THE EFFECT OF ACUTE AND CHRONIC EXERCISE ON $^{59}\text{Fe}^{2+}$-ABSORPTION IN PREVIOUSLY SEDENTARY IRON-DEFICIENT MALE BLOOD DONORS

by

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B.Ed., University of Hawaii, 1979

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The Effect of Acute and Chronic Exercise on $^{59}$Fe$^{2+}$-absorption in Previously Sedentary Iron-Deficient Male Blood Donors.

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Jan. 24, 1986
ABSTRACT

This study was designed to test a hypothesis that both acute and chronic exercise reduce iron absorption. Twenty-nine sedentary male blood donors (age 18-32) formed the main cohort of the study. They were selected on the basis of their low iron status (serum ferritin concentration (SF) < 30 \( \mu g \cdot L^{-1} \), serum iron (SI) < 11 \( \mu mol \cdot L^{-1} \) or % saturation of transferrin (%sat) < 15). Whole body retention of ferrous citrate labelled with 10 \( \mu Ci \) \({}^{59}\)Fe was measured to determine iron absorption. SF, SI, %sat, total iron binding capacity (TIBC), serum transferrin concentration (STC) and hemoglobin concentration (Hb) were measured immediately prior to ingestion of the radioiron solution. A subgroup (n=6) of the main cohort established pre-exercise absorption to be 70.8% ± 9.5 (SE).

An experimental subgroup (n=12) exercised on a cycle ergometer for one hour at 80% of their maximum heart rate (HRmax). The mean percent \({}^{59}\)Fe\(^{2+}\)-absorption following acute exercise for this group was 34.0 ± 11.6 (SE). A second experimental subgroup (n=11) trained for 6-8 weeks, completing 30 bouts of exercise at 80% HRmax for one hour on a cycle ergometer. Weekly blood measurements were taken throughout this period. A significant dissociation of TIBC and STC occurred in this group. In six subjects SF declined during the first few weeks to a steady state level. It is suggested that iron balance may have been achieved at this new lower level. Following their final exercise bout, chronically trained subjects ingested the
radioiron solution. The mean percent $^{59}\text{Fe}^{2+}$-absorption for the group was 34.2 ± 11.8 (SE). Although $^{59}\text{Fe}$-absorption reduced equally following single-bout exercise as in extended training, the mechanism responsible remains unknown.

Inconsistent correlations among experimental and control groups were noted between one or more of %sat, SI, TIBC, STC and Hb and percent $^{59}\text{Fe}^{2+}$-absorption. There was a significant trend towards faster elimination of $^{59}\text{Fe}$ from the body in subjects undergoing training. It is suggested that increased intestinal motility may have a significant effect on iron absorption and this possibility demands further study.

The results of the present study indicate the similar dose/response effect of single-bout and recurrent-bout (training) exercise on $^{59}\text{Fe}$ absorption, although the exact mode of this modification remains equivocal.
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I. INTRODUCTION

In recent years epidemiological studies of hematological status have indicated that a latent iron deficiency develops in intensely training individuals (Clement and Asmundson, 1983; Hunding et al., 1981; Nickerson and Tripp, 1983; Wishnitzer et al., 1983). This may lead to an iron deficiency anemia (Clement et al., 1982; Yoshimura, 1970) with obvious implications for oxygen delivery to working muscles and buffering capacity within the blood (Buick et al., 1980; Ekblom et al., 1972; Gardner et al., 1977). Even before a reduction in hemoglobin levels (Hb) is apparent, iron deficiency may lead to depleted myoglobin and reduction in numerous important iron containing enzymes. These include cytochromes, catalase and peroxidases as well as non-heme enzymes such as NADH-cytochrome c reductase, succinate dehydrogenase, cytochrome c reductase (Bothwell et al., 1979; Jacobs and Worwood, 1980) and α-glycerophosphate oxidase (Finch et al., 1976).

The question whether iron deficiency without anemia represents a threat to performance has yet to be confirmed in humans, although it is known that defects in the immune response, thermogenesis, intestinal function, behavior and catecholamine metabolism occur independently of anemia (Dallman, 1982). Some effects of anemia-independent iron deficiency on work capacity in man have been demonstrated (Ericsson, 1970; Ohira et al., 1979; Pollit and Leibel, 1976). Studies in experimental animals have differentiated more precisely the
degree to which various types of work performance are impaired by anemia or by decreased activity of iron-containing enzymes and iron-dependent enzymatic reactions (Dallman, 1980; Davies et al., 1982; Finch et al., 1976). Davies et al. (1982) studying iron deficient rats following a course of dietary repletion found that Hb concentration increased substantially within three days in parallel with maximal oxygen uptake (\( \text{VO}_2 \text{ max} \)), whereas there were no significant improvements in mitochondrial bioenergetic function, mitochondrial content of muscle, muscle oxidative capacity or endurance capacity until the fifth day. Finch et al. (1976) evaluated the effect of iron-deficiency apart from anemia by increasing the Hb concentration of iron-deficient rats towards normal, by exchange transfusion. This treatment revealed an underlying impaired work performance, which reversed within four days under iron treatment. If inferences can be made from these results, it certainly seems that iron deficiency as well as anemia are conditions to be avoided in the competitive endurance athlete.

Recently, doubt has been raised about the validity of clinical norms for Hb concentration and serum ferritin concentration in assessing the iron status of athletes (Hallberg and Magnusson, 1984; Magnusson et al., 1984b). It is well known that endurance training leads to an increase in the plasma volume (Brotherhood et al., 1975; Saltin et al., 1968). This factor alone could account for a lower Hb concentration, due to a dilutional effect. In fact, when the total amount of circulating Hb has been calculated, values of 20% or more above
normal have been found in well trained endurance athletes (Brotherhood et al., 1975).

Hallberg and Magnusson (1984) and others have suggested that the increase in red blood cell (RBC) 2,3-diphosphoglycerate concentration (2,3-DPG), which improves oxygen delivery to the tissues by inducing a right shift in the oxygen dissociation curve, is the cause of a lower "set point" for Hb in athletes. Thus, the cells in the kidney responsible for regulation of the erythropoietin level will sense an adequate oxygen delivery at a lower concentration of Hb. However, measurement of the $P_{50}$ of the oxygen dissociation curve has not revealed the expected right shift (Ricci et al., 1984; Shappell et al., 1971). It has also been suggested that a lower hematocrit (Hct) is a favorable adaptation, due to the lower viscosity of the blood. However, if lower Hb and Hct were adaptations, then the opposite approach seen with blood boosting (reinfusion of packed red cells previously withdrawn) should not improve performance (Buick et al., 1980; Ekblom et al., 1972). Dressendorfer et al. (1981) measured the Hb and Hct of 12 male marathon runners serially during a 20 day road race. While the Hb levels declined from 160 g·L$^{-1}$ to 134 g·L$^{-1}$, paralleled by a similar fall in Hct, running speeds were not significantly changed. Thus, although a decreased Hb concentration may not represent a favorable training adaptation i.e. an increased oxygen delivery capacity, it may well represent a normal physiological response to the rigors of heavy endurance training.
Presently, a commonly used indicator of iron status is serum ferritin concentration. Ferritin is an iron storage protein present mainly in the cells of the liver and the reticuloendothelial system (Pederson and Morling, 1978). Its appearance in the serum has been shown to be positively correlated with bone marrow stores under normal circumstances (Bezwoda et al., 1979; Harju et al., 1984; Heinrich et al., 1977). Hallberg and Magnusson (1984) suggested that low serum ferritin and bone marrow hemosiderin may not actually indicate iron deficiency in athletes. They hypothesized that due to a slightly increased intravascular hemolysis, there is a shift of the iron stores away from the reticuloendothelial cells to the hepatocytes, which take up the hemoglobin-haptoglobin complex. However, serum ferritin is also a concentration related measurement, which might fluctuate according to plasma volume levels. There is also the possibility that serum ferritin reflects a "falsely" high iron store as in the case of infection and more significantly, inflammation (Krause and Stolc, 1980). Dickson et al. (1982) found that the serum ferritin concentrations in ultramarathon athletes increased with exercise and suggested that ferritin concentration measurements may be as much as 35% in error during active training due to inflammation. Heinrich et al. (1977) recommended simultaneous measurement of white blood cell count, sedimentation rate and body temperature to counter this inaccuracy. In conclusion, any assessment of iron status in athletes should utilize a combination of tests such as the desferrioxamine (Magnusson et al., 1984a) the iron
absorption and/or the red cell protoporphyrin test (free erythrocyte protoporphyrin) (Frederickson et al., 1983; Magnusson et al. 1984a).

