Ferritin, Iron and Total Iron-Binding Capacity of the Serum from Holstein Young Steers in Prolonged Undernutrition

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ABSTRACT. Ferritin, iron and total iron-binding capacity (TIBC) of the serum from young steers were estimated during a prolonged period of caloric undernutrition and a subsequent recovering period. Holstein young steers of 3 months of age ($n=8$) received 42% ration for 5 months and 136% ration for 4 months on the basis of TDN for 0.7 kg daily weight gain. The control animals ($n=8$) received 100% ration for 9 months. The prolonged caloric restriction caused a mild normocytic and normochromic anemia without a decrease in total serum protein nor albumin. The anemia thus induced was eliminated during the recovering period. TIBC decreased gradually with the progress of the malnutritional period and was improved quickly by the increase in rations. A decrease in serum iron concentration was observed only at the end of the undernutritional period. Serum ferritin started to rise shortly after the beginning of the undernutritional condition with the highest level at the end of that period, and returned to the control level in 3 months of the recovering period. The magnitude of changes in serum ferritin was exceedingly larger than that in TIBC. Serum ferritin seems to be a sensitive indicator for the malnutritional status of steers.—**KEY WORDS:** cattle, serum ferritin, total iron-binding capacity, undernutrition.

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Concentrations of serum ferritin and transferrin have been proven to correlate with the iron status of animals and particularly with body iron stores [4, 11, 20, 24, 25]. Our previous papers [8, 9, 14–16] have shown that serum ferritin level is an indicator to evaluate the iron status in cattle and pigs.

It has been reported that serum iron and total iron-binding capacity (TIBC) of the serum of pigs progressively decrease during a long term feeding with protein-deprived or -deficient ration [3, 10]. Author has reported that serum transferrin concentration in pigs decreased after 2 week fasting [5]. Accordingly, serum transferrin level may not be only a good index of the iron status but also the nutritional status in animals. Serum ferritin level has shown to increase greatly in kwashiorkor children, with an inverse relation to serum transferrin [13], but there are few report on serum ferritin concentration in malnourished animals. Similarly, there are

many data in pigs on anemia due to malcaloric or malprotein condition [2, 22, 23], but few reports are available for cattle.

In this study, the changes of serum ferritin, TIBC and iron was determined in young steers during a period of prolonged caloric undernutrition and subsequent recovery period.

MATERIALS AND METHODS

Sixteen Holstein steers of 3 months of age averaging 100 kg body weight were divided equally into the undernourished and the control groups on the basis of body weight. Each of them was placed in an individual tie stall with a gum matted flooring. The undernourished animals ($n=8$) received for 5 months 42% of total digestible nutrient (TDN) required for 0.7 kg daily weight gain according to the U.S. National Research Council feeding standard [17]. The control animals ($n=8$) were fed for 5 months with 100% of TDN. In

order to see the growth of organs and tissues, 4 animals from each group were killed at 5 months after the start of the experiment. Then, the remaining 4 undernourished steers received 136% of TDN for 4 months (a recovery period). The 4 control animals continued to be kept on 100% of TDN for 4 months. The digestible coarse protein fed to the animals during the undernourished and the recovering periods were 44% and 140% of that to control animals. A commercial pelleted concentrate and orchard grass hay were fed to meet their dietary level of energy, protein, minerals and vitamins. The TDN ratio of concentrate and roughage was 1:3 on dry matter basis. Block salt and water were provided *ad libitum*. Iron contents were 165 ppm for concentrate, 210 ppm for orchard hay and 400 ppm for block salt. Further detail were published elsewhere [12].

Clinical observations and blood samplings were done at $\frac{1}{2}$ –1 month intervals. Blood samplings were done by jugular puncture and samples were collected into heparinized and nonheparinized tubes. Red blood cells (RBC) were counted by a TOA microcell counter (Model CC-108). Hemoglobin (Hb) and hematocrit (Ht) were determined as reported previously [8]. Serum samples separated were frozen at -20°C for later analyses for the followings: ferritin, TIBC, iron (SI), total protein (TP) and albumin (Alb). TIBC was determined colorimetrically according to the ICSH (International Committee for Standardization in Hematology) tentative magnesium carbonate method [1]. SI was determined colorimetrically according to the ICSH panel method [1] with athophenanthroline as the color reagent. Unsaturated iron-binding capacity (UIBC) was derived from SI and TIBC measurements. TP and Alb were determined as reported previously [8].

