

Changes in Serum Ferritin and Other Factors Associated with Iron Metabolism During Chronic Hyperbaric Exposure

SARA C. GILMAN, W. L. HUNTER, JR., and L. W. MOONEY

DISTRIBUTION STATEMENT A

Approved for Public Release
Distribution Unlimited

Environmental Physiology and Operational Medicine Departments, Naval Submarine Medical Research Laboratory, Groton, Connecticut 06340

GILMAN, S. C., W. L. HUNTER, JR., and L. W. MOONEY. *Changes in serum ferritin and other factors associated with iron metabolism during chronic hyperbaric exposure.* Aviat. Space Environ. Med. 50(3):223-226, 1979. *mar*

Factors related to iron metabolism were determined in 20 United States Navy divers during 8 d of air saturation-excursion hyperbaric exposures. During these simulated dives progressive and correlated increases in serum ferritin and iron occurred. No significant changes were observed in bilirubin, hemoglobin, ceruloplasmin, transferrin, copper, or total iron binding capacity. The significance of the increased serum ferritin is discussed in relation to bone marrow damage and early detection of aseptic bone necrosis.

RADIOGRAPHIC ABNORMALITIES typical of aseptic bone necrosis (ABN) have been reported in approximately 20% of compressed air workers (7), at least 2% of North Sea divers (13), and in slightly less than 2% of United States Navy divers (6). Most agree that early recognition of the disease is essential to prevent irreversible and degenerative bone damage.

At present, radiography is the primary means of diagnosis but, since X-ray changes may be variable and delayed, this technique does not allow detection of ABN in its earliest stages of development. Bone scanning techniques, using radioisotopes (^{59}Fe and $^{99}\text{Tc}^m$ -diphosphonate), are currently under evaluation and appear to detect marrow and bone injury at much earlier stages (3, 4). However, less complicated and less costly screening procedures would both detect those personnel who could be studied further and eliminate a great deal of expense and unnecessary exposure to ionizing radiation.

This study was supported by Naval Submarine Medical Research and Development Command Research Work Unit 0099-PN.001-8013, entitled "simulated saturation diving employing nitrogen-oxygen mixtures as the principal breathing gas." The opinions and assertions contained herein are the private ones of the writers and are not to be construed as officially reflecting the view of the Navy Department, the Naval Submarine Medical Research Laboratory, or the Naval Service at large.

A recent study by Gregg *et al.* (5) suggests that changes in serum ferritin may provide a sensitive biochemical indicator of bone or marrow damage at earlier stages. In that study, bone and bone marrow necrosis were artificially produced by injection of saline suspensions of glass microspheres. Subsequent changes in serum ferritin were measured since serum ferritin is closely related to body iron stores (8,12,14,15) and high serum levels of ferritin occur when the protein is released by damaged marrow cells (1,9). A significant increase in serum ferritin was associated with marrow damage, according to Gregg *et al.* (5).

The purpose of this paper is to report increases in serum ferritin and iron levels in humans observed during a series of 8-d air saturation-excursion dives. Such changes could reflect marrow damage and may thus have value as an indicator of early ABN.

MATERIALS AND METHODS

Male United States Navy divers—20 in groups of three or four were exposed to hyperbaric air for 8 d using a 10 × 30 ft steel hyperbaric chamber maintained at the Naval Submarine Medical Research Laboratory. Extensive physical and laboratory examinations of subjects prior to their participation included standard radiographic surveys for evidence of ABN; all films were interpreted as normal. The saturation depth was 18.8 MSW (60 FSW, 2.8 ATA) in all cases. Daily 8-h excursions were made by 11 men to 31.3 MSW (100 FSW, 4.0 ATA) beginning at 10 a.m., which did not require decompression upon return to saturation depth. Nine men made daily 2-h excursions to 46.9 MSW (150 FSW, 5.5 ATA), which required a 160-min staged decompression for return to saturation depth. A recirculation atmosphere control system was used to regulate PO_2 and 20.9% and $\text{PCO}_2 < 1\%$. Temperature and humidity were regulated for diver comfort. The daily schedule included numerous physiological and behavioral tests commencing at 10 a.m. and ending at 8 p.m. Decompression to the surface required 20 h and commenced at 2 p.m. on the seventh day, 40 h after the last excursion.

20060503308

Precordial Doppler monitoring for venous gas emboli (VGE) was performed periodically during and after decompression from excursions as well as from saturation depth to the surface. No evidence of VGE was detected. One case of decompression sickness (DCS) occurred. It was reported some 2 h after surfacing and responded well to a Treatment Table 5 (U.S. Navy Diving Manual NAVSHIPS 0994-001-9010).

