1 (Aneurin)	60 mg	1.4 mg	4 300
Vitamin B_2 (Riboflavin)	60 mg	1.6 mg	3 750
Vitamin B_6 (Pyridoxine)	60 mg	2.0 mg	3 000
Vitamin B_{12} (Cyanocobalamin)	60 µg	3.0 µg	2 000
Nicotinic acid	70 mg	18 mg	380
Pantothenic acid	70 mg	4-7 mg	700
Folic acid	500 ug	400 µg	130
Biotin	70 ug	100 µg	70
Vitamin C (Ascorbic acid)	850 mg	60 mg	1 400
Selenium (from Prime Yeast)	50 µg	50 µg	100
Iodine (from kelp)	150 µg	130 µg	115
Elemental Calcium	230 mg	800 mg	29
Elemental Magnesium	116 mg	350 mg	14.5
Elemental Phosphorus	116 mg	800 mg	33
Elemental Iron	13.4 mg	10 mg	134
Elemental Zinc	5.2 mg	15 mg	35
Elemental Copper	584 µg	2 mg	29

* The Recommended Daily Allowance (RDA) are those prescribed by the National Research Council, Food and Nutrition Board of the United States of America (1980).

TABLE 6.2: Contents of the test supplement for which no RDA has been established.

	Contents of capsule
Biotin	70 µg
Sodium pangamate	50 mg
Citrus Bioflavinoids	6 mg
Rutin (Buckwheat)	6 mg
Rosehip powder	6 mg
Choline (bitartrate)	60 mg
Inositol	60 mg
Siberian ginseng powder	350 mg
Elemental Potassium	32 mg
Elemental Manganese	300 µg

D. TRAINING.

Most subjects maintained their training regimes throughout the study although there was some seasonal fluctuation. The distance run, waking pulse rate, mass, hours of sleep and perceived exertion during exercise (according to a defined scale - Noakes 1986) were recorded daily in a standardized training log-book, and from these data a weekly average was calculated. Three subjects (2 from the placebo and 1 from the active group) developed serious running injuries which prevented their training in the final 3 month period and from undergoing the final performance tests. All performance data for these subjects were therefore deleted from the results, leaving performance data for 15 subjects in the active and 12 in the placebo group.

E. SIDE-EFFECTS.

During all three phases of the trial we relied on subjective reports from the athletes regarding any perceived side-effects from the supplementation.

F. DIETARY ANALYSIS.

During the study each subject completed a 5-day dietary record. Standardized apparatus consisting of a balance, measuring cup and spoon was used to measure the mass or volume of all food eaten during the 5 day period. Each food-item was then coded according to the dietary composition tables compiled by the National Research Institute for Nutritional Diseases (NRIND) (Gouws 1982). The coded data were then computer processed in order to obtain the daily average intake of all the macronutrients and the vitamins and minerals relevant to this study.

G. LABORATORY TESTING PROCEDURE.

Subjects reported to the laboratory on four occassions, specifically at 0 (base-line), 3 (post-medication for group A; post-placebo for group B), 6 (post wash-out for both groups A and B) and 9 (post-medication for group B; post-placebo for group A) months.

H. BLOOD BIOCHEMICAL AND HAEMATOLOGICAL MEASUREMENTS.

On the first day of each of the four laboratory visits, subjects reported to the laboratory at 08h00 after an overnight fast and venous blood samples were drawn for analysis of vitamin and mineral levels and for determination of haematological parameters. Sera for vitamin and haematological analysis were processed the same day; samples for mineral assays were stored frozen for subsequent analysis.

Blood levels of the following were measured:-

i. Blood minerals levels: Blood copper, zinc, magnesium and iron were measured by Atomic Absorption Spectrophotometry using a Varian Techtron Spectrophotomer according to the methods described by Varian Techtron.

ii. Blood vitamin levels: Blood thiamine levels by the method of Shouten et al. (1974); blood riboflavin levels were determined by the method of Nichoalds (1974); pyridoxine levels by the method of Chabner and Livingstone (1970); nicotinic acid levels by the methodd of Clarke et al. (1975); ascorbic acid levels by the method of Denson and Bowers (1961) and retinol and tocopherol levels by the method of Catignani and Bieri (1983).

iii. Haematological parameters: Blood samples were assayed for serum ferritin levels by immuno-assay according to the method of Addison et al. (1972), serum B12 and folate levels by radiodilution

according to the method of Raniolo et al. (1984), serum iron (SI), total iron binding capacity (TIBC) and percentage saturation were determined according to International Committee for Standardization in Haematology (ICHS) (1978 and 1980) recommendations. Haemoglobin concentration (Hb) and haematocrit (Hct) were measured by Coulter Counter according to the method of Rowan et al. (1979).

I. LABORATORY PERFORMANCE TESTS.

During the week following blood sampling all subjects reported individually to the laboratory for a maximal treadmill test.

