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Effects of Aging and Dietary Restriction on Tissue Protein Synthesis: Relationship to Plasma Insulin-like Growth Factor-1

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Insulin-like growth factor-1 (IGF-1) decreases with age in many species and appears to have an important role in the age-related decline in capacity for protein synthesis with age. The goals of these studies were to determine whether (a) ad libitum fed mice demonstrate age-related decreases in IGF-1, (b) the relationship between IGF-1 and age-related changes in protein synthetic capacity in ad libitum fed animals, and (c) whether moderate dietary restriction (which increases both life span and protein synthetic capacity) delays age-related changes in protein synthesis and plasma IGF-1. These studies indicate that (a) in ad libitum fed animals, plasma IGF-1 decreases with age between 10 and 15 months and moderate dietary restriction decreases plasma IGF-1 in young but not older animals, and (b) the temporal changes in protein synthesis are tissue specific; moderate dietary restriction either increases or prevents the age-related decline in tissue protein synthesis. Results suggest that in normal aging, decreases in IGF-1 are associated with the decline in protein synthesis but that other regulatory mechanisms appear to have an important role in this process. Dietary restriction decreases plasma IGF-1 in young animals and either increases protein synthesis or prevents the age-related decline in protein synthesis, suggesting that the effects of dietary restriction are not mediated via increases in plasma IGF-1.

SEVERAL studies have clearly shown that aging is associated with a decline in the capacity of tissues to synthesize protein (Richardson, 1981). Although the biochemical mechanisms for this age-related decline have not been fully elucidated, recent data suggest that decreases in protein synthesis are not the result of intrinsic deficits in tissue but are mediated, in part, by a decrease in the secretion of anabolic hormones that regulate the rate of protein synthesis. Growth hormone, for example, is a potent anabolic hormone that stimulates the secretion of insulin-like growth factor-1 (IGF-1) from hepatic tissue. IGF-1 circulates in blood bound to various binding proteins and stimulates DNA synthesis, amino acid uptake, and protein synthesis in many tissues (Rinderknecht and Humbel, 1978; Zapf et al., 1981, 1984; Froesch and Zapf, 1985; Froesch et al., 1985). The hypothesis that growth hormone deficiency is responsible for the decline in protein synthesis with age has gained substantial support in recent years since (a) the amplitude of growth hormone pulses decrease with age in rodents (Sonntag et al., 1980; Sonntag, 1987), nonhuman primates (Kahler et al., 1986), and man (Prinz et al., 1983; Zadik et al., 1985; (b) administration of growth hormone to old animals increases IGF-1 concentrations (Johanson and Blizard, 1981; Sonntag, unpublished observations), and (c) growth hormone increases protein synthesis in skeletal muscle of aging rodents (Sonntag et al., 1984) and muscle mass, skin thickness, and vertebral bone density in elderly men (Rudman et al., 1990). Although these results suggest that a causal relationship exists between changes in protein synthesis and the decline in IGF-1 levels with age, there have been no reports comparing plasma IGF-1 and protein synthesis in mice and no comprehensive studies comparing the rate of decline in protein synthesis in different tissues from the same animal. If the general decline in protein synthesis with age is the result of a decrease in plasma IGF-1, this decline should occur prior to, or concomitant with, the decrease in tissue protein synthesis, and cells from different tissues should exhibit a uniform decline in protein synthetic capacity with age.

In recent years, there has been increased interest in the biological effects of moderate dietary restriction. A number of laboratories have reported that this regimen increases life span compared to ad libitum fed animals (McCay et al., 1943; Nakagawa et al., 1974; Yu et al., 1982; Iwasaki et al., 1988; Weindruch and Walford, 1988). Other recent studies suggest that dietary restriction increases protein synthesis in both kidney and liver (Birchenall-Sparks et al., 1985; Ricketts et al., 1985; Ward, 1988). Although the specific mechanism for the increase in protein synthesis after dietary restriction is unclear, the important role of IGF-1 in ad libitum fed animals and the profound effects of dietary restriction on the endocrine system suggest that these effects may be mediated through hormonal factors. Currently, there are no data on age-related changes in IGF-1 in mice, and the potential role of IGF-1 in the regulation of protein synthetic activity in dietary restricted animals has not been established.

