Journal of Gerontology: BIOLOGICAL SCIENCES
1991, Vol. 46, No. 5, B180–187

Copyright 1991 by The Gerontological Society of America

Influence of Age and Long-Term Dietary Restriction on Plasma Insulin-like Growth Factor-1 (IGF-1), IGF-1 Gene Expression, and IGF-1 Binding Proteins

Charles R. Breese, Rhonda L. Ingram, and William E. Sonntag

Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University.

The purpose of these studies was to determine more accurately the relationship between IGF-1 and life span, and to determine whether moderate dietary caloric restriction alters the age-related changes in IGF-1. Studies included an assessment of plasma IGF-1, hepatic IGF-1 mRNA, and plasma IGF-1 binding proteins (IGF-1-BP). Rats (6, 12, 22, and 28 months of age) were fed ad libitum or maintained on a diet 60% of ad libitum. In ad libitum fed animals, plasma IGF-1 decreased by 20% between 6 and 28 months of age. Similar age-related declines were observed in dietary restricted animals but levels were generally 14–25% lower at each age group. IGF-1 mRNA levels demonstrated similar decreases with age in ad libitum fed animals, but in dietary restricted animals, levels plateaued at 22 and 28 months. IGF-1 binding protein analysis revealed 3 bands at approximate molecular weights of 40k, 30k, and 24k. All bands demonstrated a decrease in relative IGF-1-BP concentration with age, as well as a decrease in the 40k and 30k binding proteins after dietary restriction. These results indicate that (a) aging in ad libitum fed animals is associated with decreases in plasma IGF-1, IGF-1-BP, and IGF-1 mRNA levels; and (b) long-term dietary restriction decreases plasma IGF-1 and IGF-1-BP levels in each age group although the age-associated decreases in IGF-1 mRNA levels are prevented by dietary restriction. Our results suggest that alterations in IGF-1 in ad libitum fed animals are a contributing factor in the decline in protein synthesis with age, and long-term dietary restriction modifies the interaction between these variables.

INSULIN-LIKE growth factor-1 and 2 (IGF-1 and IGF-2) are a family of small molecular weight peptide hormones (approximately 7500k), structurally related to insulin (Rinderknecht and Humbel, 1978; Froesch and Zapf, 1985), which circulate in high concentrations in the blood. These peptide hormones have been shown to induce mitogenic activity in cultured fibroblasts and fetal cell lines (Underwood and D'Ercole, 1984; Conover et al., 1985), stimulate anabolic activity in many cell and tissue types (Van Wyk et al., 1975; Ewton and Florini, 1980; Zapf et al., 1981), and induce DNA and protein synthesis in a number of tissues (Zapf et al., 1981; Froesch et al., 1985). Recent studies indicate that these peptides may have an autocrine or paracrine role in tissue growth and hypertrophy, tissue regeneration (D'Ercole et al., 1984; Daughaday and Rotwein, 1989), cell differentiation (Ewton and Florini, 1981; Aizenman and de Vellis, 1987), and an important role in development, including aging.

Unlike most plasma peptide hormones, IGF-1 circulates in blood bound to highly specific carrier or binding protein complexes of approximately 40k and 150k (Hintz and Liu, 1977; Smith, 1984; Binoux et al., 1986; Hossenlopp et al., 1986). The larger binding protein complex has been shown to bind 85–90% of plasma IGF-1 (Zapf et al., 1984) and, similar to IGF-1, is growth hormone dependent (Hintz et al., 1981; White et al., 1981; Wilkins and D'Ercole, 1985). The 40k binding protein appears to be less growth hormone dependent (Hintz et al., 1981). Both IGF-1 and IGF-1 binding proteins (IGF-1-BP) are produced predominately in the liver (Moses et al., 1979; Schwander et al., 1983; Lund et al., 1986; Murphy et al., 1987); however, other tissues have been shown to produce similar proteins (Binoux et al., 1981; D'Ercole et al., 1984; McCusker and Clemmons, 1988; Ocrant et al., 1989; Rosenfeld et al., 1989). Binding of IGF-1 to its binding protein has been shown to prolong its apparent plasma half-life from minutes to hours (Cohen and Nissley, 1976) and influence its activity at the receptor level (Knauer and Smith, 1980; Elgin et al., 1987; DeMellow and Baxter, 1988; McCusker and Clemmons, 1988). Therefore, analysis of IGF-1-BP appears to be important in an overall assessment of IGF-1 biological activity.

In humans and animals, there is a decline in protein synthetic capacity in many tissues with age, including liver, skeletal muscle, brain, and bone (Richardson, 1981; Pluskal et al., 1984). This decline in protein synthesis appears to be closely associated with alterations in IGF-1, since plasma IGF-1 concentration has been reported to decrease with age in both animals and humans (Florini and Roberts, 1979; Florini et al., 1981, 1985), and administration of growth hormone increases plasma IGF-1 concentration in elderly men (Johanson and Blizard, 1981) and enhances protein synthesis in skeletal muscle in aging animals (Sonntag et al., 1984). Therefore, alterations in the regulation of IGF-1, either by changes in IGF-1 secretion, IGF-1 mRNA levels, or by alterations in the regulation of IGF-1 binding proteins may contribute to the age-associated decrease in tissue protein synthesis.

Recently, there has been renewed interest in the biological effects of moderate dietary restriction while still providing essential nutrients (Weindruch and Walford, 1988). Dietary

Downloaded from http://geronj.oxfordjournals.org/ at Middle Tennessee State University on March 23, 2015

restriction has been shown to prolong life span in a number of species (McCay et al., 1943; Masoro et al., 1982; Yu et al., 1982; Weindruch and Walford, 1988) and prevent the age-related decrease in protein synthesis (Birchenall-Sparks et al., 1985; Ricketts et al., 1985). While it has been shown in previous reports that plasma IGF-1 concentrations and IGF-1 mRNA levels are reduced following fasting (Maes et al., 1983; Emler and Schalch, 1987; Lowe et al., 1989), there are currently no reports on the effects of age and moderate long-term dietary restriction on IGF-1 plasma concentration, IGF-1 mRNA levels, IGF-1 binding proteins (IGF-1-BP), or the correlation of these measures with the life span as measured in an independent cohort of animals.

MATERIALS AND METHODS

Animals

Male BN × F344 rats, ages 6, 12, 22, and 28 months old, were obtained from the National Institute on Aging Project on Caloric Restriction colony at the National Center for Toxicological Research (NCTR, Little Rock, AR). Upon arrival at our institution, animals were housed in a specific pathogen free facility (the animal facility at Bowman Gray School of Medicine is fully accredited by AAALAC) and maintained on a 12:12 hour light/dark cycle (lights on at 0600h). Water was available ad libitum to all animals, and animals were fed either ad libitum rat chow (NIH-31, Purina Mills, Richmond, IN) or 60% of ad libitum food consumption (utilizing a vitamin and mineral supplemented diet). This dietary regimen, initiated at NCTR at 16 weeks of age and continued until sacrifice, has been used successfully to increase life span and study the effect of dietary restriction on longevity and disease. Animals in the dietary restricted group were supplied sufficient protein, fat, carbohydrates, and other essential nutrients as stated in the National Research Council guidelines (National Research Council, 1978). Animals in the dietary restricted group maintained body weight throughout the study (average 325g), while ad libitum animals gained approximately 300g from 6 to 28 months.

