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Effect of Age and Dietary Restriction on the Expression of α_{2u}-Globulin*

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The effect of aging on the expression of α_{2u}-globulin was studied in liver tissue from 6–30-month-old male Fischer F344 rats. The synthesis of α_{2u}-globulin by suspensions of isolated hepatocytes decreased 90% between 6 and 22 months of age. The levels of α_{2u}-globulin mRNA and the transcription of α_{2u}-globulin genes by isolated liver nuclei decreased 80–85% between 5 and 24 months of age. Because α_{2u}-globulin has been suggested to be a "senescence marker protein," the expression of α_{2u}-globulin was measured in rats fed a diet restricted in calories. This dietary restriction procedure has been shown to increase significantly the longevity of rodents. The expression of α_{2u}-globulin was compared in liver tissue from 18-month-old rats fed *ad libitum* and a restricted diet (40% restriction of total calories). The synthesis, mRNA levels, and transcription of α_{2u}-globulin were 1.8–3-fold higher for liver tissue from restricted rats compared to liver tissue from rats fed *ad libitum*. Therefore, dietary restriction alters the age-related change in the expression of α_{2u}-globulin. Our results demonstrate that the changes in α_{2u}-globulin expression that arise during aging or dietary restriction are regulated at the level of transcription.

α_{2u}-Globulin is a protein (M_r = 18,700) that is synthesized by hepatic parenchymal cells, secreted into the blood stream, and excreted in the urine of adult male rats (1, 2). The synthesis of α_{2u}-globulin is under complex multihormonal control, *e.g.* androgens (3, 4), glucocorticoids (5), thyroid hormone (6, 7), insulin (8), and growth hormone (9, 10) are stimulatory, whereas estrogens are inhibitory (11). In addition, the synthesis of α_{2u}-globulin changes as a function of malignant transformation, development, and senescence. For example, α_{2u}-globulin synthesis increases during development reaching peak values at 9–12 weeks of age (4, 12, 13), and ceases when hepatocytes undergo malignant transformation (13) or senescence (4, 14).

During the past two decades, a considerable amount of research has shown that gene expression in the liver of rodents declines with increasing age, *e.g.* the rates of protein synthesis by isolated hepatocytes (15, 16) and the transcriptional activity of liver nuclei (17) decreases between 40 and 60% with increasing age. However, very little is known about the effect of aging on the expression of specific genes. Roy *et al.* (14) proposed that α_{2u}-globulin was a "senescence marker protein" in male rats because the levels and synthesis of α_{2u}-globulin decrease dramatically with increasing age. Preliminary studies by Roy's laboratory (14, 18) suggest that the decrease in α_{2u}-globulin synthesis with age is due to changes in mRNA levels, which indicates that aging is acting at a pretranslation site in the regulation of α_{2u}-globulin expression. The age-related changes in α_{2u}-globulin mRNA levels might be a consequence of an alteration in either gene transcription, altered processing of the primary transcript, or changes in mRNA turnover. In this study, we compared the synthesis mRNA levels, and the relative transcriptional rate of α_{2u}-globulin by the hepatocytes of male rats during senescence and after dietary restriction. Dietary restriction increases the survival of laboratory rodents and appears to retard aging and senescence (19, 20). Our experiments demonstrate that the age-related decrease in α_{2u}-globulin expression occurs through the regulation of transcription of the α_{2u}-globulin genes and that dietary restriction results in an increase in the expression of α_{2u}-globulin.

MATERIALS AND METHODS

Animals—Male Fischer F344 rats were used in this study. The rats used to study the effect of aging on the expression of α_{2u}-globulin were obtained from the animal colony maintained by the National Institute on Aging. These rats are fed a commercial lab chow *ad libitum* and have a mean and maximum survival of 27 and 35 months, respectively (21). The male Fischer F344 rats used to study the effect of dietary restriction on α_{2u}-globulin expression were maintained on two diets as described by Birchenall-Sparks *et al.* (22). Briefly, rats were fed a semi-synthetic, casein diet, which was obtained from Teklad Test Diets (Madison, WI). At 6 weeks of age the rats were placed on two dietary regimens; the rats were either fed *ad libitum* or were fed 60% of the amount of diet (restricted diet) consumed by the rats fed *ad libitum*. Yu *et al.* (19) have shown that the mean and maximum survival of male Fischer F344 rats are increased approximately 40% using a dietary restriction procedure similar to that described by Birchenall-Sparks *et al.* (22).

