Effect of Dietary Restriction on Liver Protein Synthesis in Rats

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ABSTRACT At 6 wk of age, male Fischer F344 rats were fed a purified, casein-based diet either ad libitum or in the amount of 60% of the diet consumed by the rats fed ad libitum (restricted diet). Hepatocytes were isolated from the rats between 2.5 and 19 mo of age. The protein content of the hepatocytes isolated from the rats fed the restricted amount of diet was significantly lower than that of hepatocytes isolated from rats fed ad libitum. The DNA and RNA content of the hepatocytes were similar for the rats fed the two dietary regimens. The absolute rate of protein synthesis for hepatocytes isolated from rats fed ad libitum decreased 55% between 2.5 and 19 mo of age. However, the rate of protein synthesis by hepatocytes from rats fed the restricted amount of diet decreased only slightly with increasing age. At 19 mo of age, the rate of protein synthesis by hepatocytes from the rats fed the restricted amount of diet was significantly higher than the rate of protein synthesis for hepatocytes from rats fed ad libitum. Therefore, dietary restriction retards the age-related decline in liver protein synthesis.

INDEXING KEY WORDS protein synthesis • dietary restriction • hepatocytes • aging

Since the classic experiments by McCay et al. (1) in 1935, several investigators have shown that restricting the amount of diet consumed by rodents significantly increases their survival; dietary restriction increases both the mean and maximum survival (for reviews see refs. 2–4). In addition, dietary restriction has been shown to delay or decrease the incidence of a variety of diseases (2, 5).

Although dietary restriction has been shown to increase the longevity of rodents, the molecular basis for its effect is unknown. Several investigators have suggested that dietary restriction exerts its effect at the level of gene expression. In 1973, Barrows (4) proposed that dietary restriction increased longevity by reducing the use of the genetic code through a reduction of protein synthesis. More recently, Lindell (6) suggested that dietary restriction acts as a physiological "stress" that enhances gene expression. The enhanced gene expression in turn would be a significant factor in maintaining the cellular homeostasis of an aging organism.

The research described herein focuses on the effect of dietary restriction on a specific step in gene expression, protein synthesis. Although the effect of dietary restriction on a variety of biochemical processes had been studied (2–4), this is the first study on gene expression. Using isolated hepatocytes, we found that dietary restriction significantly reduced the age-related decline in the rate of protein synthesis.

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MATERIALS AND METHODS

Animals and diets. Male SPF (specific pathogen-free) Fischer F344 weanling rats were obtained from Harlan Industries (Indianapolis, IN). The rats were caged individually in metal cages (14 in. × 10 in. × 5 in.) with corn cob bedding (grade 1/2 in.) in a barrier facility with the following filtration system: 60 complete air changes per hour, positive air pressure, and a filtering system consisting of a prefILTER and HEPA (high efficiency particulate absorption) filter capable of filtering 99.9% of particulate matter greater than 0.3 μm. The temperature was maintained at 22°C–24°C with a relative humidity of 50%. The animals were maintained on a 12-h light/dark cycle (lights on at 0630 h/lights off at 1830 h). After 4 wk of age, the rats were fed the diets described below. The rats were fed the diet in powder diet feeders (Hazelton Systems, Reston, VA) and had access to the diet from 1430 h to 0630 h (16 h) each day. Dietary restriction was initiated when the rats were 6 wk old. The amount of diet given to the rats fed the restricted amount of diet was determined by measuring the feed consumption by rats fed ad libitum (group A) for two 16-h periods and using the average consumption for all the rats in group A.

The SPF status of the rat population maintained in the barrier facility was continuously monitored. External examination of each animal prior to necropsy was carried out, and the following microbial assays were performed upon necropsy: mycoplasma culturing, lung culture, bacterial examination of cecal content, and culturing of any organ, gland or tissue that showed signs of inflammation.

All rats were fed a purified diet (diet 5775) obtained from Teklad Test Diets (Madison, WI). The diet consisted of the following: 21% casein, 15% sucrose, 43.6% dextrin, 3% Solka-Floc, 5% lard, 0.15% DL-methionine, 0.2% choline chloride and mixtures of vitamins and minerals. This diet is identical to the Purina 5775 Basal Diet previously described by Yu et al. (5). At 1 mo of age, the animals were randomly divided into four groups, 45 rats were placed in each of groups A and B and 125 rats were placed in each of groups C and D. The rats in groups A and C and in groups B and D were maintained and handled identically. The hepatocytes were isolated from the animals in groups C and D. The rats in groups A and B were maintained to determine the feed consumption, weight and longevity of the rats on these two dietary regimens. The rats in groups A and C were fed ad libitum over a 16-h period as described above while the rats in groups B and D were provided, in daily allotments, 60% (by weight) of the mean amount of diet consumed by the rats in group A.