Animals were sacrificed three weeks after arrival at our facility by rapid decapitation, and blood and liver samples were collected. Since differences existed in the diurnal patterns of food consumption between ad libitum and dietary restricted groups, animals in the 6-, 12-, and 22-month age groups were divided into two groups and sacrificed four hours before (0600h) and four hours after feeding (1400h) to investigate whether feeding times influenced any of the dependent variables in this study. Heparinized plasma was separated by centrifugation at 2000 ×g and stored at −80 °C. Liver samples were placed in 500μl of RNA extraction buffer (4M guanidium thiocyanate, 25mM sodium citrate pH 7.0, 0.5% sarcosyl, 0.1M β-mercaptoethanol) and stored at −80 °C until extracted for total RNA.

IGF-1 Plasma Concentration Analysis

IGF-1 concentration in extracted plasma was measured by radioimmunoassay (RIA) as previously described (Sonntag and Boyd, 1988; Davenport et al., 1990). Briefly, plasma was acidified, extracted with 10 volumes of petroleum ether, purified on a C-18 column, and analyzed by a specific RIA using antiserum obtained from NIDDK. Thr^{59}-IGF-1 (Amgen, Thousand Oaks, CA) was radiolabeled with ^{125}I using a lactoperoxidase, glucose oxidase procedure (Sonntag et al., 1980). Data were expressed in relation to IGF-1 standards. Minimum detectable dose of this assay was 15.5 pg/tube (80% B/B_o), and 50% inhibition of tracer binding was 59.3 pg/tube. The intra-assay and interassay coefficients of variation were 4.43% and 7.00% respectively.

mRNA Analysis

Extraction of RNA from liver samples. — Total RNA from liver tissue samples was extracted by a modified acid-guanidium procedure as previously described (Chomczynski and Sacchi, 1987; Sonntag et al., 1990). Briefly, tissue (100mg in 500μl RNA extraction buffer) was homogenized with a polytron (Brinkman, setting 5, 10 sec) and 50μl 2M sodium acetate (pH 4.0), 500μl water saturated phenol, and 100μl chloroform:isoamyl alcohol were added. Samples were incubated 15 min and centrifuged (14000 ×g, 2 min). The aqueous phase was collected, combined with one volume of cold isopropanol, and the mixture incubated at −80 °C for 15 min and centrifuged. The supernatant was discarded, the pellet resuspended, and reprecipitated as above. The pellet was washed in 80% ethanol, resuspended in water, and 260/280 ratios determined. Gel electrophoresis of RNA extracted by this technique demonstrates prominent 28S and 18S ribosomal bands, 260/280 ratios of 1.9, and, after Northern transfer and hybridization to a ^{32}P-oligo $d(pT)_{16}$ probe, a high quantity of poly(A^+)RNA (size range from 0.24kb to >9.5kb).

Preparation and labeling of probes. — A cDNA probe for IGF-1 in bacterial expression vector pAT153 was generously provided by Dr. Lian J. Murphy (University of Manitoba). The insert is a 500 base pair Eco R1 fragment encoding exons 2, 3, and 5 of rat pre-pro insulin-like growth factor-1, and has been previously verified and utilized to measure IGF-1 mRNA levels in rats (Murphy et al., 1987). The plasmid was amplified in bacteria and the presence of the IGF-1 cDNA insert verified by digestion with restriction enzymes Eco R1 and Bam H1 followed by separation on a 1% agarose gel.

The cDNA IGF-1 probe was labeled by "random primed" DNA labeling. The complementary strand was synthesized using a random hexanucleotide primer, the Klenow fragment of DNA polymerase (Boehringer-Mannheim) and ^{32}P-dATP (NEN, DuPont, 3000 Ci/mmol). Approximately 50% of labeled dATP was incorporated into the probe as measured by TCA precipitation. This probe was utilized to measure total IGF-1 mRNA in hepatic tissues.

An oligonucleotide $d(pT)_{16}$ probe was 3′ end labeled with ^{32}P-dATP, separated on a C-18 column, and eluted with 50% methanol. Using this procedure, approximately 50% of ^{32}P-dATP was incorporated into the probe. Total mRNA (as poly A^+ RNA) concentrations were determined by hybridization to ^{32}P-oligo $d(pT)_{16}$.

RNA dot-blot hybridization procedures. — The RNA dot-

Downloaded from http://geronj.oxfordjournals.org/ at Middle Tennessee State University on March 23, 2015

blot procedure was adapted from the protocol recommended by Schleicher and Schuell as previously described (Sonntag et al., 1990). Serial dilutions of total RNA were blotted to nitrocellulose and prehybridized and hybridized with 4 × SSC, 5 × Denhardt's solution, 50% formamide, 0.1% SDS, 20mM sodium phosphate, 2mM EDTA, 100μg/ml sheared salmon sperm DNA, 10μg/ml yeast t-RNA, and 4×10^6 cpm/ml probe at 42 °C for 18–24 hours. Nitrocellulose was washed in 2 × SSC and 0.1% SDS at room temperature, and 3 × in 0.5 × SSC and 0.1% SDS at 60 °C. Northern analysis utilizing these stringent conditions revealed an absence of nonspecific binding to 18S and 28S ribosomal RNA. Blots were autoradiographed on Kodak X-OMAT AR x-ray film. The hybrids were eluted from the nitrocellulose and membranes reprobed with ^{32}P-oligo-d(pT)$_{16}$.

IGF-1 Binding Protein Analysis

Gel electrophoresis. — SDS-PAGE was performed according to a modified method of Laemmli (1970). A 12% continuous acrylamide running gel (0.33M Tris-HCl, pH 8.8, 0.1% SDS) was utilized in a 15 × 15 × 0.15 cm gel slab (Bio-Rad, Rockville Centre, NY). Plasma samples were diluted 1:4 with nonreducing sample buffer (10% glycerol, 62mM Tris-HCl pH 6.8, 2% SDS and 0.003% bromophenol blue), heated at 95 °C for 5 min, randomly loaded onto a 4% acrylamide stacking gel (0.125M Tris-HCl pH 6.8, 0.1% SDS) and subjected to SDS-PAGE (running buffer: 25mM Tris base, 200mM glycine, 0.1% SDS) at 80 volts (constant voltage) until the dye front reached the bottom of the gel plate (approximately 15 hrs). A pooled control plasma sample was prepared from young ad libitum animals and used as an internal standard on all gel electrophoresis runs.