There are a number of possible causes for iron deficiency in athletes. Iron balance is achieved by maintaining absorption equal to daily obligatory iron loss and/or change in demand. Normally iron loss is minimal since the body effectively conserves it. A small amount of iron is lost each day from shedding of the renal cells, skin desquamation, loss in sweat and sloughing of the mucosal cells in the feces. This amounts to approximately one mg per day in males. Ehn et al. (1980) found that athletes appear to have a faster elimination of radioactive iron from the body (biological half-life=1000 days compared with 2100 days in sedentary males). Many causes of increased iron loss with exercise have been found. Following endurance events, increased serum Hb and myoglobin concentration together with decreased serum haptoglobin concentration in athletes suggests an intravascular hemolysis has occurred (Clement and Asmundson, 1983; Davidson, 1964; Hunding et al., 1981; Lindemann et al., 1978; Magnusson et al., 1984a,b). Under these conditions haptoglobin binds to plasma Hb and myoglobin forming a complex which is taken up by the hepatocytes. Since liberated iron may be reutilized, a shortened life span of the red blood cell (normally 120 days) should not directly contribute to iron deficiency. In man, a 14 fold increase in erythropoiesis has been found to completely compensate for the reduction of mean erythron life span to ten days or less (Pollycove and Tono,
However, accompanying the shortened lifespan, there is often an inevitable loss of iron to the urine (hemoglobinuria, myoglobinuria) attributable to a lack of haptoglobin binding capacity for plasma Hb and myoglobin. Two conditions concomitantly found in endurance athletes are chronically low haptoglobin levels and excessive intravascular hemolysis (Davidson, 1964; Dufaux et al., 1981; Hunding et al., 1981; Liesen et al., 1977; Lindemann et al., 1978; Puhl and Runyan, 1980; Magnusson et al., 1984a,b; Yoshimura, 1970), which may combine to increase iron loss via the urine. However, Dufaux et al. (1981) point out that after saturation of haptoglobin, additional serum Hb could theoretically bind to hemopexin, which would not decrease as a result of exercise (Bacon and Tavill, 1984).

Although the exact cause for hemolysis observed in exercise is not known, several hypotheses have been suggested. These include: release of a hemolyzing substance from the spleen (Yoshimura, 1970), mechanical trauma (Davidson, 1964; Hunding et al., 1981) and increased osmotic and mechanical fragility (Davis and Brewer, 1935; Puhl and Runyan, 1980; Yoshimura, 1970). All of these precursor states are possibly due to the trauma of an increased circulatory rate, increased body temperature, acute exercise acidosis, elevated concentration of plasma catecholamines, physical compression of the RBCs by muscular activity or from physical forces generated in weight-bearing activities.
Other sources of increased iron loss with exercise are hematuria (whole blood released into the urine as a result of bladder trauma or bleeding from the kidney pelvis), increased fecal loss and loss through profuse sweating. Paulev et al. (1983) suggested that the primary loss of iron in male endurance athletes is through the sweat. Up to one mg. of iron or more per day could be lost in this way, which would double the average daily iron loss of a male.

In addition to an increased iron loss, an exercising individual's iron requirement may rise due to an absolute increase in synthesis of Hb, myoglobin and iron containing enzymes. Initially, iron stores would be expected to provide for the increased iron demand but unless intake was increased, iron depletion would result. In theory, iron balance could be regained by a combination of increased dietary intake and increased intestinal absorption of iron. Conversely, if an athlete failed to increase dietary intake of iron and if there were no compensatory increase in intestinal absorption, iron deficiency could be expected.

It seems unlikely that dietary intake of iron is insufficient in athletes. Many studies have included dietary analyses (Clement and Asmundson, 1983; Clement et al., 1982; De-Wijn et al., 1971; Ehn et al., 1980; Magnusson et al., 1984a; Stewart et al., 1972) and in most cases showed that iron intake was increased two to three fold above the normal daily requirement. Exceptions are found in athletes attempting to
maintain a minimum weight (the average iron content of the North American diet is approximately six mg per 4200 KJ) and in some vegetarians and females (Clement et al., 1982). Furthermore, many athletes take iron supplements amounting to several times the daily recommended amount. Some reports indicate that supplementation does little to prevent or reverse the development of iron deficiency in heavily training athletes (Banister and Hamilton, 1985; Hegenauer et al., 1983; Wirth et al., 1978).

In addressing the second possibility, several investigators have demonstrated an inverse correlation between iron stores and iron absorption. Heinrich et al. (1977) studied 158 patients whose iron status ranged from normal stores to manifest iron deficiency anemia (serum ferritin 2.7-221 μg·L⁻¹). The whole body estimation of ⁵⁹Fe-absorption was inversely correlated (r=-0.832) with the serum ferritin concentration. Bezwoda et al. (1979) studied a population whose ferritin ranged from less than 1 μg·L⁻¹ to greater than 5,000 μg·L⁻¹. These data did not follow a normal distribution so that a log transformation was necessary for their statistical analysis. In a non-athletic population, while the relationship between absorption of a three mg dose of ferrous iron and the bone marrow non-heme iron was significant (r=-0.94) and consistent throughout the whole range, the relationship between absorption and ferritin (r=-0.78) was less clear, especially in the normal range of ferritin. These studies indicate a close relationship between iron demand and iron absorption. Therefore, according to the pattern seen in
non-athletes, as an individual begins to exercise and iron demands increase, so should the absorption of iron.

In a study of swimming in rats, absorption of iron (as estimated by fecal iron) was decreased in exercised male rats compared with controls (21.3% and 42.7% respectively) (Ruckman and Sherman, 1981). There was a trend towards decreased iron and increased copper in the spleens, livers and hearts of the exercised rats, who showed the typical increase in ceruloplasmin levels, known as one of the "acute phase reactants" and postulated to be a copper carrier as well as functioning in the formation of the iron-transferrin complex (Haralambie and Keul, 1970; Liesen et al., 1977; Ruckman and Sherman, 1981). Ehn et al., (1980) measured iron absorption of an $^{59}$Fe labeled 3.45 mg test dose of ferrous sulphate and a similar dose of $^{59}$Fe labeled hemoglobin in a test meal, in eight male distance runners and eight male blood donors. The bone marrow of subjects in both groups either lacked iron completely or contained only traces. Although there were no significant differences in iron absorption between the two groups, absorption was lower in the athletes for both ferrous sulphate and hemoglobin iron (16.4% vs 30% and 13.5% vs 17.8% respectively). Initial indications from these studies are that the absorption of iron is not as high as might be expected based on iron status alone which might help to explain the cause of iron deficiency with exercise.

Although there has been recent evidence for an iron excretion mechanism (Refsum et al., 1984), it is known that body
Iron balance is maintained mainly by control of iron absorption in the intestine. Iron absorption has been shown to increase specifically in response to iron deficiency and not to reduced red blood cell count and Hb (Cox and Peters, 1980). The control of iron absorption is not fully understood, however, although several features of the system are known. The capacity for regulating iron absorption probably resides in the mucosal epithelium lining the proximal small intestine (Hallberg and Solvell, 1960; Jacobs and Worwood, 1975; Muir and Hopfer, 1985). Iron influx demonstrates many features of an active, carrier-mediated transport system (Cox and Peters, 1980). There appear to be separate control mechanisms for iron uptake by the cell and for its transfer to the plasma (Jacobs and Worwood, 1975). Initial influx across the brush border of the epithelium appears to be rate limiting (Cox and Peters, 1980). Iron absorption shows some temperature dependence and sensitivity to metabolic poisons demonstrating that the controlling step is energy requiring (Cox and Peters, 1979).

The availability of food iron is one of the major factors determining the total amount of iron absorbed (Jacobs and Worwood, 1975). It has been estimated that the maximum amount of dietary iron available for absorption is 20-25% (Jacobs and Worwood, 1975; Jacobs, 1977). This is due to variations and interactions between foods; the largest difference being iron availability as heme (more available, less affected by food interactions) versus non-heme sources of iron (less available, more affected by food interactions) (Cook et al., 1984). For
example, phytates form insoluble complexes with iron released from a non-heme source, thus inhibiting its absorption and fructose forms iron chelates which remain soluble at alkaline pH, thus enhancing its absorption (Jacobs and Worwood, 1975). Absorption of non-heme iron may vary up to tenfold depending on the content of enhancing and inhibitory factors (Cook et al., 1984). Heme iron is unaffected by such factors and it appears to enter the epithelial cell unchanged (Jacobs and Woorwood, 1975).

