Effect of Laparotomy and/or Starvation on the Plasma Rapid-turnover Protein Level in Rats

Takeshi Matsumoto,* Yasuo Iwasawa, Hideaki Shima, Hiroki Shirai, and Tetsuya Kishi

Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Company Ltd., 16–89 Kashima 3-chome, Yodogawa-ku, Osaka 532, Japan

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Plasma transferrin (Tf), transthyretin (TTR), and retinol-binding protein (RBP) have been proposed to be sensitive nutritional parameters. We investigated the effect of laparotomy and/or starvation on these plasma protein levels in rats. Starvation decreased the Tf, TTR, and RBP levels to 62.2%, 41.2%, and 52.0% of the initial values, respectively, until day 3, but then sustained the day-3 levels on day 4. In the starved and laparotomized rats, the TTR level on day 1 and the RBP level on day 2 were more decreased than those in the only-laparotomized rats. Recovery of the TTR and RBP levels in the laparotomized rats fed on a stock diet ad libitum was very slow. In conclusion, the plasma Tf, TTR, and RBP levels showed different responses according to the metabolic phase of starvation. Laparotomy affected differently each of these levels, but the decrease in these protein levels was mainly dependent on the nutritional condition of the laparotomized rats.

Nutritional therapy under the acute-phase response induced by trauma, surgical injury, and neoplastic diseases is important because the recovery from diseases depends on the nutritional condition. The metabolism of patients under the acute-phase response is altered by several mediators such as interleukin-1 and interleukin-6 released from many types of activated cells.1 Interleukin-1 induces fever, anorexia, and hyperlipidemia.2 In the liver, synthesis of positive acute-phase proteins is also induced by these mediators.3

The effect of nutritional therapy is usually quantified by a serial assessment of the body weight, nitrogen balance, plasma amino acid levels, various plasma protein levels, etc.4,5 The plasma albumin level can be easily measured, and is usually used as a parameter of protein nutrition, but the level is not sensitive enough because of its long half-life and the large extravascular pool.5 Albumin synthesis in liver has been reported to be suppressed by mediators such as interleukin-6.6,7

Plasma rapid-turnover proteins (RTPs), which are transferrin (Tf), transthyretin (TTR), and retinol-binding protein (RBP), have recently been proposed to be more sensitive parameters for estimating the status of protein nutrition than plasma albumin and total protein.7–9 However, plasma RTP levels are also affected by the acute-phase response,1,3 and it is not clear whether plasma RTP levels under the acute-phase response are useful for nutritional assessment or not. Therefore, the responses of plasma RTP levels under various stimulus-induced acute-phase responses should be characterized to use these proteins as nutritional parameters.

Animal experiments are useful for evaluating RTPs as a nutritional parameter, because the experimental conditions can be well controlled. We have previously reported the sandwich enzyme immunoassay for rat Tf,10 TTR,11 and RBP,12 and the daily changes of these protein levels under total parenteral nutrition.13

In the present paper, we describe the effect of starvation and the acute-phase response induced by laparotomy on the plasma levels of RTPs in rats.

Materials and Methods

Animals. Male rats of the Wistar strain weighing about 260 g, which had free access to a stock diet (CE-2, CLEA Japan, Tokyo, Japan) and water, were used in the experiment. They were individually housed in wire cages in an air-conditioned room (24 ± 1°C) with a 12-h light-dark cycle. The rats were divided into four groups: 1) starved (group S), 2) laparotomized (group L), 3) starved and laparotomized (group SL), and 4) initial group.

Under sodium pentobarbital anesthesia (40 mg/kg, i.p.), the rats in groups L and SL underwent laparotomy. The rats in group L had free access to a stock diet and water, and those in group SL were deprived of the diet after laparotomy. The rats in group S had been deprived of the diet and had free access only to water. The rats in initial group were used without any treatment.

These groups of rats were killed for blood sampling at 0 h and 6 h (day 0), and on days 1, 2, 3, and 4 following the treatments. The rats were killed during the period from 9:00 a.m. to 9:30 a.m. on all days except on day 0 (during the period from 6:30 p.m. to 7:00 p.m.).

Plasma proteins. Blood was withdrawn from the inferior vena cava with a heparinized syringe under sodium pentobarbital anesthesia (40 g/kg, i.p.). Plasma was obtained from the blood by centrifugation (1000 × g for 10 min). The albumin level in the plasma was measured by the method described previously,14 and the Tf, TTR,11 and RBP12 levels in the plasma were measured by the sandwich enzyme immunoassays described previously.

