PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **183**, 257–261 (1986)

Plasma Levels of Immunoreactive Ceruloplasmin and Other Acute Phase Proteins during Lactation (42415)

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Abstract. Lactation elevates plasma copper as well as oxidase activity levels of the copper-containing, acute phase protein ceruloplasmin (Cp). The present study provides an initial inquiry into the mechanisms behind these changes. Plasma obtained from 12 lactating women, 1 month postpartum, displayed a greater percentage increase in immunoreactive Cp levels (mean increase = 89%) than in plasma copper (mean = 66%) or Cp oxidase activity (mean = 42%). Lactation did not increase plasma content of C-reactive protein or α_1-antitrypsin but significantly elevated haptoglobin concentrations. Plasma α_2-macroglobulin contents correlated with immunoreactive Cp levels in lactating women but not in controls. These results strengthen the hypothesis that plasma content of individual acute phase proteins is regulated by both overlapping and individualized processes. In addition, the present findings raise the possibility that lactation increases both Cp synthesis and plasma turnover time of Cp-bound copper.

Plasma copper concentrations rise during pathological stress such as inflammation (1), infection (2, 3), and cancer (3), as well as during nonpathological stress such as chronic exercise (4), pregnancy (3), and lactation (5). In the latter situation plasma copper content is elevated to 50% above normal at 1 month postpartum. By 2 months plasma copper declines to around 20% above normal, but levels remain for 10 months significantly higher than for nonlactating women. Serum copper in nonlactating, postpartum women declines more rapidly with 1-month levels at almost normal values (6, 7).

Most increases in plasma copper have been correlated with elevations in oxidase activity of the protein ceruloplasmin (Cp) (1–5). Cp contains most of the copper in plasma (8). This protein has been proposed to act as a copper-transport agent as well as to perform enzymatic function (9). Many of the situations producing increased Cp oxidase activities also raise plasma levels of a number of other proteins known as acute phase reactants (1). Levels of Cp and these other proteins would seem to be regulated by some overlapping processes. However, not all increases in Cp oxidase activities are necessarily accompanied by increases in all the other acute phase reactants. For instance, in pregnancy Cp oxidase activities rise but those of haptoglobin and C-reactive protein remain normal (11). This pattern suggests that the regulatory mechanisms behind increases in Cp levels have both overlapping and unique aspects compared to levels of other acute phase reactants. Moreover, control and perhaps functional significance of Cp elevations could vary for different physiological situations. The present study provides initial characterization of the effects of lactation on Cp protein levels and levels of some other acute phase proteins.

Materials and Methods. Plasma was obtained from 12 lactating women, 1 month postpartum, who were recruited for another study concerning B-6 supplementation. Subjects selected for the present study all took one of three multivitamin and mineral supplements once a day with each containing 2.5 mg vitamin B-6. Only 2 women used a supplement which contained copper (2.5 mg). Nonlactating subjects were 9 young, never pregnant women, aged 20 to 32, who were believed to be disease free and were not taking oral contraceptives. For comparison purposes standard lyophylized plasma representing a pooled sample from healthy donors (Calbiochem–Behring Diagnostics) was also utilized.

Plasma copper was determined on plasma diluted 1:1 with deionized water by atomic absorbance spectometry. Cp oxidase activity toward *p*-phenylenediamine (PPD) was measured colorimetrically by the method of Rice (12). Units were arbitrarily designated as the

0037-9727/86 $1.50

TABLE I. PLASMA COPPER, PPD OXIDASE ACTIVITY, AND IMMUNOREACTIVE Cp LEVELS IN LACTATING WOMEN

	N	Plasma copper (μg/dl)	PPD oxidase (units[a]/dl)	Cp protein (mg/dl)	PPD oxidase/ Cp protein
Controls	9	88 ± 22	201 ± 42	28 ± 8	6.7 ± 1.0
Standard pool	1	85	—[b]	26	7.7
Lactating women	12	146 ± 23[c]	285 ± 52[d]	53 ± 8[c]	5.1 ± 0.8[d]

[a] Units are arbitrarily defined as $\Delta Abs_{540\ nm}$/15 min.
[b] Could not be accurately determined.
[c] $P < 0.001$ (Student's t test) compared to controls.
[d] $P < 0.01$ (Student's t test) compared to controls.

change in $Abs_{540\ nm}$/15 min. Plasma immunoreactive levels of Cp and other acute phase proteins were measured by radial immunodiffusion (RID). RID plates containing antibodies to one of each protein of interest were obtained from Calbiochem–Behring Diagnostics. Standards for the proteins were purchased from Atlantic Antibodies, Inc., and used in the concentrated form along with several dilutions. Exactly 5 μl of the plasma sample or standard was placed in each RID plate well. Precipitin rings were measured at least 48 hr later.