Once iron has been made available to the epithelium, a certain amount of it is transferred into the plasma according to the iron status of the body. The unsolved puzzle remains as to how the epithelial cells are modified to reflect iron status. There are three major theories. The first hypothesizes that the iron content of the intestinal epithelial cells regulates the amount of iron taken up. Thus, a "mucosal block" to absorption of iron is presented when iron stores are sufficient (Conrad and Crosby, 1963). Although plausible, and still supported by some findings (El-Shobaki and Rummel, 1985) this theory has been challenged (Cox and Peters, 1980). A second theory arose from the observation of structural differences between the two iron binding sites of transferrin. Fletcher and Huehns (1968) hypothesized that one specific site gave up iron preferentially either to red cell precursors or the intestinal epithelium (which were in competition), thereby linking demands in the marrow to intestinal absorption. However, no clear differences in the ability to donate iron to different tissues in vitro or in vivo in man have yet been found (Aisen and Brown, 1975;
Vander Heul *et al*., 1984). The third theory, which has been most intensively studied, implicates a humoral factor in the conveyance of information concerning body iron status to the mucosal epithelial cells. Past studies have not lent support to a humoral mechanism of regulation (Hallberg and Solvell, 1960; Hoglund and Reizenstein, 1969; Rosenmund *et al*., 1980). However, correlations have been noted between iron absorption and the plasma concentrations of ferritin (Walters *et al*., 1973), iron (Finch *et al*., 1982) and percent saturation of transferrin (Cox and Peters, 1980; Taylor and Gatenby, 1963). A possible explanation for the discrepancies between the studies could relate to a time delay in the effect of a change in iron status on the developing epithelial cells. Transferrin concentration is increased in iron deficiency anemia effectively increasing the total iron binding capacity (TIBC) and decreasing the percent saturation of transferrin (%sat) (Delpeuch *et al*., 1980). Interestingly it has been shown that supplementing the diet with valine (the N-terminal amino acid in the transferrin molecule) is more effective in increasing intestinal iron absorption than is ascorbic acid (El-Hawary *et al*., 1975). A mechanism involving humoral control, which hypothesizes reduced absorption as a possible cause of iron deficiency in athletes, is described by Banister and Hamilton (1985).

Preliminary studies by Banister and Hamilton (1985) indicated that when athletes were in heavy training, %sat and serum iron (SI) were high and serum ferritin concentration and Hb were low. The inverse was true in periods of relative rest.
The amplitude of Hb variation in response to changes in training intensity was small (as would be expected) compared with large amplitude of variation in % sat, SI and serum ferritin concentration. Although four of the five subjects in this study took iron supplements regularly, ferritin only rose into the normal range (> 20 μg·L⁻¹) in periods of relative rest. A hypothesis supporting this pattern of iron profile variation suggests that the turnover rate of RBCs, myoglobin and iron containing enzymes likely increases with intense training. Almost all of the iron from cellular breakdown is reclaimed and redistributed through one or more of iron's metabolic circuits. Plasma transferrin may be highly saturated from such reclaiming and redistribution activities and thus be unavailable to assist in increasing iron absorption from the intestine or to signal increased synthesis of the membrane carrier for iron responsible for increasing absorption. For as long as stressful training is continued, transferrin saturation will remain high, presenting the possibility that little iron will be absorbed, thus allowing a slowly developing iron-depletion anemia to develop. For this theory to be valid, iron absorption during training would indeed have to be reduced. Combining this theory with that of Hallberg and Magnusson (1984) it is possible that if the liver, which synthesizes transferrin, is sufficiently replete with iron (due to intravascular hemolysis) transferrin synthesis may not increase with exercise. This might partially explain the high % sat observed during heavy training (Banister and Hamilton, 1985). Concentration of liver iron stores appears to be a major
regulatory factor in the control of hepatic transferrin synthesis (Morton and Tavill, 1977). Thus it is important to monitor serum transferrin concentration concomitantly with percentage saturation with iron to aid in understanding the true course of events induced by exercise stress. The present study was designed to test the hypothesis regarding a reduced iron absorption with training and the possible link to serum transferrin concentration and saturation.
II. METHODS

Subjects

Previously untrained male blood donors (age 18-32) were studied for iron absorption changes which might be due to the effect of an acute exercise stimulus or to extended training. Subjects were selected on the basis of their low iron status (ferritin $< 30 \, \mu g\cdot L^{-1}$ and/or SI $< 11 \, \mu mol\cdot L^{-1}$ and %sat $< 15$) and sedentary habits in the six months preceding commencement of the study. Accordingly, they would be expected to be in a state of increased iron absorption. A control group of non-active, iron-deficient, male blood donors ($n=6$) was studied similarly for comparison of iron absorption. None of the subjects had a history of disorders that might influence iron kinetics or intestinal absorption of iron. A medical examination and questionnaire did not suggest any abnormal blood loss in the subjects and they were all declared healthy and fit to participate in the cycle ergometer exercise. Each subject understood the nature of the experiment and gave his informed consent to participate.

Treatment Groups

1. Single-bout exercise group

These subjects completed one hour of exercise on a cycle ergometer at 80% of their maximum heart rate (HR max). The pedaling rate was kept constant at 80 rpm and the braking
tension on the cycle was varied to maintain the appropriate heart rate. Initially, each subject's HR max was measured in an exhaustive incremental cycle ergometer test with a ramp slope of 16.6 watts \cdot 30\text{sec}^{-1} above a baseline work rate of 50 watts. It was assumed that HR max had been reached when the heart rate remained constant despite further increases in work rate.

Subjects ingested a small dose of radioiron solution at one of several time intervals after the one hour exercise bout.
These were:
(i) three subjects—one hour post exercise,
(ii) three subjects—four hours post exercise,
(iii) three subjects—eight hour post exercise,
(iv) three subjects—24 hours post exercise.
Immediately prior to ingesting the labelled iron solution, a blood sample was taken to establish the concomitant post exercise serum ferritin concentration, % sat, SI, TIBC, Hb and serum transferrin concentration.

2. Recurrent-bout exercise (training) group

Each subject completed 30 one hour bouts of cycle ergometer exercise at 80% of HR max, on separate occasions during a six week period. An initial test, as described above, established each subject's maximum heart rate. Subsequently, each subject exercised at 80 rpm varying the braking tension on the cycle to maintain the appropriate heart rate. As the subjects' fitness improved throughout the study, the work rate for equivalent heart rate elevation increased, thus the proportional effort
remained the same. Once each week, on random days, a modified Sjostrand PWC 170 test (Sjostrand, 1947; deVries and Klafs, 1965) was performed at the beginning of a training session to monitor serial changes in fitness.

In an attempt to maintain a "neutral" or natural iron balance status, general dietary instructions were given to each subject to encourage them to eat foods rich in iron and substances enhancing non-heme iron absorption. Subjects were dissuaded from eating iron containing foods simultaneously with those known to inhibit iron absorption and no subject took iron supplements. Blood samples were collected weekly throughout the training period to monitor for possible serial changes in serum ferritin concentration, SI, TIBC, %sat and serum transferrin concentration. Samples were collected before the start of the exercise bout on the same day of the week for all subjects and at the same time of the day for each subject (which varied between subjects).

On the final day of exercise, subjects ingested a small dose of radioiron solution at one of the following times post exercise:

(i) three subjects—one hour post exercise,
(ii) three subjects—four hours post exercise,
(iii) two subjects—eight hours post exercise,
(iv) three subjects—24 hours post exercise.

Immediately prior to ingesting the tracer dose, a blood sample was taken to establish the concomitant post exercise serum
ferritin concentration, SI, TIBC, % sat, Hb and serum transferrin concentration.

3. Control

Six sedentary iron-deficient male blood donors followed the same iron absorption and blood sampling procedures as the exercise group to establish control resting values for these indices.
Quantification of Training

In order to relate changes in iron status to an objective measure of training and the fatigue developing from it, a quantity of training called the training impulse (trimp) was calculated from the duration of a training session and the intensity (measured as a function of the average fractional elevation of the maximum heart rate range) thus:

\[
W(t) = \text{Duration} \times k_1 \times \frac{[\text{HR}_{\text{exercise}} - \text{HR}_{\text{basal}}]}{[\text{HR}_{\text{maximum}} - \text{HR}_{\text{basal}}]}
\]

\(k_1\) is a non-linear weighting coefficient dependent on exercise heart rate and is given by the equation:

\[k_1 = 0.64 \cdot e^{1.92x}\]

where \(x\) = fractional elevation of the maximum heart rate range

\(k_1\) weights brief fatiguing effort at higher heart rates positively, relative to sustained steady state effort at lower heart rates. \(k_1\) is equal to half the exponential increase in blood lactate concentration at various proportions of maximum effort (heart rate) up to a maximum in male subjects determined by Green et al. (1983).
Whenever additional training sessions were done the total training impulse was the sum of the scores for the day. Each subject completed a number of random performance tests (described previously) in order to judge the effect training was producing. The training impulses and "criterion performances" (scored on a points scale) were used in the conceptual model described to quantify fatigue.

**Conceptual Model of Training Effects**

A unit of training termed the training impulse (trimp) (Calvert et al., 1976; Banister and Hamilton, 1985) was used in a systems model of training (described above) to derive intermediate values of accrued fatigue and fitness scored in the arbitrary units measuring the trimp throughout training. The differences between these values provided a continuous estimate of performance ability. Because real performances were actually measured throughout training, iterative computer modeling of derived performances against criterion (real) performances for least squares best fit, completely defined the intermediate curves of fatigue and fitness for each subject.

Defined in this way, fatigue measured the degree of stress imposed by a training regimen upon an individual and allowed the dose/response effect of training upon iron status to be objectively studied.
Blood Measurements

Blood samples were collected in Beckton and Dickinson Vacutainers, using EDTA anticoagulant for the hemoglobin and "Serum Separator Tubes" for the separation of serum for testing. Hemoglobin concentration was measured on a "Sysmex TOA C800" counter, standardized with "Streck Tara 8" and quality controlled (QC) with "Toa QC 8" at three different levels. Typical QC and monthly coefficients of variance (CV) were < 3%.