Electrotransfer and autoradiography of IGF-1-BP. — Western ligand blotting was performed according to Towbin et al. (1979), as modified by Hossenlopp et al. (1986). Following electrophoresis, the gel was prewashed in buffer (15mM Tris base, 120mM glycine, 5% methanol) and proteins were electrotransferred to nitrocellulose in a trans-blot cell (Bio-Rad) at 300mA for 5 hours. The nitrocellulose was then washed for 30 min in 3% nonidet-40 (NP-40, Sigma) in buffered saline (0.15M NaCl, 0.01M Tris-HCl pH 7.4, 0.5 mg/ml Na Azide), followed by a 2-hour incubation with 1% BSA (Sigma) in buffer at 4 °C to reduce nonspecific binding of tracer. The nitrocellulose was then incubated with 20 ml of 0.1% tween-20 and 1% BSA in buffer with ^{125}I-IGF-1 (approximately 1×10^6 cpm), overnight at 4 °C on a shaker platform. The nitrocellulose was washed 2 × with 0.1% tween-20 in buffer and 3 × with buffer at 4 °C for 15 min each, apposed to Kodak X-OMAT AR x-ray film and placed in a film holder with an enhancing screen at − 70 °C for 4–5 hours.

Data Analysis

Autoradiographic images on x-ray film were analyzed by LKB Laser densitometer and LKB 2400 XL Gel Scan software (Turku, Finland). For dot-blot analysis, autoradiographs were analyzed at a common-fold dilution for each probe on the linear portion of the dose-response curve. Peak

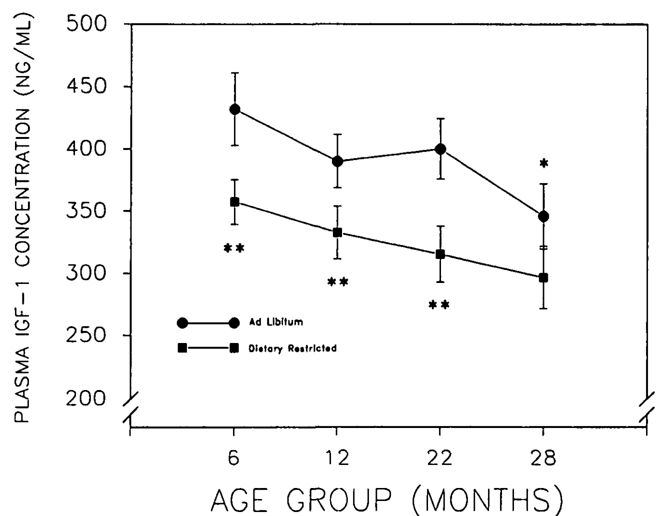

Figure 1. Comparison of plasma IGF-1 concentration in 6, 12, 22, and 28-month-old ad libitum and dietary restricted animals. Data are expressed as ng/ml and represent the mean ± *SEM* of approximately 20 animals. Closed circles represent ad libitum fed animals and closed squares represent dietary restricted animals. * $p < .05$, 6-month ad libitum fed versus 28-month ad libitum fed animals; ** $p < .05$, ad libitum versus dietary restricted animals at 4, 12, and 22 months of age.

height was calculated for each blot and expressed as relative absorbance units (AU). Data were then normalized to poly A$^+$ RNA concentration for the same dot-blot. IGF-1 binding proteins were analyzed by utilizing the area under the absorbance curve to compare samples, and individual results were expressed as a percent of the pooled control sample for each molecular weight band. All data were statistically analyzed by MANOVA using SAS (SAS Institute, Cary, NC), followed by three-way (Age × Diet × Time of Sacrifice) and/or two-way ANOVA (Age × Diet) with appropriate specific contrasts.

RESULTS

Influence of time of sacrifice. — Preliminary analyses of 6-, 12-, and 22-month-old rats from both ad libitum and dietary restricted groups revealed that time of sacrifice (4 h prior to and after feeding) had no effect on plasma levels of IGF-1 ($F(1,117) = 0.490, p = .49$), hepatic IGF-1 mRNA levels ($F(1,104) = 1.79, p = .20$), or plasma IGF-1 binding proteins levels (40k bands: $F(1,65) = 0.227, p = .60$, 30k bands: $F(1,65) = 0.960, p = .33$, 24k band: $F(1,65) = 2.174, p = .15$). Therefore, data from these groups were pooled for statistical analysis.

IGF-1 RIA analysis. — Analysis of plasma levels of IGF-1 revealed a significant main effect of age ($F(1,151) = 2.74, p < .05$) and diet ($F(1,151) = 15.80, p < .001$). As a result of age, plasma IGF-1 was significantly decreased (approximately 20%) in the ad libitum fed animals from 6 to 28 months of age (Figure 1; $p < .05$). While there were reductions at 12 and 22 months of age compared to the 6-month group, these comparisons failed to reach statistical significance. In the dietary restricted animals, there was a general decline in plasma IGF-1 concentration with age; however, no

Downloaded from http://geronj.oxfordjournals.org/ at Middle Tennessee State University on March 23, 2015

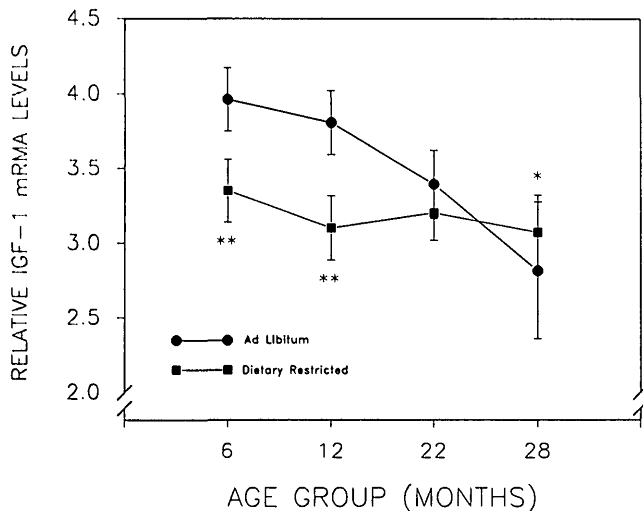

Figure 2. Comparison of relative IGF-1 mRNA levels in 6, 12, 22, and 28-month-old ad libitum and dietary restricted animals. Data are expressed as relative IGF-1 mRNA signal/poly A^+ RNA signal and represent the mean ± *SEM* of approximately 20 animals/group. Closed circles represent ad libitum fed animals and closed squares represent dietary restricted animals. * $p < .05$, 6-month ad libitum fed versus 28-month ad libitum fed animals; ** $p < .05$, ad libitum versus dietary restricted animals at 4 and 12 months of age.

significant differences were observed between 12-, 22-, and 28-month-old animals when compared to the 6-month dietary restricted age group.

Comparison of IGF-1 levels between ad libitum fed and dietary restricted animals at 6, 12, and 22 months of age revealed reductions in the dietary restricted animals of approximately 18% ($p < .05$) at each age (Figure 1). There was also a reduction in the 28-month age group as a result of dietary restriction; however, this failed to reach significance ($p = .06$).

Analysis of relative IGF-1 mRNA levels. — Analysis of IGF-1 mRNA levels revealed a significant main effect for both age ($F(3,144) = 3.39$, $p < .02$) and diet ($F(1,144) = 12.383$, $p < .001$). As demonstrated in Figure 2, there was a 29% reduction in the ad libitum fed animals in hepatic IGF-1 mRNA levels between 6 and 28 months of age ($p < .05$). Although decreases were observed at 12 and 22 months of age, these changes were not significant. In the dietary restricted groups, there were no significant differences between the 12-, 22-, and 28-month-old animals as compared to the 6-month age group.

