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EVIDENCE FOR POST-TRANSCRIPTIONAL EFFECTS OF T_3 ON HEPATIC FERRITIN SYNTHESIS

Donald B. Jump, Cary N. Mariash, J.H. Oppenheimer
Department of Medicine, University of Minnesota
Minneapolis, Minnesota 55455

Christine Conlon-Hollingshead and Hamish Munro
Physiological Chemistry Laboratories
Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02103

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SUMMARY Rat hepatic non-heme iron and ferritin were elevated 3.4-fold in hypothyroid animals over that found in normal animals. Whereas ferritin apoprotein synthesis was increased 2.1-fold in hypothyroid animals, no significant difference in hepatic mRNA levels for the two ferritin subunits (H, Mr 21,000 and L, Mr 19,000) was detected between the two thyroidal states. Thus, T_3 affects ferritin metabolism either directly or indirectly at a post-transcriptional level.

INTRODUCTION In a recent study, one of our laboratories reported that hepatic polyribosomes prepared from hypothyroid rats were contaminated with an iron-containing complex having characteristics of ferritin (1). Tissue non-heme iron levels were elevated several-fold in hypothyroid livers and kidneys but not in spleen or brain, an association that parallels the responsiveness of these tissues to thyroid hormone (T_3,3,5,3'-triiodothyronine) (1).

In this report we establish that a proportionate increase in both hepatic ferritin iron and ferritin apoprotein account for all of the increase in tissue non-heme iron in hypothyroid rat livers. As a consequence of this observation, we were interested in determining the factors responsible for elevated ferritin in the hypothyroid rat liver. Accordingly, we have measured the rates of ferritin synthesis in the two thyroidal states and related these findings to the hepatic mRNA levels for ferritin.

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MATERIALS AND METHODS Male Sprague-Dawley rats (euthyroid 2-3 months old, 300-420 g.; hypothyroid 3-4 months old, 160-210 g.) were used in all experiments. Animals were rendered hypothyroid by surgical thyroidectomy followed by administration of 100 μCi of ^{131}I. Purification of rat liver ferritin was as described previously (2). The purity of the final product was established by demonstrating only two subunits on analysis by SDS polyacrylamide gel electrophoresis which corresponded to the heavy (Mr 21,000) and light (Mr 19,000) subunits of apoferritin (2,3).

Ferritin samples were electrophoresed in 15% acrylamide slab gels with 0.1 M $NaPO_4$, pH 7.2; 6 M urea; 0.1% SDS as buffer (4). Quantitation of Coomassie blue stained subunits employed the densitometric method of Mariash et al. (5) which involves a video camera linked to a microcomputer.

Ferritin antibiodies prepared as described (2) were used to quantitate ferritin apoprotein in heated liver homogenates (70 °C) using rocket immunoelectrophoresis (6). Protein was measured using the method of Lowry et al. (7) with bovine serum albumin as standard. Non-heme iron was measured in whole liver and heated homogenates using the dipyridyl reaction (8).

Total poly (A)-containing- mRNA isolation and translation in a rabbit reticulocyte lysate system using ^{3}H-leucine was as described previously (9). Products of the translation were immune-precipitated with purified carrier ferritin (3) and characterized on two-dimensional gels; first dimension: isoelectric focusing gel electrophoresis was as described by O'Farrell (10); second dimension: SDS polyacrylamide gel electrophoresis was as described above. Gels were fixed, stained and subject to autofluorography using Enhance (New England Nuclear), SB-5 X-ray film and DuPont Cronex intensifying screens. For quantitation, the immune-precipitated products were separated by SDS gel electrophoresis. The stained subunits of ferritin were excised and hydrolyzed in 0.2 ml 70% perchloric acid and 0.4 ml 30% hydrogen peroxide for liquid scintilation counting.

Relative rates of ferritin synthesis involved the injection of euthyroid and hypothyroid animals with 500 μCi of ^{3}H-leucine two hours before death (11). Liver ferritin was immune-precipitated from heated homogenates (3) and separated in SDS polyacrylamide gels for quantitation as described above. Total perchloric acid insoluble radioactivity was quantitated by the method of Linder et al. (11). Statistical evaluation of data was carried out by a Student's t-test.

RESULTS In hypothyroid livers, non-heme iron levels in whole homogenates are elevated 3.3-fold over the euthyroid level (Table I). Tissue levels of ferritin iron and ferritin apoprotein in euthyroid livers are comparable to those previously reported (Table I) (2). However, both ferritin iron and ferritin apoprotein are elevated 3.3-and 3.8-fold, respectively, in hypothyroid livers. This observation is not due to a generalized increase in tissue protein in hypothyroid livers since the percent of total tissue protein represented by ferritin was also elevated 3.5-fold in the hypothyroid state. Thus, the increase in tissue non-heme iron in hypothyroidism is accompanied by a concomitant increase in tissue ferritin apoprotein levels as opposed to increased

Table I. Non-Heme Iron and Ferritin Levels in Hypothyroid and Euthyroid Rat Liver[a]