Serum Iron (SI), Total Iron Binding Capacity (TIBC) and percent saturation (% sat) were measured on a "Roche COBAS-BIO" by a modification of the spectrophotometric method of Carter (1971). Two levels of QC sera were run with every batch and monthly CVs were < 4% for SI and <4.5% for TIBC. Serum transferrin concentration was measured using Behring radial immunodiffusion plates and standards, using the Mancini end point precipitation method (Mancini et al., 1965). Typical monthly CVs were < 7.5%.

Serum ferritin concentration was measured using the Becton and Dickinson (BD) radioimmunoassay (RIA) kit, which employs the method of Barnet et al. (1978). Tri-level BD RIA trac quality control monthly values were < 8%.

$^{59}\text{Fe}^{2+}$-absorption Test

Subjects were instructed not to eat anything after noon on the day of the test (four hours before radioiron ingestion) and were given a list of foods (known to inhibit iron absorption) to
avoid during that day. Subjects were also asked not to eat until at least two hours after drinking the solution, at which time most of the absorbed portion of iron would have entered the blood stream (Hallberg and Solvell, 1960).

To determine iron absorption, the whole body retention of radioactive iron was measured, as described by Heinrich (1970). Each subject reported to the Nuclear Medicine Department of Lion's Gate Hospital in North Vancouver, B.C., between 3:30-5:30 pm. The purpose for this was to minimize any diurnal variation there may be in absorption of iron and in the corresponding blood indices measured (Casale et al., 1981; Sinniah and Neill, 1981).

Each subject ingested a small amount of ferrous citrate (0.16-0.31 μg Fe$^{2+}$), pH 3-5, labelled with 10 μCi $^{59}$Fe, followed by a water rinse. An identical solution (the other half of the originally prepared 20 μCi solution) was kept as a control to measure the radioactive decay and to adjust for machine variability at the various staged time intervals of repeat radioactive counts. A $2-\pi$ whole body gamma count was obtained within 30 minutes of ingestion of the radioiron solution to determine the total radioactivity of the test dose (100% reference value). The subject then returned one day, one week and two weeks post ingestion to repeat the whole body $^{59}$Fe radioactive count. The percentage retention of iron was calculated as the ratio of retained radioactivity measured 14 days after ingestion to that recorded on the initial day of
ingestion (100% reference value). This value represented the percentage iron absorbed, assuming that only a negligible proportion of that initially absorbed was lost during the two week measurement period. The subjects in the present study were instructed to cease exercising or engaging in passive activities likely to cause excessive sweating, such as taking saunas, for the whole of the two week period between the initial ingestion of $^{59}$Fe and the final gamma count. The purpose of the two week waiting period before the final whole body count is to allow excretion of unabsorbed $^{59}$Fe (usually during the first 3-7 days) and for physiological sequestration and excretion of intestinal mucosal iron (day 7-14) which may contain from .2-5% of the oral dose (Heinrich, 1970).

2-π Whole Body Radioactive Count

Since a whole body counter was not available for this study, a 2-π whole body count was measured using a Nuclear Data Inc. 62 gamma counter (NaI crystal). The counting efficiency was .014-.028% and the chi square value for repeated measures was between 0.90 and 0.10 ($x^2=11.7-27.2$), indicating a well functioning machine (Bernier et al., 1981). Before each count, the machine was calibrated using a standard Cs$_{137}$ source.

Placement of the subject was calculated to minimize the distance from the counter (therefore maximize counting efficiency), while including the whole body in the field of the NaI crystal. Marks were put on the floor so that the chair and
probe were in the same relative position for each of the repeat counts of each subject. Tapes were hung from the collimator (as illustrated in fig. 1) to ensure that the collimator height and distance from the subject was the same for each recount. The following formula was used to calculate the height of the collimator and the distance from the face of the collimator to the zyphoid process of the subject:

\[
B = \left( \frac{2.9 \times A}{5.8} \right) - 4.2
\]

\[
C = (A + 2) + (14.8 - 4.2)
\]

Each of the counts (standard, background and subject) were measured twice for ten minutes. The average of the two background counts was subtracted from the average of the two standard counts. The subject was counted for ten minutes with his left side closest to the collimator and for a second ten minutes with his right side closest to the collimator. The average background measurement was subtracted from the average of the two ten minute counts of the subject. The background-corrected standard and subject counts on the first measurement day were used as the 100% reference values and on subsequent re-counts, the subject's count was corrected by the ratio of the current day's standard to that of the 100% reference standard count. This decay-corrected value was then
divided by the 100% reference count of the subject, which then
determined the percent of the total dose remaining in the whole
body count for that day.

Figure 1: Experimental set-up for $^{59}$Fe counts where $A$ is the
sitting height of the subject, $B$ is the distance from the
zyphoid process of the subject to the face of the collimator and
$C$ is the distance from the bottom of the collimator to the
floor.
**Statistical Analysis**

The significance of the difference between $^{59}$Fe$^{2+}$-absorption in the control, single-bout exercise and recurrent-bout exercise groups was established by using Dunn's Multiple Comparison Procedure (Kirk, 1982). The data did not follow a normal distribution due to the 0-100 limits of percentage measurements, so that an arcsin transformation was performed before statistical analysis. $P$, the probability of the difference being due to chance, was obtained from tables for the test. A T-test was used to establish correlations between the various blood concentrations and with $^{59}$Fe$^{2+}$-absorption and an analysis of covariance tested the effect of the blood measurements on $^{59}$Fe$^{2+}$-absorption in each group. A least square best fit line was calculated for the relationship between serum transferrin concentration and TIBC in the exercising subjects and in all subjects before commencement of exercise. Arithmetical means and standard errors of the mean (SE) were calculated for the blood values (according to treatment group) for each subject at the time of iron ingestion.
# III. RESULTS

### $^{59}$Fe Absorption and Corresponding Blood Parameters

#### Control group

The mean percentage absorption of $^{59}$Fe for the control group was $70.8 \pm 9.5$ (SEM). One subject's absorption was 16% and $^{59}$Fe absorption in the remaining five controls ranged between 78-84%. The percentage $^{59}$Fe absorbed within the control group correlated with serum transferrin concentration and TIBC ($r=.629$ and $.626$ respectively) although these were not significant.

#### Single-bout exercise group

The mean percentage absorption of $^{59}$Fe within this group was $34.0 \pm 11.6$ (SEM). The t statistic of Dunn's multiple comparison (Kirk, 1982) comparing the mean $^{59}$Fe absorption in the control and acute group was 3.65 ($p \leq .005$). After accounting for differences between the blood values of the single-bout exercise and control groups, (i.e. treating the blood values as covariates) there remained a significant effect of the exercise stimulus on the absorption of $^{59}$Fe (t statistic = $-3.036$ $p \leq .01$). Within the single-bout exercise group serum transferrin concentration and SI correlated positively ($r=.58$ and $r=.69$ respectively and Hb correlated negatively ($r=-.59$) with % absorption of iron. All three correlations were significant at
the .05 level. TIBC and % sat showed a non-significant positive correlation with $^{59}$Fe absorption. There was no significant effect of the timing of post-exercise $^{59}$Fe ingestion on its subsequent absorption.

**Recurrent-bout exercise (training) group**

The mean percentage absorption of $^{59}$Fe within this group was 34.2 ± 11.8 (SEM). The t statistic of Dunn's multiple comparison (Kirk, 1982) comparing the mean $^{59}$Fe absorption in the control and training group was 3.22 ($p \leq .006$). There was no significant difference between the training and control group in % absorption of $^{59}$Fe. As may be seen in table 1, the means of some of the blood parameters for the training group were different, although not significantly, from means within either the control or single-bout exercise group. Subsequently, when the $^{59}$Fe absorption was adjusted for the covariates (blood parameters), the effect of training was judged to be of a less direct influence on reduced $^{59}$Fe absorption, suggesting that perhaps the effect of training acts through changing one or more of the covarying factors. Within the training group TIBC correlated significantly with % $^{59}$Fe absorption ($r = .59 \ p \leq .05$). No other blood index correlated significantly with iron absorption in this group and there was no significant effect of the timing of post-exercise $^{59}$Fe ingestion on its subsequent absorption.
# Table 1: Mean blood indices and $^{59}$Fe absorption

