

THE EFFECT OF VARYING LEVELS OF DIETARY PROTEIN ON
CARCASS COMPOSITION OF ELEVEN- AND EIGHTEEN-MONTH-OLD
MALE RATS

By

Lisa K. Linley

Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University in
partial fulfillment of the requirements for the degree
of

MASTER OF SCIENCE

in

Human Nutrition and Foods

Approved:

S.J. Ritchey, Chairman

R.E. Webb

G.E. Bunce

May, 1988

Blacksburg, Virginia

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(ABSTRACT)

Carcass composition of male Sprague-Dawley rats, aged 11 and 18 months, in response to varying levels of dietary protein was determined. Groups of ten rats of each age were fed diets containing from 1.53 to 8.05 percent protein as casein supplemented with d-l-methionine for five weeks. The 8.05% protein groups were used as controls. Carcasses were analyzed for total nitrogen and percent protein, fat, and water. Liver composition and total serum protein values were also determined. Two-way analysis of variance and Student's t-tests were used to determine significant age and diet effects. Differences in the response of the two age groups of rats were evident. Eighteen-month-old rats required more protein than the younger animals for the maintenance of body weight.

When compared to control values, older rats also needed a higher level of dietary protein to maintain normal total carcass nitrogen. Fatty livers in older rats persisted at higher dietary protein levels than fatty livers in 11-month-old rats, indicating that 18-month-old rats required more protein to support adequate liver lipoprotein synthesis. These findings suggest that 18-month-old rats have a higher dietary protein requirement than 11-month-old rats. High serum protein values for older rats at lower protein levels, however, do not support this conclusion. The increased body weight and proportionally greater fat mass of older animals was a complicating factor in this study. Further research is needed to more clearly define changes in protein requirements during aging. For future studies, using rats of a more advanced age and three, rather than two, different age groups is recommended.

ACKNOWLEDGEMENTS

The author wishes to express sincere thanks and appreciation to Dr. S.J. Ritchey, major professor, for his advice and encouragement throughout the completion of this project. The author also wishes to thank the following people:

Dr. R.E. Webb for his valuable recommendations.

Dr. G.E. Bunce for his suggestions, encouragement, and understanding.

Leslie K. Reynolds for sharing her expert knowledge of laboratory procedures, and for being there to provide advice, assistance, and support throughout all phases of this study.

Dr. M. Lentner and Tesa Leon for assistance with statistical interpretation of data.

Suzanne Linley for inspiring in the author the motivation and confidence to pursue her educational goals.

Lastly, special thanks are extended to the author's fiance, Wes Brubacher, whose patience and moral support made possible the completion of this work.

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INTRODUCTION

Aging is a complex process during which many structural and functional changes occur (Masoro, 1976). With advancing age in humans, a decline in lean body mass, which comprises the bulk of actively metabolizing body tissue, has been observed (Forbes and Reina, 1970). Whole body protein synthesis and degradation rates have been reported to be lower in elderly compared to young adult individuals (Winterer et al., 1976). Total body potassium content has also been found to decrease with age, indicating a decline in body cell mass and total body protein content. It appears that the intensity of whole body protein metabolism in man declines throughout life (Young et al., 1975).

These age-related changes in protein metabolism suggest that dietary protein needs may change with advancing age in humans. Other factors, such as decreased basal metabolic rate, decreased physical activity, increased prevalence of diseases and disabilities, and a decreased energy intake with advancing age would also tend to alter protein needs (FAO/WHO, 1973). Studies to assess the protein needs of older adults have yielded conflicting results. Protein requirements of the

elderly have been reported to be lower (Scrimshaw et al., 1976), higher (Uauy et al., 1978a), and the same as (Cheng et al., 1976) those of young adults.

The adequacy of the current Recommended Dietary Allowance (RDA) for meeting the protein needs of the elderly has recently been evaluated (Gersovitz et al., 1982). These researchers report that the current National Research Council (NRC) RDA of 0.8g of protein per kg body weight per day for ages 51 and older was not sufficient to maintain positive nitrogen balance in many men and women 70 years or older. It was concluded from this study that specific allowances for protein may be needed for age groups within the broad 51 years and older category used by the NRC. The elderly now comprise at least 10% of the population and this percentage is expected to increase. Further research to assess the actual protein requirements of older individuals seems justified (Baldwin and Griminger, 1985).

Rats are being heavily relied upon as model systems for the study of aging (Gibson et al., 1979). Like humans, rats undergo age-related changes in body composition and physiological systems which may alter protein needs. Both a decline (Fujita and Ichikawa, 1986) and no change (Lesser et al., 1973) in rat lean body mass with increasing age have been reported. An age-related

decrease in the rates of protein synthesis of muscle (Pluskal et al., 1984), liver (Cook and Buetow, 1981), kidney (Ricketts et al., 1985) heart (Geary and Florini, 1972), and brain tissue (Ekstrom et al., 1980) have been found. These findings suggest that maintenance protein requirements may decrease with increasing age in rats.