Isolation of Hepatocytes and Protein Synthesis—Hepatocytes were obtained by the *in situ* collagenase perfusion of liver as described by Engelmann *et al.* (23). Hepatocyte viability, as determined by trypan blue exclusion, was consistently greater than 90%, and the number of hepatocytes was determined using a hemocytometer. Protein synthesis was determined using a system similar to that described by Ricca *et al.* (24). Hepatocytes (3 million/ml) were suspended in Eagle's minimal essential medium (Sigma) and incubated for 15 min at 37 °C under an atmosphere of oxygen/carbon dioxide (95:5). Either [^{35}S]L-methionine (1000 Ci/mmol) or [^{3}H]L-valine (10 Ci/mmol) was added to the suspension at a concentration of 20 or 50 μCi/ml, respectively. The hepatocytes were incubated at 37 °C under an atmosphere of oxygen/carbon dioxide (95:5) for 120 min unless otherwise indicated. The reaction was terminated by placing the cells on ice and pelleting

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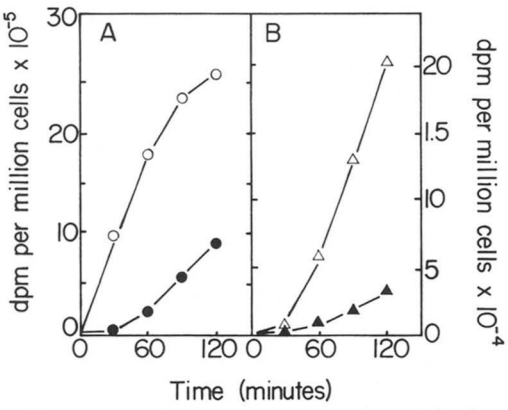

FIG. 1. **Time course for the synthesis of proteins by suspensions of hepatocytes.** Hepatocytes were isolated from a 5-month-old rat. *A* shows the incorporation of [^{3}H]valine into intracellular (○) and extracellular (●) proteins. *B* shows the incorporation of [^{3}H]valine into albumin (△) and α_{2u}-globulin (▲) in the extracellular medium. Proteins in the extracellular medium were subjected to SDS-polyacrylamide gel electrophoresis, and the radioactivity migrating as albumin or α_{2u}-globulin was determined.

the cells by low-speed centrifugation. The incorporation of radioactivity into either extracellular protein (supernatant) or intracellular protein (cell pellet) by hepatocytes is shown in Fig. 1*A*. There is a short lag period of 30–45 min before radioactivity appears in extracellular proteins; this corresponds to the time to synthesize and transport extracellular proteins (25). The synthesis of α_{2u}-globulin by suspensions of hepatocytes was determined by taking the extracellular supernatant from hepatocytes incubated with either [^{35}S]methionine or [^{3}H]valine and separating the proteins by SDS[1]-polyacrylamide slab gel electrophoresis (26). In a few cases, the isoelectric variants of α_{2u}-globulin were analyzed by two-dimensional gel electrophoresis using the protocol described by O'Farrell (27). In all cases, equal amounts of radioactively labeled proteins were placed on the gels. The gels were either analyzed by fluorography (28) or the amount of radioactivity incorporated into α_{2u}-globulin measured as follows. α_{2u}-Globulin standards were added to the gels, and after electrophoresis the band migrating as α_{2u}-globulin was removed, digested with tissue solubilizer, and suspended in liquid scintillation solvent. The radioactivity incorporated into α_{2u}-globulin was determined with a liquid scintillation counter and corrected for background by measuring the radioactivity migrating as α_{2u}-globulin in samples of medium taken immediately after the addition of the [^{3}H]valine. Fig. 1*B* shows the time course for the incorporation of [^{3}H]valine into albumin and α_{2u}-globulin by hepatocytes in suspension. The appearance or radioactivity in α_{2u}-globulin is linear between 45 and 120 min. Therefore, when measuring α_{2u}-globulin synthesis by hepatocytes, the amount of radioactivity incorporated into α_{2u}-globulin was determined after incubating hepatocytes with the radioactive amino acid for 120 min. Approximately 1% of the total radioactivity incorporated into proteins by hepatocytes is incorporated into α_{2u}-globulin (Fig. 1), which is identical to the level of α_{2u}-globulin synthesis reported *in vivo* (13).