Serum ferritin concentration was determined by a two-site immunoradiometric assay as reported previously [8]: Bovine ferritin was prepared from the spleen. The antisera a-

gainst purified bovine spleen ferritin were obtained from rabbits received repeated subcutaneous injections of the purified ferritin emulsified in complete Freund's adjuvant. The IgG fraction from the rabbit antisera was separated by fractionation with Na_2SO_4 , followed by affinity chromatography on a liver ferritin-coupled CNBr-activated Sepharose 4B column. Iodination of the purified anti-ferritin antibody was performed by the method of Niitsu *et al.* [18]. A standard curve was obtained in ferritin concentration from 0.1 to 500 ng/ml. All samples were analyzed at least in triplicate.

RESULTS

Body weight changes during the periods of caloric undernutrition and recovery are presented in Fig. 1. The undernourished animals maintained around 95% of the initial body weight during the 5-month period of undernutrition. Notable clinical signs of malnutrition (Wrinkled skin, partial loss of hair, dermatitis and protrusion of anus) were gradually developed. During the 4 months of the recovery period, compensatory growth at a rate of 1.15 kg per day occurred. The average daily weight gain in the control animals was 0.68 kg/day. An increased intake of block salt was observed in the undernourished animals.

Changes in the hematological measures are shown in Figs. 1 and 2. One to 3 months after the start of the undernutritional condition, RBC counts, Ht and Hb elevated to significantly higher ($P < 0.01$) levels than those in the control group, but those levels fell to significantly lower ($P < 0.01$) levels at 5-month period of the undernutrition. Levels of RBC counts, Ht and Hb in the undernourished group reached to those in the control animals at 3 months after the recovering treatment. They were significantly lower ($P < 0.05$) in undernourished than control groups from 1 to 3-month period of recovery. RBC counts, Ht and Hb concentrations in the

Fig. 1. Effects of prolonged undernutrition on body weight, red blood cell counts (RBC), hemoglobin (Hb), hematocrit (Ht), serum total protein (TP) and albumin (Alb) of young steers. After the period of undernourishment for 5 months, animals in this group were fed for 4 months (the recovery period). Mean values of 8 animals by 5th month or 4 animals after 5th month are presented.

Fig. 2. Effects of prolonged undernutrition on mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) of red cells of young steers. After the period of undernourishment for 5 months, animals in this group were fed for 4 months (the recovery period). Mean values of 8 animals by 5th month or 4 animals after 5th month are presented.

Fig. 3. Effects of prolonged undernutrition on serum iron (SI), total iron-binding capacity (TIBC) and unsaturated iron-binding capacity (UIBC) of young steers. After the period of undernourishment for 5 months, animals in this group were fed for 4 months (the recovery period). Mean $\pm$ standard error of 8 animals by 5th month or 4 animals after 5th month is presented.

Fig. 4. Effects of prolonged undernutrition on serum ferritin concentration of young steers. After the period of undernourishment for 5 months, animals in this group were fed for 4 months (the recovery period). Mean $\pm$ standard error of 8 animals by 5th month or 4 animals after 5th month is presented.

control animals remained at almost constant levels during 9 months of the experimental period. Any reduction of TP and Alb or any sign of edema was not found during the caloric undernutritional period. However, during the recovering period from 1/2 to 2 months, Alb was significantly lower ($P < 0.05$). The malnutrition did not give any

remarkable effect on the mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC) and the mean corpuscular hemoglobin (MCH) as shown in Fig. 2.

Changes in SI, TIBC and UIBC values are shown in Fig. 3. SI was markedly reduced from the 4th to 5th month of the undernutri-

tional period, but progressively increased during the following recovery period. TIBC and UIBC were gradually decreased; their values from the 2nd to 5th month of the undernutritional period were significantly lower ($P < 0.01$) than those in the control animals. The lowest TIBC value, less than half of the initial value, was found at the 5th month of the undernutritional state. With half a month delay TIBC and UIBC increased sharply and attained to the level of the control animals at the 3rd month of recovering period.

Changes in serum ferritin during the periods of undernutrition and recovery are presented in Fig. 4. Serum ferritin levels rose rapidly soon after the onset of restricted caloric intake (9-fold increase in 5 months). During the subsequent 4 months of recovering period, serum ferritin concentration decreased progressively to the level of control animals. Serum ferritin concentration in the control animals almost unchanged throughout the period of 9 months.

DISCUSSION

The caloric undernutritional condition for 5 month resulted in a mild nutritional anemia in young steers after showing hemoconcentration during the initial 3 months. This normochromic and normocytic anemia was eliminated with a progress of compensatory body growth during the recovery period. The characteristic of this anemia is almost in agreement with that of pigs in undercaloric conditions [22, 23]. Meanwhile, a low protein diet to miniature pigs has been shown to cause microcytic and normochromic anemia [2].