Maximal oxygen uptake ($VO_2max.$) was measured using a continuous horizontal testing protocol as described previously (Scrimgeour et al. 1986). Body weights, including the weight of running shoes and running attire, since these contribute to the running work-load, were measured on a Seca 770 Alpha Personal Scale (Vogel and Halke, Hamburg, Germany). The test began with a 5 minute warm-up and familiarization at 10 $km.hr^{-1}$ while running on a Quinton air-cooled transformer type BA-1 treadmill (Tierney Electrical Motor Co., Seattle, U.S.A.). Following a 5 minute rest period the test was started at 10 $km.hr^{-1}$ (6 $mins.km^{-1}$) with speed increments of 0,5 $km.hr^{-1}$ every 30 secs until the subject volitionally terminated the test.

All subjects ran with a Model no. 2766 Counterbalanced head support holding a Model no. 2700 Rudolph valve (both by Hans Rudolph, Inc.,

Kansas City, Kansas). A nose-clip prevented nasal breathing. Air was exhaled through clear-bore 35mm tubing into a 15 litre perspex mixing chamber with baffles. Expired air from the mixing chambers was continuosly sampled through Drierite anhydrous $CaSo_4$ (Vacumed Inc., Ventura, California) to the pick-up heads of a Beckman OM-11 O_2 Analyzer Model 242 B and a Beckman LB-2 Medical Gas Analyzer Model 240M (Beckman Instruments Inc., Illinois). The outputs from the analyzers were recorded on a Beckman Respiratory Recorder RR-2. Both analyzers were calibrated before and after each test using gases of known composition that had been calibrated previously using the Haldane Technique.

Exercising heart rates were measured using the Medi-Trace pellet electrodes in the CM 5 position, and recorded on a Life-Trace Monitor (Albany Insruments Ltd., London). Inspiratory volume and respiratory rate were from a Morgan Ventilation Monitor (P.K. Morgan Ltd., Kent). The ventilation monitor was calibrated using a Collins chain-compressed gasometer (Collins Inc., Braintree, Massachussets). During exercise testing heart rate, ventilation ($l.min^{-1}$) and respiration ($breaths.min^{-1}$) were recorded at the end of each minute. FEO_2 and $FECO_2$ were recorded continuously on the paper recorder.

Rates of oxygen uptake (VO_2), carbon dioxide production (VCO_2) and respiratory quotient (RQ) were calculated on a Sperry computer using conventional equations (Jones and Campbell 1982).

Prior to commencement of the test, a Jelco I.V. catheter placement unit (Critikon, Tampa, Florida) was inserted into a subcutaneous forearm vein. The catheter was then connected, via heparin flushed tubing to an

Eyele microtube pump MP-3 (Tokyo, Rikakikai Co. Ltd, Japan). A lml blood sample was taken every minute into a test-tube containing 2 ml ice-cold 70% perchloric acid. Samples were centrifuged immediately following the test, weighed and the supernatant decanted and frozen for later analysis of lactate levels according to the method of Gutmann and Wahlefeld (1974). Venous lactate concentrations at each speed were plotted and the 'lactate turnpoint' was determined visually. The 'lactate turnpoint' was defined as the treadmill running speed at which the first blood lactate level was clearly elevated above the preceeding values.

J. FIELD PERFORMANCE TESTS:

Four separate 15 km time-trials were performed in the week following the four laboratory treadmill tests. The first and fourth of these trials were club-organized official road-races. The second and third trials were run on the same route as the fourth trial, but with only the 30 subjects in the study competing. The two courses were of similar difficulty. The first trial was run in warm, windy conditions, but the remaining three were in cooler, still conditions with light rain in the final trial. Subject compliance was relatively poor for the field tests; 65 % of the group completed all four trials so that the data for these tests include only these 20 subjects.

K. STATISTICAL ANALYSIS:

Differences between the groups, active (A) and placebo (B) were measured using the Student's T-test for unpaired data (Cohen and Holliday 1979). One-way-analysis-of-variance (ANOVA) (Siegal 1976) was used to examine the pooled data for differences in all parameters for the four tests. The Scheffe post-hoc test was then used to determine where the significant difference lay in those parameters found to be significantly different using the one-way ANOVA. The accepted level of significance was $p < 0.05$ for all statistical tests. Correlation coefficients were used to establish relationships between the haematological parameters, dietary intake and blood vitamin and mineral levels (Siegal 1976).

CHAPTER SEVEN

RESULTS.

Group A were the group initially on the active agent, and Group B were those initially on the placebo, where n = 15 for both groups except for the performance data where n = 14 and 13 respectively. After statistical analysis had determined that there were no significant differences between the 2 groups nor in the way they responded to the intervention, the data for both groups were pooled. The mean age of the subjects was 31.9 ± 10.6 years; the mean height was 1,79 ± 5.6 m. and the mean mass was 70.2 ± 6.5 kg.

7.1 BLOOD BIOCHEMICAL AND HAEMATOLOGICAL MEASUREMENTS.

i) Blood mineral levels.