Comparison of dietary restricted with ad libitum fed animals revealed reductions in both the 6- and 12-month age groups ($p < .05$), with an average reduction of approximately 20% (Figure 2). However, in the older age groups (22 and 28 months), IGF-1 gene expression was similar to that of ad libitum fed animals. Hepatic IGF-1 gene expression was correlated with plasma IGF-1 concentration (Spearman's correlation coefficient $r = .240$, $p < .004$).

IGF-1 binding protein analysis. — A typical migration pattern of plasma IGF-1-BP following SDS-PAGE, electrotransfer, and application of ^{125}I-IGF-1 tracer is shown in

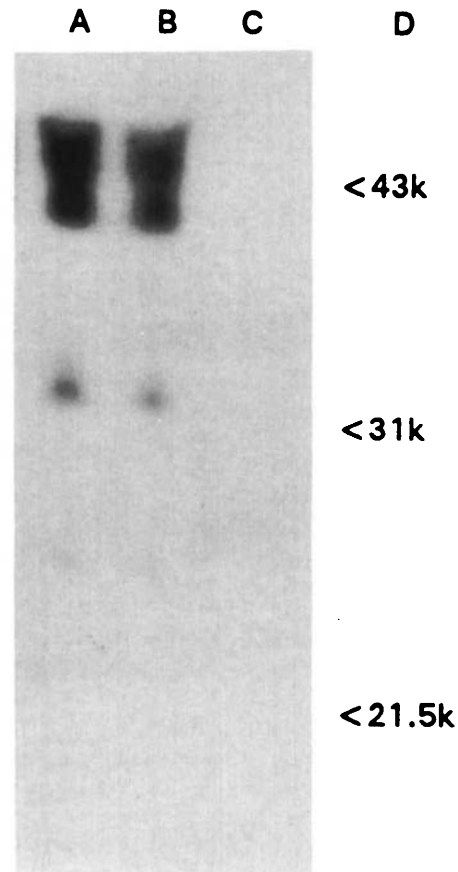

Figure 3. Example of SDS-PAGE of plasma from pooled young ad libitum (lane A) and pooled dietary restricted animals (lane B), followed by ligand blotting with 1×10^6 cpm of ^{125}I-IGF-1, and autoradiography. Lanes A and B demonstrate the three major bands at apparent molecular weights of 40k, 30k, and 24k. Lane C demonstrates incubation with an excess of cold IGF-1 (2μg). Molecular weight markers (Bio-Rad) are shown in lane D: ovalbumin (43k), carbonic anhydrase (31k), and soybean trypsin inhibitor (21.5k).

Figure 3A. Autoradiographs revealed three bands of IGF-1-BP, which are in agreement with other studies on IGF-1-BP in the rat. The greatest amount of tracer binding occurred at 2 distinct bands corresponding to apparent molecular weights of 43k and 39k (subsequently referred to as 40k binding protein). A fainter doublet occurred at approximately 34k and 29k (subsequently referred to as 30k binding protein) and a single faint band at approximately 24k. These bands were not resolved sufficiently to allow an examination of individual components and were therefore grouped for densitometric analysis, as in other studies on IGF-1 binding proteins (Clemmons et al., 1989). Increasing concentration of IGF-1 (0.5ng-2.0ng) inhibited tracer binding to all IGF-1 binding protein bands in a dose-dependent manner and an excess of cold IGF-1 (2ng) completely inhibited binding (Figure 3C).

Analysis of age and dietary status on IGF-1-BP. — There was a significant effect of age on each of the 3 major bands (40k bands, $F(3,83) = 4.34$, $p < .007$; 30k bands, $F(3,75)$

Downloaded from http://geronj.oxfordjournals.org/ at Middle Tennessee State University on March 23, 2015

= 8.11, $p < .001$; 24k bands, $F(3,83) = 4.98$, $p < .003$). The greatest differences occurred between the 6-month and the 22- and 28-month ad libitum fed age groups for all three IGF-1-BP bands (Figure 4). However, reductions were also observed in the 40k binding protein band by 12 months of age ($p < .05$) when compared to the 6-month age group (average reduction approximately 18%).

As with the ad libitum fed animals, there was also a general decline in the binding protein with age in the dietary restricted group. There were statistically significant reductions in the dietary restricted group at the 22-month age group when compared to the 6-month age group for each of the three binding proteins ($p < .05$). Furthermore, the 30k binding protein analysis revealed significant reductions by 12 and 28 months of age ($p < .05$).

Comparison of ad libitum fed and dietary restricted animals revealed statistically significant reductions in the IGF-1-BP (see Figure 3A, B) for the 40k binding protein ($F(1,83) = 39.8$, $p < .001$), and the 30k binding protein ($F(1,75) = 18.15$, $p < .001$) at all ages ($p < .05$). The 24k IGF-1-BP demonstrated a modest reduction at 6 and 22 months of age; however, these changes failed to reach significance ($F(1,83) = 3.57$, $p = .06$). The changes in IGF-1-BP closely followed the IGF-1 plasma concentrations (Spearman's correlation coefficients, 40k bands, $r = .565$, $p < .001$; 30k bands, $r = .388$, $p < .001$; 24k bands, $r = .329$, $p < .003$). There were similar correlations between relative IGF-1 mRNA levels and plasma IGF-1 binding protein concentration.

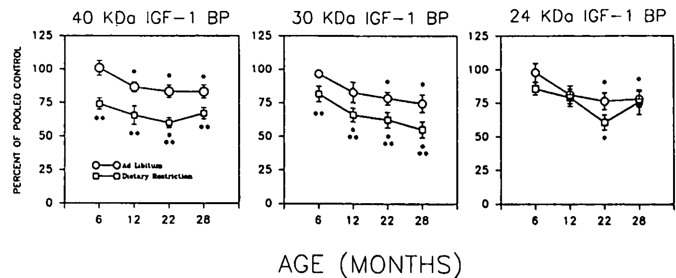

Figure 4. Comparison of relative plasma IGF-1 binding protein concentration in 6, 12, 22, and 28-month-old ad libitum and dietary restricted animals. Binding proteins were separated by SDS-PAGE, blotted to nitrocellulose and probed with ^{125}I-IGF-1. Upon autoradiography, three distinct bands of apparent molecular weights of 40k, 30k, and 24k were densitometrically analyzed. Data are expressed as the relative percent of IGF-1-BP levels of a pooled ad libitum plasma sample and represent the mean *SEM* of approximately 10 animals/group. Open circles represent ad libitum fed animals and open squares represent dietary restricted animals. * $p < .05$, 6-month versus 12, 22, and 28-month age group of the same dietary status; ** $p < .05$, ad libitum versus dietary restricted animals at each age group.