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<tr>
<th></th>
<th>Control n=6</th>
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<th>Acute n=12</th>
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<th>Chronic n=11</th>
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<td></td>
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<td>SE</td>
<td>mean</td>
<td>SE</td>
<td>mean</td>
<td>SE</td>
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<tr>
<td>Hemoglobin (g·L$^{-1}$)</td>
<td>149.5</td>
<td>3.4</td>
<td>143.8</td>
<td>4.2</td>
<td>144.7</td>
<td>4.2</td>
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<td>Ferritin (ug·L$^{-1}$)</td>
<td>24.5</td>
<td>4.9</td>
<td>25.5</td>
<td>6.0</td>
<td>31.2</td>
<td>6.1</td>
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<td>13.6</td>
<td>1.9</td>
<td>12.5</td>
<td>2.3</td>
<td>17.0</td>
<td>2.3</td>
</tr>
<tr>
<td>TIBC (umol·L$^{-1}$)</td>
<td>59.2</td>
<td>2.9</td>
<td>59.1</td>
<td>3.6</td>
<td>58.9</td>
<td>3.7</td>
</tr>
<tr>
<td>% saturation of transferrin</td>
<td>23.5</td>
<td>3.2</td>
<td>21.3</td>
<td>3.9</td>
<td>28.5</td>
<td>3.9</td>
</tr>
<tr>
<td>Transferrin (ug·L$^{-1}$)</td>
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<td>0.18</td>
<td>3.15</td>
<td>0.22</td>
<td>3.09</td>
<td>0.23</td>
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<tr>
<td>% $^{59}$Fe remaining day 1</td>
<td>86.8</td>
<td>8.5</td>
<td>70.1</td>
<td>10.4</td>
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<td>10.6</td>
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<tr>
<td>final % absorption</td>
<td>70.8</td>
<td>9.5</td>
<td>*34.0</td>
<td>11.6</td>
<td>*34.2</td>
<td>11.8</td>
</tr>
</tbody>
</table>

(* significantly different from control)
TIBC, Transferrin Concentration and %Sat interactions

The correlation between TIBC and serum transferrin concentration on the day of the $^{59}$Fe test was significant for the control group ($r = .94$, $p \leq .01$) and for the single-bout exercise group ($r = .61$, $p \leq .05$) but not for the training group ($r = .53$, NS). The correlation between % sat and serum transferrin concentration in the control, single-bout exercise and training groups were not significant ($r = -.64$, .09, .17 respectively) however, %sat in the control group showed an expected tendency towards an inverse correlation with serum transferrin concentration.

**Percent of $^{59}$Fe in the Whole Body Count After One Day**

Of all the covarying factors the percent of the $^{59}$Fe dose remaining after one day was a good predictor of final % absorption for the training group ($t$ statistic $= 2.9$, $p \leq .02$) and the correlation between day one and day 14 values was also significant ($r = .72$, $P \leq .01$). Such was not the case for either the single-bout exercise or control groups ($r = -.04$ and $r = .49$ respectively).
Recurrent-Bout Exercise (training)

General

Co-operation in every aspect of the study on the part of the subjects was very good. The major motivation of the previously sedentary subjects was to gain fitness in a supervised setting and motivation was maintained at a high level by frequent feedback on progress, evidenced by the increasing load tolerated at the same working heart rate (80 % HR max) and improvements in the weekly PWC 170 tests.

The effect of training on blood indices

Figures 2 to 12 show the training impulse, criterion performance levels, derived fatigue and blood indices of the 11 subjects throughout training. Blood results from the initial screening test (taken before the commencement of training) are shown with blood results for the two week period of non-activity during the iron absorption measurement period allowing a "before" and "after" comparison to be made for each subject.

Training Impulses

Training impulses are plotted for each subject on the graph in the lower right corner of figures 2 to 12. As previously described, the training impulse was calculated from the duration of training and the average fractional elevation of the maximum heart rate range. The aim for each of the 30 sessions was to have the subject maintain 80 % HR max for 60 minutes. In general
this was the case. Most subjects reported that the effort of maintaining 80% HR max became more difficult in the latter three weeks of the training period. Subject 366 began a running program at the same time as he commenced cycling, thus the pattern of his training impulses varies from the rest of the group, who rarely completed any training in addition to the cycling. A general plan involved daily training Monday to Friday with rest during the weekend, however due to the subjects' individual schedules occasional days were missed. All 30 sessions were completed within eight weeks for all of the subjects.

Criterion Points

Figures 2 to 12 (top right) show criterion points vs predicted performance for each subject. As stated previously, a PWC 170 test was completed weekly (in most cases) in order to model "real" performance against predicted performance, which was derived from training. In general, the test results showed a small improvement over the course of the training. These were able to be patterned closely by predicted performance modeled from the degree of training undertaken during the period. When this was done, both the accompanying fatigue and fitness curves were quantitatively defined for each subject.
**Hemoglobin**

Figures 2 to 12 show regression of hemoglobin concentration (Hb) on derived fatigue (top left) and Hb on predicted performance (right center). In eight of the eleven subjects there is a decline of Hb (quite dramatic in some), from the pre-training value to that obtained on the final day of training. During the two week rest period, Hb concentration began to revert towards the pre-training levels or higher, in some cases. Hb concentration increased, in all subjects, from its level on the final day of training after either one or two weeks of rest. The pattern of this change corresponds closely with both the time course of increase in predicted performance and the increase in Hb concentration for most of the subjects.

**Serum Ferritin Concentration**

Figures 2 to 12 (center right) show the regression of serum ferritin concentration on derived fatigue. In six of the eleven subjects serum ferritin concentration declined during the course of the training. One subject's serum ferritin concentration increased and four others showed no change. During the two week rest period serum ferritin concentration increased in seven of the subjects, continued falling in two and remained the same in two others.
Total Iron Binding Capacity (TIBC)

Figures 2 to 12 (bottom left) show the regression of TIBC on derived fatigue. During the training period, TIBC was variable in five subjects, while the remaining six subjects showed a decline in their values. Conversely, TIBC increased in six subjects during the two week recovery period and was variable in the five others.

Serum Transferrin Concentration

Figures 2 to 12 (bottom center) show the variation of serum transferrin concentration with derived fatigue. In seven subjects, serum transferrin concentration increased slightly, in phase with the increasing fatigue, whereas the pattern was variable in the remaining four subjects. During recovery, serum transferrin concentration increased then declined in five cases, and varied randomly in the remaining six cases.

Percent Saturation of Transferrin (%sat) and Serum Iron Concentration (SI)

Figures 2 to 12 (center top and center) show variation of %sat and SI respectively, with derived fatigue. There seemed to be no coherent response pattern induced by training or recovery in the group throughout the period for either of these variables.
Figure 14 and 15 show TIBC plotted against serum transferrin concentration for the training and single-bout sub-groups respectively, while figure 13 shows the relation for the control sub-group and for the single-bout and training subjects prior to the commencement of any experimental procedure. Blood samples for the single-bout exercise subjects were taken immediately prior to ingestion of the radioiron solution, therefore the time between the end of exercise and the blood sample varied accordingly (see table 4). Blood samples for the training subjects were taken in the rested state, prior to the commencement of an exercise bout (usually 24 hours after the previous day's training). There was no difference in the regression equations when the data for early and late training were analyzed separately. The regression of transferrin on TIBC was different following a single-bout of exercise (figure 15) and in the rested state during training (figure 14), from that in the control sub-group (figure 13).
Figure 2: Showing serial hemoglobin, serum ferritin, total iron-binding capacity (TIBC), % saturation of transferrin, serum iron and serum transferrin measurements respectively (filled circles) patterned against derived fatigue (thin line) in the left and center graphs, predicted performance (thin line) modeled to criterion points (filled circles) in the top right graph, hemoglobin concentration (filled circles) patterned against predicted performance in the center right graph and training impulses in the bottom right graph, for subject 363. Iron status indices are plotted throughout the training period. A single measurement prior to commencement of exercise and two measurements (one and two weeks following cessation of training) are also plotted. The calendar date is shown as a six digit number (YY MM DD) along the abscissa as well as the number of days elapsed from the onset of training, defined as day zero (0).
Figure 3: Iron status measurements patterned with derived fatigue and predicted performance curves for subject 364. Legend otherwise as in figure 2.
Figure 4: Iron status measurements patterned with derived fatigue and predicted performance curves for subject 365. Legend otherwise as in figure 2.
Figure 5: Iron status measurements patterned with derived fatigue and predicted performance curves for subject 366. Legend otherwise as in figure 2.
Figure 6: Iron status measurements patterned with derived fatigue and predicted performance curves for subject 367. Legend otherwise as in figure 2.
Figure 7: Iron status measurements patterned with derived fatigue and predicted performance curves for subject 368. Legend otherwise as in figure 2.
Figure 8: Iron status measurements patterned with derived fatigue and predicted performance curves for subject 369. Legend otherwise as in figure 2.
Figure 9: Iron status measurements patterned with derived fatigue and predicted performance curves for subject 370. Legend otherwise as in figure 2.
Figure 10: Iron status measurements patterned with derived fatigue and predicted performance curves for subject 380. Legend otherwise as in figure 2.
Figure 11: Iron status measurements patterned with derived fatigue and predicted performance curves for subject 381. Legend otherwise as in figure 2.