Table 7.1 contains the results of the blood mineral assays. There was no significant change in any of the mineral levels, with all levels remaining in the high-normal range throughout the trial. However, there was a non-significant drop in the serum iron levels after active medication but these levels rose again after the placebo medication.

ii) Blood vitamin levels.

The blood vitamin levels during the study are listed in Table 7.2. Supplementation caused a significant rise in the blood levels of riboflavin and pyridoxine. The blood levels of thiamine, nicotinic acid, ascorbic acid or tocopherol did not change significantly although the mean ascorbic acid levels rose to high levels after supplementation. Retinol levels fell after active medication, but

decreased more significantly in the washout phase and had not returned to control levels by the end of the trial. The data for serum B_{12} and serum and red cell folate levels appear in the following section (Table 7.3).

TABLE 7.1: Blood mineral levels during the 9 month study.

Mineral	Normal ranges	Values ($mol.l^{-1}$) during the study			
		Control	Active	Washout	Placebo
Copper	12-24 $mol.l^{-1}$	16.03 3.5	17.59 3.2	16.48 2.6	17.82 3.0
Zinc	8.3-30 $mol.l^{-1}$	17.82 2.6	17.92 3.3	19.54 3.3	18.1 3.3
Magnesium	0.7-1.0 $mol.l^{-1}$	0.82 0.1	0.79 0.1	0.82 0.1	0.78 0.1
Iron	8-30 $mol.l^{-1}$	18.04 7.2	15.57 4.5	19.8 5.7	20.98 7.4

Values expressed as Mean SD.

TABLE 7.3: Blood vitamin levels during the 9 month study.

Vitamin	Normal ranges	Values during the study			
		Control	Active	Washout	Placebo
Transketolase+	30-50 units.l^{-1}	57.14 ±10.6	64.68 ±11.7	57.35 ±10.2	58.86 ±9.7
Thiamine pyr-ophosphate+	0 - 25%	11.85 ±5.1	11.29 ±5.3	16.83 ±11.9	14.2 ±6.5
Riboflavin++	> 1.15	1.02 ±0.1	0.89* ±0.1	1.06 ±0.1	1.02 ±0.1
Nicotinic acid	5 - 20 mg.ml^{-1}	12.93 ±1.4	12.97 ±1.6	11.65 ±1.5	12.19 ±1.3
Pyridoxine	6 - 20 ng.ml^{-1}	13.24 ±4.4	86.48* ±45.5	19.43 ±20.8	19.21 ±15.6
Ascorbic acid	0.25-1.25 mg.ml^{-1}	1.01 ±0.3	1.35 ±0.3	1.06 ±0.3	1.05 ±0.3
Retinol	> 20 mg.ml^{-1}	85.6 ±20.1	66.66 ±16.1	51.88** ±14,6	68.25 ±14.3
Tocopherol	> 6 mg.ml^{-1}	13.97 ±3.6	18.52 ±6.3	14.11 ±3.3	13.8 ±3.1

Values expressed as Mean ± SD.
* $p < 0,05$ active vs. control ** $p < 0,05$ washout vs. control

NOTE:

+ Transketolase and thiamine pyrophosphate values are indicators of the blood thiamine status.

++ The erythrocyte glutathionine reductase activity coefficient (EGRAC) method which was used to measure blood riboflavin levels is an enzyme-coenzyme stimulation test (Nichoalds 1974). Thus the decrease in the EGRAC value after active medication indicates an increase in the blood riboflavin level.

iii) Haematological parameters: Table 7.3 lists the data for the haematological parameters measured during the study. There were no significant changes in the Hb, Hct, TIBC, % saturation, serum iron, serum B_{12}, serum ferritin, serum or red cell folate levels at any time during the study. There was also no evidence of iron-deficiency anaemia in any of the subjects at any stage during the trial.

TABLE 7.3: Haematological parameters during the 9 month study.

Parameter	Normal ranges	Values during the study			
		Control	Active	Washout	Placebo
Haemoglobin	12-16 $g.dl^{-1}$	15.43 ±0.9	15.45 ±1.0	14.87 ±0.8	15.48 ±1.0
Haematocrit	40-52 %	45.24 ±2.7	44.76 ±3.2	44.0 ±2.5	45.22 ±3.0
TIBC	264-376 $\mu g.dl^{-1}$	360.75 ±33.8	366.39 ±53.7	379.18 ±36.6	357.07 ±35.3
% Saturation	18-52%	28.64 ±8.1	31.96 ±15.5	28.39 ±9.5	31.61 ±8.3
Serum Iron	46-173 $\mu g.dl^{-1}$	102.54 ±30.4	107.39 ±38.3	107.68 ±35.4	111.57 ±32.5
Serum Ferritin	20-300 $ng.ml^{-1}$	71.32 ±43.6	80.86 ±61.1	72.07 ±46.9	80.25 ±39.3
Serum B_{12}	180-710 $ng.ml^{-1}$	465.86 ±78.6	496.21 ±141.6	470.34 ±160.9	479.14 ±90.9
Serum Folate	$>2.5 ng.ml^{-1}$	4.89 ±1.9	6.63 ±3.4	5.49 ±2.6	6.3 ±1.9
Red Cell Folate	230-710 $ng.ml^{-1}$	305.69 ±65.4	348.76 ±107.4	329.45 ±113.4	321.62 ±78.4

Values are expressed as Mean ± SD.