Discussion

The purpose of this study was (a) to examine the effects of aging and long-term dietary restriction on the regulation of plasma IGF-1 concentrations, hepatic IGF-1 mRNA levels, and plasma IGF-1 binding proteins (IGF-1-BP), and (b) to correlate these findings with life span as measured in an independent cohort of animals. Previous studies have indicated that plasma IGF-1 declines with age in both animals and man (Florini and Roberts, 1979; Florini et al., 1981, 1985) and suggested that these changes may be associated with the decline in protein synthesis normally observed in tissues from aging animals. Our results confirmed the decrease in plasma IGF-1 concentration with age, indicating that there was an approximate 20% decrease in IGF-1 plasma concentration between 6 and 28 months of age in BN × F344 rats. We observed similar age-related reductions in IGF-1-BP, suggesting that a close correlation exists between the age-related declines in IGF-1 and IGF-1-BP in ad libitum fed animals. The decrease in IGF-1 appears to be the result of a decline in hepatic IGF-1 gene expression since the reductions were both qualitatively and quantitatively similar over the life span of these animals, and greater than 85% of plasma IGF-1 is derived from hepatic tissue (Zapf et al., 1984). Therefore, these results clearly suggest that a close relationship exists between age-related changes in plasma IGF-1, plasma IGF-1-BP, IGF-1 mRNA levels, and probability of survival in ad libitum fed animals.

Previous studies have found an age-related decline in many anabolic processes in mammals, including protein synthesis (Richardson, 1981; Pluskal et al., 1984). Specific mechanisms for the reduction in protein synthesis with age are poorly understood but may be associated with a decrease in both mRNA levels and a reduction in the efficacy of the ribosomal complex necessary to initiate and maintain protein synthesis (Burini et al., 1984). We and others have hypothesized that the age-related decline in protein synthesis may be closely associated with alterations in IGF-1, because (a) IGF-1 has been shown to be widely distributed in liver, bone, skeletal muscle, and other organ systems; and (b) several laboratories have reported that this hormone has an important role in the normal regulation of tissue growth, hypertrophy, and cell differentiation as well as maintenance of protein synthesis (Underwood and D'Ercole, 1984; Zapf et al., 1984; Daughaday and Rotwein, 1989). In addition, administration of growth hormone, which stimulates IGF-1 secretion, increases plasma IGF-1 concentrations in elderly men (Johanson and Blizard, 1981) and enhances protein synthesis in skeletal muscle from aging animals (Sonntag et al., 1984). Although the alterations in plasma IGF-1 levels in the present study are only 20% between 6 and 28 months of age, the coordinate decrease in IGF-1-BP, which has been shown to have an important role in IGF-1 activity, may exacerbate the age-related declines in hormone levels. Other studies in Sprague-Dawley and Fischer 344 rats have suggested that greater reductions in IGF-1 occur with age in rats (Florini and Roberts, 1979; Sonntag, unpublished data), but these strains have shorter life spans than the strain under investigation (Nakagawa et al., 1974; Iwasaki et al., 1988; NCTR, unpublished data). Parallel studies conducted at NCTR indicate that the ad libitum fed BN × F344 rats have a mean life span of 32 months as measured in an independent cohort of animals (Figure 5). Since our studies were conducted at 28 months, we expect that as animals approach maximum life span, IGF-1 levels continue to decline and that these changes directly contribute to the age-related, progressive reduction in protein synthesis.

It has been demonstrated previously that moderate dietary restriction prolongs the life span of several species (McCay et al., 1943; Masoro et al., 1982; Yu et al., 1982; Weindruch and Walford, 1988) including the BN × F344 strain (Figure 5). The life span augmenting effects of dietary restriction

Downloaded from http://geronj.oxfordjournals.org/ at Middle Tennessee State University on March 23, 2015

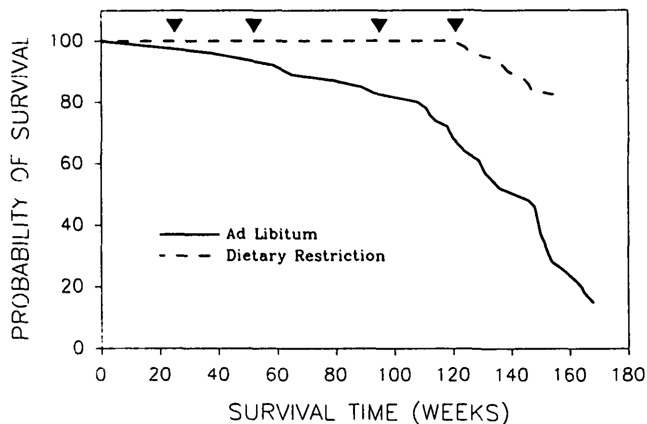

Figure 5. Comparison of the probability of survival of ad libitum fed and dietary restricted Brown Norway × Fischer 344 rats as measured in an independent cohort of animals at the facilities of NCTR. Solid line represents ad libitum fed animals, dashed line dietary restricted animals; arrows denote age of the animals used in this study, 6, 12, 22, and 28 months. Data were collected at the National Center for Toxicological Research (used with permission).

appear to depend upon overall energy (caloric) restriction rather than a restriction of specific dietary components (fats, carbohydrates, protein, vitamins or minerals) (Nakagawa et al., 1974; Feldman et al., 1982; Iwasaki et al., 1988; Weindruch and Walford, 1988). One possible mechanism for the increase in life span of dietary restricted animals may be that these animals demonstrate a delay in the normal age-related decline in protein synthesis compared to ad libitum fed controls (Richardson, 1981; Birchenall-Sparks et al., 1985; Ricketts et al., 1985). We have hypothesized that moderate dietary restriction, although initially lowering plasma IGF-1 concentrations (Maes et al., 1983; Emler and Schalch, 1987; Lowe et al., 1989), would delay the age-related decrease in plasma IGF-1 concentration over the life span of these animals, thereby ameliorating the loss in anabolic activity which is normally observed in aged ad libitum fed animals. The results of the present study demonstrated a reduction in plasma IGF-1 concentrations at 6, 12, and 22 months of age due to dietary restriction. By 28 months of age, however, there were no statistically significant differences between ad libitum and dietary restricted groups. There was also a corresponding reduction in plasma IGF-1-BP concentration in dietary restricted animals in the 40k and 30k binding protein as compared to ad libitum fed animals of the same age, an effect not found in the 24k binding protein. While plasma IGF-1 and IGF-1-BP were reduced in the dietary restricted animals, IGF-1 mRNA levels appeared to stabilize at 22 and 28 months of age while levels in ad libitum fed animals continued to decline. By 22 months, no effect of diet was observed, and at 28 months of age, IGF-1 mRNA levels were greater in the dietary restricted animals. The significance for the disparity between IGF-1 mRNA levels and plasma IGF-1 concentration is unknown but may be associated with changes in the plasma half-life of IGF-1 between these groups. In addition, many tissues are known to express IGF-1 activity and although the relative significance of the paracrine activity of IGF-1 remains to be established, it does appear that dietary restricted animals retain the capacity to synthesize IGF-1 mRNA. This may prevent the age-related reduction in protein synthesis via a local paracrine or autocrine action on tissues. Further studies on the interaction of age and dietary restriction on IGF-1 gene expression in various tissues will be required to understand the actions of dietary restriction on IGF-1 gene expression and the significance of these results for the aging animal.

