IRON STATUS AND THE EFFECT OF IRON SUPPLEMENTATION IN THAI MALE BLOOD DONORS IN NORTHERN THAILAND

SUKANYA LINPISARN, WARUNEE KUNACHIWA,* THEERA LAOKULDILOK, JARUNEE LAOKULDILOK, RASSAMEE KEAWVICHIT and PANJA KULAPONGS**

Research Institute for Health Sciences, *Department of Clinical Immunology, Faculty of Associated Medical Sciences, **Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.

INTRODUCTION

Iron is an essential trace element for human life. The total body iron content of a man with an adequate intake of dietary iron is about 4 g (Skikne *et al.*, 1984). Most of this is in the forms of haemoglobin for oxygen transport and myoglobin for oxygen storage. Small but physiologically important quantities of iron are present in various tissue enzymes and bound to transferrin in the circulation. The remaining iron is stored as ferritin or haemosiderin.

Several factors can contribute to the development of iron deficiency anaemia, for example, chronic blood loss or bleeding which results in mobilization of iron from the body store. Although iron balance may be achieved by increasing the efficiency of dietary iron absorption, if the iron intake is adequate (Magnusson *et al.*, 1981). In Thailand inadequate food iron intake and low bioavailability of dietary iron are the major causes of the high prevalence of iron deficiency anaemia (Hallberg *et al.*, 1974 and 1977). The aim of the present study was to evaluate the iron status of two groups of male blood donors, the volunteers and professionals. Opportunity was also taken to evaluate, the effect of iron supplementation in male professional donors. The study was conducted in the northern Thai population.

MATERIALS AND METHODS

Subjects: Two groups of male subjects were included for evaluation of iron status. Voluntary donors were polytechnic and university students who donated their blood to a mobile unit of the Chiangmai Red Cross Blood Banks. Professional donors were recruited from men who appeared at the Blood Transfusion Unit, Chiangmai University Hospital and were offered monetary reward in return of their blood donation. In both cases, all donors were willing to give approximately 1 unit of blood (450 ml).

Male professional donors who had given blood consecutively 4 times or more, each with an interval of 6 months or less were included in the supplementation study.

Donors were selected on the basis of body weight, (> 50 kg), haemoglobin concentrations (>12 gm%). The whole blood specific gravity method, using copper sulphate solution as recommended by the American Red Cross and the American Association of Blood Banks was used in the assessment of Hb status (Miller, 1977).

Evaluation of iron status: Subjects were divided into sub-groups according to the number of blood donations. Voluntary donors were divided into three groups. Group 1 consisted of subjects who had never donated blood previously. Group 2 and Group 3 had already donated blood twice and three times per year for one year respectively, prior to entry into the study. In the professional donors, Group 1 were those who had never been phlebotomised before. Group

2, 3 and 4 had donated blood twice, three and four times or more respectively, and the intervals between each donation were less than six months.

Iron supplementation study: Subjects were randomized into two groups: the placebo (control) group and the iron supplemented group. 69 subjects were enrolled in the placebo group and 72 subjects in the iron supplemented group. Of these, 51 subjects in the placebo group and 47 subjects in the iron supplemented group completed the study. The iron employed in the supplementation study was purchased from the Government Pharmaceutical Organization, Bangkok Metropolis, Thailand. Each tablet contains approximately 56 mg of elementary iron and this was also confirmed by analysis. The 90 tablets were given to each subject after phlebotomy and instructed to take each tablet daily after meals. The period of supplementation was 95.5 ± 9.1 and 97.1 ± 10.8 days in the placebo and iron supplemented group respectively.

Iron status was evaluated both before and after supplementation which consisted of haemoglobin level, serum iron, total iron binding capacity (TIBC) and serum ferritin.

