Elevation of Hemoglobin and Work Tolerance in Iron-Deficient Subjects

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Summary Selected parameters related to work tolerance were measured in 31 adult subjects with hemoglobin (Hb) concentration from 2.5 to 14.0 g/100 ml. Work tolerance was closely related to Hb concentration (r = 0.74) regardless of the adequacy of storage iron level. One male and six females with a mean Hb of 3.5 g/100 ml (27–55 years old) were studied before and 24 hr after transfusing 570 ml of whole blood. The mean maximal work load tolerated increased 83% within 24 hr after transfusion in these seven subjects. Post-exercise venous blood lactate was markedly lower after transfusion. Work tolerance of these subjects within 24 hr after transfusion was the same as in other subjects who had had the equivalent Hb level as the post-transfusion subjects presumably for at least several weeks. These data suggest that the decrement in work performance capacity in iron-deficient and anemic subjects is, in large part, a reflection of the level of anemia rather than other non-Hb related biochemical changes that could accompany prolonged iron deficiency anemia.

Key Words iron deficiency anemia, hemoglobin, transfusion, work capacity, heart rate, lactate

The extent to which an abnormally low hemoglobin (Hb) concentration may limit physical work capacity has been previously investigated in humans (1, 2) and in rats (3, 4). The improvement of physical work capacity in iron-deficient anemic subjects was observed following the elevation of Hb levels after the administration of iron (5–7). Woodson et al. (8) have reported an immediate decrement in the work
performance capacity of humans after lowering the Hb concentration by more than 30%. However, the effect of elevating the oxygen-carrying capacity of the blood of iron-deficient and anemic subjects on work performance is not clear. For example, it has been reported that elevation of the Hb of an iron-deficient rat from about 6 to almost 12 g/100 ml has no beneficial effect on work tolerance (9). The implications of this result are rather profound in terms of the clinical ramifications because it not only means that non-Hb iron is a major element in the clinical manifestations of iron deficiency anemia, but also because many people presumably are iron deficient but not anemic. The current investigation deals with the maximal physical work capacity and selected physiological responses related to work in normal adult subjects and in severely iron-deficient and anemic adult subjects and suggests that iron deficiency does not affect work capacity as much as the level of anemia.

METHODS

Age, sex, height, weight, Hb, serum iron and total iron-binding capacity (TIBC) of 31 adult Sri Lankan subjects are shown in Table 1. These subjects were divided into four groups. A normal group (n=11) consisted of subjects who had Hb of 13 g/100 ml or greater and were actively working on a nearby tea estate at the time they were studied. A second group (tea estate moderately anemic) consisted of subjects with a mean Hb concentration of 6.3 g/100 ml (n=5) and who were actively working on the tea estate when their anemia was detected. A third group (hospital moderately anemic) consisted of subjects with a mean Hb of 5.6 g/100 ml, (n=8) who had been admitted to the Kandy General Hospital of Sri Lanka for various dietary deficiencies and hookworm infestation (12). Presumably the subjects identified in the hospital had not been as active during the preceding week(s) as those that had a similar Hb level but had remained on their job. A fourth group (n=7) consisted of subjects admitted to the hospital after their anemia was determined to be severe (hospital severely anemic). Within 24 hr of their admittance, these subjects were transfused with 570 ml of whole blood which had been stored for less than 24 hr. After transfusion their Hb level approximated that of the tea estate

Table 1. Characteristics of subjects.

| Group         | Age (years) | n (sex) | Height (cm) | Weight (kg) | Hb (g/100 ml) | Serum iron (µg/100 ml) | TIBC (µg/100 ml) | Sat (%) |
|---------------|-------------|---------|-------------|-------------|---------------|------------------------|-----------------|---------|
| HSA           | 41 ± 5      | 6 (F), 1 (M) | 143 ± 3     | 37 ± 2      | 3.5 ± 0.4    | 28 ± 6                | 454 ± 29        | 6       |
| HMA           | 39 ± 3      | 6 (F), 2 (M) | 151 ± 2     | 37 ± 2      | 5.6 ± 0.3    | 51 ± 15               | 443 ± 44        | 12      |
| TMA           | 39 ± 7      | 5 (F)    | 146 ± 4     | 38 ± 3      | 6.3 ± 0.1    | 40 ± 11               | 508 ± 118       | 8       |
| TN            | 34 ± 3      | 1 (F)    | 147 ± 2     | 39 ± 2      | 13.8 ± 0.3   | 143 ± 15              | 517 ± 46        | 28      |

Mean ± SEM. HSA, hospital severely anemic; HMA, hospital moderately anemic; TMA, tea estate moderately anemic; TN, tea estate normal; Sat, saturation of iron.