It has been shown in several studies that IGF-1 circulates in blood bound to highly specific binding protein complexes (Hintz and Liu, 1977; Smith, 1984; Binoux et al., 1986; Hossenlopp et al., 1986). However, to date, few studies have been undertaken to assess the effects of age and long-term dietary restriction on IGF-1-BPs. These binding proteins have been shown to prolong IGF-1 plasma half-life (Cohen and Nissley, 1976), increase the activity of IGF-1 at the receptor (Knauer and Smith, 1980; Elgin et al., 1987; DeMellow and Baxter, 1988), and may have an additive effect on the regulation and physiological response of tissue to IGF-1. Only recently have investigators begun to study the regulation of IGF-1-BP. Previous reports have shown that plasma IGF-1-BP concentrations are acutely affected by fasting and glucose infusion (Busby et al., 1988; Cotterill et al., 1988; Suikkari et al., 1988; McCusker et al., 1989), and other studies suggest that IGF-1 may regulate plasma concentration of its binding protein in the absence of growth hormone (Clemmons et al., 1989). These results suggest that there may be a specific feedback system which controls the release of IGF-1 binding proteins into the plasma. The results of our study indicate that the reductions in the 40k and 30k plasma IGF-1-BP concentrations closely correlate with the reductions in plasma IGF-1 concentration, implying that these peptides are regulated by similar influences.

Previous studies indicate that both IGF-1 and IGF-1-BP are regulated by growth hormone release from the anterior pituitary. IGF-1 has also been shown to be a mediator of the actions of growth hormone on many tissues (Hintz et al., 1981; White et al., 1981; Wilkins and D'Ercole, 1985). Experiments have also demonstrated a decline in the amplitude of growth hormone release in both animals and man with age (Sonntag et al., 1980; Prinz et al., 1983; Kahler et al., 1986; Takahashi et al., 1987), as well as decreases in the 24-hour integrated release of growth hormone in humans between 20 and 40 years of age (Zadik et al., 1985). The hypothesis that the age-related decrease in IGF-1 and protein synthesis is due to a decrease in growth hormone secretion is supported by at least two studies: (a) administration of exogenous growth hormone restores plasma levels of IGF-1 in aged animals (Takahashi and Meites, 1987) and elderly men (Johanson and Blizard, 1981), and (b) administration of exogenous bovine growth hormone to aged rats increases relative rates of protein synthesis to levels observed in young animals (Sonntag et al., 1984). These results indicate that alterations in growth hormone secretory dynamics in aged animals are, in part, responsible for diminished IGF-1 gene expression, the concomitant reduction in plasma IGF-1, and IGF-1-BP concentrations and contribute to the reduction in protein synthesis with age.

In recent years, there has been intense interest in the relationship between the age-related decrease in growth hor-

Downloaded from http://geronj.oxfordjournals.org/ at Middle Tennessee State University on March 23, 2015

mone (and subsequently IGF-1) (Sonntag, 1987) and the deterioration of tissue function associated with aging. A decline in plasma concentrations of growth hormone and IGF-1 has the potential to influence wound healing, bone mass, lipid metabolism, renal plasma flow, immune function, general tissue protein synthesis, and possibly brain aging. To date, studies on the effects of growth hormone and/or IGF-1 replacement are limited but indicate that these hormones restore skeletal muscle protein synthesis (Sonntag et al., 1984), immune function (Kelley et al., 1986), and aortic elastin mRNA (Foster et al., 1990) in animals; and skin thickness, vertebral bone density in man, and reduce adiposity (Rudman et al., 1990). Our results on the effects of long-term dietary restriction and aging on plasma IGF-1 do not support the hypothesis that dietary restriction increases life span by maintaining plasma IGF-1 concentrations. However, these results do not preclude an important role for IGF-1 in the mechanism of action of dietary restriction. It is well-known that IGF-1 has an important autocrine/paracrine role in many tissues and the prevention of the age-related decrease in IGF-1 mRNA levels after dietary restriction suggests that alterations occur in the paracrine activity of this hormone. Alternatively, dietary restriction may modify tissue response to IGF-1 by increasing levels of IGF-1 receptors, thereby ameliorating the age-related decline in plasma IGF-1 levels. These possibilities are currently under investigation.

Acknowledgments

This research was supported by grant AG-07752 to Dr. Sonntag. IGF-1 antibody was kindly provided by The National Pituitary Agency (NIDDK) and Dr. Louis Underwood. The cDNA probe for IGF-1 was generously provided by Dr. Lian Murphy. Animals used in this study were obtained from the Project on Caloric Restriction at the National Center for Toxicological Research.

Address correspondence to Dr. William E. Sonntag, Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Medical Center Boulevard, Winston-Salem, NC 27157.

References

Aizenman, Y.; de Vellis, J. Brain neurons develop in a serum and glial free environment: Effects of transferrin, insulin, insulin-like growth factor-1 and thyroid hormone on neuronal survival, growth, and differentiation. Brain Res. 406:32–42; 1987.

Binoux, M.; Hossenlopp, L.; Lassarre, C.; Hardouin, S. Production of insulin-like growth factors and their carriers by rat pituitary gland and brain explants in culture. FEBS Lett. 124:178–184; 1981.

Binoux, M.; Hossenlopp, L.; Hardouin, S.; Seurin, D.; Lassarre, C.; Gourmelen, M. Somatomedin (insulin-like growth factors)-binding proteins: molecular forms and regulation. Hormone Res. 24:141–151; 1986.

Birchenall-Sparks, M. C.; Roberts, M. S.; Staecker, J.; Hardwick, J. P.; Richardson, A. Effect of dietary restriction on liver protein synthesis in rats. J. Nutr. 115:944–950; 1985.

Burini, R. C.; Pluskal, M. G.; Wei, I. W.; Young, V. R. Protein synthesis studies in skeletal muscle of aging rats II. In vitro studies with 0.5M potassium chloride washed polyribosomes. J. Gerontol. 39:392–397; 1984.

Busby, W. H.; Snyder, D. K.; Clemmons, D. R. Radioimmunoassay of a 26000-dalton plasma insulin-like growth factor-binding protein: control by nutritional variables. J. Clin. Endocrinol. Metab. 67:1225–1230; 1988.

Chomczynski, P.; Sacchi, N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162:156–159; 1987.

Clemmons, D. R.; Thissen, J. P.; Mead, M.; Ketelslegers, J. M.; Underwood, L. E. Insulin-like growth factor I (IGF-1) infusion into hypophysectomized or protein-deprived rats induces specific IGF-binding proteins in serum. Endocrinology 125:2967–2972; 1989.

Cohen, K. L.; Nissley, S. P. The serum half-life of somatomedin activity: evidence for growth hormone dependence. Acta Endocrinol. (Kbh) 83:243–258; 1976.

Conover, C. A.; Hintz, R. L.; Rosenfeld, R. G. Comparative effects of somatomedin-C and insulin on the metabolism and growth of cultured human fibroblast. J. Cell. Physiol. 122:133–141; 1985.

Cotterill, A. M.; Cowell, C. T.; Baxter, R. C.; McNeil, D.; Silinik, M. Regulation of the growth factor-binding protein in children. J. Clin. Endocrinol. Metab. 67:882–887; 1988.