7.2 LABORATORY PERFORMANCE TESTS.

The results of the laboratory performance tests are detailed in Table 7.4. There was no significant change in the maximal oxygen consumption, maximum heart rate, peak treadmill running speed or treadmill speed at the lactate turnpoint, lactate concentration at the lactate turnpoint or the peak blood lactate concentration at any time during the study.

TABLE 7.4: Physiological variables measured during maximal treadmill testing.

	Values measured during the study			
Physiological variable	Control	Active	Washout	Placebo
VO_2 max. ($ml.O_2\ kg.^{-1}min^{-1}$)	65.45 ±6.9	65.4 ±5.9	61.94 ±7.1	62.76 ±7.2
Maximal Heart rate ($beats.min^{-1}$)	183.27 ±8.8	182.77 ±9.2	183.00 ±9.5	183.86 ±8.9
Peak Treadmill Speed ($km.h^{-1}$)	20.85 ±1.5	20.78 ±1.4	20.59 ±1.6	20.89 ±1.7
Lactate Turnpoint (LTP) ($km.hr^{-1}$)	15.55 ±1.4	15.65 ±1.7	15.5 ±2.0	15.35 ±1.9
Blood lactate conc. at LTP ($mmol.l^{-1}$)	1.73 ±0.6	1.71 ±0.5	1.54 ±0.5	1.48 ±0.3
Peak blood lactate conc. ($mmol.l^{-1}$)	9.93 ±3.6	8.37 ±2.2	8.75 ±2.3	8.1 ±2.1

Values expressed as Mean ± SD.

7.3 FIELD PERFORMANCE TESTS.

There was no significant change in the times for the 15 km time-trial among those 20 subjects who completed all four time-trials (Table 7.5).

TABLE 7.5: 15 km time-trial time measured during the 9 month study.

	Values during the study			
	Control	Active	Washout	Placebo
15 km Time-trial time (mins)	57.89 ±5.0	57.93 ±4.5	58.62 ±3.8	58.32. ±4.6

Values expressed as Mean ± SD. n = 20.

7.4 TRAINING VARIABLES AND RACING PERFORMANCE.

Weekly mass, waking pulse rate and hours of sleep did not change during the trial (Table 7.6). There was a significant decrease in weekly training distance during the washout phase which can be attributed to the usual decrease in training during the winter months. There was no significant change in the perceived effort ratings at any stage during the trial, even during the period of reduced training distance.

43% (n=13) of the athletes ran personal best times for either the 56km Two Oceans marathon, the 90km Comrades Marathon or a 42km standard marathon during the trial period. 61% (n=8) of these athletes were on active medication at the time they ran their best time, while 23% (n=3) were on the placebo agent and 8% (n=1) were on no medication (wash-out phase).

TABLE 7.6: Training variables and perceived effort ratings during the trial.

Variable	Values during the trial		
	Active	Washout	Placebo
Mass (kg)	68.9 ±6.5	69.4 ±7.1	69.6 ±7.5
Training distance ($km.wk^{-1}$)	90.9 ±34.7	70.1 ±19.9	82.5 ±32.5
Pulse ($beats.min^{-1}$)	51.1 ±6.3	52.4 ±7.6	51.6 ±6.0
Sleep ($hrs.wk^{-1}$)	52.2 ±2.9	52.2 ±3.1	50.6 ±3.2
Effort-rating (0-10)	5.4 ±0.9	5.5 ±0.9	5.7 ±0.7

Values expressed as Mean ± SD.

7.5 REPORTED SIDE EFFECTS.

Four subjects (13%) experienced varying degrees of diarrhoea including one severe case, during the three months on the active agent. This could be attributed to the laxative effect of ascorbic acid and iron intolerance, particularly in the first few days of high-dose administration (Marks 1984). 20% complained of periodic constipation during the placebo medication. Whether this can be attributed directly to the supplementation is not clear.

During the 9 months of the study, 50% of the total group experienced a minimum of 3-4 days of illness, ranging from mild colds to severe influenza. 20% of the group who developed these infections were sufficiently

ill to be in bed for 2-3 days and they did not run for at least 10 days. Of the 50% who were ill during the trial, 74% were ill during the wash-out period, 13% during the active phase, and 13% during the placebo phase.

7.6 DIETARY ANALYSIS.

The mean values for the dietary intake of the macronutrients, vitamins and minerals are presented in Table 7.7. The mean daily intake of all the vitamins and minerals with the exception of pyridoxine, folic acid and zinc was above the RDA as defined by the United States Food and Nutrition Board (1980).