Hematocrit and plasma glucose. Hematocrit was measured by the micro-method, using a capillary tube for microhematocrit (Drummond Scientific Co., PA, U.S.A.). Glucose in the plasma was measured by the method described previously.14

Urinary nitrogen. The urine from each rat was collected daily (for 24 h) during the experimental period, except for day 0, when the urine from

* To whom correspondence should be addressed.

Abbreviations: Glc, glucose; RBP, retinol-binding protein; RTP, rapid-turnover protein; Tf, transferrin; TTR, transthyretin.
each rat was collected for 6 h after surgical treatment. These urine samples were analyzed for total nitrogen by a fully automatic nitrogen and carbon analyzer (SUMIGRAPH NC-800, Sumitomo Chemical Co., Osaka, Japan).

Statistical analysis. The significance of the difference between the means was assessed by an analysis of variance (one-way ANOVA), \( p < 0.05 \) being considered to be significant.

Results

Body weight

Daily changes in body weight are shown in Fig. 1. The body weight of the group S rats gradually decreased during the experimental period. On day 4, the body weight of the group S rats was 77.9% of the initial weight. The change for the group SL rats was no different from that of the group S animals during the experimental period. The body weight of the group L rats, which were fed on a stock diet ad libitum, did not change until day 2, after which the body weight increased and was significantly higher than that of the initial group on day 4.

Daily changes of hematocrit and plasma glucose

Daily changes of hematocrit and plasma glucose are shown in Table I.

The values of hematocrit on days 3 and 4 for group S were significantly higher than those for the initial group. On the other hand, hematocrit for group SL slightly increased, but did not significantly change. Hematocrit for group L did not significantly change either.

The plasma glucose levels on days 1, 2, and 3 for group S were significantly decreased. On the contrary, the levels for group SL were significantly lower than those for the initial group only on day 2.

Nitrogen excreted into urine

The nitrogen excreted into urine from the starved rats with or without laparotomy (groups S and SL) is shown in Table II.

The mean excretion per hour into urine for group S was at a maximum level on day 0, and then gradually decreased. Laparotomy on the starved rats (group SL) significantly increased the nitrogen excretion only on day 0 (for 6 h after laparotomy).

Plasma level of albumin

Daily changes of the plasma albumin level are shown in Fig. 2. The albumin level was not significantly changed by

Table I. Daily Changes of Hematocrit and Plasma Glucose

<table>
<thead>
<tr>
<th></th>
<th>Hematocrit (%)</th>
<th>Plasma glucose (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>41.3 ± 0.4</td>
<td>182 ± 5</td>
</tr>
<tr>
<td>Day 0 S</td>
<td>42.7 ± 0.6</td>
<td>194 ± 5</td>
</tr>
<tr>
<td>L</td>
<td>41.7 ± 0.4</td>
<td>179 ± 2</td>
</tr>
<tr>
<td>SL</td>
<td>41.7 ± 1.1</td>
<td>190 ± 4</td>
</tr>
<tr>
<td>Day 1 S</td>
<td>41.5 ± 0.8</td>
<td>93 ± 4*</td>
</tr>
<tr>
<td>L</td>
<td>39.6 ± 0.6</td>
<td>155 ± 6</td>
</tr>
<tr>
<td>SL</td>
<td>40.8 ± 0.6</td>
<td>111 ± 8</td>
</tr>
<tr>
<td>Day 2 S</td>
<td>44.4 ± 0.3</td>
<td>98 ± 9*</td>
</tr>
<tr>
<td>L</td>
<td>38.1 ± 1.0</td>
<td>160 ± 3</td>
</tr>
<tr>
<td>SL</td>
<td>41.8 ± 0.7</td>
<td>92 ± 10*</td>
</tr>
<tr>
<td>Day 3 S</td>
<td>47.0 ± 0.4*</td>
<td>97 ± 8*</td>
</tr>
<tr>
<td>L</td>
<td>38.4 ± 0.6</td>
<td>153 ± 2</td>
</tr>
<tr>
<td>SL</td>
<td>44.8 ± 1.0</td>
<td>105 ± 9</td>
</tr>
<tr>
<td>Day 4 S</td>
<td>47.4 ± 0.4*</td>
<td>113 ± 8</td>
</tr>
<tr>
<td>L</td>
<td>37.9 ± 0.8</td>
<td>195 ± 46</td>
</tr>
<tr>
<td>SL</td>
<td>45.8 ± 1.5</td>
<td>101 ± 4</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM: S, for starved rats; L, for laparotomized rats; SL, for starved and laparotomized rats.

The numbers of rats in each group are indicated in Fig. 1.

* Significantly different from the value for the initial group, \( p < 0.05 \).