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Figure 12: Iron status measurements patterned with derived fatigue and predicted performance curves for subject 382. Legend otherwise as in figure 2.
Figure 13: Correlation of serum transferrin concentration and TIBC of sedentary male subjects at rest.
Figure 14: Correlation of serum transferrin concentration and TIBC of training male subjects at rest.
Figure 15: Correlation of serum transferrin concentration and TIBC in males at rest following a single bout of exercise.
IV. DISCUSSION

Limitation of Method

The present study was limited technically, somewhat, by lack of a whole body counter, statistically, by the small number of subjects necessarily investigated by reason of the invasive nature of the involved technique, and medically by restrictions placed on the study design by the potential radioactive hazard of the analytical method. Ideally the design should have allowed an iron absorption count on each subject both in the sedentary state prior to any exercise treatment and again following either an acute exercise stimulus or at the end of training. This would have been possible if radioiron absorption had been estimated from two isotopes of iron ($^{59}$Fe and $^{55}$Fe) ingested on separate occasions (Heinrich, 1970). Such a design allows a subject to act as his own control, so that it would not have been necessary to study as large a number subjects to increase statistical power of any test of significance.

However, there are several possible sources of error even with this seemingly ideal approach. First, in this method the total blood volume of a subject must be estimated, either from body surface area or by a dilutional technique, to allow calculation of total blood radioactivity from a small blood sample. In the present study, total blood volume estimated from body surface area would be unreliable, especially in the case of a subject who might reduce his body surface area due to chronic
training. The latter perturbation, paradoxically, has also been shown to stimulate an increase in total blood volume (Saltin et al., 1968). Measurement of total blood volume by the isotope dilution principle would have involved introduction of another radioisotope into each subject, in addition to the $^{59}\text{Fe}$ and $^{55}\text{Fe}$ necessary for iron absorption estimation. Ethically this would have been undesirable. Secondly, an important assumption of the double isotope method is that a major portion of absorbed iron is incorporated directly into red blood cells, thus the absorbed radioiron will be detectable in the blood (Cook, 1977). In fact, red cell utilisation of $^{59}\text{Fe}$ ranges between 80 to 100% (Cook, 1977) and the % incorporation of $^{59}\text{Fe}$ may vary appreciably between subjects (Bezwoda et al., 1979) as well as between successive measurements on the same subject throughout a given period. Exercise might well exacerbate this effect. A third possible source of error might be due to the extended elapsed time between pre and post-exercise measurement in chronic exercise trainees. After 6 to 8 weeks of training, intra-individual changes in iron absorption might be due to day to day variability or to one or more other factors, occurring during the time period, quite unrelated to any direct effect of exercise. Although this latter point also remains as an unknown factor in the methodology of the present study, a whole body radioactive count, which does not require a two isotope design, was decided to be a more reliable test of $^{59}\text{Fe}$ absorption and was used in the present study.
Unfortunately, a 4π whole body counter was not available and a 2π counter was used instead. The counting efficiency of a 4π whole body count is approximately 20%, appreciably greater than the .014 to .028% efficiency of the 2π whole body counts measured in this study. However, the reliability of the Nuclear Data 62 gamma counter is high. Thus despite low counting efficiency preventing absolute measurement of the total amount of absorbed iron, relative 59Fe percent absorption was effectively and reliably measured among the experimental and control subjects.

Another factor which must be considered in iron absorption studies is the method of administration of the 59Fe. In the past, studies have used both intrinsic and extrinsic labelling of foods in a test meal and have varied both the amount of 59Fe and carrier iron ingested (Cook et al., 1977). There is no agreement on the standard amount of carrier iron to be used, although 1 to 3 mg. is common in most studies (Bezwoda et al., 1979). However, Heinrich et al., 1977 point out that because of the existing dose/absorption relationship for iron in relation to existing iron stores, smaller doses of 59Fe (e.g. 0.56 mg. in their study) are more suitable for a non-overlapping separation of iron absorption capacity between subjects than are larger doses of several mg. In the present study, a quite small amount of ferrous citrate (0.16-0.31 μg) labelled with 10 μCi 59Fe was ingested by each subject. It is difficult to speculated on the consequences of this rather nonpharmacological dose, although the wide variability in iron absorption of the subjects
resulting from this amount (3-84% in the present study) eliminates one obvious confounding consequence i.e. that all subjects would absorb the total dose. Thus it must be appreciated that iron absorption studies are fraught with non-standard methodology. If useful comparison of absorbing power is to be made, therefore, there is a need to titrate the dose of radio labelled iron administered close to the perceived (best judged) iron absorbing power of the individual under study at the time of the dose administration.

Iron balance during training

Normally the body maintains a relatively large reserve of iron in the bone marrow. If there is loss of blood, a temporary reduction in dietary iron intake or intestinal absorption or an increase in iron demand, the iron reserve may be used to cover the imbalance. Since iron stores in the subjects of this study were quite low to begin with, presumably due to blood donations, increases in iron demand would have to be met by an increased intestinal absorption of dietary iron. The subjects did not take iron supplements, rather they were given instructions on maximizing the bioavailability of iron from their normal diet. The magnitude of iron depletion from exercise either in individuals or groups remains equivocal, although Ehn et al. (1980) found the biological half-life of iron to be 1000 days in male runners (estimated from radio iron elimination curves) compared with 2100 days in male non-runners. Adjustments in the
blood parameters measured in this study may indicate attempts to meet the iron demand of exercise.

**Hemoglobin concentration (pre and post exercise)**

Hemoglobin concentration (Hb) declined from its pre-training value by the end of training in eight of the eleven trained subjects (Table 2 and figures 2-12). This decrease in Hb paralleled a decrease in hematocrit (Hct) and red blood cell count without any accompanying significant change in mean cell volume (MCV) or mean corpuscular hemoglobin concentration (MCHC) except in one subject (#382). It is known that endurance training leads to an increase in plasma volume (Brotherhood *et al.*, 1975; Saltin *et al.*, 1968). Thus assuming that the reduced Hct was due to hemodilution, post-training Hb concentration values were normalized to the value corresponding to pre-training Hct (Table 2). In this way an authentic decrease in Hb concentration was demonstrated only by subject 382, whose Hct increased while the corresponding Hb value decreased. In this subject, although the corresponding MCV was increased, MCHC was decreased, which may indicate a deficiency of Hb in the red blood cell after training.

Conversely, a normal Hb concentration was attained in all subjects after the first and second week of rest following training, when fatigue was declining. The absolute hemoglobin levels returned to pre-training levels and even increased in some subjects (363, 364, 367, 369). Thus any erythropoiesis
<table>
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<th>Subject</th>
<th>Hct(%) pre training</th>
<th>Hct(%) post training</th>
<th>Hb(ug·L⁻¹) pre training</th>
<th>Hb(ug·L⁻¹) post training</th>
<th>Hb(norm) pre training</th>
<th>Hb(norm) post training</th>
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<td>147</td>
<td>154</td>
<td>43.2</td>
<td>43.5</td>
</tr>
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Hct - hematocrit
Hb - hemoglobin concentration
Hb(norm) - Hb normalized to the pre-training Hct
induced by training was probably sufficient to maintain Hb at a
constant absolute level and to cause a small "overshoot", in
phase with predicted performance (center right panel of figures
2 to 12), following the abrupt cessation of training. This
effect is analogous to a process called "peaking", well known to
athletes preparing to maximize their performance before
competition. It is not possible to deduce from present data
whether enhanced erythropoiesis is needed to maintain a constant
Hb since no measurements of concomitant hemolysis accompanying
training were taken.

*Serum ferritin concentration during training*

During the first few weeks of training, serum ferritin
concentration declined to a steady state level in 6 subjects.
Similarly, growth of fatigue modeled from training impulse
scores, increased steeply at the beginning of training and less
steeply when subjects adapted somewhat to the training stress.
Subjective comments at this time indicated that the effort of
maintaining a workload sufficient to raise their heart rate to
80% of it's maximum rate became more difficult during the latter
weeks of the training period. This was probably due to an
increase in stroke volume accompanying training so that the
cardiac output for a similar workrate was met by a reduced heart
rate (Ekblom, 1969). Thus in order to maintain a constant
training impulse (product of duration of training and fractional
elevation of heart rate) a higher average power output needed to
be maintained in a training session, held constant at 60 minutes
for the duration of the training experiment. Thus perception of effort might well dramatically increase if regional adaptation to training in the working muscle did not parallel the time course of adaptation in the central circulatory system. A lag in adaptation of working muscle could well be due to the beginning effect of iron depletion on reduction of myoglobin and iron-containing enzymes of the aerobic pathways.