This study was designed to assess two important issues: (a) Do ad libitum fed mice exhibit age-related declines in plasma IGF-1 and are these changes associated with the decline in protein synthesis in tissues from different organs? and (b) Does moderate dietary restriction increase protein synthesis in tissues, and are these changes associated with increases in plasma IGF-1 in aged animals?

Materials and Methods

Animals

Male C57/BL6 mice, ages 5, 10, 15, and 24 months, were obtained from the National Center for Toxicological Re-

search, Program on Caloric Restriction (NCTR, Jefferson, AK). Upon arrival at our institution, animals were housed in single cages in a specific pathogen free facility 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, which was initiated at NCTR at 14 weeks of age and continued until sacrifice, has been used successfully to increase life span (Figure 1) and to study the effects 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 this group were fed between 1100 and 1300h daily and maintained body weight throughout the study (average 22g) while ad libitum fed animals ranged from 35g (5 months) to 40g (24 months). After three weeks of adaptation to our facilities, animals were subdivided into two groups for study of protein synthesis (n = 5/group) or IGF-1 concentrations (n = 15/group) and sacrificed between 0900 and 1030h.

IGF-1 Radioimmunoassay

IGF-1 concentrations were measured in plasma after extraction as previously described (Sonntag and Boyd, 1988). Briefly, plasma was acidified, extracted in 10 volumes of petroleum ether, purified on a C-18 column (Prep-sep, Fisher Scientific, Atlanta, GA) and analyzed by a specific radioimmunoassay using antiserum obtained from the National Pituitary Program and NIDDK. Thr^{59}IGF-1 (Bachem, Inc., Torrance, CA) was radiolabeled with ^{125}I using a lactoperoxidase, glucose oxidase procedure (Sonntag et al., 1980). Data were expressed in relation to the IGF-1 standards. The minimum detectable dose of this assay was 17 pg/tube (80% B/B_0), and 50% inhibition of tracer binding was 60.5 pg/tube. The intra- and interassay coefficients of variation were 5.1% and 8.6%, respectively.

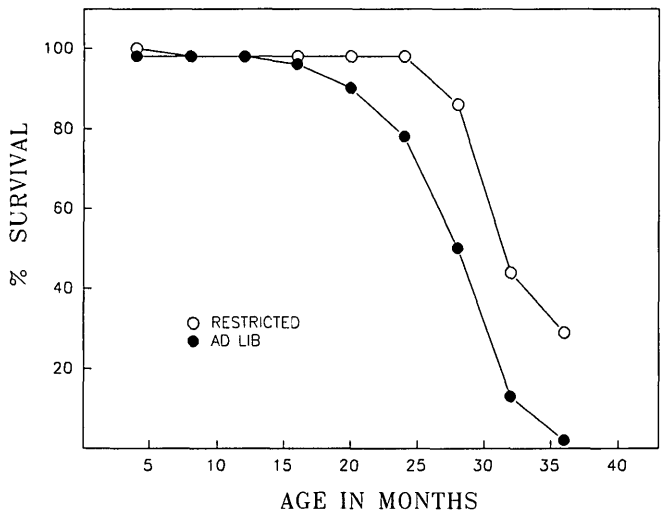

Figure 1. Effect of dietary restriction on life span of C57/BL6 male mice. Mean life span was 28 months in ad libitum fed mice and 33 months in dietary restricted animals. These data were summarized from information obtained from the Program on Caloric Restriction at The National Center for Toxicological Research and are used with permission. Data represent the life span beginning with 56 animals/group.