The mean energy intake (10 364 $kJ.day^{-1}$) was lower than that recommended for sedentary males aged 23-50 years (11 340 $kJ.day^{-1}$). 60% of food energy was derived from carbohydrate, 21% from fat and 19% from protein.

There was no correlation between the dietary intake and the serum levels of any of the vitamins and minerals measured. However 3 of the subjects with low serum iron and ferritin levels (46 $mg.l^{-1}$ and 32 $ng.ml^{-1}$ respectively) had the lowest dietary iron intake (8 -10 $mg.day^{-1}$).

Table 7.7: Average daily intake of vitamins, minerals and macro-nutrients of the athletes studied.

	Mean daily intake	Std Dev	RDA
Thiamine	1,51 mg	0,5	1,4 mg
Riboflavin	1,79 mg	1,8	1,6 mg
Nicotinic acid	20,4 mg	5,6	18,0 mg
Pyridoxine	1,69 mg	0,6	2,2 mg
Cyanocobalamin	4,95 µg	3,2	3,0 µg
Folate	264,6 µg	100,1	400 µg
Ascorbic acid	108,7 mg	56,6	60 mg
Retinol	7911,7 i.u.	5084,9	5000 i.u.
Tocopherol	19,8 mg	16,1	10 mg
Iron	14,9 mg	4,2	10 mg
Copper	2,1 mg	0,6	2 mg
Zinc	13,2 mg	3,3	15 mg
Magnesium	371,9 mg	122,3	350 mg
Calcium	0,99 g	0,4	0,8 g
Phosphorus	1,554 g	0,4	0,8 g
Potassium	3 249 mg	0,9	-
Sodium	2 255 mg	0,9	-
Energy	10364 kJ	2328,3	13400 kJ
Protein	88,1 g	21,4	-
Fats	96,6 g	32,1	-
Carbohydrate	277,8 g	76,3	-
Fibre	25,2 g	10,8	-

CHAPTER EIGHT

DISCUSSION.

This study was designed to to address three major questions regarding chronic vitamin and mineral supplementation in athletes, specifically: (i) Does vitamin and mineral supplementation above the RDA enhance athletic performance ? (ii) Do athletes require an increased intake of vitamins and minerals because of their increased daily energy expenditure and (iii) what are the toxic side-effects, if any, of chronic vitamin and mineral supplementation ?

I considered it relevant to study these questions as the use of vitamin and mineral supplements is believed to be widespread in the general public and possibly even more so amongst competitive athletes who believe that such supplemention is necessary for optimal health and athletic performance (Barnett and Conlee 1985; Van der Beek 1985; Williams 1976). In addition it has become apparent that the prolonged consumption of large doses of vitamins may have toxic side-effects (Alhadeff et al. 1984; Barness 1977; Fumig and Esser 1982; Hornig 1981; Korner and Weber 1972; Marks 1984; Schaumberg et al. 1983) and could thus impair rather than enhance athletic performance.

(i) <u>The effects of vitamin and mineral supplementation on athletic performance.</u>

Three months of vitamin supplementation did not influence either the maximal oxygen consumption, the blood lactate turnpoint, the peak treadmill running speed or performance in a 15 km time-trial (Tables 7.4 and 7.5). As competitive running performance is related to these laboratory-measured variables (Scrimgeour et al. 1986), it must be concluded

that the mineral supplementation used in this study failed to enhance athletic performance to a degree that could be identified by the methods used in this study.

This conclusion is in keeping with the majority of studies which have shown that the ingestion of vitamins and minerals, both individually and in multi-vitamin complexes, exert no measurable ergogenic effect (Barnett and Conlee 1984; Costill 1982; Haralambie 1975; Keys and Henschel 1941 and 1942; Keys et al. 1945; Shepherd et al. 1974; Van der Beek et al. 1984; Van der Beek 1985; Williams 1976). Those studies in which an ergogenic effect has been shown have not established either the subjects' vitamin or mineral status or both prior to supplementation, and thus may have used nutritionally-deficient subjects (Archdeacon and Murlin 1944; Barborka et al. 1943; Buzina et al. 1982; Consolazio 1956; Suboticanec-Buzina et al. 1984; Van der Beek et al. 1984; Van der Beek 1985; Williams 1976). Such subjects would be expected to show an improved performance capacity with supplementation that corrected their deficient vitamin or mineral status. However, our athletes had normal vitamin and mineral levels on commencement of the study (Tables 7.2 and 7.3); thus this conclusion is in keeping with the finding that vitamin and mineral supplementation is without ergogenic effect in persons who were not initially vitamin or mineral deficient.

(ii) <u>The need for vitamin and mineral supplementation in the athletes studied.</u>

Throughout the nine-month trial period there was no biochemical evidence

of vitamin or mineral deficiencies in these subjects whose blood vitamin and mineral levels (Tables 7.1 and 7.2) and haematological parameters (Table 7.3) remained well within the normal ranges. In addition, analysis of the five-day dietary record revealed that the mean intake of all the vitamins and minerals studied with the exception of pyridoxine, folic acid and zinc was above the RDA for adult males. This supports the belief that vitamin and mineral supplements are generally unnecessary for persons consuming a well-balanced diet (Barnett and Conlee 1984; Costill 1982; Nelson 1975; Van der Beek 1985; Williams 1976).