The maintenance protein requirement for adult rats has been widely investigated (Bricker and Mitchell, 1947; Hartsook and Mitchell, 1956; Sheehan et al., 1981). This requirement level has been estimated to be in the range of 3.18 (Hartsook and Mitchell, 1956) to 4.07% (Sheehan et al., 1981) dietary protein as casein supplemented with methionine. The National Research Council currently recommends 4.8% casein plus methionine as adequate for tissue maintenance in adult rats.

Very few studies have investigated changing maintenance requirements for protein with progressing age towards senescence. In the studies which have investigated the protein needs of old or senescent rats in comparison to those of young mature animals, conflicting reports have appeared. Fischer and Canolty (1983) found that 25-month-old rats had lower mean maintenance requirements for protein than 15-month-old rats. Baldwin and Griminger (1985), however, report that old rats (24 months) required more protein than mature (12

months) animals for the maintenance of nitrogen equilibrium. The nature and extent of the effect of aging on the protein requirement of rats has not been clearly demonstrated.

The objective of this study was to further clarify the relationship between protein needs and aging in the rat. Total carcass nitrogen and percent carcass protein, water, and fat content were determined for mature (11-month-old) and old (18-month-old) male rats fed diets containing 1.53 to 8.05% casein supplemented with methionine for five weeks. Liver composition and total serum protein values were also determined. Two-way analysis of variance and Student's t-tests for pairwise comparisons were used to determine the effects of age and varying levels of dietary protein on carcass composition of each age group of rats.

REVIEW OF LITERATURE

Body Composition Changes During Aging Important in the Determination of Protein Needs

Age-associated change in body composition and alteration of physiological systems may have a considerable impact on protein metabolism in man and other organisms (Masoro, 1980). Lean body mass (LBM) and total body water and fat content are body constituents thought to be altered during aging (Forbes and Reina, 1970; Lesser et al., 1980; Gommers et al., 1983). It is generally believed that a loss of muscle mass accompanies aging (Gutmann and Hanzlikova, 1976).

Several studies have been conducted to investigate the relationship between age and lean body mass (Forbes and Reina, 1970; Lesser et al., 1973; Yu et al., 1982). In a small longitudinal study, Forbes and Reina (1970) used ^{40}K to estimate the LBM of six adult males. Estimates were calculated based on the assumption that LBM potassium content remains constant. In four of the six subjects studied, a gradual decline in LBM with increasing age was observed. In one subject a slight increase was seen, and for another the results were difficult to interpret.

Forbes and Reina compared their results to those of other longitudinal and cross-sectional studies, comprising a total of almost 9000 male and 3000 female human subjects. For males, there was a consistent trend with LBM highest in the third decade, followed by two decades of gradual decline, after which a more rapid decline was seen. For females, a consistent but less rapid rate of decline was observed. This slower rate of decline is not surprising considering that the LBM of adult females is only two-thirds that of adult males. A progressive increase in body fat content was found to accompany the decline in LBM.

In a conflicting study, Lesser and Markofsky (1979) have reported that the fat-free body mass (FFB) of elderly men and women, aged 60 to 80 years, was comparable to that of young adults of the same height and sex. In this study, FFB was estimated from in vivo measurements of body fat. Disagreement among findings may result from the wide variation in subjects and methodologies used in human studies to determine LBM changes during aging.

Yu et al. (1982) report a life span study of specific pathogen free (SPF) Fischer 344 male rats fed ad libitum or restricted diets. By subtracting total fat mass from total body mass, the LBM of each rat was

determined several times during its life span. Fat mass was indirectly determined by measuring the uptake of cyclopropane, an inert, highly fat-soluble gas. In rats fed ad libitum, LBM increased for the first 75% of the life span, after which it remained unchanged. The mean life span of these animals was approximately 23 months. In the six longest-lived rats, a decline in LBM was observed subsequent to 90% of the life span, which averaged approximately 26 months.

These results are in agreement with Lesser et al. (1973) who report that LBM remains stable in male Sprague-Dawley rats into senescence. Lesser et al. suggest that the decline in LBM observed in very late age results from the onset of the terminal disease process. In contrast to these findings, Fujita and Ichikawa (1986) have reported decreased urinary creatinine excretion in aging Wistar rats. This decreased creatinine excretion is indicative of skeletal muscle loss. The rats in this study, however, were fed moderately restricted diets (80% ad libitum) and McCarter et al. (1982) have pointed out that the muscle mass of aging rats is greatly influenced by food intake.

Age-related changes in individual muscle composition and mass have been widely investigated (Mohan and Radha, 1975; Yu et al., 1982; McCarter et al., 1982). In

their life span study, Yu et al. (1982) measured the mass and protein and phospholipid content of the gastrocnemius muscle of rats ranging in age from 6 to 27 months. Gastrocnemius muscle mass reached a maximum by 12 months of age and markedly declined after 18 months. Protein content significantly declined between 12 and 27 months, while phospholipid per gram of muscle remained unchanged. A decrease in total protein per unit wet weight in red and white skeletal muscle with increasing age in Wistar strain rats has been reported (Mohan and Radha, 1975). Lesser et al. (1980), however, found no age-related change in the protein content or mass of the psoas muscle in male Sprague-Dawley rats.