Fasting blood samples were obtained daily at 7 a.m. from each subject three times during the pre-dive period, during the 8-d dive period (including decompression), and on the first 3 d of the post-dive period. Subjects were given a general diet throughout the study. After collection, serum was frozen for subsequent determination of ferritin, iron, and other substances related to iron metabolism.

The following methods were used to evaluate changes in iron metabolism: (a) serum ferritin was assayed by a competitive binding radioimmunoassay which utilized a precipitating antiserum reagent to separate antibody-bound tracer from unbound tracer (GammaDab, Clinical Assays, Cambridge, Ma); (b) serum iron and bilirubin were assayed using a computer-controlled biochemical analyzer (SMAC, Technicon, Tarrytown, NY); (c) serum transferrin was measured using a nephelometer with a laser light source (LAS-R, Hyland, Costa Mesa, Ca); (d) copper levels were determined by atomic absorption spectroscopy (Perkin-Elmer, Norwalk, Ct); (e) ceruloplasmin level was determined by the technique of radial immunodiffusion (Meloy Labs, Springfield, Va); (f) hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin content, and red cell counts were measured on an automatic hemocytometer (Coulter Model S, Coulter Electronics, HiLaleah, Fl); and (g) total iron binding capacity (TIBC) was measured by protein precipitation (American Monitor, Indianapolis, In).

In order to limit statistical analyses to differences occurring across the three test condition (pre-dive, dive, and post-dive), the mean of the separate collection values for each subject within each of the three test conditions was determined first. An analysis of variance for repeated measures (same subjects) was applied to the distributions obtained for the three conditions. Differences between conditions were then tested for significance using *t* tests for correlated samples.

RESULTS

Fig. 1 shows the ferritin means and standard errors during the pre-dive, dive and post-dive periods. Analysis of variance revealed significant differences in serum ferritin levels across the three test conditions ($F = 9.95$; $df = 2.15$; $p < 0.01$). No significant difference was found between the pre-dive and overall dive period ($t = 1.60$; $p > 0.1$). However, as can be seen in Fig. 1, the ferritin level increased progressively during the dive. Further, a comparison of late dive day means (days 7 and 8) and pre-dive means demonstrates that the ferritin level during the late dive days was significantly higher ($t = 3.87$; $p < 0.01$). Ferritin levels remained elevated during the immediate post-dive period.

Fig. 2 shows the means and standard errors for serum

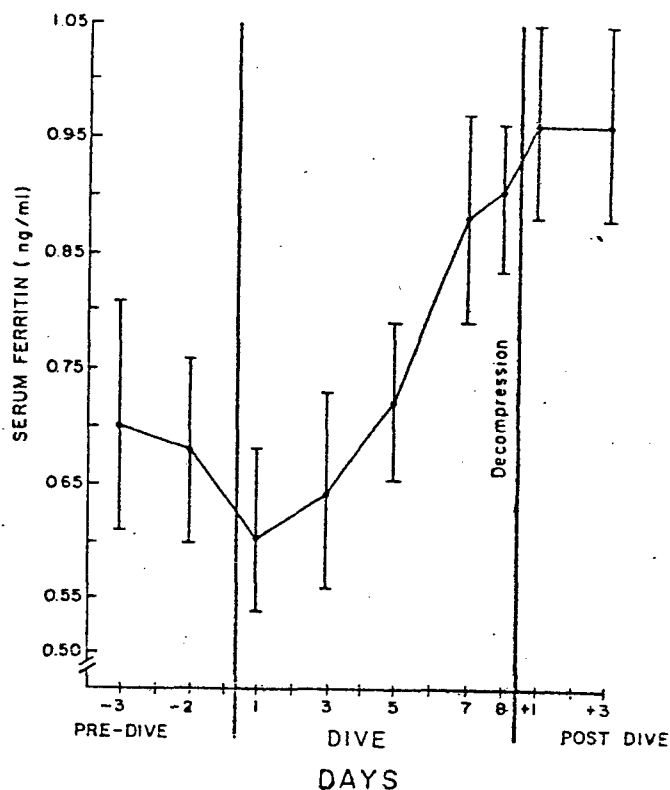


Fig. 1. Means and standard errors for iron level of the divers during the 8 d of hyperbaric exposure.