(iii) The response of blood vitamin and mineral levels and haematologcal parameters to multi-vitamin and mineral supplementation.

There was a variable response of blood vitamin and mineral levels and haematological parameters to supplementation. After three months of supplementation with the active agent, the blood levels of riboflavin and pyridoxine increased significantly while the blood levels of thiamine, nicotinic acid, cyanocobalamin, folate, tocopherol, copper, zinc magnesium and iron did not change. The blood levels of retinol decreased significantly in the washout phase and increased again in the placebo phase.

There is good evidence that amino-acid chelated mineral compounds are more readily absorbed and metabolized than are non-chelated mineral salts (Ashmead 1973). As the minerals in the active agent used in this trial were all in the amino-acid chelated form it can be assumed that the bio-availibilty of the minerals was optimal. Therefore the failure of blood levels of copper, zinc, magnesium and iron to increase

following supplementation may not be attributed to poor bio-availability per se. Furthermore, with regard specifically to the blood magnesium levels, it must be borne in mind that blood and erythrocyte magnesium levels are not necessariy valid indicators of magnesium tissue levels (Shil s 1969). Thus it has been stated that definite evidence of magnesium status in athletes must be obtained by measurement of the muscle magnesium levels or by loading with magnesium salts (Haralambie 1981a).

A possible explanation for the failure of the majority of blood vitamin and mineral levels to increase after supplementation would be the variable interaction that vitamins and minerals are known to have with each other (Herbert et al. 1982; Hines 1975; Kondo et al. 1982; Milne and Omaye 1980; Monson and Cook 1976). It is well known that there is a complex interaction between iron, ascorbic acid, copper and zinc (Milne and Omaye 1980). Ascorbic acid enhances iron absorption and its incorporation into ferritin; copper is important for maintaining iron in its ferric state and also for its absorption and mobilization (Bush et al. 1956; Milne and Omaye 1980). Ascorbic acid however exaccerbates copper deficiency as does zinc (Milne and Omaye 1982). Calcium and phosphorus salts may combine to inhibit iron absorption, possibly through the formation of an insoluble complex (Monson and Cook 1976). Absorption of non-heme iron is also influenced by a number of dietary factors including tannin and phytates (Brodan et al. 1968; Elwood et al. 1968; Hallberg et al. 1979; Monson et al. 1978).

High doses of ascorbic acid (> 50 mg) may adversely affect the availability of cyanocobalamin so that even high doses of supplemental

cyanocobalamin will not protect against deficiency when large doses of ascorbic acid are ingested simultaneously (Hines 1975). It has also been reported that cyanocobalamin metabolism is adversely affected by the anti-oxidant action of ascorbic acidand iron when cyanocobalamin is taken in a multi-vitamin supplement (Herbert et al. 1982; Hines 1975; Kondo et al. 1982). Also cyanocobalamin absorption depends on the body cyanocobalamin status, so that the greater the cyanocobalamin stores the less the absorption even at high oral doses (Reccuglia et al. 1969). This may explain why the blood levels of cyanocobalamin did not change during the trial. There is also evidence that retinol and tocopherol mutually effect each others absorption (Jenkins and Mitchell 1975; Yang and Desai 1977), and this may explain the small decrease in the mean blood retinol levels after active supplementation, but it does not explain the significant decrease in retinol levels during the washout phase.

It is possible therefore that optimal absorption of all nutrients is not possible when a multivitamin and mineral complex such as the one tested in this study and which contains vitamins and minerals at concentrations well above the RDA, is used. Multiple nutritional interactions between the various vitamins and minerals would explain the variable response of blood vitamin and mineral levels to supplementation.

(iv) Haematological and iron status of the athletes studied.

The haematological and iron status of athletes as reviewed in Chapter 3, has received considerable attention and the results of this study appear somewhat contrary to previous findings. There was no evidence of iron-

deficiency anaemia in any of the subjects at any stage of the trial, with haemaglobin and haematocrit levels remaining well within the normal range. This despite the fact that they were competitive athletes who had been training high mileages for at least three years, and who thus thus, according to previous studies, would be most likely to develop iron-deficiency anaemia (Noakes 1986).

While the inherent variabilty of serum iron (Sinniah et al. 1973; Statland et al. 1976 and Statland 1977; Wiltink et al. 1973) limits its usefulness as an indicator of iron status, the percentage saturation is regarded as the most reliable single index of iron status (Statland 1977). This value correlated closely with serum ferritin levels in this study ($r = 0.99$); thus I have considered it to be the most significant indicator of iron status in this group of runners.