Age-related change in the composition of the lateral omohyoideus (LOMO) muscle in adult rats was investigated by McCarter et al. (1982). Muscle fiber diameter was found to decrease with increasing age from 6 to 27 months, but the number of fibers increased sufficiently to prevent a change in muscle mass. Tauchi et al. (1971) report that the number of red muscle fibers, rather than white, decreases with age in the tibialis anterior muscle of rats. The LOMO is composed primarily of white muscle fibers (McCarter et al., 1982).

Because of the varying effects of age on individual muscle mass, it is not possible to interpret individual

muscle mass changes in terms of LBM gain or loss. The LOMO may not be representative of the majority of rat skeletal muscles because it is used throughout the life of the rat. Reports that the LBM of rats does not decline with age (Lesser et al., 1982; Yu et al., 1982) indicate that hindleg muscles, rather than the LOMO or psoas, may be unique. Hindleg muscles such as the gastrocnemius are susceptible to a decline in functional activity resulting from decreased locomotion with age (McCarter et al., 1982). This decreased activity and not an inherent effect of age may be responsible for the loss of mass observed in hindleg muscles. Yu et al. (1982) suggest that the loss of individual muscle mass would be compensated for by an increase in the mass of other muscles, organs such as viscera, or extracellular volume.

Lesser and his group (1980) have conducted an in-depth investigation of the compositional changes in fat-free body mass (FFB) during aging in male Sprague-Dawley rats. Age-related changes in intracellular, extracellular, and total body water content were also determined. Representative animals were sacrificed for analysis at about 100 day intervals from 297 to 888 days of age. Total FFB increased 8% from 362 to 579 days, remained stable from 579 to 715 days, and decreased thereafter.

This decrease in late age was not attributed to an age-related loss of lean tissue, but to selective longevity of smaller animals (Lesser et al., 1973).

The proportion of total skeletal muscle and total fat-free skin comprising FFB remained constant. Fat-free masses of visceral organs (heart, kidneys, lungs, liver, brain, spleen, and testes) increased from 362 to 518 days, then remained stable. Extracellular water (ECW) increased significantly ($p < 0.02$) from 362 to 579 days. This increase was, for the most part, accounted for by alterations in the masses and extracellular volumes of individual tissues. Mean ECW at later ages was not significantly different. No significant trend in intracellular water (ICW) content was observed. Total body water increased parallel to FFB from 362 to 579 days, then remained stable.

Total carcass nitrogen content was determined by a micro-Kjeldahl method, with protein calculated as $N \times 6.25$. Protein/FFB was 182, 198, and 196 g/kg at 297, 421, and 518 days, respectively. At 715, 800, and 888 days values for protein/FFB were 194, 189, and 184 g/kg, respectively. These values were not significantly different from each other. A uniquely high value of 210 g/kg was observed at 638 days. This value was significantly different from those determined at any other age.

Whether this value represented an age-related phenomenon or an isolated finding is unknown. Lesser et al. conclude from these findings that body cell mass in healthy rats is maintained into senescence.

Bertrand et al. (1980) examined the change in adipose mass and cellularity throughout the life span of male Fischer 344 rats. The epididymal and perirenal fat depots were greatest in 18-month-old animals and decreased at older ages. The decrease in adipose mass resulted from a decreased mean adipocyte volume, not a reduction in the number of adipocytes. The decline in adipose mass could not be related to a general loss of body mass since, in some rats, body mass continued to increase while fat mass declined. In a similar study (Gommers et al., 1983), morphological alterations of epididymal tissue in aging male Wistar rats were determined. Body weight of 30-month-old animals was significantly lower ($p < 0.05$) than 6- and 24-month-old rats. This decreased body weight corresponded to a loss of fat weight, particularly epididymal adipose tissue weight.

Consistent with the findings of Bertrand et al. (1980), the total number of adipocytes remained constant, but adipocyte volume decreased. These researchers, in agreement with Lesser et al. (1980), propose that smaller body size may have contributed to

longevity (Lesser et al., 1973). The mortality rate of animals in this study was 90% at 30 months of age, with an average life span of 22 months of age.

Age-related Alterations in the Rates of Protein Synthesis and Degradation

In addition to age-related changes in body composition, alterations in protein biosynthesis may play a role in the aging process. Most studies have reported a decline in the rates of protein synthesis with advancing age (Pluskal et al., 1984; Bailey and Webster, 1985; Geary and Florini, 1972). The effect of age on skeletal muscle protein synthesis has been studied using a cell-free system containing polyribosomes and pH 5 enzyme fraction (Pluskal et al., 1984). The cell-free systems were isolated from mixed hindleg muscles of young (2 months), mature (12 months), and aged (22 to 24 months) Sprague-Dawley rats. Total muscle protein content continued to increase from growth through maturity and senescence. This increase in total protein resulted in a decreased RNA concentration, probably through a dilution of ribosomes in the muscle cell.