Protein Synthesis

General. — In vivo protein synthetic activity was determined by modification of procedures previously described (Garlick et al., 1980; Sonntag et al., 1984). Unanesthetized animals (5, 15, and 24 months of age; 5/group) were injected with ^{3}H-phenylalanine (150uCi with 3mM phenylalanine, ip) and 10 minutes later sacrificed by decapitation. Heart, liver, and diaphragm were excised, washed in saline, and frozen at −80 °C. Tissue was thawed, homogenized (Polytron, level 5, 20s) in 25mM Tris (pH 7.4), 10mM $MgCl_2$, and 25mM Sucrose and aliquoted for determination of incorporation into total protein or determination of free amino acid specific activity. DNA concentrations were determined using the diphenylamine procedure (Burton, 1956) and protein concentrations determined by the Bradford assay (Bradford, 1976).

Incorporation into total protein. — Aliquots of homogenate were pipetted onto Whatman GF/C filters (24mm) in triplicate and protein precipitated with 10% trichloroacetic acid (TCA) containing 100mM phenylalanine (10ml/disk). The TCA/phenylalanine solution was replaced, the solution heated to 90 °C followed by another cold TCA/phenylalanine wash. Disks were dehydrated with ethanol/ether followed by 100% ether and counted in a scintillation counter. DPM were calculated, averaged, and normalized to DNA concentrations.

Free amino acid specific activity. — Because of interconversion of phenylalanine to tyrosine, the specific activity of each amino acid was determined in cytosol after derivatization of primary amino acids with ophthalaldehyde/2-mercaptoethanol (OPA/ME) followed by reverse phase HPLC (Turnell and Cooper, 1982; Bidlingmeyer et al., 1984). Aliquots of homogenate were precipitated with equal volumes of acetonitrile and centrifuged at 16,000g (5 min). The supernatant was separated, dried in a speed-vac concentrator, and reconstituted with water. Iodoacetic acid and OPA/ME (Pierce Chemical Co., Rockford, IL) were added to the mixture and immediately injected onto the HPLC column using a 20 μl loop. Internal standards (homocysteic acid and homoserine) were included in each run. Derivitized amino acids were separated using a microsorb 5u C-18 column (250 × 4.6mm) with water/sodium propionate/acetonitrile (72/80/2) and water/acetonitrile/methanol/dimethyl sulfoxide (42/30/25/3) as the mobile phase. OPA amino acids were detected using a fluorescence detector (Beckman) with excitation and emission wavelengths of 230 and 418nM, respectively. Area under the curve and peak height were calculated using a Shimadzu integrator. Preliminary studies indicated clear separation of phenylalanine and tyrosine from other amino acids (Figure 2) and established dose-response curves for each amino acid. These curves were linear in the concentration range of phenylalanine and tyrosine found in cytosol. Specific activity was determined by dividing dpm in the

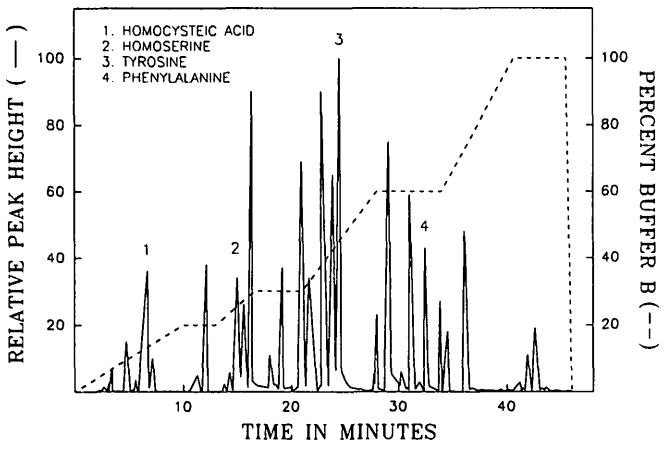

Figure 2. Separation of OPA derivitized amino acids by reverse phase HPLC. Amino acids were detected by fluorescence using excitation at 230 and emission at 418nm. Relative peak height for amino acids is expressed versus retention time. Dashed line represents percent buffer B. Phenylalanine and tyrosine eluted at 24.3 and 33.1 minutes, respectively.