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moderately anemic and hospital moderately anemic groups. All subjects on the tea estate were identified by a routine Hb check of the entire estate work force.

All moderately anemic subjects admitted to the study were identified during routine preliminary clinical examination for general patient admission. The entire experimental protocol was explained and voluntary consent was obtained from all subjects. Any subject was permitted to withdraw at any time from the study. Immediately following this study, treatment of the moderately and slightly anemic subjects was continued with a single dose of iron dextran (Imferon) and their progress was followed for at least 2 weeks (7). The severely anemic subjects were also treated with iron dextran 1 week after receiving the whole blood transfusion. Blood was withdrawn from the brachial vein and analyzed for Hb (cyanmethemoglobin method), serum iron and TIBC (10) and lactate (11).

A transthoracic electrocardiogram (ECG, V5-RV6) was continually monitored using an oscilloscope and paper recorder while the subjects were resting (supine) and walking. The walking consisted of a progressive treadmill test in which increasing load increments occurred every 2 min until either the subject chose to step from the treadmill belt, the subject reached near-maximal heart rate, or cardiac ischemia was indicated by ECG S-T segment changes. In one subject the maximal work load reached before transfusion was simply standing. The work loads on the treadmill and their relative rating in terms of work intensity are shown in Table 2 and have been reported by Ohira et al. (7). The maximum work load attainable was limited by the capacity of the treadmill (6.4 km/hr and 20% grade).

Normal fresh whole blood (less than 24 hr old) was transfused into the brachial

Table 2. Work loads on motor-driven treadmill.
The numbers as the relative work load are used in Figs. 1B, 2, and 3. Oxygen consumption ($\dot{V}_{O_2}$) was obtained in five normal sedentary females.

| Relative work load | Time (min) | Speed (km/hr) | Grade (%) | $\dot{V}_{O_2}$ (ml/kg/min) |
|--------------------|------------|---------------|-----------|-----------------------------|
| 0 (standing)       | 0-2        | 1.1           | 0         | 8.0±0.4                     |
| 1                  | 2-4        | 1.1           | 10        | 10.1±0.5                    |
| 2                  | 4-6        | 1.6           | 10        | 12.1±0.3                    |
| 3                  | 6-8        | 2.4           | 10        | 13.6±0.6                    |
| 4                  | 8-10       | 3.2           | 10        | 15.8±0.8                    |
| 5                  | 10-12      | 4.0           | 10        | 18.4±0.9                    |
| 6                  | 12-14      | 4.8           | 10        | 21.6±1.2                    |
| 7                  | 14-16      | 4.8           | 13        | 25.0±1.6                    |
| 8                  | 16-18      | 4.8           | 16        | 28.5±2.1                    |
| 9                  | 18-20      | 4.8           | 20        | 31.7±2.8                    |
| 10                 | 20-22      | 5.6           | 20        | 35.3±2.8                    |
| 11                 | 22-24      | 6.4           | 20        | 37.9±2.7                    |

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vein several hours after the subjects had performed the initial tests noted above. In order to assure the patient tolerance to the transfusion, heart rate was monitored regularly. In three of these subjects ECG was recorded continuously immediately before the transfusion process began and for the next 3 hr. The ECG was recorded on Holter recorders (Del Mar Engineering Laboratories, Los Angeles, California, U.S.A.) secured to the waist with a custom-made belt. On the following morning, resting and post-exercise blood samples were taken and analyzed for the same parameters listed above except serum iron. Blood samples were also taken 3 min after exercise. In one subject, heart rate was monitored for 24 hr, beginning just before transfusion.

Previous tests had shown that subjects could be tested up to at least three times before training or the adapting effect of the heart rate to the treadmill test occurred. Therefore adaptation could not explain the improved work performance capacity of the transfused subjects. To approximate the energy cost of each relative work load, oxygen consumption at each work load was measured in five normal but relatively sedentary females, 20 to 30 years old, from Los Angeles, as described by Ohira et al. (7). The mean (+SEM) Hb level of these subjects was 11.4 ± 0.4 g/100 ml.