Results. Table I shows that as in a previous study (5), lactating women at 1 month postpartum displayed elevated plasma copper and Cp oxidase activity levels. The mean plasma copper value for the 12 nursing mothers was 66% higher than the mean for the 9 controls. However, the mean control reading obtained here as well as the reading for the standard plasma pool fell into the lower end of what is considered the normal range (13). PPD oxidase from the control subjects could not be compared to that for the standard pool since the samples were stored differently. In this laboratory, standard plasma, reconstituted from the lyophylized state, gave a PPD oxidase reading of 120 units/dl as compared to an average of 201 for freshly frozen plasma from the control subjects.

Immunoreactive Cp protein levels were increased in the lactating women by an average of 89% over the mean for controls (Table I). The mean control value was very close to that found for the standard plasma pool. The average percentage increase in Cp protein for lactating females exceeded that for PPD oxidase activity (42%) and plasma copper (66%). In addition, each individual sample from the nursing mothers displayed a greater percentage increase in Cp protein than in PPD activity. As a result, the amount of oxidase activity per milligram of Cp protein was significantly lower in the lactating mothers than in the controls (Table I). The percentage increase in Cp protein also exceeded that for plasma copper in nearly all of the lactating females. The ratio of plasma copper to Cp protein was also lower in lactating mothers than in controls (2.7 ± 0.4 versus 3.0 ± 0.6), but the difference was not statistically significant. However, this ratio reflects total plasma copper, not just Cp-bound copper. A pair of lactating mothers took daily supplements containing 2.5 mg of copper. The various measurements for these two subjects were close to the means for the lactating women.

TABLE II. ACUTE PHASE PROTEINS IN PLASMA FROM LACTATING WOMEN, 1 MONTH POSTPARTUM

Sample group	N	Haptoglobin (mg/dl)	α_1-Antitrypsin (mg/dl)	C-Reactive protein (mg/dl)
Standard pool	1	100	184	<0.5
Controls	9	106 ± 25	227 ± 25	<0.5
Lactating	12	191 ± 48[a]	228 ± 23	<0.5

Note. Values for controls and lactating women represent the means ± SD.
[a] $P < 0.001$ (Student's t test) compared to controls.

The response of three non-Cp acute phase proteins varied (Table II). Plasma α_1-antitrypsin concentrations were similar in controls and nursing mothers. On the other hand, haptoglobin levels in the lactation group showed a higher mean value than did the controls. However, two individuals in the lactation group did not show elevated plasma haptoglobin content. The control mean measurement for the haptoglobin closely matched that found for the standard plasma pool. Plasma C-reactive protein content was undetectable in virtually all plasma samples examined. In contrast, the standards produced easily measurable precipitin rings.

Plasma levels of α_2-macroglobulin responded to lactation in a variable manner (Table III). This protein is considered an acute phase protein in rats though not in humans (10). In the present study eight lactating subjects had plasma α_2-macroglobulin contents which exceeded the highest value obtained for nine controls. However, four lactating females displayed α_2-macroglobulin levels in the lower half of the control range. Interestingly, these four subjects also showed four of the five lowest increases in immunoreactive Cp levels. In general, lactating mothers showed a significant correlation between plasma Cp and α_2-macroglobulin protein concentrations ($p = 0.708$, $p = 0.01$) (Fig. 1) while controls did not.

Discussion. Percentage increases in plasma copper might be expected to match those for Cp protein and oxidase activity since Cp contains most of the copper in plasma (8). However, this was clearly not the case for lactation (Table I). The percentage increase in Cp protein exceeded that for plasma copper in most of the lactating women. This pattern occurred even though the control mean value obtained here for comparison purposes fell into the lower end of what is considered the normal range (13). The mean percentage increase in Cp protein (89%) also exceeded that for Cp oxidase activity (42%). This pattern held for each individual nursing mother with the mean oxidase value per milligram of Cp protein significantly lower than the mean control value (Table I). Cp oxidase activity depends on the presence of copper within the Cp protein (9). Thus, a decrease in Cp oxidase activity per milligram of Cp protein implies the presence of apoCp not fully saturated with copper. Such accumulation of this apoprotein would require its generation at a fairly rapid rate since apoCp is presumed to be degraded faster than normal Cp (14). Possibly, lactation accelerates both the rate of Cp protein synthesis and the rate of copper release from Cp. The latter could result from copper incorporation into milk. Interestingly, for the time points studied so far plasma copper and milk copper both reach highest levels at about the same time (5, 15). Cancer, a very different physiological situation than lactation, also produces elevations in plasma Cp protein and oxidase activity. However, the percentage increases in Cp protein do not consistently exceed those for the Cp oxidase (2) (R. A. DiSilvestro and E. D. David, unpublished results). Hence, the regulation and function of increased plasma Cp may be different for cancer than for lactation.