RNA Isolation and RNA/cDNA Hybridization—Rats were killed by decapitation and the livers immediately removed and frozen in liquid nitrogen. The RNA was isolated from the liver tissue by homogenization in guanidium thiocyanate and cesium chloride density gradient centrifugation as described by Chirgwin *et al.* (29). The levels of α_{2u}-globulin mRNA in the RNA preparations were determined by dot blot hybridization as described by Thomas (30) using a cDNA probe which had been labeled with ^{32}P radioactivity by nick translation (31). The cDNA probe (SGII) for α_{2u}-globulin was obtained from Dr. Philip Feigelson (Columbia University) and has been described previously (32). Northern blot analysis of the RNA was accomplished as described by Rutherford *et al.* (33) using the method of Southern (34) to transfer the RNA to nitrocellulose.

Transcription by Isolated Nuclei—Nuclei were isolated from hepatocytes by a slight modification of the procedure described by Lamers *et al.* (35). Hepatocytes were lysed in a buffer (0.3 M sucrose, 5 mM dithiothreitol, 5 mM $MgCl_2$, 2% Triton X-100, and 10 mM Tris, pH 7.5) using a Vortex mixer. The nuclei were obtained by low-speed centrifugation, resuspended in buffer (50% glycerol, 5 mM MgCl, 0.1 mM EDTA, and 50 mM Hepes, pH 7.5), and stored at −80 °C until used for the transcription assays.

The *in vitro* transcription of α_{2u}-globulin was measured using isolated nuclei pooled from four animals for each age studied. The procedure for nuclear transcription, isolation of RNA, and hybridization is similar to that described by Lamers *et al.* (35). Nuclei (3×10^7) were incubated in triplicate in a 200-μl assay, which contained 25% glycerol, 2.5 mM $MgCl_2$, 0.05 mM EDTA, 75 mM Hepes (pH 7.5), 100 mM KCl, 4 mM dithiothreitol, 40 μg/ml creatine kinase, 8.8 mM creatine phosphate, and the following nucleotides: 0.5 mM GTP, 1.0 mM ATP, 0.5 mM CTP, and 25 μCi of [α-^{32}P]UTP. After incubating for 60 min, the reaction was terminated, and the RNA isolated from the nuclear pellet by digestion with proteinase K and phenol extraction. The RNA was digested with RNase-free DNase (15 μg/ml) and re-extracted with phenol. Eight μg of either the cDNA containing plasmid or pBR322 was fixed to nitrocellulose as described by Harplod *et al.* (36). The prehybridization and hybridization conditions were identical to those described by Lamers *et al.* (35). The filters were air-dried, and the radioactivity associated with the filters was determined by liquid scintillation counting. The amount of radioactivity that hybridized to the pBR322 DNA was used as the background and was subtracted from the radioactivity that hybridized to the cDNA-containing plasmids. The transcription of α_{2u}-globulin was expressed as parts per million as defined by Lamers *et al.* (35): (counts/min (SGII-pBR322)/counts/min in total RNA) × (100/efficiency of hybridization) × 1300/1224, where 1300 is the length of the α_{2u}-globulin mRNA in nucleotides and 1224 is the length of the SGII in base pairs, and the efficiency of hybridization is 56%.

RESULTS

Changes in Synthesis of α_{2u}-Globulin—The effect of age on the synthesis of α_{2u}-globulin was studied in suspensions of hepatocytes isolated from rats of various ages. The autoradiogram in Fig. 2 shows that α_{2u}-globulin can be resolved by SDS-gel electrophoresis from other extracellular proteins synthesized by hepatocytes. It is also evident from Fig. 2 that the incorporation of [^{35}S]methionine into α_{2u}-globulin decreases markedly with age. The synthesis of α_{2u}-globulin was quantified by measuring the amount of [^{3}H]valine incorporated into α_{2u}-globulin by suspensions of hepatocytes isolated from 5–