Using this criterion, four subjects with a mean percentage saturation value of 20 % and with serum ferritin levels below $40ng.ml^{-1}$ were considered to be border-line iron deficient and not anaemic, as all had normal blood haemoglobin levels. The dietary iron intake of these four athletes was below the recommended daily allowance of $10mg.day^{-1}$ for adult males. The response to the iron-containing active agent was the same as that for the subjects with normal iron status; that is their haematological status including serum iron and serum ferritin levels did not change.

This failure to respond to supplementation raises the question of iron absorption and iron kinetics in these athletes. According to the

recent hypothesis of Hallberg and Magnusson (1985) and Magnusson et al. (1985a and b), low serum ferritin levels and low percentage saturation in runners are not indicative of iron deficiency as the iron storage occurs predominantly in the liver and not in the bone marrow, due to a shift in red catabolism in athletes. It is hypothesized that red blood cells undergoing intravascular haemoloysis are catabolozed in the hepatocytes and not the reticulo-endothelial system. Thus, according to this theory, these subjects are not iron deficient but show an adaptive response to endurance training. However the fact that in this study the majority of the subjects all of whom were all equally well-trained did not show this adaptation indicates that it is not a universal phenomenon.

This failure to respond to oral iron therapy has been demonstrated previously by other workers. Thus Brotherhood et al. (1975) reported unaltered serum iron levels in athletes following oral iron therapy, and Hoglund (1970) found that serum iron or transferrin levels did not change in normal men despite an increased rate of iron absorption with oral iron supplementation. Banister and Hamilton (1985) have proposed that supplemental oral iron intake is ineffective in correcting iron deficiency during heavy training, due to the elevated transferrin saturation (percentage saturation) values which have been demonstrated in response to exercise (Dickson et al. 1982; Liesen et al. 1977). In contrast Matter et al. (1986) showed that iron and folate supplementation significantly improved the serum iron and folate levels of previously iron- and folate-deficient female marathon runners.

Thus it would thus appear that the iron absorption of all the athletes in the study was inhibited in some way so that taking the supplemental oral iron did not alter either their serum iron or serum ferritin levels. This could have occured either because the athletes were iron-replete or because of the known complex interactions of the various vitamins and minerals contained in this supplement.

(v) Evidence of vitamin toxicity.

Throughout the trial blood vitamin and mineral levels of all substances except pyridoxine and ascorbic acid remained well within the normal range and there was no clinical evidence of serious vitamin toxicity. The mean blood levels of pyridoxine increased significantly to levels above the normal range after active medication, with the percentage increase over control values being 555%. This vitamin is however water-soluble and thus has a high safety ratio (Alhadeff et al. 1984; Marks 1984). It is rapidly metabolized and excreted so that even though the blood levels were abnormally high following active medication, they returned to near control values after the 3 month washout-phase (no medication) and remained at the same level on the placebo medication. While the rise in blood ascorbic acid levels after active supplementation is not significant, the high levels measured 1,35 $mg.ml^{-1}$ indicate a fully saturated ascorbic acid pool as, even with very large doses it is virtually impossible to elevate and maintain blood ascorbic acid levels above the value of 1,25 $mg.ml^{-1}$ (Kallner et al. 1979; Labadarios 1986). When supplementation ceased, ascorbic acid levels returned to near base-line values and there was no evidence of "rebound scurvy".

It would therefore appear that three months of supplementation with this particular preparation did not result in the development of any vitamin toxicity. Blood levels of the fat soluble vitamin remained within the normal range even after 3 months of high dose supplementation.

Furthermore, it is unlikely that chronic supplementation over a longer period would have resulted in higher blood pyridoxine and riboflavin levels as both are water-soluble and the blood levels had presumably reached a steady state after three months of active supplementation. Blood levels of this vitamin returned rapidly to normal after withdrawal of supplementation.

I conclude that provided multi-vitamin and mineral supplements similar to this preparation are taken intermittently in the recommended doses, there is no risk of toxic side-effects developing in endurance athletes such as those studied in this trail.

CHAPTER NINE

CONCLUSIONS.

The results of this study support the conclusions of the limited previous studies of the effects on athletic performance of multi-vitamin and mineral supplemention (Barnett and Conlee 1985; Costill 1982; Williams 1976; Van der Beek 1985). Thus it was found that although the three months of active supplementation significantly increased most serum vitamin levels, such supplementation did not significantly alter any physiological variable associated with running performance, not did it alter running performance over 15 km.

The diets of these athletes were entirely normal as was their vitamin status, with no evidence of vitamin or mineral deficiencies either at the start of the trial or subsequently. Only four athletes showed evidence of possible iron deficiency and there was no evidence for the high incidence of iron-deficiency and anaemia as suggested in many previous studies of endurance athletes. The failure of blood measures of iron status to respond to iron supplementation is in agreement with previous findings.

Blood vitamin and mineral levels and haematological parameters showed a variable response to vitamin and mineral supplementation and it is possible that in a vitamin and mineral complex such as the one studied, optimum absorption of the constituents is affected by multiple vitamin and mineral interactions.