Table II. Nitrogen Excretion into Urine from the Starved Rats with or without Laparotomy

<table>
<thead>
<tr>
<th>Laparotomy</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without</td>
<td>9.0 ± 0.5</td>
<td>6.4 ± 0.3</td>
<td>6.0 ± 0.3</td>
<td>5.5 ± 0.3</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>With</td>
<td>12.0 ± 1.2</td>
<td>7.3 ± 0.5</td>
<td>6.1 ± 0.8</td>
<td>5.6 ± 0.5</td>
<td>5.0 ± 0.2</td>
</tr>
</tbody>
</table>

The value is shown as the mean nitrogen excretion rate for 24 h, except for day 0 (6 h). Each value represents the mean ± SEM \( (n = 5) \).

* Significantly different from the value for the starved rats without laparotomy, \( p < 0.05 \).

Fig. 2. Daily Change in Plasma Albumin Level for the Starved and/or Laparotomized Rats during the Experimental Period.

Values are the mean ± SEM: open circles, for group S; closed circles, for group SL; closed squares, for group L. The numbers of rats in each group are the same as those shown in Fig. 1.

* Significantly different from the value for the initial group: \( p < 0.05 \); \( * p < 0.01 \).

Significantly different from the value for group L: \( p < 0.05 \); \( * p < 0.01 \).
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Fig. 3. Daily Change in Plasma Transferrin Level for the Starved and/or Laparotomized Rats.
Values are the mean ± SEM: open circles, for group S; closed circles, for group SL; closed squares, for group L. The numbers of rats in each group are the same as those shown in Fig. 1.
Significantly different from the value for the initial group: *p < 0.05; **p < 0.01.
Significantly different from the value for group L: *p < 0.05; **p < 0.01.

Fig. 4. Daily Change in Plasma Transthyretin Level for the Starved and/or Laparotomized Rats.
Values are the mean ± SEM: open circles, for group S; closed circles, for group SL; closed squares, for group L. The numbers of rats in each group are the same as those shown in Fig. 1.
Significantly different from the value for the initial group: *p < 0.05; **p < 0.01.
Significantly different from the value for group L: *p < 0.05; **p < 0.01.

starvation during the experimental period. On the contrary, the levels for group SL were significantly lower than those for the initial group during the experimental period. Moreover, the levels for both groups SL and L on day 2 were significantly lower than those for group S.

Plasma levels of RTPs

Plasma Tf levels are shown in Fig. 3. The level for group S was significantly decreased to 62.2% of the initial group level on day 3, while the level on day 3 was sustained to day 4. The Tf levels for group SL changed similarly to those for group S. The levels for both groups S and SL were significantly lower than those for the initial group on and after day 1, while the level for group L hardly changed until day 2, after which it rapidly increased.

Plasma TTR levels are shown in Fig. 4. The level for group S decreased to 41.2% of the initial group level on day 3, this level then being sustained on day 4. The level for group SL on day 1 was significantly lower than that for group L, while the levels for both groups S and SL were significantly lower than those for group L on and after day 3. For group L, the TTR level slightly but significantly decreased until day 2, the level on day 4 still being lower than that for the initial group.

Plasma RBP levels are shown in Fig. 5. The level for group S decreased to 52.0% of the initial group level on day 3. Similar to the levels of Tf and TTR, the RBP level on day 3 was also sustained to day 4. The level for group SL on day 2 was significantly lower than that for group L, while the levels for groups S and SL were significantly lower than those for group L on day 3. Recovery of the RBP level for group L was also slow.

Discussion

Effect of starvation on plasma rapid-turnover proteins

Three phases of starvation have been characterized by changes in the protein and lipid metabolism of laboratory rats. Phase 1 is a period of adaptation marked by a reduction in net protein breakdown. Goodman et al. have shown that the protein and RNA contents in liver and heart were gradually decreased by starvation for a few days (phase 1). Phase 2 is a long period of economy, during which proteins are efficiently spared, and most of the energy is derived from lipid oxidation. During phase 3, net protein breakdown increases. Cherel et al. have characterized the three phases of starvation by changes in the specific daily loss in body weight and the energy metabolism of rats, and showed that the duration of phase 1 was 2 days and that of phase 2 was 6 days. Under our conditions, the decrease (22.1%) in body weight of the rats starved for 4 days is similar to that (23.9%) for the rats under phase 2, which was reported by Cherel et al. (Fig. 1). The plasma glucose level in the starved rats significantly decreased until day 3. The level on day 4 was higher than that on days 1, 2, and 3, which may have resulted from the change in energy metabolism (Table 1). These results show that our experimental period included phase 1 (from day 0 to day 3) and phase 2 (on day 4).