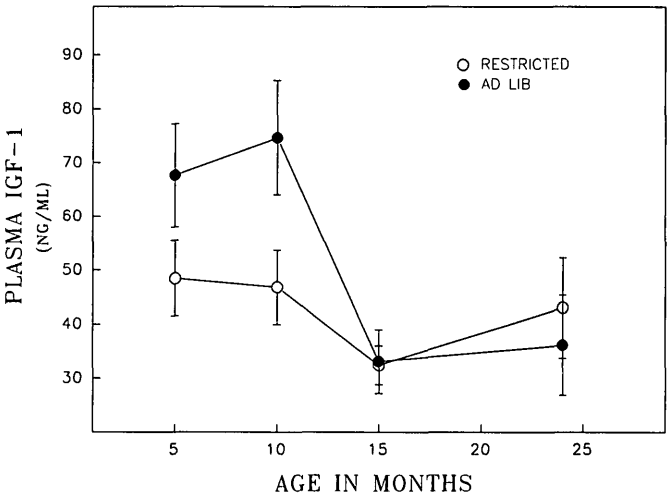

Figure 3. Age-related changes in plasma IGF-1 levels in ad libitum and dietary restricted animals. Values (n = 15/group) are expressed as mean ± *SEM*.

cytosol by the concentrations of phenylalanine and tyrosine (from integration of the area under each peak and correction to the standard curve).

Data Analysis

Results of the plasma IGF-1 study were analyzed by ANOVA (using a 2 × 4, Diet × Age design). Relative protein synthesis was measured as DPM/μg DNA corrected for free amino acid specific activity for tissue samples from heart, liver, and diaphragm. Data from the protein synthesis measures were analyzed by multivariate ANOVA using PC-SAS (SAS Institute, Cary, NC). Initial analysis of the later experiment revealed significant variance between error terms using the F_{max} test (Weiner, 1971); therefore, further analysis was done after log transformation of data. Multivariate ANOVA revealed significant effects for diet [$F(3,22) = 6.25$, $p < .05$] and Age × Diet [$F(3,23) = 2.95$, $p < .05$]. As the experiment-wise error rate was significant for the later experiment, univariate ANOVAs were analyzed (using a 2 × 3, Diet × Age design). The Duncan's Multiple Range Test was used subsequent to ANOVA when appropriate.

RESULTS

Insulin-like growth factor-1. — Analysis of plasma IGF-1 levels revealed a significant effect of both age [$F(3,89) = 2.97$, $p < .05$] and diet [$F(1,89) = 5.67$, $p < .05$]. In ad libitum fed animals, plasma levels of IGF-1 varied between 68 and 75ng/ml in 5- and 10-month-old animals (Figure 3) and, at 15 and 24 months of age, levels decreased by 56 and 52%, respectively, compared to 10-month-old animals ($p < .05$). Dietary restriction decreased IGF-1 levels by 30% in young animals ($p < .05$); however, the age-related decrease in IGF-1 was not apparent. At 15 and 24 months of age, no differences in plasma IGF-1 levels were observed between old ad libitum fed and dietary restricted animals.

Relative protein synthesis. — A significant main effect of age [$F(2,23) = 4.62$, $p < .05$] was observed for protein