RESULTS

The mean ± SEM of Hb and maximum relative work load tolerated (see Table 2) of all subjects tested are shown in Figs. 1A and 1B. The post-transfused, tea estate moderately anemic and hospital moderately anemic subjects performed equally well and each load was significantly greater than the work loads reached for the severely anemic subjects prior to transfusion. The subjects with a normal Hb performed significantly better than any of the other three groups with lower Hb concentrations. The correlation coefficient between work tolerance and Hb in all anemic subjects was 0.74 (p < 0.001) and is shown in Fig. 2. On the 7th day after transfusion three of the subjects were tested. The mean Hb level (6.2 g/100 ml) and the relative work load (6.7) were the same as 24 hr after transfusion.

Mean heart rates at various work loads are shown in Fig. 3. There was no significant difference in the heart rate before and after transfusion although it appears to be higher at the higher work loads before rather than after transfusion. Generally, at any given work load the heart rate was higher in the groups with the lower Hb concentrations. Figure 3 shows that the subjects with the lowest Hb reached their maximum heart rate at the lowest work load. The maximal work load attainable with the treadmill being used was not sufficient to induce a maximal heart rate in the normal subjects. Before transfusion of the severely anemic subjects, five of the seven were stopped from continuing to walk because of S-T segment depression. This occurred in only one patient after transfusion even though their work tolerance was elevated markedly. Also, it should be noted that standing was the maximum work load tolerable to one subject while the slowest walk at 0° grade was reached by another.
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Fig. 1. Resting hemoglobin (A) and maximal work load reached (B, expressed in relative numbers, see Table 2) are shown prior to and 1 day after transfusion of 570 ml of whole blood (mean ± SEM). The maximum work load refers to the maximum speed and grade attainable on the treadmill used in this study. n represents the number of subjects in each case. HSA, hospital severely anemic (pre- and post-transfusion); HMA, hospital moderately anemic; TMA, tea estate moderately anemic; TN, tea estate normal subjects. The * p<0.05, ** p<0.01 and *** p<0.001 were obtained by paired or unpaired t-tests between pre-transfusion and each group. Only TN group had significantly higher values than HSA post both in Hb and maximal work load tolerated (p<0.001 by unpaired t-test).

Before and during the process of whole blood transfusion ECG was recorded continuously for 3 hr in three subjects and in one subject for 24 hr after transfusion. In one subject (Hb: 2.5 to 5.0 g/100 ml), the heart rate ranged from 75 to 90 beats/min with an average of 80 during the first 35 min of transfusion. For the next 2.5 hr it remained consistently around 70 beats/min. In another subject (Hb: 3.5 to 5.5 g/100 ml), the heart rate reached 100 for a few minutes but ranged from 70 to 85 for the next few hours. A third subject (Hb: 4.2 to 5.8 g/100 ml) had an initial heart rate of 120 for 5 min. After 1 hr it was 90 and remained between 75 and 85 thereafter. In the one subject (Hb: 2.8 to 4.6 g/100 ml) whose heart rate was monitored for 24 hr, the heart rate ranged from 75 to 85.

Venous blood lactate seemed to be relatively independent of Hb concentration.
Fig. 2. The relationship between resting hemoglobin (Hb) concentration and work tolerance. The linear regression line is represented by max work load = 1.18 (Hb). HSA, hospital severely anemic; HMA, hospital moderately anemic; TMA, tea estate moderately anemic; TN, tea estate normal subjects. Eleven normal subjects from the tea estate were not included in the calculation of correlation coefficient because all of them reached the work load of 12 which was the maximum load attainable on the treadmill available for this study.

when the subjects were at rest (Fig. 4). However, the subjects with the normal Hb did have significantly lower lactate than the other groups (p < 0.05). Blood lactate in response to work was reduced significantly within 24 hr after transfusion, in spite of the work load tolerated being 83% greater than on the pre-transfusion test (Fig. 1B). The post-exercise blood lactate was similar for the post-transfusion (Hb: 5.9 g/100 ml) and the hospital moderately anemic subjects (Hb: 5.6 g/100 ml). The post-exercise venous blood lactate concentration in tea estate normal subjects was similar to that of the subjects 1 day after transfusion even though the normal subjects worked longer and reached a higher work load (Fig. 4).