Thyroidal Status	Non-heme Iron: Whole Homogenate	Non-heme Iron: Ferritin[b]	Ferritin Apoprotein	% of Total Tissue Protein as Ferritin[c]	Shell Composition[d] Subunit Ratio
	mg Iron/g liver		mg Protein/g liver		
Euthyroid	59.8±3.4	48.7±10.6	247.5±47.0	0.10	0.50
Hypothyroid	200.1±20.8*	163.0±6.0*	929.2±79.0*	0.35	0.24

[a]The results are the mean ± S.D. of eight determinations
[b]Ferritin iron was measured in ferritin immunoprecipitates
[c]Hypothyroid and euthyroid liver contained 263±82 mg protein/g liver
[d]Assuming 24 subunits per apoprotein shell, heavy subunit (H) M_r 21,000; light subunit (L) M_r 19,000
*P<.001

iron saturation of a constant pool of ferritin protein. This is reflected in the lack of a significant difference in the ratio of ferritin iron to apoprotein, (0.18 vs. 0.20) in euthyroid and hypothyroid hepatic tissue, respectively. Although the subunit composition of liver ferritin does not show striking differences between the two thyroidal states, the slight enrichment of hypothyroid liver ferritin with the light chain (Table I) may reflect the increased tissue level of iron, an established response to iron excess (2).

We next examined the rates of liver ferritin synthesis in euthyroid and hypothyroid animals. As shown in Table II, the *in vivo* rate of ferritin protein synthesis relative to total liver protein

Table II. *In Vivo* Ferritin Synthesis and Ferritin mRNA Levels in Rat Liver

Thyroidal Status	*In Vivo* Rate of Ferritin Synthesis % of Total Protein Synthesis	*In Vivo* Synthesized Subunits (H/L)[a]	Total mRNA Coding for Ferritin Subunits % of Total mRNA	*In Vitro* Translated Subunit Ratio (H/L)[a]
Euthyroid	0.19±0.06 (4)	0.71	0.20±0.04 (3)	1.2
Hypothyroid	0.39±0.06 (3)*	0.49	0.18±0.05 (3)	0.9

The results are the mean ± S.D. of the number of experiments indicated in parenthesis
[a]See Table I.
*P<.005

synthesis is elevated by 2.1-fold in the hypothyroid animals as compared to the euthyroid animals. Euthyroid ferritin synthesis rates agree favorably with previously reported values (11). Thus, the increase in tissue ferritin levels can, at least in part, be accounted for by increased synthesis of ferritin apoprotein in the hypothyroid liver.

The hepatic level of ferritin mRNA was measured in an in vitro heterologous reticulocyte lysate translation system programmed with total rat liver poly (A)-containing mRNA. Ferritin mRNA levels in euthyroid rat liver are comparable to previously reported values (12-14). Whereas, a significant increase in both ferritin protein mass and synthesis of ferritin apoprotein is found in the hypothyroid state (Tables I and II), no significant increase could be detected in the mRNA levels for ferritin (Table II). In fact, ferritin mRNA levels are 11.4% higher in euthyroid livers than in hypothyroid livers.

Taking advantage of the fact that each subunit contains nearly equal amounts of leucine i.e. 20H and 23L residues, respectively (15), we found that both mRNAs appear to be in nearly equal abundance (Table II). In contrast, both the tissue level of the two subunit proteins and the relative rates of synthesis (Tables I and II, respectively) favor an enrichment of ferritin with the light subunit.

DISCUSSION In this report, we establish that elevated non-heme iron levels in hypothyroid livers reflect a corresponding increase in ferritin apoprotein. The elevated hepatic ferritin level is, in part, due to a 2-fold increase in the synthesis of the two subunits of ferritin. However, hepatic mRNA levels coding for the two subunits did not show a corresponding increase, suggesting that tissues levels of ferritin may be controlled at a post-transcriptional level.

Since an increased rate of ferritin synthesis may not account entirely for elevated levels of ferritin, it is possible that the fractional turnover of ferritin may also be impaired in hypothyroidism. Decreased fractional rates of ferritin degradation that occur with aging

in rats leads to an accumulation of ferritin in hepatic tissue (16). Although the hypothyroid rats used in this study were older than euthyroid rats, age per se does not appear to be a factor in contributing to the higher levels of ferritin in hypothyroid livers. Previous studies showed similar elevations in non-heme iron in younger thyroidectomized rats, and administration of T_3 (200 ug for 4d) to such animals decreased liver non-heme iron to euthyroid levels (1). Additional studies involving the direct measurement of ferritin turnover will be required to determine the precise contribution of diminished turnover to the steady state ferritin level in hypothyroidism.