Lactation also seems to raise Cp levels via mechanisms different from those occurring during pregnancy, though some of the increase could simply represent an incomplete reversal of pregnancy effects (6, 7). However, pregnancy-induced increases in Cp are considered to result primarily from estrogen effects (16). Acute phase proteins other than Cp respond differently to these two states. Plasma levels of haptoglobin and α_2-macroglobulin are normal at the end of pregnancy (10) but were elevated in most of the lactating females studied here (Tables II and III). In contrast, α_1-antitrypsin concentrations are twice normal at the end of pregnancy but had returned to normal by 1 month postpartum in the lactating women (Table II). These results strengthen the hypothesis that acute phase protein levels are

TABLE III. α_2-MACROGLOBULIN LEVELS IN LACTATING WOMEN, 1 MONTH POSTPARTUM

Sample group	N	α_2-Macroglobulin (mg/dl) Mean ± SD	Range
Standard pool	1	173	—
Controls	9	175 ± 13	150–190
Lactating women			
All	12	219 ± 52[a]	151–297
Those > 190 mg/dl	8	248 ± 38[b]	199–297

[a] $P < 0.05$ (Student's t test) compared to controls.
[b] $P < 0.001$ (Student's t test) compared to controls.

regulated through both overlapping and individualized mediation processes. This notion is further supported by the behavior of C-reactive protein levels. Plasma concentrations of this protein, as of Cp, rise during inflammation (17). However, neither lactation (Table II) nor pregnancy (1) increases C-reactive protein levels.

Many of the increases in Cp and other acute phase protein levels have been attributed to the direct or indirect actions of monokine hormones such as interleukin 1 and hepatocyte-stimulating factor (18, 19). Interleukin 1 has recently been shown to alter gene expression of some acute phase proteins in human hepatoma cells (20). The variety of responses of acute phase proteins to pregnancy and lactation suggests that plasma levels of these proteins can also be elevated through monokine-independent processes. Alternatively, these monokines could represent a family of structurally similar proteins with some differences in secretion rate controls and regulatory actions.

The increase in α_2-macroglobulin levels in 8 of 12 lactating women was surprising since this protein is considered an acute phase protein in rats but not in people (10). In the present study α_2-macroglobulin levels correlated with those of Cp protein in the lactating females but not in control subjects. The 4 lactating women showing normal α_2-macroglobulin concentrations did show increased Cp protein levels but the percent increases were among the lowest found for the nursing mothers. Possibly, lactation elevates plasma Cp through more than one mediation process. α_2-Macroglobulin levels would be influenced by one of the processes which would occur to varying extents in different lactating women. Possibly, one of these processes does not result from lactation but rather just from the recovery from pregnancy which to some extent temporarily prevents the return of copper metabolism to normal (6, 7).

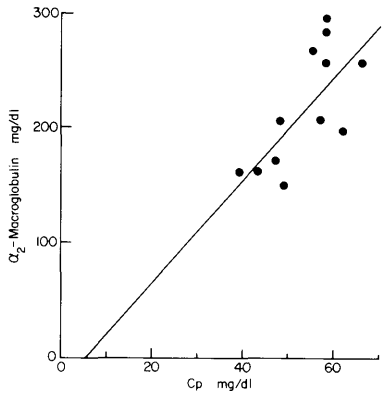

FIG. 1. Correlation of α_2-macroglobulin levels with ceruloplasmin levels in lactating women ($r = 0.708$, $P < 0.01$).

This work was supported by funds from the Showalter Endowment. The author thanks Mary Frahm, Renee Massad, and Lynn Yoder for their excellent technical assistance. The author also thanks Isobel Miller, Mihirra Kara, and Dr. Avanelle Kirksey for providing the plasma from the lactating mothers.

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Received April 8, 1986. P.S.E.B.M. 1986, Vol. 183.
Accepted July 24, 1986.