Daughaday, W. H.; Rotwein, P. Insulin-like growth factor I and II. Peptide, messenger ribonucleic acid and gene structures, serum and tissue concentrations. Endocr. Rev. 10:68–91; 1989.

Davenport, M. L.; D'Ercole, A. J.; Underwood, L. E. Effect of maternal fasting on fetal growth, serum insulin-like growth factors (IGFs), and tissue IGF messenger ribonucleic acids. Endocrinology 126:2062–2067; 1990.

DeMellow, J. S.; Baxter, R. C. Growth hormone-dependent insulin-like growth factor (IGF) binding protein both inhibits and potentiates IGF-I-stimulated DNA synthesis in human skin fibroblast. Biochem. Biophys. Res. Commun. 156:199–204; 1988.

D'Ercole, A. J.; Stiles, A. D.; Underwood, L. E. Tissue concentrations of somatomedin-C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. Proc. Natl. Acad. Sci. USA 81:935–939; 1984.

Elgin, R. G.; Busby, W. H.; Clemmons, D. R. An insulin-like growth factor binding protein enhances the biologic response to IGF-1. Proc. Natl. Acad. Sci. USA 84:3254–3258; 1987.

Emler, C. A.; Schalch, D. S. Nutritionally induced changes in hepatic insulin-like growth factor I (IGF-1) gene expression in rats. Endocrinology 120:832–834; 1987.

Ewton, D. Z.; Florini, J. R. Relative effects of the somatomedins, multiplication-stimulating activity, and growth hormone on myoblast and myotubes in culture. Endocrinology 106:577–583; 1980.

Ewton, D. Z.; Florini, J. R. Effects of somatomedins and insulin on myoblast differentiation in vitro. Dev. Biol. 86:31–39; 1981.

Feldman, D. B.; McConnell, E. E.; Knapka, J. J. Growth, kidney disease, and longevity of syrian hamsters fed varying levels of protein. Lab. Anim. Sci. 32:613–618; 1982.

Florini, J. R.; Roberts, S. B. Effect of rat age on blood levels of somatomedin-like growth factor. J. Gerontol. 35:23–30; 1979.

Florini, J. R.; Harned, J.; Richman, R. A.; Weiss, J. P. Effect of rat age on serum levels of growth hormone and somatomedins. Mech. Ageing Dev. 15:165–176; 1981.

Florini, J. R.; Prinz, P. N.; Vitiello, M. V.; Hintz, R. L. Somatomedin-C levels in healthy young and old men: Relationship to peak and 24-hour integrated levels of growth hormone. J. Gerontol. 40:2–7; 1985.

Foster, J. A.; Rich, C. B.; Miller, M.; Benedict, M. R.; Richman, R. A.; Florini, J. R. Effect of age and IGF-1 administration on elastin gene expression in rat aorta. J. Gerontol. Biol. Sci. 45:B113–B119; 1990.

Froesch, E. R.; Zapf, J. Insulin-like growth factors and insulin: comparative aspects. Diabetologia 28:485–493; 1985.

Froesch, E. R.; Schmid, C. H.; Schwander, J.; Zapf, J. Actions of insulin-like growth factors. Ann. Rev. Physiol. 47:443–467; 1985.

Hintz, R. L.; Liu, F. Demonstration of specific plasma protein binding sites for somatomedins. J. Clin. Endocrinol. Metab. 45:988–995; 1977.

Hintz, R. L.; Liu, F.; Rosenfeld, R. G.; Kemp, S. F. Plasma somatomedin-binding proteins in hypopituitarism: changes during growth hormone therapy. J. Clin. Endocrinol. Metab. 53:100–104; 1981.

Hossenlopp, P.; Seurin, D.; Segovia-Quinson, B.; Hardouin, S.; Binoux, M. Analysis of serum insulin-like growth factor binding proteins using Western blotting: Use of the method for titration of the binding proteins and competitive binding studies. Anal. Biochem. 154:138–143; 1986.

Iwasaki, K.; Gleiser, C. A.; Masoro, E. J.; McMahan, C. A.; Seo, E. J.; Yu, B. P. Influence of the restriction of individual dietary components on longevity and age-related disease of Fischer rats: The fat component and the mineral component. J. Gerontol. Biol. Sci. 43:B13–B21; 1988.

Johanson, A. J.; Blizard, R. M. Low somatomedin-C levels in older men rise in response to growth hormone administration. Johns Hopkins Med. J. 149:115–117; 1981.

Kahler, L. W.; Gliessman, P.; Craven, J.; Hill, J.; Critchlow, V. Loss of

Downloaded from http://geronj.oxfordjournals.org/ at Middle Tennessee State University on March 23, 2015