The physiological parameters leading to increased hepatic ferritin in hypothyroidism are not fully understood. Increased intracellular non-heme iron has been reported to stimulate ferritin apoprotein synthesis by inducing a translocation of ferritin mRNA from a cytoplasmic mRNP pool to free polyribosomes (12-14) as well as by stimulating apoferritin biosynthesis (17). The sex differences in hepatic ferritin synthesis between male and female rats are believed to be due to an increased availability of intracellular iron in livers as a result of increased dietary iron absorption (18) in the female rats rather than a direct effect of estrogens on liver (11). Alternatively, factors affecting iron utilization may indirectly contribute to iron storage and ferritin synthesis. Hypothyroidism in man (19,20) and rat (21) is accompanied by anemia. Both in vivo (22) and in vitro (23) studies indicate that erythropoesis is significantly suppressed in hypothyroidism and corrected by administration of T_3. Since animals cannot excrete excess iron, the decreased hemoglobin pool associated with hypothyroidism would be expected to lead to storage of the excess iron in body tissues. Thus, the effect of T_3 on erythropoesis may indirectly influence ferritin synthesis in livers by increasing intracellular non-heme iron. A direct effect of T_3 on hepatic apoferritin synthesis appears unlikely because this would lead to a decrease in the iron: apoprotein ratio which is contrary to our observation (Table I).

Finally, T_3 is known to stimulate or inhibit the formation of specific cellular mRNA's (24). Previous studies have also provided several examples in which T_3 regulates specific proteins and mRNA sequences in a parallel fashion (25-27). In this report, we clearly demonstrate that cellular levels of the mRNA species coding for the ferritin subunits do not reflect the mass of these subunits in the cell or the in vivo rate of their synthesis. Therefore, T_3 appears to affect hepatic ferritin levels at a post-transcriptional level. Thus, alterations in a given cellular protein in varying thyroidal states cannot automatically be assumed to be a consequence of a direct effect of T_3 on the expression of the gene coding for that protein.

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REFERENCES

1. Winkelmann, J.C., Mariash, C.N., Towle, H.C., and Oppenheimer, J.H. (1981) Science 213:569.
2. Bomford, A., Conlon-Hollingshead, C., and Munro, H.N. (1981) J. Biol. Chem. 256:948.
3. Arosio, P., Adelman, T.G., and Drysdale, J.W. (1978) Biol. Chem. 253: 4451.
4. Technical Report: Biologue 1. (1981) Bethesda Research Lab, Bethesda, MD.
5. Mariash, C.N., Seelig, S.A., and Oppenheimer, J.H. (1981) Clin. Res. 29:708A.
6. Laurell C.B. (1966) Anal. Biochem. 15:45.
7. Lowry, O.H., Rosebrough, H.J., Farr, A.L., and Randall, A.J. (1951) J. Biol. Chem. 196:265.
8. Drysdale, J.W. and Munro, H.N. (1965) Biochem. J. 96:851.
9. Towle, H.C., Mariash, C.N., and Oppenheimer, J.H. (1980) Biochem. 19: 579.
10. O'Farrell, P.J. (1975) J. Biol. Chem. 250:4007.
11. Linder, M.C., Moor, J.R., Scott, L.E. and Munro, H.N. (1973) Biochem. et Biophys. Acta 279:70.
12. Zahringer, J., Konihin, A.M., Baliga, B.S. and Munro, H.N. (1975) Biochem. Biophys. Res. Comm. 65:583.
13. Zahringer, J., Baliga, B.S., and Munro, H.N. (1976) Biochem. Biophys. Res. Comm. 66:1088.
14. Zahringer, J., Baliga, B.S. and Munro, H.N. (1976) Proc. Natl., Acad. Sci. U.S.A. 73:857.
15. Hollingshead, C. unpublished observation.
16. Ove, P., Obenrader, M., and Lansing, A. (1972) Biochem. et Biophys. Acta 277:211.
17. Drysdale, J.W. and Shafritz, D.A. (1975) Biochem. Biophys. Acta 383:97.
18. Hershko, C. and Eilon, L. (1974) Brit. J. Hematol. 28: 471.

19. Horton, L., Coburn, R.J., England, J.M., Himsworth, R.L. (1975) Quart. J. Med. 45:177.
20. Larsson, S.L. (1957) Acta. Med. Scand. 157:349.
21. Gordon, A.S., Kadow, P.C., Finkelstein, G., Charipper, H.A. (1946) Amer. J. Med. Sci. 212:385.
22. Das, K.C., Mukherjee, M., Sarker, T.K., Dash, R.J., Rastogi, G.K. (1975) J. Clin. Endo. Meta. 40:211.
23. Popovio, W.J., Brown, J.E., Adamson, J.W. (1977) J. Clin. Invest. 60: 907.
24. Seelig, S.A., Liaw, C., Towle, H.C., and Oppenheimer. (1981) J.H. Proc. Natl. Acad. Sci. U.S.A. 78:4733.
25. Seo, H., Vassart, G., Brocas, H., and Refetoff, S. (1977) Proc. Natl. Acad. Sci. U.S.A. 74:2054.
26. Martial, J.A., Baxter, J.P., Goodman, H.M., and Seeberg, R.H. (1977) Proc. Natl. Acad. Sci. U.S.A. 74:1816.
27. Samuels, H.H., Klein, D., Stanley, F., and Casanova, J. (1978) J. Biochem. 253:5895.