It is well known that serum protein concentration varies to some extent with the change in nutrient status. In particular, lowered serum protein concentration has been reported for pigs fed with low protein or protein free diet [19]. The present result in steers indicates that TP and Alb levels in the serum were poorly indicating the malnutritional condition

of animals as compared with RBC counts, Ht and Hb. Alb decreased to a lower level only during the recovery period without an incidence of edema. At that time, blood glucose was depressed, whereas blood urea was greatly elevated [12]. These findings were in agreement with those in calorie-deprived pigs [22].

Since TIBC decreased linearly with the progress of malnutritional condition and that increased with the recovery of caloric intake, it seems likely that circulating transferrin is strongly influenced by the nutritional status of animals. This finding in steers is consistent with that in pigs fed with rations deprived of protein [10], but conflicting with the fact of calorie-deprived pigs [22].

UIBC decreased almost linearly in parallel with TIBC. Since UIBC plays an important part to prevent infections from bacteria by its capacity to bind iron [7], its decrease would enhance the danger of infections in undernourished animals. SI level was not always correlated to TIBC of the serum. It decreased only after a long duration of undernutrition. In contrast of TIBC, an elevation of serum iron occurred soon after the recovery of caloric intake. This may be due to an immediate increase in the absorption of iron from the intestine.

Serum ferritin levels of control animals are almost in agreement with those of calves, heifer and young bulls [14–16]. Serum ferritin concentration increased linearly after the initiation of the undernutritional condition and reached an extraordinarily high level in 5 months. A high animal-to-animal variability developed with the progress of the undernutritional period. This coincides with the result by McFarlane [13] on kwashiorkor children. Generally, a consistent inverse relationship between serum ferritin and transferrin was observed in iron deficient, overloading or various disease states [1]. In this study, also, a clear inverse relationship between serum ferritin and transferrin level was observed in

steers. In the undernutrition period, the elevation of serum ferritin preceded the decrease in TIBC. Furthermore, in the recovery period, serum ferritin decreased more sharply than TIBC. Thus, serum ferritin is one of the most rapidly responding protein to a malnutritional condition.

Although lowered level of serum Alb in malnutritional condition is considered due to a decreased synthesis of Alb in the liver [21], the reason why serum ferritin and transferrin behave in relation to undernutritional condition of animals is not unequivocally settled at present. Previous papers on pigs has shown that an excess in red blood cells due to fasting caused a marked diminution in erythropoiesis and that this depressed erythropoiesis in turn restricts the amount of iron incorporated into red cells, resulting in an increase of the iron storage in the liver and the spleen [6]. It has also been reported that iron accumulation occurred in the liver of the protein starved pigs [10]. It is considered that the increase in iron storage is one of the reason for elevated serum ferritin and reduced TIBC levels in the condition of caloric malnutrition. In addition, elevated ingestion of block salt during the undernutritional period in this study might enhance the iron deposit.

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要 約

低栄養時におけるホルスタインおす子牛血清のフェリチン、鉄および鉄結合能の変動: 古郡 浩 (北海道農業試験場)——長期にわたる低栄養とその後の栄養回復が血清フェリチン、鉄結合能および血清鉄に及ぼす影響を明らかにする目的で、3カ月齢の乳用おす子牛16頭を低栄養区と対照区に分け、つぎの実験をおこなった。低栄養区では、対照区の日増体量 0.7 kg の TDN 要求量の42%を5カ月間給与し、体重を実験開始時の約95%に維持したところ、末期には正球性、正色素性の栄養性貧血を発症した。ついで4カ月間にわたり TDN 要求量の136%を給与て、栄養回復をはかったところ、代償成長の発現後に栄養性貧血は消失した。低栄養時に、血清の蛋白質量とアルブミン量は低下しなかった。鉄結合能と不飽和鉄結合能は、低栄養が進行すると有意に低下したが、栄養回復とともに急速に上昇した。血清鉄の変動は鉄結合能のそれとは必ずしも平行しなかったが、低栄養末期には、有意な低下が認められた。血清フェリチン量は低栄養開始後、急速に上昇し、低栄養末期には最高値を示したが、栄養回復とともに直線的に低下した。血清フェリチン量と鉄結合能との間には、常に相反する関係が認められたが、低栄養時には血清フェリチンの上昇は鉄結合能よりも先行し、栄養回復期には急速に低下した。