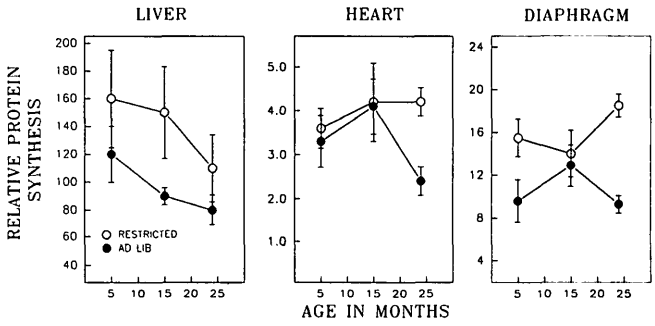

Figure 4. Age-related changes in relative protein synthesis in liver, heart, and diaphragm of ad libitum fed and dietary restricted animals. Protein synthesis was calculated as incorporation into total protein/μg DNA and corrected for the specific activity of the amino acid precursor. Values represent mean ± *SEM* for 5 animals/group.

synthesis in liver, but neither the effects of diet [$F(1,23) = 2.46$, $p = .107$] nor the interaction of Age × Diet [$F(2,23) = 1.11$, $p = .35$] was significant. Specific comparisons between treatment groups revealed a significant decrease in ad libitum fed animals with age (5 vs 24 months, $p < .05$). Moderate dietary restriction increased protein synthesis in liver in all age groups; however, only the differences at 15 months reached statistical significance (ad libitum vs dietary restricted, $p < .05$).

In the heart, the main effect of diet was statistically significant [$F(1,23) = 4.29$, $p < .05$] but the effect of age and the interaction of Age × Diet were nonsignificant [$F(2,23) = 2.56$, $p = .09$; $F(2,23) = 2.06$, $p = .15$, respectively]. Protein synthesis decreased with age in 24-month-old ad libitum fed compared to 15-month-old animals ($p < .05$, Figure 4). Dietary restriction had little effect on protein synthesis at either 5 or 15 months of age, but the decrease in protein synthetic capacity between 15 and 24 months was completely prevented by dietary restriction (ad libitum vs dietary restricted at 24 months, $p < .05$).

Analysis of data from protein synthesis in diaphragm revealed a significant effect for diet [$F(1,23) = 13.61$, $p <$

.005]; however, neither the age nor the Age × Diet interaction reached statistical significance [$F(2,23) = .30, p = .75$; $F(2,23) = 3.35, p = .07$, respectively]. In ad libitum fed animals, protein synthetic rate increased between 5 and 15 months before declining and, by 24 months, rates were similar to those observed at 5 months of age. None of the latter changes reached statistical significance. Significant increases in protein synthesis were observed at both 5 and 24 months of age in diaphragm in response to dietary restriction ($p < .05$). As in other tissues, dietary restriction prevented the reduction in protein synthesis observed between 15 and 24 months.

Discussion

The results of our study provide clear evidence that insulin-like growth factor-1 decreases with age in ad libitum fed mice. Previous studies in several other rodent strains (Roberts, 1979; Sonntag et al., 1980; Florini et al., 1981; Breese et al., 1991) as well as nonhuman primates (Kahler et al., 1986) and man (Johanson and Blizard, 1981; Rudman et al., 1981; Prinz et al., 1983; Zadik et al., 1985) have indicated that the amplitude of growth hormone pulses decreases with age, resulting in a decline in plasma levels of IGF-1. Since IGF-1 is a potent anabolic hormone, it was hypothesized that a causal relationship may exist between the age-related decrease in IGF-1 and the well-known decline in protein synthetic capacity with age (Richardson, 1981). Subsequent studies supported this hypothesis since it was found that administration of growth hormone increased plasma IGF-1 levels in man (Johanson and Blizard, 1981; Rudman et al., 1990) and total protein synthesis in rat skeletal muscle (Sonntag et al., 1984). In addition, IGF-1 administration to aging rats increases cardiac heavy chain myosin (Florini and Ewton, 1989) and restores aortic elastin levels (Foster et al., 1990), suggesting that IGF-1 may have effects on specific proteins that decline with age. Therefore, age-related decreases in IGF-1 have been found in all mammals tested to date and appear to be an important correlate of the aging process.