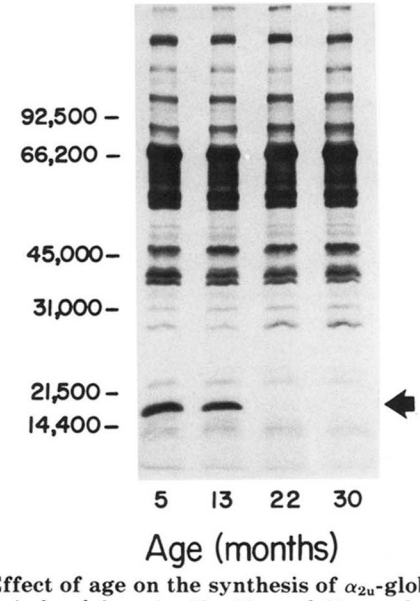

FIG. 2. **Effect of age on the synthesis of α_{2u}-globulin.** Hepatocytes were isolated from 5-, 13-, 22-, and 30-month-old rats and incubated with [^{35}S]methionine. The culture supernatants from four animals for each age were pooled. The proteins in the supernatants were separated by SDS-polyacrylamide gel electrophoresis, and the gel was analyzed by fluorography. The migration of protein standards of known molecular weights is shown, and the *arrow* indicates the migration of α_{2u}-globulin.

[1] The abbreviations used are: SDS, sodium dodecyl sulfate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

30-month-old rats (Table I). The synthesis of α_{2u}-globulin decreased over 90% between 5 and 22 months of age. No change in α_{2u}-globulin synthesis was observed after 22 months of age.

Changes in Levels and Transcription of α_{2u}-Globulin mRNA—The levels of α_{2u}-globulin mRNA in liver RNA isolated from 5–29-month-old rats was measured to determine whether the age-related decline in α_{2u}-globulin synthesis arose from pretranslational changes. Fig. 3 shows that the cDNA probe to α_{2u}-globulin hybridized to a 1.2–1.3-kilobase RNA species, which is identical to the size of α_{2u}-globulin mRNA reported in rat liver by Unterman *et al.* (37). Although the hybridization of the cDNA probe to the total RNA decreased markedly with age, no change in the size of the α_{2u}-globulin mRNA was observed. In addition, there was no evidence for an accumulation of α_{2u}-globulin mRNA precusors or a degradation of α_{2u}-globulin mRNA with increasing age (Northern blot in Fig. 3). Identical results were obtained with poly(A)$^+$ RNA isolated from the livers of 5–29-month-old rats (data not shown). Therefore, the decline in α_{2u}-globulin mRNA levels is not correlated with a loss in the poly(A) sequence at the 3′-end of the mRNA.

The autoradiograph of the dot blot in Fig. 3 shows that α_{2u}-globulin mRNA was essentially undetectable in the livers of 29-month-old rats. It also should be noted that the levels of α_{2u}-globulin mRNA in 24-month-old rats were quite variable; in some rats it was relatively high, whereas in others it was undetectable. Table I gives the relative levels of α_{2u}-globulin mRNA in total RNA isolated from rats of various ages. The level of α_{2u}-globulin mRNA in hepatic RNA decreased over 85% between 6 and 24 months of age. Similar results were observed with RNA isolated from young and old male C57B1/6J mice (data not shown). To determine whether the change in α_{2u}-globulin mRNA levels was common to other RNA species, the levels of 28 S rRNA and the mRNAs for albumin and α_1-antitrypsin were determined (data not shown). The relative levels of 28 S rRNA did not change significantly with age, whereas the relative levels of both albumin and α_1-antitrypsin increased significantly.

TABLE I

Age-related changes in the expression of α_{2u}-globulin

Age	Synthesis[a]	mRNA levels[a]	Transcription[b]
months	*dpm/10^3 cells*	*area/8 μg RNA*	*ppm*
5–6	25.2 ± 1.8	2.07 ± 0.06	1420
12–14	17.8 ± 1.4	1.46 ± 0.08	1056
22–24	3.3 ± 0.4	0.29 ± 0.13	298
29–30	3.3 ± 0.4	0.09 ± 0.01	

[a] Values represent the mean ± S.E. of data from four animals for each age. Values for synthesis and mRNA levels were obtained from two different groups of animals. The decrease in the synthesis of α_{2u}-globulin and α_{2u}-globulin mRNA levels between 5 and 14 months and between 14 and 24 months was significant at the $p < 0.001$ level.

[b] Values represent the mean of triplicate samples obtained from nuclei pooled from three animals for each age. These data were obtained with a group of rats that were different from the rats used to measure synthesis or mRNA levels.