To minimize the variation in feeding patterns between the two groups of rats, hepatocytes were isolated from rats after fasting. The rats fed the two regimens had access to their diets for a period of 5 h after the initiation of the dark cycle. During this time period, the restricted rats consumed all of their diet, and the amount of diet consumed by the rats fed ad libitum was similar to that consumed by the restricted rats. The rats were then fasted for 18 h before they were used in the experiments.

Protein synthesis assay. Hepatocytes were obtained by the in situ collagenase perfusion of the liver as described by Engelmann et al. (7). Hepatocyte viability, as determined by trypan blue exclusion, was consistently greater than 90%. The number of hepatocytes was determined by using a hemocytometer. The DNA, RNA and protein content of the hepatocytes were determined as described by Setaro and Morley (8), Munro and Fleck (9) and Peterson (10), respectively. Protein synthesis was measured by using the system described previously by Ricca et al. (11). Approximately 6 million cells (3 million/ml) were incubated in a modified Eagle's Minimal Essential Medium (12) supplemented with 1 mM pyruvate, a vitamin mixture (12), 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) buffer at pH 7.4, and two times the level of essential and nonessential amino acids (omitting L-valine) described by Eagle (12). L-[2,3-3H]-Valine (0.85 mCi/mmol) was added to the mixture at a concentration of 1.6 mM. Protein synthesis was terminated after 30 min of incubation, by the addition of the cell suspension to an equal volume of 10% trichloroacetic acid. The resulting acid-insoluble material was washed as described by
Khairallah and Mortimore (13), and the acid-insoluble material was then air-dried and dissolved in 0.3 N NaOH. The radioactivity present in the NaOH solution was determined with a liquid scintillation counter. The specific activity of the extracellular acid-soluble valine was determined as described by Ricca et al. (11), and the rate of protein synthesis was determined by dividing the radioactivity incorporated into the acid-precipitable material by the specific activity of the extracellular amino acid pool.

Statistical analyses. The data were analyzed statistically by using the two-way analysis of variance (ANOVA) followed by least significant differences (LSD) comparison when significant differences were observed by the two-way ANOVA.

RESULTS

Figure 1 shows the amount of diet consumed by rats fed ad libitum or the restricted amount of diet. Feed consumption increased up to 35 wk of age and then remained relatively constant. As one would expect, the body weight of the rats fed the restricted amount of diet was consistently lower than the body weights of the rats fed ad libitum (fig. 2). However, the general growth pattern was similar for the rats on the two dietary regimens; the body weight increased rapidly during the first 20 wk of age and increased at a lower rate thereafter. Figure 3 shows the survival curve for the rats on the two dietary regimens. The survival of the rats at 100 wk of age was 77–82%; there was no statistically significant difference in the survival of the rats on the two dietary regimens. Unfortunately, the study was terminated when all the rats died at 100 wk of age as a result of a malfunction in the heating system.

Table 1 gives the DNA, RNA and protein content of hepatocytes isolated from rats maintained on the two dietary regimens. The protein content of hepatocytes increased significantly with age (P < 0.01), which has been reported previously (8, 14). More importantly, ANOVA showed that the protein content of the hepatocytes isolated from the restricted rats was significantly lower (P < 0.01) than the hepatocytes isolated from rats fed ad libitum; however, the differences between the restricted and ad libitum-fed groups were not significant when each age was compared individually by the LSD procedure.

No significant difference was observed in either the RNA content of the hepatocytes as a function of age or the RNA content of hepatocytes from the rats on the two dietary regimens. The DNA content increased significantly (P < 0.01) after 12 mo of age for hepatocytes isolated from both groups of rats. The increase in DNA content probably represents an increase in ploidy, which is a characteristic change of the liver with aging and has been observed by several laboratories (7, 14, 15). At 19 mo of age, the DNA
content of hepatocytes isolated from rats fed ad libitum was significantly \((P < 0.01)\) greater than that of hepatocytes isolated from the diet-restricted rats.