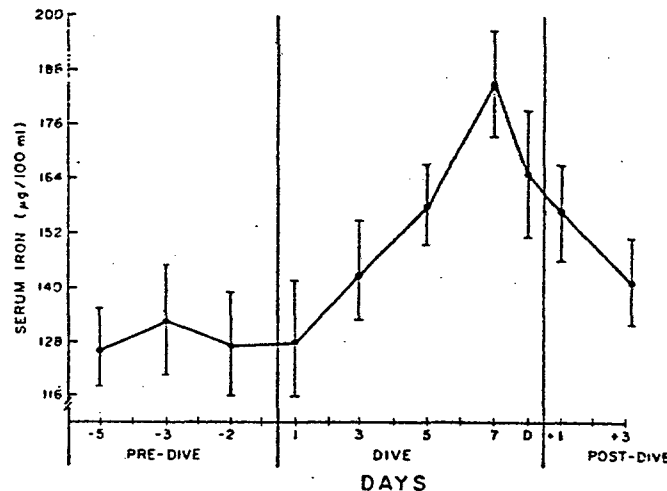


Fig. 2. Means and standard errors for ferritin levels during the 8 d of hyperbaric exposure.

iron level across the three test conditions. Analysis of variance showed significant differences occurring across the iron means ($F = 6.57$; $df = 2.19$; $p < 0.01$). Subsequent *t* tests demonstrated that pre-dive iron levels differed significantly from both dive and post-dive levels (pre-dive $\times$ dive $t = 4.49$; $p < 0.01$; pre-dive $\times$ post-dive $t = 2.49$; $p < 0.05$). No significant difference was found between dive and immediate post-dive iron levels ($t = 0.56$; $p > 0.5$). These results indicate that iron levels during the dive and immediate post-dive conditions were significantly higher than during the pre-dive (control) period. Regression analysis demonstrated significant

TABLE I. EFFECTS OF LONG-TERM HYPERBARIC EXPOSURE ON VARIOUS PARAMETERS ASSOCIATED WITH IRON METABOLISM.*

MEASUREMENT	NUMBER	MEAN VALUES $\pm$ STANDARD ERROR		
		PRE-DIVE	DIVE	POST-DIVE
Bilirubin (mg/dl)	20	0.6 $\pm$.08	0.8 $\pm$.06	0.7 $\pm$.05
Hemoglobin (g/dl)	20	15.5 $\pm$.10	15.5 $\pm$.07	13.4 $\pm$.95
Ceruloplasmin (mg/dl)	9	42.8 $\pm$.10	37.4 $\pm$ 3.52	58.1 $\pm$ 4.0
Transferrin (mg/dl)	7	207.9 $\pm$ 7.08	218.1 $\pm$ 2.03	212.5 $\pm$ 3.60
Copper (mg/dl)	3	95.9 $\pm$ 2.45	102.8 $\pm$ 3.90	108.4 $\pm$ 2.10
TIBC (mg/dl)	6	264.2 $\pm$ 5.60	263.5 $\pm$ 4.91	255.7 $\pm$ 7.20

* Measures made before, during, and after 8 d of exposure to 20.9% O₂ at 2.8 ATA.

correlations between the mean changes in serum ferritin and iron during pre-dive, dive, and post-dive periods ($r = 0.63$; $p < 0.05$).

Table I also shows the means and standard errors for various other parameters associated with iron metabolism across the three test conditions. No significant changes were found in any of these substances during the 8 d of hyperbaric exposure.

DISCUSSION

The data obtained during these dives indicates a pattern of consistent, progressive increases in serum ferritin and iron in human subjects during air saturation-excursion dives at relatively shallow depths. These increases were apparent by the third dive day for iron and the seventh dive day for ferritin. No changes were found in other factors associated with iron metabolism.

In analyzing the data, we considered all aspects of iron metabolism. Red cell lysis (hemolysis) would have produced increases in bilirubin (11,16), biliverdin (11), ceruloplasmin (10), copper (10), free hemoglobin, and urinary hemosiderin (16). Changes would also have been seen in mean corpuscular volume, mean corpuscular hemoglobin content, red cell count and volume of packed cells (16). Although several factors were not measured, the absence of changes in others favors some other explanation. In addition, the elevations in ferritin cannot be accounted for by red cell hemolysis.

Serum iron increases could result from increased net intake of iron, because of either increased dietary supply or increased absorption in the gut (2,17). The subjects were on a general hospital diet without vitamin or mineral supplements, so that increased supply and absorption seemed unlikely. And, as in the case of hemolysis, increased intake of net iron would not explain the observed increases in ferritin.

The most likely source of the increased amounts of serum ferritin and iron found during these dives appears to be iron storage sites. The body maintains iron reserves, in cells of the liver and bone marrow, in the form of an iron-ferritin complex. Damage to these cells could result in release of stored reserves into the gen-

eral circulation with elevation in levels of both ferritin and iron, and could account for changes seen.