Part of the hypothesis examined in this series of studies was that the age-related decreases in protein synthesis are regulated by plasma IGF-1. If the declines in growth hormone and IGF-1 were the primary factors contributing to the decrease in protein synthesis, a coordinate decline in protein synthesis would be expected in all tissues between 10 and 15 months. Our results, however, indicate that age-related decreases in protein synthesis in tissues are highly individualized, occurring at various rates and times that are unique to the tissue being studied. For example, heart demonstrates minor changes in protein synthetic capacity between 5 and 15 months but thereafter demonstrates a substantial decline; diaphragm protein synthesis is relatively stable throughout life, whereas protein synthesis in liver steadily declines beginning at 5 months of age. Although the specific mechanisms for the variation in protein synthesis between tissues are unclear, they most likely reflect functional requirements of the growing organism and the paracrine actions of several growth factors. In addition to intrinsic mechanisms within the cell that have an important role in maintaining protein synthetic capacity, it is well-known that cells produce both IGF-1 and type 1 IGF receptors as well as other growth factors that contribute to the regulation of tissue protein synthesis. Since total protein synthesis within tissues represents the result of a coordination of intrinsic cellular regulation with paracrine regulation, as well as endocrine regulation, the decline in plasma IGF-1 alone cannot be responsible for the characteristic age-related decline in tissue protein synthesis. Rather, other endocrine, paracrine, and intrinsic mechanisms appear to compensate for the decline in plasma IGF-1. Our data suggest that there may be a differential ability of tissues to compensate for the decline in IGF-1 or differences in the signaling mechanisms which regulate protein synthesis in response to decreases in IGF-1. Therefore, in ad libitum fed animals, plasma IGF-1 appears to be only a single component of a complex series of events which regulate tissue protein synthesis.

In agreement with previously published data (Birchenall-Sparks et al., 1985; Ricketts et al., 1985; Ward, 1988), we have found that dietary restriction increases protein synthesis in a number of tissues. In both liver and diaphragm, increases were evident at multiple ages, whereas in cardiac tissue, dietary restriction appeared to prevent the age-related decrease in protein synthesis between 15 and 24 months. The specific mechanisms responsible for the increases in protein synthesis after moderate dietary restriction are unknown. Severe dietary restriction decreases plasma IGF-1 (Emler and Schalch, 1987; Lowe et al., 1989; McCusker et al., 1989), and we hypothesized that moderate dietary restriction may elevate or prevent the age-related decline in plasma IGF-1, resulting in increased protein synthesis. In the present study, plasma levels of IGF-1 decreased by approximately 30% in young animals, but levels were stable thereafter and the age-related decline in IGF-1 was not evident in animals on this feeding regimen. At later ages, there were no differences between IGF-1 concentrations in ad libitum fed or dietary restricted animals. Since our study indicated that IGF-1 levels were reduced in younger animals and no differences were observed at older ages, it appears that plasma IGF-1 levels alone cannot account for the increased protein synthetic capacity in dietary restricted animals. Our results suggest that the effects of dietary restriction on tissue protein synthesis are mediated by processes other than regulation of plasma IGF-1.

Since there is strong evidence linking age-related changes in protein synthesis with decreases in plasma IGF-1 in ad libitum fed animals, it is possible that moderate dietary restriction increases protein synthesis by increasing the paracrine activity of IGF-1 or enhancing IGF-1 mediated events in tissue. Several studies have reported that IGF-1 is produced by both peripheral tissue (Binoux et al., 1981; D'Ercole et al., 1984; Murphy et al., 1987) and vasculature (Hansson et al., 1989; Delafontaine et al., 1991) and exerts paracrine effects either directly or by interaction with other growth factors. The actions of IGF-1 are mediated by binding to specific type 1 IGF receptors which regulate intracellular processes through a tyrosine kinase dependent mechanism (Chernausek et al., 1981; Czech, 1982). Additional studies will be necessary to analyze the effects of aging and moderate dietary restriction on tissue IGF-1 levels, type 1 IGF receptors, and IGF regulated tyrosine kinase activity.

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