This study therefore fails to support the belief that athletes eating a well-balanced diet will enhance their performances or even their vitamin and mineral status by multi-vitamin and mineral supplementation. I

therefore conclude that athletes eating a normal balanced diet and who have normal blood vitamin and mineral levels, do not require vitamin and mineral supplementation.

There was no clinical evidence of toxic side-effects of three months of multi-vitamin and mineral supplementation apart from well-documented mild and transient gastro-intestinal disturbances. The concentrations of vitamin and mineral supplements in the active preparation tested in this study were well above the RDA but did not exceed the documented safety ratio. Although the mean blood levels of pyridoxine were significantly elevated above normal levels following active medication, these levels returned to normal on cessation of the medication and caused no obvious toxic side-effects. Although blood riboflavin levels also rose significantly with supplementation these remained well within the normal range. The mean blood vitamin and mineral levels of the remainder of the vitamins and minerals investigated did not change significantly and remained within the normal ranges throughout the trial. It is unlikely that toxic effects would occur even if this preparation were to be taken continuously for prolonged periods.

These findings therefore argue against any ergogenic benefit to athletic performance from multivitamin and mineral supplementation, although there may be some psychological benefit which we cannot exclude. There is also no evidence of toxic side-effects from reasonably prolonged multi-vitamin and mineral supplementation at levels moderately in excess of the RDA.

APPENDIX I

ANALYTICAL METHODS.

1) BLOOD MINERAL LEVELS.

These were analyzed in the Chemical Pathology Department, Groote Schuur Hospital under the supervision of Dr. Peter Berman. The assay methods used were those described for the Varian Techtron Atomic Absorption Spectrophotometer.

2) BLOOD VITAMIN LEVELS.

The vitamin assays were performed in the Metabolic Unit, Tygerburg Hospital, under the supervision of Dr. Dimitri Labadarios. The assay methods were as follows:-

i)) Thiamine: Transketolase (Trans K) content of red blood cells is measured by the in vitro addition of thiamine phosphate (TPP) as a measure of thiamine status (Shouten et al. 1974).

ii) Riboflavin: Assay of erythrocyte glutathione reductase activity.

iii) Nicotinic acid: Flourometric determination of N^1-Methylnicotinamide and nicotinamide in serum (Clark et al. 1975).

iv) Pyridoxine: Enzymatic assay of pyridoxal phosphate (PLP) using L-tyrosine-^{14}C and PLP dependent tyrosine apodecarboxylase from S. faecalis (Chabner and Livingstone 1970).

v) Ascorbic acid: The determination of ascorbic acid, dehydroascorbic acid and diketoglutaric acid by coupling with 2-4 dinitro-phenolydiozide (Denson and Bowers 1961).

vi) Retinol and Tocopherol: High power Liquid Chromatography (HPLC) and ultra-violet light is used to determine retinol and ▮-tocopherol simultaneously in serum or plasma (Catagnini and Bieri 1983).

3. HAEMATOLOGICAL PARAMETRS.

These were performed in the Department of Haematology, U.C.T. Medical School by Mr John Graves. The methods used are as referenced in the text.

4. LACTATE LEVELS.

1.2 ml of blood was placed in a previously weighed plastic test tube containing 2 ml of 0,6N perchloric acid (PCA). The tube was agitated to mix thoroughly, weighed and placed in a fridge. Within two hours the lactate was spun at 2000 RPM for 15 mins and the supernatant decanted off and stored in the fridge (4°C) until the assays were performed.

Assay method.

Cuvettes were made up using blanks and 2 standards as follows:

	Std	Blank	Test
Hydrazine buffer	1,0 ml	1,0 ml	1,0 ml
NAD	0,1 ml	0,1 ml	0,1 ml
PCA	-	0,1 ml	-
LDH	0,01 ml	0,01 ml	0,01 ml
Supernatant	-	-	0,1 ml
Standard (Std)	0,1 ml	-	-

The solutions were mixed (vortex mixer Super-Mixer No. 1291, Lab-Line Instruments, Inc., Melrose Park, llinois.) and allowed to equilibrate to room temperature for 30 mins.

The absorption at 340 nm was then read using a spectrophotometer (Beckman Instruments, Spectrophotemeter Model 35), zeroed against distilled water.

The following calculation was then used:

$$\frac{\text{Total volume in cuvette}}{\text{Volume of supernatant}} \times \frac{\text{vol. of P.C.A. + blood}}{\text{vol. blood}} \times \frac{1}{6,22}$$

6,22 = molar extinction coefficient of NADH to give the concentration of lactate in $mole.ml^{-1}$.

APPENDIX 2

STATISTICAL ANALYSIS

APPENDIX 2

STATISTICAL ANALYSIS.

The correlation coefficient was calculated using the following formula:-

$$r = \frac{\Sigma\ (x - \bar{x})\ (y - \bar{y})}{\sqrt{\Sigma(x - \bar{x})^2\ \Sigma(y - \bar{y})^2}}$$

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