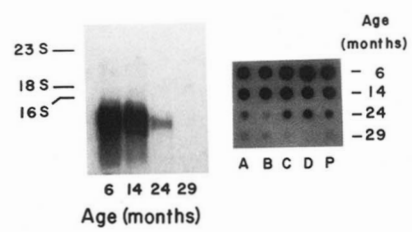

FIG. 3. **Assay of α_{2u}-globulin mRNA levels in the livers of rats of various ages.** The hybridization of the ^{32}P-labeled α_{2u}-globulin cDNA probe to a Northern blot is shown on the *left*. Five μg of RNA, which was pooled from four rats for each age, was applied to the gel, and the migration of rRNA standards is shown. The hybridization of the ^{32}P-labeled probe to RNA blots (8 μg of RNA) from four individual rats (*A*, *B*, *C*, and *D*) for each age and from a pooled sample (*P*) is shown on the *right*.

The decline in α_{2u}-globulin mRNA levels could arise from age-related changes in either the transcription of α_{2u}-globulin genes, the processing of initial α_{2u}-globulin transcript, or the turnover of α_{2u}-globulin mRNA. To elucidate the mechanism responsible for the age-related decline in α_{2u}-globulin mRNA levels, the transcription of α_{2u}-globulin mRNA was measured using isolated nuclei. Table I shows that the synthesis of RNA transcripts that hybridize to the α_{2u}-globulin cDNA probe decreased 80% between 5 and 23 months of age.

Effect of Dietary Restriction on Expression of α_{2u}-Globulin—In this series of experiments, the expression of α_{2u}-globulin in two groups of 18-month-old rats was measured: rats fed *ad libitum* and rats that were placed on a restricted diet (60% of *ad libitum*) at 6 weeks of age. It should be noted that at 18 months of age a significant decline in α_{2u}-globulin expression was observed (Table I). Fig. 4 (*panel 1*) shows that dietary restriction reduced the levels of protein in the urine. This observation agrees with previous studies, which showed that dietary restriction reduced the age-related increase in proteinuria (38). In addition, it is evident that the level of α_{2u}-globulin in the urine of the restricted rats was markedly greater than that found in the urine of rats fed *ad libitum*. Using suspensions of hepatocytes, the synthesis of α_{2u}-globulin by hepatocytes isolated from rats fed *ad libitum* and the restricted diet was measured (Fig. 4, *panel 2*, and Table II). The incorporation of [^{3}H]valine into α_{2u}-globulin was significantly greater for hepatocytes isolated from the restricted rats. Roy *et al.* (14) reported that five major isoelectric variants of α_{2u}-globulin could be detected by two-dimensional gel electrophoresis, and the synthesis of all isoelectric variants decreased with

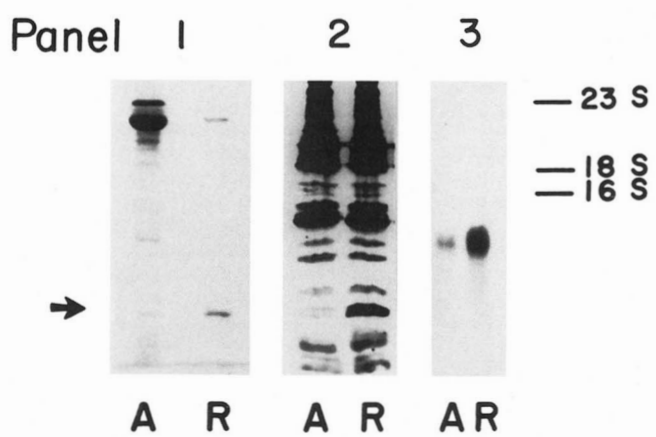

FIG. 4. **Effect of dietary restriction on the expression of α_{2u}-globulin.** Expression of α_{2u}-globulin was compared for 18-month-old rats fed *ad libitum* (*A*) or a restricted diet (*R*). *Panel 1* shows the resolution of urinary proteins by SDS-polyacrylamide gel electrophoresis. Urine was collected over a 24-h period as described by Ricketts *et al.* (38). The urine was pooled from four animals for each group of rats and was diluted to the same volume. Equal volumes of the urine were subjected to electrophoresis, and the gels were stained with Coomassie Blue. The *arrow* shows the migration of α_{2u}-globulin. *Panel 2* shows the fluorograph of a SDS-polyacrylamide gel of the supernatants from hepatocytes incubated with [^{35}S]methionine. Supernatants were pooled from four animals for each group of rats. The migration of α_{2u}-globulin in the gel is shown by the *arrow* the *left*. *Panel 3* shows the hybridization of the ^{32}P-labeled α_{2u}-globulin cDNA probe to a Northern blot, which contained RNA (5 μg) pooled from four animals for each group of rats. The migration of rRNA standards is shown on the *right*.