Haemoglobin concentration was measured by the cyanmethaemoglobin method, and haematocrit by centrifugation in a capillary microhaematocrit tubes (Cartwright, 1968). Serum iron and TIBC were measured as recommended by the International Standardization Committee (1971). Serum ferritin levels were measured by an enzyme linked immunosorbent assay employing horseradish peroxidase as an enzyme label and microtitre plate as solid phase (Linpisarn *et al.*, 1981). A haemoglobin value below 12 g/dl, and a haematocrit below 38% were regarded as indications of iron deficiency anaemia. Transferrin saturation of less than 16% and a serum ferritin level of less than 15 µg/l were considered as iron deficiency erythropoiesis and iron depletion respectively.

Statistical analysis: Serum ferritin values were first converted into logarithm (since they did not follow the Gaussian distribution) prior to analysis for means and S.D. of mean as recommended by Simon *et al.*, (1981). All results were compared using student's t-test, and p-value of less than 0.05 were considered significant.

RESULTS

Haematological values of 71 voluntary donors who had donated blood once to three times within one year period are summarized in Table 1. With an increasing frequency of

Table 1

Haematological data of male voluntary donor group.

	Number of donations/year		
	1	2	3
Number of subjects	28	23	20
Age	18.6 ± 2.0*	20.9 ± 5.1	20.7 ± 2.4
Weight	52.0 ± 4.4	56.1 ± 7.3	59.2 ± 12.8
Haemoglobin (g/dl)	15.21 ± 0.84	15.28 ± 0.86	15.27 ± 1.12
Haematocrit (% vol.)	45.89 ± 2.39	45.96 ± 2.16	45.30 ± 3.80
Transferrin saturation(%)	37.36 ± 15.40	35.22 ± 14.58	35.41 ± 13.89

*±S.D.

blood donation, there were no significant differences in haemoglobin, haematocrit and transferrin saturation levels in those who had previously donated once, twice or three times. On the other hand, the mean serum ferritin levels showed a steady decline, and there was a statistically significant difference between the first time donors and those who donated three times per year ($p < 0.05$, Fig. 1).

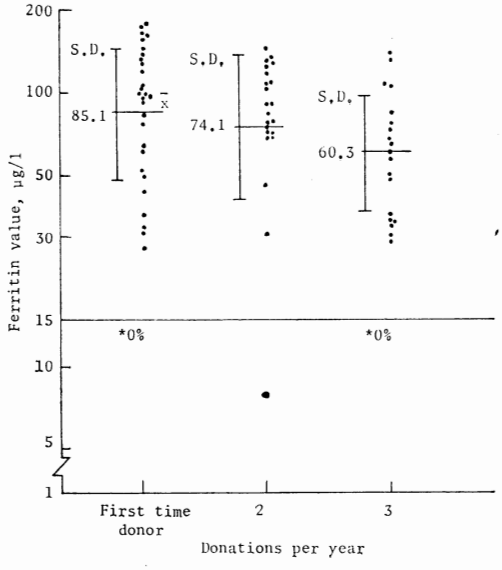

Fig. 1—Serum ferritin levels in male voluntary donor group. ($\bar{X}$) indicates the median and (*) indicates the percentage of donors with serum ferritin level lower than 15 µg/l.

Similar results were observed among the professional donors, that is, reduction of the mean serum ferritin values appeared to be related to the frequency of blood donations (Fig. 2). A reduction in mean serum ferritin levels was observed after two or three blood donations. Only with a more frequent donation, i.e after four times or more, that the decline in mean serum ferritin levels became significant when compared with the first time donors ($p < 0.001$). Furthermore, with the exception of Group 3, an increase in the frequency of donation also led to an