Changes in serum ferritin concentrations cannot be accounted for by plasma volume expansion. Thus, Hct in subject 369 declined from 48% to 43% while serum ferritin concentration increased from a pre-training level of 32 μg·L⁻¹ to a post-training level of 45 μg·L⁻¹. Training of the severity and rapidity of onset imposed on the trainees in this study, may have led to a transient muscle cell necrosis and loss of cell constituents to the extracellular and vascular space accompanied by local inflammation and soreness (Friden et al., 1984). Inflammation is known to result in increased ferritin synthesis in the liver, prior to changes in iron levels, analogous to an acute phase inflammatory reaction (Halliday and Powell, 1984). Dickinson et al. (1982) found that the serum ferritin levels in endurance runners, training exhaustively, declined by an average of 37% after two weeks of rest, thus they concluded that serum ferritin levels were probably elevated (in relation to the iron store they are thought to reflect) due to the tissue damage encountered during training.
A possible way to test for ferritin of tissue origin would be to determine the iron content of serum ferritin. Tissue ferritin is normally much higher in iron content than serum ferritin (Bacon and Tavill, 1984). Isozymes of ferritin, determined by the proportion of H and L type subunits, may be identified by electrophoresis and isoelectric focusing (Halliday and Powell, 1984; Zuyderhoudt and Linthorst, 1984). Ferritins of liver and spleen origin may be identified in this way since they are more basic due to a greater proportion of the L subunit. Heart and kidney ferritins are more acidic due to a greater proportion of the H subunit. There is evidence to suggest that serum ferritin concentration is regulated by the liver. Studies by Mack et al. (1981) of an isolated perfused rat liver preparation using normal, iron-loaded and iron-deficient liver and serum have shown that the perfusate attains a ferritin level appropriate to the iron stores of the animal from which the liver was taken. Thus, theoretically if tissue damage results in an acute release of ferritin into the blood stream, the liver would soon take up excess ferritin and re-establish a level appropriate to its stores.

In contrast to the suggestion of Dickinson et al. (1982), Magnusson et al. (1984b) suggest that serum ferritin concentration may underestimate actual iron stores. These authors suggest that serum ferritin reflects the content of iron in the reticuloendothelial system and that iron stores may shift to the hepatocytes of the liver, which take up the hemoglobin-haptoglobin complex that results from intravascular
hemolysis. It would be interesting to assess liver iron by computed tomography (Roudot-Thoraval et al., 1983) at the beginning of an athlete's career and then several years later, in conjunction with serum ferritin measurements, to see if such a shift in iron stores really does occur. Although all of the subjects were sedentary at the beginning of the present study and had been so for at least the prior six months, it is not known what continuing effect past athletic activity may have had on distribution of their iron stores. Thus interpretation of the meaning of serum ferritin in heavily training individuals is uncertain and the decline in serum ferritin concentration in six of eleven subjects during the course of this study may not be taken as conclusive evidence of a negative iron balance.

Milman and Sondergaard (1984) measured serum ferritin concentration in 1348 male blood donors. Serum ferritin showed a moderate fall up to the fourth donation and thereafter showed only minor insignificant changes. A similar levelling off of serum ferritin (after two donations) was observed in blood donors evaluated by Pederson and Morling (1978), who speculated that donors seem to regulate iron at this new lower level. A similar phenomenon may have occurred in the subjects of this study. If an increase in iron absorption does not begin before the iron stores decline to some critical level, this might explain the initial decline in ferritin values, followed by regulation at a new, lower level. It seems wise and necessary that iron absorption should not increase until a low, critical threshold body ferritin store is reached, especially since the
toxic action of excess iron is a greater threat than is a small deficit. This fact may also explain the apparent resistance to iron supplements exhibited by individuals with low serum ferritin concentration, who may actually be regulating quite adequately at these low levels (Banister and Hamilton, 1985). At high concentrations of iron in the intestine, a non-energy dependent uptake process appears to prevail which does not show saturation kinetics (Linder and Munro, 1977) so that large doses of iron may overcome the "mucosal block" and raise iron stores (Worwood, 1977).

\textit{Serum transferrin concentration, %sat and serum iron}

The concentration of liver iron stores appears to be a major regulatory factor in the control of hepatic transferrin synthesis (Morton and Tavill, 1977). Normally the iron donating cells of the liver, gut mucosa and reticuloendothelial system are triggered simultaneously to replace iron taken up by the erythron for increased erythropoiesis and by the muscle cells for synthesis of myoglobin and iron-containing enzymes of the oxidative pathway (Bhargava and Gabbe, 1984). Thus if iron demand is increasing during training, serum transferrin concentration should also be increasing. In this study no definite pattern of increase in serum transferrin concentration accompanied training. The small increase observed in seven subjects did follow a pattern of increasing fatigue (bottom center panel, figures 2 to 12) but could be explained by the coefficient of variance of measurement (<7.5%) and changes in
hematocrit (reflecting hemodilution) of between -10.4 to +2.5%.
The serum iron concentration (SI) and percent saturation of transferrin (%sat) seemed to show no coherent dose/response pattern with the fatigue accompanying training and no correspondence with changes induced in serum transferrin concentration by training.

In a previous study, Banister and Hamilton (1985) observed an increased saturation of transferrin and increased SI during periods of heavy training, with a lower %sat and SI during periods of relative rest. A possible explanation would be reduced release of transferrin into the serum during heavy training (higher saturation of a lesser amount of transferrin) and an increased release of transferrin into the serum during recovery (lower saturation of a higher amount of transferrin). O'Shea et al. (1973) observed a decline in plasma transferrin levels accompanying inflammation. In the presence of inflammation there is elevation of certain serum glycoproteins known collectively as "acute phase reactants" (APR) most of which are of hepatic origin (Liesen et al., 1977). Transferrin is not classified as an acute phase reactant, rather its rate of synthesis remains the same and its catabolic rate is increased during inflammatory conditions (Schreiber et al., 1982). Intravascular hemolysis, implicated by reduced haptoglobin levels in exercise (Magnusson et al., 1984a) may also lead to a return of iron to the liver (Magnusson et al., 1984b) and reduce the stimulus to synthesize transferrin. Thus the interaction of inflammatory processes and intravascular hemolysis, both
encountered during exhaustive training, tends to suppress transferrin synthesis despite an increased extra-hepatic demand for iron, which normally results in an iron release from the liver, and a stimulation of transferrin synthesis. This would lead to the conclusion, as in this study that serum transferrin is unresponsive to a recurrent-bout exercise stimulus.

Dissociation of TIBC and serum transferrin concentration

Interestingly, a deviation in correspondence between serum transferrin concentration and total iron binding capacity (TIBC) was observed in the training sub-group. A least squares fit of regression of serum transferrin concentration on TIBC gave a linear relationship, shown in figures 13, 14 and 15. The derived equation for sedentary subjects of this study at rest was similar to those of previous investigations (Table 3). However, the equation derived for regression of serum transferrin concentration on TIBC of training subjects at rest was different from any of those found in the literature and the correlation coefficient was not as high \( r^2 = 0.29 \), indicating that iron binding capacity was affected by changes in factors induced by training other than changed transferrin concentration. The correlation coefficient was low as well \( r^2 = 0.32 \) after only a single bout of exercise (figure 15). Shamberger (1980) suggested that the iron-poor ferritin released into the serum during inflammatory states may be capable of taking up iron, thus increasing the iron binding capacity of the blood in conjunction
TABLE 3: Comparison of regression equations of serum transferrin concentration (ST) on total iron binding capacity (TIBC) in patient populations of previous studies and male blood donors of the present study

<table>
<thead>
<tr>
<th>Reference</th>
<th>Formula</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackburn <em>et al.</em>, 1977</td>
<td>$ST = 0.80 \times \text{TIBC} - 43.0$</td>
<td>40</td>
</tr>
<tr>
<td>Rajamaki <em>et al.</em>, 1979</td>
<td>$ST = 0.68 \times \text{TIBC} + 19.9$</td>
<td>96</td>
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<tr>
<td>Miller <em>et al.</em>, 1981</td>
<td>$ST = 0.68 \times \text{TIBC} + 21.0$</td>
<td>125</td>
</tr>
<tr>
<td>Short <em>et al.</em>, 1984</td>
<td>$ST = 0.94 \times \text{TIBC} - 32.8$</td>
<td>91</td>
</tr>
<tr>
<td>Present Study</td>
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</tr>
<tr>
<td>Sedentary</td>
<td>$ST = 0.82 \times \text{TIBC} + 55^*$</td>
<td>17</td>
</tr>
<tr>
<td>Training</td>
<td>$ST = 0.47 \times \text{TIBC} + 160^*$</td>
<td>**87</td>
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* not in SI units for the purpose of comparison
** observations
with a decline in serum transferrin concentration. Inflammation may also result in increased synthesis and release of the acute phase reactant, ceruloplasmin. Ceruloplasmin is thought to function in the formation of the Fe(III)-transferrin complex (Ruckman and Sherman, 1981) and has been shown to promote incorporation of Fe(III) into apoferritin (Boyer and Schou, 1983). An increase in serum ceruloplasmin resulting from physical exercise (perhaps mediated by inflammation) has been reported in both humans (Haralambie and Keul, 1970) and in rats (Ruckman and Sherman, 1981). Thus, an increase in the levels of ceruloplasmin might partially offset a decline in serum transferrin synthesis accompanying inflammation, by increasing iron binding capacity. Conversely, Dowdy and Burt (1980) found that serum ceruloplasmin declined during the second month of a training regimen in members of a men's swim team and remained depressed for the remainder of the study (5 months). One might speculate therefore that a reduced TIBC would result from such an effect.