TABLE II

Expression of α_{2u}-globulin by liver of 18-month-old rats fed ad libitum and a restricted diet

The values for the synthesis of α_{2u}-globulin and α_{2u}-globulin mRNA levels represents the mean ± S.E. of data from four rats. The data for the transcription of α_{2u}-globulin represent the mean of triplicate samples from liver nuclei pooled from four rats for each dietary regimen.

Diet	Protein synthesis	mRNA levels	Transcription
	dpm/10^3 cells	*area/8 μg RNA*	*ppm*
Ad libitum	7.5 ± 2.4	0.37 ± 0.08	554
Restricted	22.0 ± 4.6[a]	0.66 ± 0.12[b]	1306

[a] Value is significantly greater than *ad libitum* value ($p < 0.05$).
[b] Value is significantly greater than *ad libitum* value ($p < 0.001$).

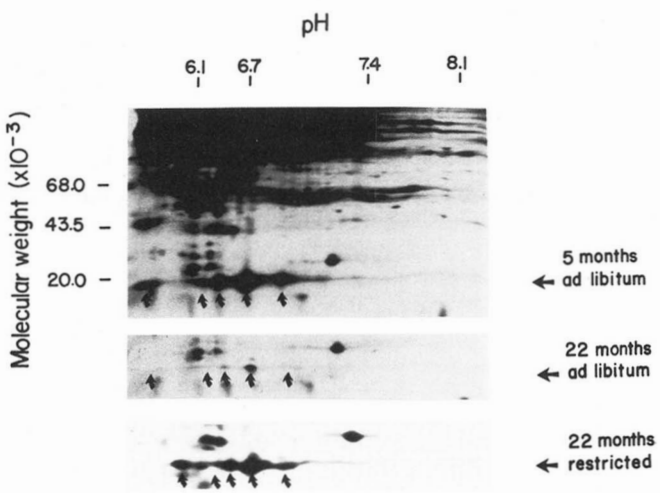

FIG. 5. **Effect of dietary restriction on the synthesis of isoelectric variants of α_{2u}-globulin.** Hepatocytes isolated from 5- and 22-month-old rats fed *ad libitum* and 22-month-old rats fed a restricted diet were incubated with [^{35}S]methionine. The culture supernatants from four animals for each group were pooled, equal amounts of radioactively labeled proteins from the supernatants were simultaneously subjected to two-dimensional gel electrophoresis, and the gels were analyzed by fluorography. The entire gel from the 5-month-old rats is shown, and the regions of the gels containing the isoelectric variants of α_{2u}-globulin are shown for the 22-month-old rats fed *ad libitum* and the restricted diet. The fluorographs were overexposed to enhance the detection of the isoelectric variants of α_{2u}-globulin. The migration of protein standards of known molecular weights and pI values is shown. The isoelectric variants of α_{2u}-globulin, which are shown by the *arrows*, were identified according to the description given by Roy *et al.* (14). It should be noted that the migration of the proteins at the acidic end of the gel for the restricted rats was slightly different from the gels for rats fed *ad libitum*.

increasing age. Therefore, we were interested in determining whether the increase in α_{2u}-globulin synthesis by restricted rats was due to an increase in the synthesis of all isoelectric variants or whether the synthesis of only a few variants was affected by dietary restriction. The data in Fig. 5, which confirm the study by Roy *et al.* (14), show that all five isoelectric variants of α_{2u}-globulin decreased with age. In addition, Fig. 5 shows that the increase in α_{2u}-globulin synthesis in restricted rats was due to an increase in the synthesis of all five isoelectric variants.

The effect of dietary restriction on the expression of α_{2u}-globulin was also studied at the transcriptional level. Fig. 4 (*panel 3*) shows that dietary restriction had no effect on the size of the α_{2u}-globulin mRNA; however, the levels of α_{2u}-globulin mRNA in the livers of restricted rats were 1.8-fold greater than the levels in the liver of rats fed *ad libitum* (Table II). Table II also shows that the synthesis of RNA transcripts that hybridized to the α_{2u}-globulin cDNA probe was almost 2-fold greater for liver nuclei isolated from the restricted rats.