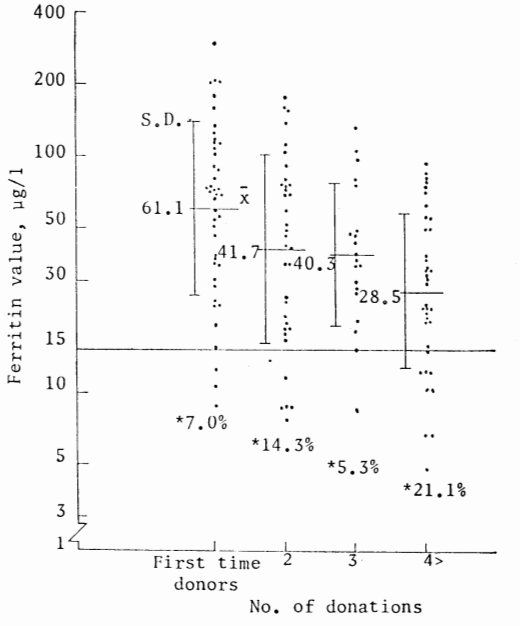

Fig. 2—Serum ferritin levels in male professional donor group. ($\bar{X}$) = the median and (*) = percentage of donors with serum ferritin level lower than 15 µg/l.

increase in the percentage of those with serum ferritin levels of less than 15 µg/l, a level generally accepted as indicating iron depletion. The percentage of iron depleted subjects was 7% in the first time donors compared to 21.1% in the group who donated four times or more. The other haematological parameters investigated were not significantly altered (Table 2).

The iron status of the two donor groups, namely voluntary and professional group, were compared. Haemoglobin and haematocrit levels were similar; but the mean transferrin saturation and mean serum ferritin levels were significantly lower in the professional donor group ($p < 0.005$ and $p < 0.001$ respectively). Moreover, the percentage of subjects who had serum ferritin level of less than 15 µg/l was found to be 7.0% in the professional donors, but none in the voluntary group.

Table 2

Haematological indices of male professional donor group who donated consecutively at intervals not more than six months apart.

	Number of blood donations			
	1	2	3	4>
Number of subjects	43	35	19	38
Age	23. 7 ± 5.2	23.5 ± 5.2	22.7 ± 3.1	24.7 ± 4.7
Haemoglobin (g/dl)	14.97 ± 1.28	15.15 ± 1.26	15.06 ± 1.52	15.21 ± 0.92
Haematocrit (% vol.)	45.53 ± 3.04	46.34 ± 3.60	46.68 ± 4.75	46.53 ± 2.72
Transferrin saturation(%)	28.07 ± 9.70	31.94 ± 14.76	30.10 ± 12.58	26.65 ± 14.31

Table 3

Haematological indices before and after iron supplementation in male professional donor group.

	No. of Subjects	Initial			Final		
		Hb g/dl	Hct %	Transferrin saturation %	Hb g/dl	Hct %	Transferrin saturation %
Placebo group	51	15.16 ± 1.19	46.53 ± 3.43	32.06 ± 15.84	15.12 ± 1.16	47.59 ± 3.51	29.93 ± 13.75
Iron supplemented group	47	14.93 ± 1.13	46.34 ± 3.17	27.17 ± 15.16	15.19 ± 1.22	48.34 ± 3.12	31.70 ± 14.59

Table 3 shows the initial and final mean and standard deviation of mean of haemoglobin, haematocrit and tansferrin saturation levels in the placebo and iron supplemented group. Supplementation with oral iron over a period of an approximately three months showed a definite tendency of improvement in haemoglobin, haematocrit level and transferrin saturation, though the increments were not statistically significant. In contrast, in the placebo group, no tendency toward an increase in any of these parameters was observed. In fact, the mean transferrin saturation was even lower after placebo supplementation.