DISCUSSION

These data are consistent with previous findings in humans (1, 13–15) and in rats (3, 16) that a close relationship exists between work tolerance and Hb concentration (Figs. 1A, 1B and 2). It has also been reported that iron treatment of anemic subjects results in an improvement in work performance at a rate similar to
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Fig. 3. Mean heart rate during maximal exercise in subjects with various hemoglobin (Hb) levels. The shade shows the area of mean ± SEM in oxygen consumption (Table 2). The * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ are results of unpaired t-tests between the values 1 day after transfusion and in each group at a given work load. No significant change was observed in the pre-exercise and maximal heart rates. Although recovery heart rate is not shown in the figure, it was significantly lower ($p<0.05$) 1 day after transfusion than before transfusion (during the first 2 min of recovery). HSA, hospital severely anemic; HMA, hospital moderately anemic; TMA, tea estate moderately anemic; TN, tea estate normal subjects.

the rate of elevation of Hb level (6, 7). However, the current study is not consistent with the finding that work performance of iron-deficient anemic rats does not improve at all when Hb is elevated with transfusion (9). It was suggested that a decrement in the rate of phosphorylation with $\alpha$-glycerophosphate in muscle might explain the non-Hb related effect (9, 17). But when the fatigue ability of muscles with either a high or relatively low dependence on oxidative metabolism is tested with the blood supply intact, it does not differ between iron-deficient and normal rats when the blood has the same Hb levels (17). But if the Hb levels are different the fatigue ability is less in the rats with the higher Hb levels (16). Also, in the present study, there was no suggestion of an excessive metabolic stress due to the exercise. In fact, the venous blood lactate levels were very moderate compared to that which occurs in response to intense exercise (18). This was also true in iron-deficient rats which ran poorly (17).

There could be some non-Hb related effects of iron treatments in either iron-
deficient or normal subjects that could affect work performance. One suggestion of such an effect has been demonstrated by a reduction in the heart rate response to exercise by iron treatment in iron deficiency (19) and in subjects with normal iron stores (unpublished data) beyond that which can be accounted for by the elevation in Hb. However, unlike the findings of Finch et al. (9, 17), our data suggest that the Hb level is far more critical to work tolerance than the non-Hb effects regardless of the iron store status. A major factor may be age. Finch et al. (9, 17) have used 4-week-old rats which become iron-deficient and anemic when placed on an iron-deficient diet. An adult rat will not become anemic and myoglobin and cytochromes will not decrease when it is placed on an iron-deficient diet (20).

There are several potentially complicating factors that could alter our conclusion that the oxygen-carrying capacity of the blood is a much more critical factor of work capacity than non-Hb related factors. Although the blood that was transfused had been collected within 24 hr, some iron may have been released from fragmented red blood cells and thereby could have altered the iron status. But this is unlikely because 1) up to 2 days after iron treatment of iron-deficient anemic subjects no change in maximum work capacity, heart rate (7), or level of voluntary activity (21) is evident, 2) in the study by Finch et al. (9), no change in work performance was seen in iron-deficient rats transfused with blood every 2 days for 9

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85 days. For these reasons we have assumed that iron deficiency cannot be corrected within 24 hr by whole blood transfusion.

It cannot be excluded that deficiencies other than iron deficiency existed in these subjects. However, it is unlikely that their nutritional status other than the iron deficiency anemia would be altered significantly by 570 ml of blood within a 24-hr period. There were no overt symptoms of protein deficiency. Muscle mass could not have changed significantly within 24 hr. Also, we have administered egg protein to 16 anemic subjects with mean Hb of 6.6 g/100 ml for 4 weeks without any effect on their Hb or serum iron (unpublished data).

The improvement in work capacity could have resulted from an elevated blood volume (22). But on the other hand, the additional volume could have decreased work tolerance due to the additional volume and overloading of the heart. It should be noted, however, that the heart rate, even during transfusion, remained well within the normal range for subjects with normal Hb levels.

In summary, these data demonstrate a close relationship between work tolerance and Hb concentration even in iron-deficient subjects. The fact that the rapid correction of anemia to the level equivalent to other moderately anemic subjects resulted in an improvement in performance equal to the moderately anemic subjects suggests that anemia was the major factor that caused the reduced work tolerance of iron-deficient and anemic subjects.

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REFERENCES

1) Gardner, G. W., Edgerton, V. R., Senewiratne, B., Barnard, R. J., and Ohira, Y. (1977): Physical work capacity and metabolic stress in subjects with iron deficiency anemia. *Am. J. Clin. Nutr.*, 30, 910-917.

2) Viteri, F. E., and Torun, B. (1974): Anaemia and physical work capacity. *Clin. Haematol.*, 3, 609-626.