Polyribosome protein synthesis activity was reduced by 64% in aged and 20% in mature rats compared to young

animals. No significant difference, however, was found between the polyribosome activity of mature versus aged animals. The aminoacylation activity of the pH 5 enzyme material decreased from young to mature animals, but no significant further decrease was observed between the mature and aged groups. The researchers conclude that the age-related decline in the efficiency of protein synthesis is associated with both the ribosome and soluble fractions of the cell.

As a follow-up study, Burini et al. (1984) used 0.5M potassium chloride (KCL) to wash polyribosomes in order to remove non-ribosomal fractions from the cell-free preparation. The ability of these salt wash fractions from young, mature, and aged animals to stimulate protein synthetic activity of young cell-free salt washed polyribosomes was determined. Mature and aged salt wash fractions were found to have a significantly lower stimulatory effect ($p < 0.05$) than young fractions, thus confirming the results of Pluskal et al. (1984). These findings also indicate that the decline in protein synthesis with increasing age may be related to changes in initiation/elongation factor activity since these factors are found in the non-ribosomal protein fraction of the cell.

Ricca et al. (1978) measured the uptake of L-[³H]

valine by hepatocytes from 1.5- and 18-month-old female Sprague-Dawley rats. Protein synthesis by isolated hepatocytes was found to decrease by 64% during this age span, with the decline occurring primarily between the ages of 6 and 12 months. Coniglio et al. (1978) used [³H] valine incorporation into protein to measure the rate of protein synthesis by intact liver parenchymal cells isolated from male Fischer F344 rats ranging in age from 2 to 30 months. A 44% decrease in the rate of protein synthesis was observed from 2.5 to 18 months of age, after which a slight increase (18%) was observed from 18 to 30 months of age. The researchers propose that this increase in late age may be related to the role of the liver in serum protein synthesis.

Similar results were reported by Van Bezooijen et al. (1977) who found protein synthesis by isolated rat liver parenchymal cells to decrease from 3 to 12 months, remain constant from 12 to 24 months, and increase from 24 to 36 months of age. These researchers propose that the increase in late age may result from increased proteolysis or compensation by the liver for elevated excretion of protein caused by renal insufficiency.

Ricketts et al. (1985) found the rate of protein synthesis by suspensions of rat kidney cells to decline 63% between 4 and 23 months of age with no significant

continuing decline from 23 to 31 months. Proteinuria increased over six fold between 5 and 35 months, thus supporting the conclusion of Van Bezooijen et al. (1977) that increased liver protein synthesis by very old rats may result from a more pronounced proteinuria.

Bailey and Webster (1984) measured the rate of protein synthesis in isolated mitochondria from livers and kidneys of female C57BL/6J mice of various ages. Mitochondria from livers of 23- to 27-month-old mice showed a 48% decrease in the rate of protein synthesis when compared to mitochondria from 6-month-old mice. Protein synthesis by kidney mitochondria was greater than that of liver mitochondria but an age-related decrease in synthesis rate was observed. Between 3 and 6 months, a 30% decrease occurred and a further 22% decrease was seen between 6 and 23 to 27 months of age. These results demonstrate that there is an age-related decline in protein synthesis by the translational system of the mitochondria as well as by the cytoplasmic ribosomal system.

Cook and Buetow (1981) used an in vitro protein synthesizing system consisting of adult (10- to 13-month-old) or senescent (24- to 30-month-old) rat liver polysomes, transfer RNAs, and aminoacyl transfer RNA synthetases to compare liver protein synthesis of these two age groups. The rate and extent of protein

synthesis were decreased by 44% in the senescent rat liver system. Decreased protein synthesis by the senescent system was not due to a change in protease and/or ribonuclease activities, levels of free amino acids, or in the proportion of messenger RNA attached to ribosomes.

In a study to clarify the mechanism by which protein synthesis is reduced in old cell-free preparations, Sojar and Rothstein (1986) used a system from rat liver which utilizes monosomal ribosomes and poly(U). Single ribosomes were used to control for differences in the number and aggregation of ribosomes between young and old preparations. In this study, preparations from young (8 to 10 month) and old (28 to 30 month) male Fischer rats were used.

Mixing experiments were conducted in which high salt extracts of young and old polysomes were added to both young and old unwashed ribosomes. The results indicated that part of the age-related decrease in cell-free protein synthesis may be due to a soluble factor which is present in young, but deficient in old ribosomal extracts. While this factor has not been identified, it does not appear to be one of the components involving initiation or elongation.

The effect of age on total protein synthesis in

hearts of C57Bl/6J mice has been investigated by Geary and Florini (1972). The rate of protein synthesis was measured by ^3H -leucine incorporation into protein in isolated perfused hearts. When incorporation data were corrected for variations in the unlabeled leucine pool, protein synthesis was found to decrease slightly from 1 to 2.8 months, markedly increase from 8 to 9 months, then decrease substantially in animals 25 to 27 months of age. The rate of protein synthesis in the oldest animals, while 50% lower than that of 8- to 9-month-old mice, was still higher than that of the 1- to 2.8-month-old mice.