The previously cited work of Gregg *et al.* (5) showed increased serum ferritin levels after histologically-verified bone marrow necrosis. The magnitude of the increases they observed in their animals was large (as much as 50 times reference values), while those we are reporting, although statistically significant, were relatively small (15-30% elevation). Although our findings are consistent with damage to bone marrow cells, it is not possible to definitely exclude hepatic involvement or to confirm bone marrow injury. Changes were observed in 17 of the 20 subjects, possibly indicating that such biochemical alterations occur much more frequently than ABN. It is of interest that no VGE were heard at any time during the dives, and the biochemical patterns seen in the one diver who developed DCS did not differ from the 19 who did not.

In summary, we found progressive, statistically significant elevations of serum iron and ferritin in a group of 20 divers participating in shallow air saturation-excursion dives. These elevations were unaccompanied by changes in other factors associated with iron metabolism. The most likely source seemed to be damaged bone marrow cells. It was concluded that serum ferritin measurements may prove to be useful as a screening technique for early detection of bone marrow damage and aseptic bone necrosis.

ACKNOWLEDGMENT

The authors wish to thank Ms. Pauline K. Sokoiski for her valuable assistance in the preparation of this manuscript. We also wish to thank Lakeville Medical Laboratories, New Hyde Park, NY, for technical assistance and support.

REFERENCES

1. Eastham, E. J., J. E. Bell, and A. P. Douglas. 1976. Serum ferritin levels in acute hepatocellular damage from paracetamol overdose. *Br. Med. J.* 1:750-731.
2. Forth, W., and W. Rummel. 1973. Iron absorption. *Physiol. Rev* 53:724-792.
3. Gorton, R. J., and R. N. Cooley. 1974. Bone scans with fluorine-18 in diagnosing osteonecrosis in divers. In: E. L. Beckman and D. H. Elliott (Eds.) *Dysbarism-*

- Related Osteonecrosis. Proceedings of a symposium on Dysbaric Osteonecrosis. Washington, DC, U.S. Department of Health, Education, and Welfare.
4. Gregg, P. J. 1977. The use of radioisotopes in aseptic bone necrosis. In: A. Evans and D. N. Walder (Eds.) Aseptic Bone Necrosis. Proceedings of a symposium EUBS meeting, Newcastle, September 1976. CIRIA Underwater Engineering Group Technical Note 12.
5. Gregg, P. J., E. J. Eastham, J. E. Bell, and D. N. Walder. 1977. Serum ferritin and dysbaric osteonecrosis. *Undersea Biomed. Res.* 4:75-79.
6. Hunter, W. L., R. J. Biersner, R. L. Sphar, and C. A. Harvey. 1978. Aseptic bone necrosis among U.S. Navy divers: Survey of 934 non-randomly selected personnel. *Undersea Biomed. Res.* 5:25-36.
7. McCallum, R. I. 1975. Dysbaric osteonecrosis: Aseptic necrosis of bone. P. B. Bennett and D. H. Elliot (Eds.), *The Physiology and Medicine of Diving and Compressed Air Work*, 2nd Ed. London: Bailliere, Tindall.
8. Powell, L. W., E. Alpert, K. J. Isselbacher, and J. W. Drysdale. 1975. Human isoferritins: Organ specific iron and apoferritin distribution. *Br. J. Haematol.* 30:47-55.
9. Prieto, J., M. Barry, and S. Sherlock. 1975. Serum ferritin in patients with iron overload and with acute and chronic liver diseases. *Gastroenterology* 68:525-533.
10. Roeser, H. P., G. R. Lee, S. Nacht, and G. E. Cartwright. 1970. The role of ceruloplasmin in iron metabolism. *J. Clin. Invest.* 49:2408-2417.
11. Routh, J. E. 1976. Liver function. In: Tietz, N. W. (Ed.) *Clinical Chemistry*. Philadelphia: W. B. Saunders.
12. Sorbie, J., L. S. Valberg, W. E. N. Corbett, and J. Ludwig. 1975. Serum ferritin, cobalt excretion, and body iron status. *C.M.A. J.*, 112:1173-1178.
13. Walder, D. N. 1976. Human aseptic bone necrosis. In: Suk Ki Hong (Ed.), *International Symposium on 'Man in the Sea'*. Bethesda, Md; Undersea Medical Society.
14. Walters, G. O., F. M. Miller, and M. Worwood. 1973. Serum ferritin concentration and iron stores in normal subjects. *J. Clin. Pathol.* 26:770-772.
15. Willekens, F. L. A. 1975. Serum ferritin, iron stores, and iron metabolism. *Neth. J. Med.* 18:89-93.
16. Wintrobe, M. E. 1969. Hemolytic anemias. In: W. E. Wintrobe *Clinical Hematology*. Philadelphia: Lea and Fibarger.
17. Worwood, M. 1977. The clinical biochemistry of iron. *Sem. Hematol.* 14:3-30.