The distribution of serum ferritin level before and after 3 months supplementation is illustrated in Fig 3. Although the mean

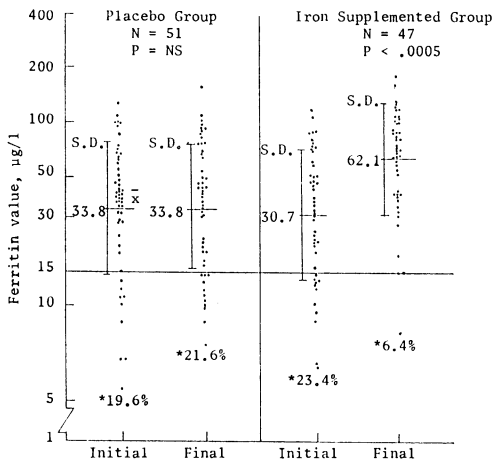

Fig. 3—Changes in serum ferritin level after three months of iron supplementation. ($\bar{X}$) = median and (*) = the percentage of donors with serum ferritin level lower than 15 µg/l.

initial ferritin levels were similar in both groups, the iron supplemented group showed a significant increase ($p < 0.05$) in the final serum ferritin level. In contrast, there was no significant difference in the placebo group. As expected, the percentage of subjects who had a serum ferritin level of less than 15 µg/l did not change after placebo treatment. Nonetheless, after supplementation with iron there was a reduction in the percentage of subjects who had developed iron depletion from 23.4% to 6.4%.

DISCUSSION

Regular blood donation has been recognized as a cause of diminishing iron stores (Simon *et al.*, 1981; Pedersen and Morling, 1978; Birgegard *et al.*, 1978). Each unit of blood (approximately 450 ml.) corresponds to 236 mg of iron or 6% of the total body iron in the male. Thus, an additional daily iron requirement of 0.65 mg should be provided to compensate for this loss in order to maintain the body iron balance (Finch *et al.*, 1977).

This study shows that, in general, the body iron stores of professional donors are lower to those of voluntary donors. To a certain extent, this may be due to a difference in socioeconomic situation, since the majority of the professional donors are laborers or farmers while voluntary donors were either polytechnic or university students.

The supplementation study shows that no alteration were observed in the mean serum ferritin level or the percentage of iron depleted subjects after three months administration of placebo. This suggested that a new balance is established between iron intake and iron loss at a low level of storage iron (Pendersen and Morling, 1978; Birgegard *et al.*, 1978). Supplementation with iron at a total dose of approximately 5,000 mg significantly increases the mean serum ferritin and transferrin saturation. However, the mean serum ferritin was still lower than that of the voluntary first time donors. Since it is unlikely that the dose is insufficient to replenish body iron stores to normal level, one possible explanation is that the study subjects may have had a lower initial body iron stores. Birgegard *et al.*, (1980) also reported this phenomenon by demonstrating smaller amount of stainable iron in bone marrow when compared with a normal population.

In Thailand, little attention has been paid to the effect of phlebotomy on the reduction of the total body iron. The majority of blood banks still use the copper sulphate gravity test for estimation of the haemoglobin level as a screening procedure for the ability to donate blood. However, this measurment, as expected, is not a guarantee against iron depletion (Olsson, 1972). At present, the health authority has recommended an interval of at least 3 months in men between donations. Even if this recommendation is followed, blood donations at a less frequent interval may still cause depletion of body iron stores in subjects with already low body iron storage or in those with a low iron absorption. The ethical acceptance of depleting iron stores in blood donors should be considered. A possible method of preventing iron depletion in the donor population is to use a more sensitive screening method, such as measuring serum ferritin level or red blood cell zinc protoporphyria (Pintar *et al.*, 1982; Schifman *et al.*, 1982). However, this would drastically reduce the number of potential donors and might thus adversely affect the nation's blood supply. The other approach would be to prescribe iron supplementation. The present study has indicated that iron supplementation is beneficial, even though total-compliance cannot be assessed.

SUMMARY

The iron status of voluntary and professional male donor groups was investigated.