This study demonstrates the importance of the age groups of animals used in experimental research. Different conclusions could have been made, that myocardial protein synthesis increases or decreases with age, depending upon whether the oldest mice were compared with the 2.8- or 9-month-old age groups. Geary and Florini suggest that the decrease in cardiac protein synthesis from adult to old mice may represent a loss of ability of the heart to respond to stress or damage through replacement of heart proteins.

A more recent study has supported the findings of Geary and Florini (Starnes et al., 1983). Using labeled phenylalanine and a method which eliminates the uncer-

tainty of precursor metabolism and pool size, Starnes et al. (1983) measured the rates of protein synthesis in isolated perfused hearts of 9- and 25-month-old Sprague-Dawley rats. The rate of cardiac protein synthesis was found to be significantly lower ($p < 0.01$) in the aged rats when compared to the younger adult animals.

Alteration in brain protein synthesis during senescence in male Fischer 344 rats has been investigated (Ekstrom et al., 1980). Brains obtained from 6- to 30-month-old rats were assayed for protein synthesis using a cell-free system derived from the post mitochondrial supernatant (PMS). The cell-free protein synthetic activity of the brain decreased by 56% between the ages of 6 and 32 months. The greatest decrease occurred from 6 to 14 months of age. The decreased protein synthetic rate could not be attributed to an increase in ribonuclease activity, but the biological activity of ribosomes obtained from 6-month-old rats was greater than that obtained from 14- to 28-month-old rats. The researchers concluded that the decrease in cell-free brain PMS protein synthesis is partially associated with the ribosome.

Studies comparing in vivo rates of protein synthesis during aging have also been reported (Kanugo et al., 1970; Ove et al., 1972). Kanugo et al. (1970) found

decreased protein synthesis by the skeletal muscle and brain of old rats in vivo. Liver RNA synthesis in vivo also decreased with increasing age. A conflicting study by Ove et al. (1972) reports no difference in the in vivo incorporation of [^{14}C] leucine into protein by livers of 1- and 17-month-old rats.

The slowing of protein synthesis with age may result in an increase in the half-life of proteins in older cells (Reff, 1985). Unfortunately, limited studies are available on changes in the rate of protein degradation or half-lives of proteins during aging in higher organisms. Protein degradation rates in female C57BL/6J mice aged 4, 18, and 28 months were determined using $\text{NaH}^{14}\text{CO}_3$ (Lavie et al., 1982). An age-related increase in the half-lives of short-lived proteins in the nuclear, mitochondrial, lysosomal, 100,000g supernatant, and total acid-precipitable proteins was found.

A decreased rate of protein degradation with increasing age has been reported for rat skeletal muscle (Millward, 1978). Meerson et al. (1978) found that cardiac protein degradation was significantly lower in rats 23- to 24-months-old compared to 3- to 4-month-old rats. In contrast, Barrows and Roeder (1961) found no age-related change in cardiac protein degradation between 12- and 24-month-old rats. Neither of these studies

accounted for the possible reutilization of labelled amino acids.

Menzies and Gold (1971) have reported no age-dependent change in the half-life of mitochondrial protein in liver, testes, brain, intestine, kidney, or lung of rats aged 12 to 24 months. As reported by Makrides (1983), Wiederanders (1981), using the $\text{NaH}^{14}\text{CO}_3$ -pulse labelling method, found a 50% increase in the half-lives of liver cytosol proteins in rats 21- and 27-months-old compared to 4- to 5-month-old animals. In this study, lung and ovary tumors were present in some of the older animals and may have affected results. The various methods of analysis used in these studies may have contributed to the contradictory findings reported. Makrides (1983) proposes that altered endocrine status of the animals used, rather than defective protein degradative pathways, could have caused some of the age-related alterations in protein degradation rates.

It should be noted that there are shortcomings associated with both in vitro and in vivo studies involving protein synthesis and degradation rates during aging. In vitro experiments may not be representative of changes occurring in the whole body. For example, cell-free protein synthesis by the liver is only 1% of the rate in vivo. On the other hand, it is difficult to

control experimental variables in whole animals for in vivo studies (Coniglio et al., 1979).

Age-associated alteration of total body protein turnover may be more effective for predicting protein needs than changes in protein synthesis of individual tissues. There is a limited amount of literature available concerning changes in whole body protein metabolism during aging (Young et al., 1975; Winterer et al., 1976; Uauy et al., 1978; Fujita and Ichikawa, 1986). Young et al. (1975) studied total human protein synthesis in relation to protein requirements of young male and female adults (20 to 23 years) and elderly females (69 to 91 years). A constant isotope method using ^{15}N labelled glycine was used to estimate whole body protein synthesis. Protein synthesis, per unit of body weight, by elderly subjects was 63% that of the young adults. This decrease was partially attributed to age-related changes in body composition.