The absolute rates of protein synthesis by hepatocytes isolated from rats on the two dietary regimens are given in table 2. Because the protein content was different for hepatocytes isolated from the two groups of animals (table 1), the rates of protein synthesis are expressed per million cells. The rate of protein synthesis for rats fed ad libitum decreased 55\% between 2.5 and 19 mo of age; this decrease was highly significant \((P < 0.001)\). On the other hand, the rate of protein synthesis by hepatocytes isolated from 19-mo-old rats fed the restricted diet was only 16\% less than that for hepatocytes isolated from 2.5-mo-old rats. No significant age-related change in the rate of protein synthesis by hepatocytes isolated from restricted rats was observed when the data were analyzed by ANOVA (in this analysis we also included the rate of protein synthesis by the 2.5-mo-old rats fed ad libitum).

At 12 and 19 mo of age the rate of protein synthesis by the restricted rats was significantly \((P < 0.01)\) greater than that of the ad libitum–fed rats. Therefore, it is evident from the data in table 2 that dietary restriction significantly retarded the age-related decline in protein synthesis in the liver.

**DISCUSSION**

It appears that one characteristic feature of the aging process is a decline in protein synthesis. Protein synthesis has been observed to decrease with increasing age in a variety of organisms ranging from fungi and insects to mammals (16, 17). The effect of aging on protein synthesis has been studied most in the livers of laboratory rodents. Our laboratory has studied protein synthesis as a function of age by using suspensions of hepatocytes isolated from rats of various ages because experimental conditions can be carefully controlled in suspensions of hepatocytes and because isolated hepatocytes mimic the in vivo situation, e.g., the rate of protein synthesis by hepatocytes is comparable to that observed for perfused liver and the liver in vivo (11). To measure protein synthesis accurately in whole cells or tissues by the incorporation of radioactively labeled amino acids into protein, it is essential that the specific activity of the amino acid precursor pool be determined. The intracellular and extracellular valine pools rapidly equilibrate in suspensions of hepatocytes, and under

**TABLE 1**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>Month of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Protein content, mg/million cells</td>
<td>Ad libitum(^8)</td>
<td>1.40 ± 0.10 (10)</td>
</tr>
<tr>
<td></td>
<td>Restricted(^8)</td>
<td>1.43 ± 0.11 (9)</td>
</tr>
<tr>
<td>RNA content, pg/million cells</td>
<td>Ad libitum</td>
<td>14.7 ± 1.1 (10)</td>
</tr>
<tr>
<td></td>
<td>Restricted</td>
<td>15.7 ± 1.3 (9)</td>
</tr>
<tr>
<td>DNA content, pg/million cells</td>
<td>Ad libitum(^8)</td>
<td>12.6 ± 1.1 (8)</td>
</tr>
<tr>
<td></td>
<td>Restricted</td>
<td>11.7 ± 0.6 (7)</td>
</tr>
</tbody>
</table>

\(^8\) Each value represents the mean ± SEM of data collected from the number of animals shown in parentheses. • Significant main effect of age at \(P < 0.01\) based on two-way analysis of variance (ANOVA). • Significant main effect of diet at \(P < 0.01\) based on two-way ANOVA.
TABLE 2

Protein synthesis by isolated hepatocytes

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Ad libitum</th>
<th>Restricted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol valine/(min · million cells)</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>150.4 ± 11.2 (5)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>138.0 ± 8.7 (11)</td>
<td>139.4 ± 9.1 (7)</td>
</tr>
<tr>
<td>7</td>
<td>125.2 ± 12.1 (5)</td>
<td>140.5 ± 12.2 (5)</td>
</tr>
<tr>
<td>12</td>
<td>106.0 ± 4.5 (9)</td>
<td>124.9 ± 6.1 (9)</td>
</tr>
<tr>
<td>19</td>
<td>88.2 ± 5.2 (4)</td>
<td>125.8 ± 7.9 (5)</td>
</tr>
</tbody>
</table>

*Each value represents the mean ± SEM of data collected from the number of animals shown in parentheses. **Significant main effect of age (P < 0.001) based on two-way ANOVA. ***Significantly different from restricted rats of the same age at P < 0.01 based on least significant differences.

conditions that our laboratory has established, the specific activities of the valine pools can be maintained constant for at least 60 min of incubation while the incorporation of valine into protein is linear with respect to time (11).