enhanced nocturnal growth hormone secretion in aging rhesus monkeys. Endocrinology 119:1281–1284; 1986.
Kelley, K. W.; Brief, S.; Westley, H. J.; Novakofski, J.; Bechtel, P. J.; Simon, J.; Walker, E. B. GH_3 pituitary adenoma cells can reverse thymic aging in rats. Proc. Natl. Acad. Sci. USA 83:5666–5667; 1986.
Knauer, D. J.; Smith, G. L. Inhibition of biologic activity of multiplication stimulating activity by binding to its carrier protein. Proc. Natl. Acad. Sci. USA 77:7252–7256; 1980.
Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685; 1970.
Lowe, W. L.; Adamo, M.; Werner, H.; Roberts, C. T.; LeRoith, D. Regulation by fasting of rat insulin-like growth factor I and its receptor: effects on gene regulation and binding. J. Clin. Invest. 84:619–626; 1989.
Lund, P. K.; Moats-Staats, B. M.; Hynes, M. A.; Simmons, J. G.; Jansen, M.; D'Ercole, A. J.; Van Wyk, J. J. Somatomedin-C/Insulin-like growth factor-1 and insulin-like growth factor-2 mRNA's in rat fetal and adult tissues. J. Biol. Chem. 261:14539–14544; 1986.
Maes, M.; Underwood, L. E.; Ketelsegers, J. M. Plasma somatomedin-C in fasted and refed rats: close relationship with changes in liver somatogenic but not lactogenic binding sites. J. Endocrinol. 97:243–252; 1983.
Masoro, E. J.; Yu, B. P.; Bertrand, H. A. Action of food restriction in delaying the aging process. Proc. Natl. Acad. Sci. USA 79:4239–4241; 1982.
McCay, C. M.; Sperling, G.; Barnes, L. L. Growth, aging, chronic diseases and life span in rats. Arch. Biochem. Biophys. 2:469–479; 1943.
McCusker, R. H.; Clemmons, D. R. Insulin-like growth factor binding protein secretion by muscle cells: effect of cellular differentiation and proliferation. J. Cell. Physiol. 137:505–512; 1988.
McCusker, R. H.; Campion, D. R.; Jones, W. K.; Clemmons, D. R. The insulin-like growth factor-binding proteins of porcine serum: endocrine and nutritional regulation. Endocrinology 125:501–509; 1989.
Moses, A. C.; Nissley, S. P.; Passamani, J.; White, R. M. Further characterization of growth hormone-dependent somatomedin-binding proteins in rat serum and demonstration of somatomedin-binding proteins produced by rat liver cells in culture. Endocrinology 104:536–546; 1979.
Murphy, L. J.; Bell, G. I.; Friesen, H. G. Tissue distribution of insulin-like growth factor I and II messenger ribonucleic acid in the adult rat. Endocrinology 120:1279–1282; 1987.
Nakagawa, I.; Sasaki, A.; Kajimoto, M.; Fukuyama, T.; Suzuki, T.; Yanada, E. Effect of protein nutrition on growth, longevity, and incidence of lesions in the rat. J. Nutr. 104:1576–1583; 1974.
National Research Council. Nutrient requirements of laboratory animals, 3rd ed. Washington, DC: National Academy of Science; 1978.
Ocrant, I.; Pham, H.; Oh, Y.; Rosenfeld, R. G. Characterization of insulin-like growth factor binding proteins of cultured rat astroglial and neuronal cells. Biochem. Biophys. Res. Commun. 159:1316–1322; 1989.
Pluskal, M. G.; Moreyra, M.; Burini, R. C.; Young, V. R. Protein synthesis studies in skeletal muscle of aging rats. I. Alterations in nitrogen composition and protein synthesis using a crude polyribosome and pH 5 enzyme system. J. Gerontol. 39:385–391; 1984.
Prinz, P. N.; Weitzman, E. D.; Cunningham, G. R.; Karacan, I. Plasma growth hormone during sleep in young and aged men. J. Gerontol. 38:519–524; 1983.
Richardson, A. The relationship between aging and protein synthesis. In: Florini, J.R., ed. Handbook of biochemistry in aging. Boca Raton, FL: CRC Press, 1981:70–101.
Ricketts, W. G.; Birchenall-Sparks, M. C.; Hardwick, J. P.; Richardson, A. Effect of age and dietary restriction on protein synthesis by isolated kidney cells. J. Cell. Physiol. 125:492–498; 1985.
Rinderknecht, E.; Humbel, R. E. The amino acid sequence of human insulin-like growth factor I and its structure homology with proinsulin. J. Biol. Chem. 253:2769–2776; 1978.
Rosenfeld, R. G.; Pham, H.; Oh, Y.; Ocrant, I. Characterization of insulin-like growth factor-binding proteins in cultured rat pituitary cells. Endocrinology 124:2867–2874; 1989.
Rudman, D.; Feller, A. G.; Nagraj, H. S.; Gergans, G. A., et al. Effects of human growth hormone in men over 60 years old. N. Engl. J. Med. 323:1–6; 1990.
Schwander, J. C.; Hauri, C.; Zapf, J.; Froesch, E. R. Synthesis and secretion of insulin-like growth factor and its binding protein by the perfused liver in organ culture. Endocrinology 113:297–305; 1983.
Smith, G. L. Somatomedin carrier proteins. Mol. Cell. Endocrinol. 34:83–89; 1984.
Sonntag, W. E. Hormone secretion and action in aging animals and man. Rev. Biol. Res. Aging 3:299–335; 1987.
Sonntag, W. E.; Boyd, R. L. Chronic ethanol feeding inhibits plasma levels of insulin-like growth factor-1. Life Sci. 43:1325–1330; 1988.
Sonntag, W. E.; Steger, R. W.; Forman, L. J.; Meites, J. Decreased pulsatile release of growth hormone in old male rats. Endocrinology 107:1875–1879; 1980.
Sonntag, W. E.; Hylka, V. W.; Meites, J. Growth hormone restores protein synthesis in skeletal muscle of old male rats. J. Gerontol. 40:689–694; 1984.
Sonntag, W. E.; Boyd, R. L.; Booze, R. M. Somatostatin gene expression in hypothalamus and cortex of aging male rats. Neurobiol. Aging 11:409–416; 1990.
Suikkari, A. M.; Koivisto, V. A.; Rutanen, E. M.; Yki-Jarvinen, H.; Karonen, S. L.; Seppala, M. Insulin regulates the serum levels of low molecular weight insulin-like growth factor-binding protein. J. Clin. Endocrinol. Metab. 66:266–272; 1988.
Takahashi, G. S.; Meites, J. GH binding to liver in young and old female rats: relation to somatomedin-C secretion. Proc. Soc. Exp. Biol. Med. 186:229–233; 1987.
Takahashi, G. S.; Gottschall, P. E.; Quigley, K. L.; Goya, R. G.; Meites, J. Growth hormone secretory patterns in young, middle-aged and old female rats. Neuroendocrinology 46:137–142; 1987.
Towbin, H.; Staehelin, T.; Gordon, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. USA 76:4350–4354; 1979.
Underwood, L. E.; D'Ercole, A. J. Insulin and insulin-like growth factors/somatomedins in fetal and neonatal development. In: Daughaday, W. H., ed. Clinics in endocrinology and metabolism, tissue growth factors. Philadelphia: W.B. Saunders, 1984; 13:69–89.
Van Wyk, J. J.; Underwood, L. E.; Baseman, J. B.; Hintz, R. L. Explorations of the insulin-like and growth promoting properties of somatomedins by membrane receptor assays. Adv. Metab. Disord. 8:127–150; 1975.
Weindruch, R.; Walford, R. L. The retardation of aging and disease by dietary restriction. Springfield, IL: Charles C Thomas; 1988.
White, R. M.; Nissley, S. P.; Moses, A. C.; Rechler, M. M.; Johnsonbaugh, R. E. The growth hormone dependence of a somatomedin-binding protein in human serum. J. Clin. Endocrinol. Metab. 53:49–59; 1981.
Wilkins, J. R.; D'Ercole, J. Affinity-labeled plasma somatomedin-C/Insulin-like growth factor I binding proteins: evidence of growth hormone dependence and subunit structure. J. Clin. Invest. 75:1350–1358; 1985.
Yu, B. P.; Masoro, E. J.; Murata, I.; Bertrand, H. A.; Lynd, F. T. Life span study of SPF Fischer 344 male rats fed ad libitum or restricted diets: Longevity, growth, lean body mass and disease. J. Gerontol. 37:130–141; 1982.
Zadik, Z.; Chalew, S. A.; McCarter, R. J.; Meistas, M.; Kowarski, A. A. The influence of age on the 24-hour integrated concentration of growth hormone in normal individuals. J. Clin. Endocrinol. Metab. 60:513–516; 1985.
Zapf, J.; Froesch, E. R.; Humbel, R. E. The insulin-like growth factors (IGF) of human serum: chemical and biological characterization and aspects of their physiological role. Curr. Top. Cell Regul. 19:257–309; 1981.
Zapf, J.; Schmid, C. H., Froesch E. R. Biological and immunological properties of insulin-like growth factor-1 and insulin-like growth factor-2. In: Daughaday, W.H., ed. Clinics in endocrinology and metabolism, tissue growth factors. Philadelphia: W.B. Saunders, 1984; 13:3–30.

Received September 1, 1990
Accepted December 20, 1990

Downloaded from http://geronj.oxfordjournals.org/ at Middle Tennessee State University on March 23, 2015