Mean total body protein synthesis in relation to energy intake and dietary protein allowances was determined. When dietary protein allowances (g/kg/day) for the two groups, 0.57 for adults and 0.42 for elderly, were compared to the decline in intensity of whole body protein metabolism, no difference was found in the amount of utilizable protein required to support body

protein synthesis. These results suggest that the efficiency of utilization of total dietary nitrogen is similar for these two age groups, and that the variation in protein needs, per kg body weight, is related to the amount of protein synthesized per unit time.

Winterer et al. (1976) used ^{15}N -glycine infusion to estimate rates of total body protein synthesis and degradation in young adult (18 to 25 years) and elderly (65 to 91 years) subjects. The rates of protein synthesis, expressed per unit body weight were lower for females than for males. Elderly females had significantly lower ($p < 0.1$) rates of synthesis and breakdown than young women. The differences between young and elderly men, however, were not significant.

The effect of age-related changes in body composition on whole body protein turnover were also determined. Urinary creatinine excretion was used as an index of muscle mass. Lower values for the elderly versus young subjects were indicative of a decline in muscle mass with aging. When expressed per unit creatinine excretion, rates of protein synthesis and breakdown were significantly higher ($p < 0.1$) for elderly males and females in comparison to young adults. These findings indicate that there is a redistribution of protein synthesis with advancing age in which the visceral organs

contribute to a progressively greater proportion of whole body protein turnover. Protein synthesis and breakdown rates were also expressed per kilogram of body cell mass. Expressed in this way, rates for elderly men were significantly higher ($p < 0.1$) than those of young men. While rates for elderly versus young women were also lower, this difference was not significant.

Uauy et al. (1978b) investigated whole body protein metabolism during aging by using urinary 3-methylhistidine excretion as a quantitative index of muscle protein breakdown. Body cell mass, measured by ^{40}K counting, was lower in elderly (68 to 91 years) compared to young adult (18 to 25 years) subjects. In agreement with Forbes and Reina (1970), the decrease in body cell mass was attributed to a decline in total body protein mass. Urinary creatinine excretion was also lower in the elderly subjects, indicating decreased muscle mass. The rate of whole body protein breakdown was determined by ^{15}N -glycine infusion. Protein breakdown per unit of body weight was lower in the elderly than in the young adults, thus supporting the results of Winterer et al. (1976).

When expressed per unit body cell mass and per unit metabolic rate, however, no differences between the two age groups were observed. Urinary 3-methylhistidine

output, per unit body weight, was significantly higher in the young adult compared to the elderly subjects. Mean rates of muscle protein breakdown based on 3-methylhistidine excretion were 0.76 and 0.64 gm/kg/day for young men and women, respectively. For elderly men and women, the values were 0.53 and 0.31 gm/kg/day, respectively. The differences between age groups were significant ($p < 0.01$). Muscle protein breakdown accounted for 27% of whole body protein breakdown in young adults. In the elderly, muscle contributed to only 20% of whole body protein degradation. These findings confirm the results of Young et al. (1975) that with increasing age, skeletal muscle plays a diminishing role in whole body nitrogen turnover in humans.

An age-related decrease in protein synthesis by rats at the whole body level has been reported (Waterlow, 1967). Fujita and Ichikawa (1986), however, report that whole body nitrogen metabolism in rats does not consistently change with age, but instead, cyclic fluctuations in nitrogen balance occur. These fluctuations were attributed to age-related changes in the protein metabolism of individual tissues.

Age-related Changes in the Regulation of Protein Metabolism

The regulation of protein synthesis and degradation is a complex process involving subcellular level mechanisms and coordinated actions between tissues and cells. At the cellular level, protein synthesis may be regulated through ribosome function, mRNA, and post-translational factors. Some aspects of the effect of age on subcellular regulatory mechanisms have been presented in a previous section of literature. Findings reported in the literature suggest that the decreased rate of protein synthesis in various tissues with age results from alterations in ribosomes (Ekstrom et al. 1980; Pluskal et al., 1984), initiation/elongation factors (Burini et al., 1984), and/or deficiency of an unidentified soluble factor (Sojar and Rothstein, 1986).

Hormones play an important role in the regulation of protein synthesis in the whole animal (Manchester, 1970). In this respect, hormones function by influencing protein metabolism in the cells of target tissues. It has been hypothesized that at least part of the reduction in muscle protein synthesis with increasing age is due to a decline in growth hormone (GH) secretion (Sonntag et al., 1985). Rudman et al. (1981), using

human subjects aged 21 to 86 years, have demonstrated that growth hormone and somatomedin C (SmC) secretion progressively decline from the third to ninth decade. One of the functions of growth hormone is to stimulate protein synthesis in lean body mass. Growth hormone secretion in young (4 to 5 months) and old (18 to 20 months) Sprague-Dawley rats has been compared (Sonntag et al., 1980). Pulsatile release of GH was lower in old versus young animals. Growth hormone serum concentrations were also significantly lower ($p < 0.01$) in the old compared to the young rats.