3) Edgerton, V. R., Bryant, S. L., Gillespie, C. A., and Gardner, G. W. (1972): Iron deficiency anemia and physical performance and activity of rats. *J. Nutr.*, 102, 381-399.

4) Wranne, B., and Woodson, R. D. (1973): A graded treadmill test for rats: Maximal work performance in normal and anemic animals. *J. Appl. Physiol.*, 34, 732-735.

5) Anderson, H. T., and Barkve, H. (1970): Iron deficiency and muscular work performance. *Scand. J. Clin. Lab. Invest.*, 25 (Suppl. 114), 1-62.

6) Gardner, G. W., Edgerton, V. R., Barnard, R. J., and Bernauer, E. M. (1975): Cardiorespiratory, hematological and physical performance responses of anemic subjects to iron treatment. *Am. J. Clin. Nutr.*, 28, 982-988.

7) Ohira, Y., Edgerton, V. R., Gardner, G. W., Senewiratne, B., Barnard, R. J., and Simpson, D. R. (1979): Work capacity, heart rate and blood lactate responses to iron
treatment. *Br. J. Haematol.*, **41**, 365–372.

8) Woodson, R. D., Wills, R. E., and Lenfant, C. (1978): Effect of acute and established anemia on $O_2$ transport at rest, submaximal and maximal work. *J. Appl. Physiol.*, **44**, 36–43.

9) Finch, C. A., Miller, L. R., Inamdar, A. R., Person, R., Seiler, K., and Mackler, B. (1976): Iron deficiency in the rats. Physiological and biochemical studies of muscle dysfunction. *J. Clin. Invest.*, **58**, 447–453.

10) Levy, A. L., and Vitacca, P. (1961): Direct determination and binding capacity of serum iron. *Clin. Chim.,* **7**, 241–248.

11) Gutmann, I., and Wahlefeld, A. W. (1974): L-(+)-Lactate determination with lactate dehydrogenase and NAD, in Methods of Enzymatic Analysis, Vol. 3, 2nd ed., ed. by H. U. Bergmeyer, Academic Press, New York, pp. 1464–1468.

12) Senewiratne, B., Hettiarachchi, J., and Senewiratne, K. (1974): Some problems in the management of anaemia in tea-estate workers in Sri Lanka. *J. Trop. Med. Hyg.*, **77**, 177–181.

13) Davies, C. T. M., Chukweumeka, A. C., and Van Haaren, J. P. M. (1973): Iron deficiency anemia. Its effect on maximum aerobic power and responses to exercise in African males aged 17–40 years. *Clin. Sci.*, **44**, 555–562.

14) Ericsson, P. (1970): Total hemoglobin and physical work capacity in elderly people. *Acta Med. Scand.*, **188**, 15–23.

15) Vellar, O. D., and Hermansen, L. (1971): Physical performance and hematological parameters: With special reference to hemoglobin and maximal oxygen uptake. *Acta Med. Scand.* (Suppl. 522), 1–40.

16) Edgerton, V. R., Diamond, L. B., and Olson, J. (1977): Voluntary activity, cardiovascular and muscular adjustments to anemia in rats. *J. Nutr.*, **107**, 1595–1601.

17) Finch, C. A., Gollnick, P. D., Hlastala, M. P., Miller, L. R., Dillmann, E., and Mackler, B. (1979): Lactic acidosis as a result of iron deficiency. *J. Clin. Invest.*, **64**, 129–137.

18) Karlsson, J. (1971): Lactate and phosphagen concentrations in working muscle of man. *Acta Physiol. Scand.* (Suppl. 358), 1–72.

19) Ohira, Y., Edgerton, V. R., Gardner, G. W., Senewiratne, B., and Simpson, D. R. (1978): Non-hemoglobin related effects on heart rate in iron deficiency anemia. *Nutr. Rep. Int.*, **18**, 647–651.

20) Koziol, B. J., Ohira, Y., Simpson, D. R., and Edgerton, V. R. (1978): Biochemical skeletal muscle and hematological profiles of moderate and severely iron deficient and anemic adult rats. *J. Nutr.*, **108**, 1306–1314.

21) Edgerton, V. R., Gardner, G. W., Ohira, Y., Gunawardena, K. A., and Senewiratne, B. (1979): Iron-deficiency anaemia and its effect on worker productivity and activity patterns. *Br. Med. J.*, **2**, 1546–1549.

22) Oscai, L. B., Williams, B. T., and Hertig, B. A. (1968): Effect of exercise on blood volume. *J. Appl. Physiol.*, **24**, 622–624.