The study indicated that serum ferritin level was lower significantly in those who donated three times per year compared to the first time donors ($p < 0.05$) in voluntary donor group. Similar results were observed among the professional donors. Whereas haemoglobin, haematocrit and transferrin saturation were not altered by donating blood in both groups. It also showed that, in general, the body iron stores of professional donors were lower to those of voluntary donors. Supplementation with iron over a period of three months produced a rise in serum ferritin levels and the percentage prevalence of iron depleted subjects decreased from 23.6% to 6.4%. Haemoglobin, haematocrit and transferrin saturation levels also improved.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the cooperation of Chiangmai Red Cross Blood Banks and the Blood Transfusion Unit, Chiang Mai University Hospital in supplying donor blood for this study. They also wish to thank Dr. Kosin Amatayakul, Research Institute for Health Sciences, Chiang Mai University, Thailand, for textual criticism, and Professor Yoshio Yoshino, Department of Biochemistry II, Nippon Medical School, Tokyo, Japan for invaluable discussions.

The study was supported by grant from the Royal Thai Government given to the Research Institute for Health Sciences.

REFERENCES

BIRGEGARD, G., HOGMAN, C., KILLANDER, A. and WIDE, L., (1978). Serum ferritin levels in male blood donors. *Vox. Sang.*, *34* : 65.

BIRGEGARD, G., HOGMAN, C., JOHANSSON, A., KILLANDER, A., SIMONSSON, B., and WIDE, L., (1980). Serum ferritin in the regulation of iron therapy in blood donors. *Vox. Sang.*, *38* : 29.

CARTWRIGHT, G.E., (1968). *Diagnostic Laboratory Hematology*, ed. 4th, Grune & Stratton, New York.

FINCH, C.A., COOK, J.D., LABBE, R.F., and CALALA, M., (1977). Effect of blood donation on iron stores as evaluated by serum ferritin. *Blood, 50* : 441.

HALLBERG, L., GARBY, L., SUWANIK, R., and BJORN-RASMUSSEN, E., (1974). Iron absorption from Southeast Asian diets. *Amer. J. Clin. Nutr., 27* : 826.

HALLBERG, L., BJORN-RASMUSSEN, E., ROSSANDER, L., and SUWANIK, R., (1977). Iron absorption from Southeast Asian diets. II. Role of various factors that might explain low absorption. *Amer. J. Clin. Nutr., 30* : 539.

INTERNATIONAL COMMITTEE for STANDARDIZATION in HEMATOLOGY, (1971). Proposed recommendations for measurement of serum iron in human blood. *Blood., 37* : 598.

LINPISARN, S., KRICKA, L.J., KENEDY, J.H., and WHITEHEAD, T.P., (1981). Sensitive sandwich enzyme immunoassay for serum ferritin on microtitre plates. *Ann. Clin. Biochem., 18* : 48.

MAGNUSSON, B., BJORN-RASMUSSEN, E., HALLBERG, L., and ROSSANDER, L., (1981). Iron absorption in relation to iron status. Model proposed to express results of food iron absorption measurement. *Scand. J. Haematol., 27* : 201.

MILLER, M.V., (1977). Technical Manual of the American Association of Blood Banks, ed. 7th, J.B., Lippincott Co., Philadelphia.

OLSSON, K.S., (1972). Iron stores in normal men and male blood donors. *Acta. Med. Scand., 192* : 401.

PEDERSEN, N.S. and MORLING, N., (1978). Iron stores in blood donors evaluated by serum ferritin. *Scand. J. Haematol., 20* : 70.

PINTAR, J., SKIKNE, B.S. and COOK, J.D., (1982). A screening test for assessing iron status. *Blood, 59* : 110.

SCHIFMAN, R.B., RIVERS, S.L., FINLEY, P.R. and THIES, C., (1982). RBC zinc protoporphyrin to screen blood donors for iron deficiency anemia. *JAMA., 248* : 2012.

SIMON, T.L., GARRY, P.J., and HOOPER, E.M., (1981). Iron stores in blood donors. *JAMA., 245* : 2038.

SKIKNE, B., LYNCH, S., BOREK, D. and COOK. J., (1984). Iron and blood donation. *Clin. Haematol., 13* : 271.