To test the relationship between GH and protein synthesis during aging, bovine GH or L-dopa was administered to old (19 to 21 month) male rats. L-dopa was used because it is known to increase circulating levels of growth hormone. The rate of ^3H -phenylalanine incorporation into diaphragm muscle in treated old rats was compared to young rats treated with vehicle. Protein synthesis by the diaphragm muscle of old rats was restored to the level of young rats through the administration of bovine GH. It appears that the decrease in GH secretion from the anterior pituitary may be partially responsible for the decline in protein synthesis with advancing age.

Florini and Roberts (1981) have observed an age-

related decline in plasma levels of somatomedin-C. The action of pituitary growth hormone on muscle may be mediated by somatomedins (Florini et al., 1977). In this study, three age groups, young (2 to 6 month), middle-aged (12 to 16 month), and old (24 to 28 month), of Fischer 344 rats were used. A bioassay based on myoblast proliferation in culture was used to measure the activity of somatomedin-like growth factors in serum. The activity of serum somatomedin decreased 10% between the young and middle-aged rats, and 20% between the middle-aged and old rats. This decrease was not found to result from an increased circulating concentration of somatomedin inhibitor or a decreased sensitivity of muscle to somatomedin-like agents. These results support the findings of Sonntag et al. (1980) by indicating that the age-related decline in muscle protein synthesis may be related to decreased circulating levels of somatomedin-like growth factors.

Age-related changes in the hormones insulin and glucagon, which also function in amino acid metabolism, have been investigated (Elahi et al., 1982; Berger et al., 1978; Dudl and Ensinnck, 1977). Elahi et al. (1982) measured fasting plasma levels of insulin and glucagon in 186 male subjects ranging from 23 to 93 years of age. When younger (18 to 55 years) obesity-matched controls

were compared to older (56 to 83 years) participants, no age-related difference in fasting plasma insulin concentration was observed. Age was also not significantly correlated with fasting plasma levels of glucagon.

In contrast, Berger et al. (1978) have reported that mean plasma glucagon levels increased during the third and fourth decades, but did not change thereafter. In agreement with Elahi et al. (1982), mean fasting insulin levels did not change with age. This study involved 263 male and female subjects, ranging in age from 20 to 69 years.

In a similar study, Dudl and Ensinck (1977) examined the effect of aging on basal levels of insulin and glucagon in 44 volunteers aged 22 to 81 years. Percent adiposity was found to increase with age, but no age-dependent changes in insulin or glucagon release were found. Alterations in plasma levels of insulin and glucagon do not appear to influence the rates of protein synthesis or degradation during aging.

Protein Requirements in Relation to Age

In adult humans, the physiological requirement for protein is that which is necessary to maintain total body protein mass (Young, 1984). Age-related changes in

body protein metabolism suggest that dietary protein needs may change with advancing age in humans. Minimum physiologic needs for protein in adult humans have generally been determined by one of two nitrogen balance methods, the factorial approach and the nitrogen balance response curve. The factorial method determines the requirement as the amount of high quality protein necessary to balance obligatory nitrogen losses. The nitrogen balance response curve measures nitrogen balance response to graded protein intakes. The requirement level is determined as the protein intake needed to just maintain nitrogen balance.

In a short-term study, Uauy et al. (1978) measured nitrogen balances of elderly men (68 to 74 years) and women (70 to 84 years) in response to graded levels of egg protein. Subjects received a protein-free diet for one day, then were randomly assigned to one of three levels of egg protein for a 10-day diet period. The mean protein requirement for elderly women was 0.83g of egg protein per kg per day. For male subjects, individual variation in nitrogen balance response prevented a reliable prediction of the protein requirement. The protein requirement determined for elderly females was twice the level of 0.42 g/kg determined in a previous study by the factorial method (Scrimshaw et al., 1976).

This discrepancy indicates that the factorial method may underestimate protein requirements. Uauy et al. (1978) conclude that the protein requirement for elderly females is either the same as or higher than that of young females.

As a follow-up to these findings, Gersovitz et al. (1982) conducted a study to evaluate the current recommended daily protein allowances of the Dietary Allowances Committee of the Food and Nutrition Board (1980). In this study, the response of 7 elderly men (75 +/- 4 years) and 8 elderly women (78 +/- 9 years) to the current recommended allowance was determined during a 30-day metabolic N balance period. Subjects received 0.8 g/kg/day of egg protein throughout the 30 days of the study. The 30-day study period was divided into three 10-day diet periods, with nitrogen balances determined during the last five days of each diet period. For the last five days of the 30-day study period, three of the seven male and four of the eight female subjects were in negative nitrogen balance.

These results indicated that 0.8g egg protein/kg/day was not adequate to maintain positive nitrogen balance in many subjects 70 years or older, even after a 30-day adaptation period. It is proposed that the broad age categories used in the current RDAs

for protein do not account for possible requirement differences among older age groups within these categories. In light of the findings of Gersovitz et al. (1982), specific allowances may need to be considered for age groups within the 51 years and older category.