Under these conditions, the plasma albumin level was not significantly changed by starvation (Fig. 2). On the other hand, the plasma RTP level was decreased by starvation.
until day 3, but subsequently, the level did not change (Fig. 6). These results indicate that the RTP level decreased in phase 1 (adaptation phase), but hardly changed in phase 2 (period of economy).

The half-life (6.5 h) of rat RBP is shorter than that (10.6 h) of TTR (18). However, the change in RBP level by starvation was less than that of TTR (Fig. 6). RBP synthesis has been reported to be up-regulated by glucocorticoid hormones or glucagon, while the syntheses of TTR and albumin are slightly up-regulated or not at all.12,20,21 It has also been reported that the glucocorticoid level in blood increased by starvation22 and that the hormone plays an important role in regulation energy metabolism.23 These results show that the lower sensitivity of plasma RBP to starvation than that of plasma TTR may have been due to the induction of synthesis by hormones in the liver.

Effect of the acute-phase response on plasma rapid-turnover proteins

The acute-phase response affects the nitrogen balance and levels of many plasma proteins, including α2-macroglobulin and fibrinogen.30 The synthesis of albumin in the liver is strongly suppressed by interleukin-1, interleukin-6, and TNF-α, which are released from activated cells under the acute-phase response.1)

It is known that various types of tissue damage such as that from surgery, burns, and infection markedly increase body protein loss, and that the metabolic response can be characterized by the increased urinary excretion of nitrogen and by changes in the plasma protein levels.24) In the present study, laparotomy significantly increased the nitrogen excretion into the urine of starved rats on day 0 (for 6 h after laparotomy) (Table II). The plasma albumin level was transiently affected by the laparotomy-induced acute-phase response, whether the laparotomized rats were fed on a stock diet or not (Fig. 2). We have previously reported that the albumin level in a surgically injured rat infused with a saline-solution transiently decreased similarly to the present result, but that the level in the rats infused with a solution (total parenteral nutrition solution, TPN solution) containing glucose and amino acids did not change.13 These results indicate that the plasma albumin level may be sensitive to the acute-phase response under malnutrition.

Under the acute-phase response, Tf synthesis in the human hepatocyte is suppressed by such mediators as interleukin-6.31) On the contrary, the Tf level in serum and its mRNA level in liver were increased by the turpentine-induced acute-phase response in rats.3) We have reported that the plasma Tf level in injured rats immediately increased under parenteral nutrition.13) In the present study, when the laparotomized rats were fed on a stock diet, the plasma Tf level did not significantly change until day 2, after which it increased (Fig. 3). The change in Tf level for the group with starvation and laparotomy was similar to that for the group that was only starved (Fig. 3). These results indicate that the effect of laparotomy on the plasma Tf level was a little.

The rat TTR level in serum and its mRNA level in liver are rapidly decreased by a turpentine treatment, and remain at a low level.3) In the present study, the TTR level was significantly decreased by starvation and by laparotomy. The effect of starvation on the laparotomized rat was only apparent for the TTR level on day 1 (Fig. 4). Smale et al.25) have reported that the TTR level in macaques was not altered by 7 days of starvation, and that the addition of surgical stress significantly decreased the TTR level. The cause of the difference in TTR response to starvation and surgical stress between the rat and macaque is not clear. The additional effect of starvation on the RBP level of the laparotomized rat was only observed on day 2. These results indicate that depression of the plasma TTR and RBP levels by laparotomy was weak, and that the response of TTR level was more rapid than that of RBP level under starvation.

The recovery of the TTR and RBP levels from the decreases induced by laparotomy was very slow in the laparotomized rats fed on a stock diet ad libitum, although the body weight and plasma Tf level had already increased (Figs. 1, 3, 4, and 5). Wade et al.26) also reported that the serum TTR level in 24-h starved rats remained low after 2 days of refeeding. On the other hand, we have reported that the plasma RTP level in injured rats under parenteral nutrition rapidly increased according to the nutritional conditions.13) These results indicate that the nutritional response of plasma TTR and RBP levels in injured rats under enteral nutrition may be different from that under parenteral nutrition.

In conclusion, the plasma levels of rapid-turnover proteins were decreased in normal and laparotomized rats by starvation. However, the recovery of the transthyretin and retinol-binding protein levels was slow in laparotomized rats fed on a stock diet ad libitum. To clarify the usefulness of these proteins as nutritional parameters, further investigations under several different conditions are needed.

References

6) T. Peters, Jr., in "The Protein Molecules," Vol. 1, ed. by F. W. Putnam,
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