By using experimental conditions that allowed us to accurately measure protein synthesis, we studied the effect of life-long dietary restriction on the age-related decline in protein synthesis. A 50% decrease in the rate of protein synthesis was observed between 2.5 and 19 mo of age for hepatocytes isolated from rats fed ad libitum. This is similar to the decrease in protein synthesis that we previously observed for rats fed a commercial nonpurified diet ad libitum (11, 18). Therefore, the purified casein-based diet did not appear to have any gross effect on the age-related decline in protein synthesis. On the other hand, the rate of protein synthesis by hepatocytes isolated from rats fed the restricted amount of diet did not change significantly with age. In fact, at 19 mo of age, the rate of protein synthesis by hepatocytes isolated from restricted rats was 85% greater than the rates of protein synthesis from rats fed ad libitum. Therefore, life-long dietary restriction retarded the age-related decrease in protein synthesis that occurred between 2.5 and 19 mo of age in rats fed ad libitum.

Since McCay's (1) initial studies in 1935, several investigators have shown that the survival of laboratory rodents can be increased significantly by a variety of methods that reduce the dietary intake of laboratory rodents (3). Recently, Masoro's laboratory showed that both the mean and maximum survival of male Fischer F344 rats was increased over 40% by feeding the rats 60% of the amount of diet consumed by rats fed ad libitum (5). In our study, we used the same strain of rats, diet and restriction regimen as was used by Masoro's laboratory (5). At the time our study was terminated (100 wk of age), no difference in the survival of the rats fed ad libitum or the restricted amount of diet was observed (fig. 3). However, the survival curve for the rats up to 100 wk of age was similar to the survival curve observed by Masoro's laboratory (5). Therefore, based on data from Masoro's laboratory (5) we believe that the dietary restriction regimen used in our study would have significantly increased the survival of the male Fischer F344 rats.

Dietary restriction is a very important tool in studying the aging process because it increases both the mean and maximum survival of rodents (2, 3). At the present time, no information is available on the effect of dietary expression on protein synthesis in aging organisms. Several investigators have studied the effect of dietary restriction on the levels of a variety of enzymes, and no clear trend has been observed (4, 19-21). However, one cannot assess how dietary restriction affects protein synthesis by measuring enzyme levels, because the levels of enzymes are determined by both the synthesis and degradation of the enzymes. Our results (table 2) clearly demonstrate that dietary restriction, which was initiated at 6 wk of age, retarded the age-related decline in protein synthesis. The effect of dietary restriction on protein synthesis did not arise indirectly from differences in the survival of the rats because the survival of the rats on the two dietary regimens was similar during the period studied (fig. 3).

The fact that dietary restriction altered the age-related decline in protein synthesis supports the concept that the decline in protein synthesis is involved in the biological mechanism underlying the aging process. Richardson and Cheung (22) proposed that the decline in protein synthesis is physiologically important because it is involved in a decrease in protein turnover. The decrease

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in protein turnover with aging could be very important to an organism because it could affect the ability of an organism to respond to cellular and environmental stimuli. Preliminary studies from several laboratories indicate that the turnover of protein decreases significantly with increasing age (22). Although only the rates of protein synthesis were measured in our study, it is possible to predict what effect dietary restriction might have on protein turnover. Even though the rates of protein synthesis were higher in hepatocytes from rats on the restricted dietary regimen, the protein content of the hepatocytes from the restricted rats was significantly less than that of hepatocytes from rats fed ad libitum (table 1). Johnson and Barrows (23) also found that dietary restriction resulted in a significant decrease in size of kidney cells from mice. If the protein content of hepatocytes is less while protein synthesis is higher for hepatocytes from rats fed the restricted diets, the turnover of protein in hepatocytes from the rats fed the restricted amount of diet would be expected to be higher than that of hepatocytes from rats fed ad libitum.

Although the effect of dietary restriction on the survival of rats has been studied in detail, the biological mechanism underlying its effect on longevity is unknown. Barrows (4) proposed in 1972 that dietary restriction increased longevity by reducing the use of the genetic code through a reduction in protein synthesis. The results of our study show conclusively that dietary restriction does not increase longevity by this mechanism; dietary restriction results in an increase in protein synthesis. More recently, Lindell (6) suggested that dietary restriction is a physiological “stress” that enhances gene expression. Lindell (6) proposed that the enhanced gene expression is a significant factor in maintaining cellular homeostasis in an organism as it ages. Our data tend to support this hypothesis. The protein synthetic activity of hepatocytes from older rats fed the restricted amount of diet was significantly higher than that of the rats fed ad libitum.

If a higher rate of protein turnover is important in maintaining cellular homeostasis as proposed by Richardson and Cheung (22), it is possible that the mechanism underlying the effect of dietary restriction on longevity is at least partially due to the effect of dietary restriction on protein synthesis and protein turnover.

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LITERATURE CITED