DISCUSSION

We have demonstrated that the age-related decline in α_{2u}-globulin expression arises predominantly through the regulation of α_{2u}-globulin transcription. An excellent correlation was found between the age-related decline in the synthesis, mRNA levels, and transcription of α_{2u}-globulin genes in liver (Table I). Because these three parameters were measured in three different groups of rats at different times, the correlation is even more striking. Recently, Kulkarni *et al.* (32) showed that the control of α_{2u}-globulin expression during development and hormonal stimulation occurred predominantly through the regulation of the transcription of α_{2u}-globulin genes. Thus, our results and the data by Kulkarni *et al.* (32) show that the regulation of α_{2u}-globulin expression occurs primarily at the level of transcription.

If α_{2u}-globulin is a senescence marker protein, as proposed by Roy *et al.* (14), one should be able to change the age-related decline in α_{2u}-globulin expression by procedures that alter the aging process, *i.e.* senescence. Dietary restriction (underfeeding not malnutrition) is the only experimental manipulation known to increase the longevity of mammals (20, 39, 40). The classic experiments by McCay *et al.* (41) in 1935 showed that a drastic restriction of total calories increased the survival of rats. Later studies by Berg (42, 43) showed that the longevity of rats could be increased by dietary restriction procedures less severe than those used by McCay *et al.* (41). Because dietary restriction has consistently been shown to increase the life expectancy of rodents, it has been suggested that this intervention retards the aging process. However, increasing life expectancy of an organism does not necessarily indicate that an intervention has influenced the process by which the organism ages. Several lines of evidence strongly suggest that the increase in longevity resulting from dietary restriction is the result of a retardation of the aging process (39), *e.g.* dietary restriction (*a*) increases the maximum as well as the mean survival, (*b*) retards the incidence and severity of a wide variety of diseases, (*c*) retards the decline in a variety of physiological processes, and (*d*) retards the decline in the function of a variety of organs/tissues.

In our study, we used a restriction regimen similar to that described by Yu *et al.* (19). They showed that this dietary restriction regimen increased the mean and maximum survival of male Fischer F344 rats 45–50%. We compared the expression of α_{2u}-globulin by hepatic tissue from rats fed *ad libitum* and rats fed the restricted diet at 18 months of age. In rats fed *ad libitum*, we observed a dramatic (80–90%) decrease in the expression of α_{2u}-globulin by 22 months of age (Table I). In contrast, at 18 months of age, rats maintained on the restricted diet had higher levels of α_{2u}-globulin in their urine, and the expression of α_{2u}-globulin by liver tissue from the restricted rats was higher than rats fed *ad libitum* (Fig. 4, Table II), *e.g.* the synthesis, mRNA levels, and transcription of α_{2u}-globulin were 1.8–3-fold higher for hepatic tissue from the restricted rats. Thus, our study supports the suggestion by Roy *et al.* (14) that α_{2u}-globulin can serve as a senescence marker protein because dietary restriction, which increases the longevity of the rats, retards the age-related decline in α_{2u}-globulin expression.

The data obtained in this study clearly show that the regulation of α_{2u}-globulin expression during senescence and dietary restriction occurs predominantly at the level of transcription. However, the molecular basis for the changes in α_{2u}-globulin transcription is unknown. Because α_{2u}-globulin

is regulated by a variety of hormones, the changes in α_{2u}-globulin expression during senescence and dietary restriction could arise from age-related changes in the hormonal status of the animal. On the other hand, the decrease could arise from changes at the cellular level, *i.e.* cells could be unable to express α_{2u}-globulin.

Although it is well documented that dietary restriction increases the longevity of rodents, the mechanism underlying the effect of dietary restriction on longevity is not understood. Because the methodology used to increase longevity involves restricting calories, most investigators have assumed that dietary restriction acts through some type of metabolic mechanism. However, research conducted during the past 5 years shows that the increase in longevity is not due to retardation of development/maturation (40, 44, 45), reduced adiposity (46, 47), or reduced metabolic rate (39). Because dietary restriction has a profound effect on most tissues and diseases, the mechanism must involve a process that has the potential to impact on most, if not all, cells in an organism. The regulation of gene expression represents one site of regulation common to all cells in a living organism, and changes in gene expression can have a profound effect at both the cellular and organismic level. Our study is the first evidence to show that dietary restriction has an effect at the level of gene expression, specifically transcription. Future studies should be conducted with other cDNA probes and other tissues to determine whether changes in the expression of other genes occur with dietary restriction.

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