It is known that each specific site of transferrin requires a suitable anion, ordinarily bicarbonate or carbonate, for its Fe(III)-binding activity to be displayed (Aisen, 1984; Marx, 1984). A reduction in the iron binding capacity of transferrin might occur if serum bicarbonate content were reduced, due to the need to buffer increasing \([H^+]\) produced regularly during recurrent-bout exercise. The reversibility of such inhibition and the duration of the effect of reduced serum bicarbonate upon TIBC is presently unknown. Also, a variety of other anions may
function as bicarbonate analogs (Aisen, 1984).

In this study, TIBC declined in six subjects. The pattern was particularly distinct in subject 368, whose TIBC declined steeply as serum transferrin concentration increased. It is of interest to note that within the training group, TIBC was the only blood measurement that correlated significantly with % absorption of $^{59}$Fe.

**Correspondence of $^{59}$Fe absorption with changes in iron status variables**

Table 1 shows the mean and standard error within the experimental groups for each of the blood parameters measured at the time of $^{59}$Fe ingestion. Banister and Hamilton (1985) hypothesised that %sat correlates inversely with % iron absorption and that training leads to high %sat, perhaps due to a lower serum transferrin concentration. In the present study, while the %sat mean of the training group was marginally higher than either the control or single-bout group, %sat did not correlate significantly with % $^{59}$Fe-absorption in any of the three groups, although within the single-bout group the correlation was almost significant ($r = 0.55$). However, except for subject 366, the average amount of training that the subjects in this study were doing (approximately 125 trimps per day) was lower than the subjects in the study of Banister and Hamilton (1985) (175 trimps per day), who also trained every day of the week in contrast to five days per week by the subjects in the present study, and had a developed habit of training.
extending over several years. Subject 366, whose absorption of $^{59}\text{Fe}$ was only 6% following training, began running daily at the same time as he commenced cycling and thus sustained a considerable daily training impulse of close to 175. This subject's SI and %sat values were much higher than the training group mean, and closer to the pattern observed in the female track runners undertaking a similar quantity of training. Sport specificity has been demonstrated by several investigators (Dufaux et al., 1981; ). Reduced iron stores are observed more often in runners than rowers, swimmers or cyclists so that cycle training undertaken in this study may not have had the same effect as running in the subjects of Banister and Hamilton's study (1985).

Within the single-bout exercise group, Hb correlated negatively with $\%^{59}\text{Fe}$-absorption. If hemoglobin levels were not back to "normal" following blood donation in some of the subjects with the lowest levels, any exercise effect reducing absorption may have been dampened by an erythropoietic stimulus in addition to the stimulus of low iron stores. However, the effect of erythropoiesis as a stimulus to iron absorption in humans has been disputed (Bezwoda et al., 1979; Eschbach et al., 1977).

In the control group, both serum transferrin concentration and TIBC correlated positively with $\%^{59}\text{Fe}^{2+}$-absorption, although neither were significant ($r= 0.629$ and 0.626 respectively) due to the small number of subjects and large.
variance within the group. In the single-bout exercise group,
serum transferrin, but not TIBC correlated significantly with %
$^{59}\text{Fe}^{2+}$-absorption. This lack of synchronous effect is presumably
due to the lower correlation between TIBC and serum transferrin
concentration (discussed earlier) in the single-bout exercise
group ($r = 0.61$) compared with the control group ($r = 0.94$).
Conversely, TIBC and not serum transferrin concentration
correlated significantly with % $^{59}\text{Fe}^{2+}$-absorption in the
training group. The TIBC-serum transferrin concentration
correlation was even lower in this group ($r = 0.53$).

While it is interesting to speculate on the significant
correlations noted between serum iron status measurements and
$^{59}\text{Fe}$-absorption there is currently no direct evidence to suggest
that any of these modifies the absorptive behavior of the
intestinal cells directly (Hallberg and Solvell, 1960; Hoglund
and Reizenstein, 1969; Rosenmund et al., 1980). In order to
establish a connection conclusively it will be necessary to
study iron transport in the intestinal epithelium before and
after standard amounts of exercise, concomitantly with iron
status measurements.

**Timing of post-exercise $^{59}\text{Fe}$ ingestion on iron absorption**

There was no significant effect of the timing of post-exercise
$^{59}\text{Fe}$ ingestion on its subsequent absorption in either the
single-bout exercise or training group however, the number of
subjects was too small and the variance too large to establish
this point conclusively. Although it may be speculated from data of this study that absorption continues for longer than 24 hours following either a single bout of exercise or prolonged training, the problem needs further study. Knowledge of the time course of recovery of iron absorptive power would provide important information on the appropriate time to ingest iron containing meals or iron supplements in order to maximize absorption during different training protocols.

\( \% {^{59}}\text{Fe in the whole body count after one day} \)

It has been proposed that exercise increases intestinal motility by an increase in parasympathetic dominance (Van Liere et al., 1954). Rapid intestinal transit time has been implicated in malabsorption syndromes (Bond and Levitt, 1977), although actual studies have not confirmed this effect (Molla et al., 1983). In this study the whole body \( \% {^{59}}\text{Fe} \) retention after one day was a good predictor of final \( \% \) absorption for the training group and the mean whole body \( \% {^{59}}\text{Fe} \) retention after one day was significantly lower in the training group than in the control group. While this may imply a faster transit time, it may also be a reflection of the lower final absorption of iron attained by the training subjects. However, the single-bout group mean of \( \% {^{59}}\text{Fe} \) remaining after one day was not as low as the training group mean (70.1\% and 61.0\% respectively), whereas both groups reached the same mean final absorption (34.0\% and 34.2\%). Also, the subject in the control group who absorbed only 16\% according to the final \( {^{59}}\text{Fe} \) count still had 81\% of the \( {^{59}}\text{Fe} \) left after one
day. Thus it may be speculated that the faster disappearance rate of $^{59}\text{Fe}$ in the training subjects was due to an increase in intestinal motility and that at least part of the lower final $^{59}\text{Fe}$ absorption in these subjects may have been due to reduced transit time. In future, transit times could be measured directly (by administering a charcoal marker or a radionuclide). Alternatively, a second control group of non-exercisers may be used to estimate the amount of $^{59}\text{Fe}$ expected to remain in the whole body count after one day in subjects with lower final counts. However, this would still not account for other factors which might coincide with decreased absorption and increased motility, such as changes in intralumenal factors.

**Single-bout exercise versus training**

Two treatment groups (single bout and repeated bout) were employed to answer the questions, "If absorption is reduced with exercise, is it due to a cumulative effect?" i.e. will absorption be reduced in the training group and not the single-bout group, or "Is reduced absorption the result of successive acute stimuli?" i.e. will absorption be reduced in both exercise groups. The results of this study suggest the latter case is correct, in that the mean $\%$ $^{59}\text{Fe}^{2+}$ final absorption was almost identical for both groups. However, differences between the groups, such as in the mean $\%$ $^{59}\text{Fe}$ remaining after one day and the correlation of this value with the final $\%$ $^{59}\text{Fe}^{2+}$-absorption measured, indicates that although $^{59}\text{Fe}$-absorption was reduced similarly following a single bout of
exercise and an extended period of training, the mechanisms responsible may have been different. For instance, it would not have been possible, within 24 hours after a single bout of exercise, to alter the population of the absorptive cells in the intestine (Refsum and Schreiner, 1984). However, other studies have shown immediate effects of increased absorption (Finch et al., 1982; Hallberg and Solvell, 1960) so that there may be at least two mechanisms operating in the control of iron absorption; one to deal with acute changes and one to deal with chronic adaptation. Separate mechanisms operating in the single-bout and training groups to reduce absorption would then explain the results of this study.

Summary

Whole body retention of ferrous citrate labelled with 10 μCi $^{59}$Fe was found to be decreased following both acute (34.0% ± 11.6) and chronic (34.2% ± 11.8) exercise compared with a control group (70.8% ± 9.5) of similar iron-deficient male blood donors. Although $^{59}$Fe-absorption reduced equally following single-bout exercise as in extended training, the mechanism responsible remains unknown.

Weekly blood measurements were taken throughout the 6-8 week training period. A significant dissociation of total iron binding capacity (TIBC) and serum transferrin concentration (STC) occurred in this group. In six subjects serum ferritin concentration (SF) declined during the first few weeks to a
steady state level. It is suggested that iron balance may have been achieved at this new lower level.

SF, TIBC, STC, serum iron (SI), % saturation of transferrin (%sat) and hemoglobin concentration (Hb) were measured immediately prior to ingestion of the radioiron solution. Inconsistent correlations among experimental and control groups were noted between one or more of %sat, SI, TIBC, STC and Hb and % $^{59}$Fe$^{2+}$-absorption. There was a significant trend towards faster elimination of $^{59}$Fe from the body in subjects undergoing training. It is suggested that increased intestinal motility may have a significant effect on iron absorption and this possibility demands further study.

The results of the present study indicate the similar stimulus/response effect of single-bout and recurrent-bout (training) exercise on $^{59}$Fe absorption, although the exact mode of this modification remains equivocal.
REFERENCES