It has been well established that the protein requirement for rats begins to decline after weaning (National Academy of Sciences, 1978). The extent to which this decrease continues, however, is not well documented. In mature animals, with the exception of pregnant and lactating females, protein is required for the maintenance of body tissues (Said and Hegsted, 1969). Several studies have been reported which examine the dietary protein needs of mature rats (Bricker and Mitchell, 1947; Goettsch, 1951; Hartsook and Mitchell, 1956; Smith and Johnson, 1967; Sheehan et al., 1981). Table 1 contains a summary of data pertaining to protein requirements of adult rats.

In an early study, Bricker and Mitchell (1947) used nitrogen balance periods of 12 days to determine protein requirements of adult male rats weighing 294 to 408 grams. Dietary levels of 3.6% egg protein and 4.3% milk protein were adequate for the maintenance of nitrogen equilibrium. Smith and Johnson (1967) also found a level of 3.6% whole egg protein adequate to maintain

Table 1 Estimated Maintenance Protein Requirements of the Adult Rat

Rat Characteristics		Strain	Number of observations (n)	Protein Requirement (% of diet)	Source of Dietary Protein	Method of Analysis	References
Age (months)	Sex						
Adult (294-408g)	M	albino	10	3.6	egg	N balance	Bricker and Mitchell (1947)
Adult (294-408g)	M	albino	10	4.3	milk	N balance	Bricker and Mitchell (1947)
8-9	M	wistar	10	3.6	whole egg	N balance	Smith and Johnson (1967)
Adult (300g)		albino	12	8.3	rice-beans-casein	Body weight	Goettsch (1951)
10	M	albino	7	3.18	casein	N balance	Hartsook and Mitchell (1956)
Adult (200g)	F		5	3.39	casein	Carcass water	Said and Hegsted (1969)
12	F	Sprague-Dawley	7-21	3.91-4.19	casein	Carcass N	Sheehan et al. (1981)

nitrogen equilibrium in 8- to 9-month-old male rats.

Goettsch (1951) determined the minimum protein requirement of adult rats weighing 300 grams. In this study, 28-day periods of body weight maintenance were used to determine the minimum requirement level. The rice-beans-casein diets ranged in total nitrogen content from 0.8 to 1.4%. A level of 53.4 mg N per 100g body weight was found to be the minimum maintenance requirement level. Based on the net utilization of protein in this diet of 63, the requirement level of an ideal protein would be 34 mg N per 100g of body weight (53.4×0.63). Using body weight as the criteria for determining protein needs does not account for weight change resulting from loss or gain of non-nitrogen body constituents. Goettsch et al. also reported that this requirement level was 1.6 times higher than the level required for the maintenance of nitrogen equilibrium in these same animals.

Hartsook and Mitchell (1956) reported that a 3.18% casein diet maintained nitrogen equilibrium in adult rats. In this study, 300-day-old male rats weighing 308 to 401 grams were used. Casein supplemented with methionine was fed at 0, 1.6 to 2, or 4 to 4.5 percent of the diet for 14-day balance periods. Linear regression was used to determine the point of nitrogen equilibrium. Said and Hegsted (1969) determined maintenance protein

requirements of 200 gram adult female rats. Casein was fed at 0.85, 1.70, or 2.50% of the diet for 31 days. At the end of the experimental period, carcasses were analyzed for total water and nitrogen content.

Change in carcass water was determined by comparison to initial values of a baseline group. Because it is highly correlated with carcass nitrogen, change in carcass water was used as the dependent variable in linear regression to predict the maintenance protein requirement. A dietary casein level of 0.387g per day, or 3.39% of the diet, was determined as the requirement level. Unfortunately, in several of these earlier studies the maturity of rats is expressed in terms of weight, rather than age which makes results difficult to interpret in terms of changing protein requirements with age.

In a more recent study, Sheehan et al. (1981) used carcass nitrogen to predict the protein requirement of mature (12-month-old) female rats. Rats were fed casein supplemented with methionine at 0.84, 1.86, 2.81, 3.77, and 4.69% of the diet for 4, 8, or 12 weeks. A baseline group was sacrificed and a concurrent control group was fed 9.90% protein for comparative purposes. Total carcass nitrogen and water were determined. Using carcass N as the dependent variable in linear regres-

sion, a dietary protein requirement of 3.91 to 4.19% was predicted.

Carcass nitrogen analysis resulted in narrower confidence intervals compared to carcass water and therefore appears to be a more reliable predictor of maintenance protein requirements. Feeding experimental diets for 12 instead of 8 weeks did not result in further changes in protein nutriture. The protein requirement predicted by Sheehan et al. (1981) is lower than the maintenance requirement of 4.8% casein currently recommended by the National Research Council for adult rats (National Academy of Sciences, 1978).

A limited number of studies have investigated changes in protein requirements from maturity to senescence. In the literature available, conflicting results have been reported (Fischer and Canolty, 1983; Baldwin and Griminger, 1985). Fischer and Canolty (1983) fed 15- and 25-month-old male Sprague-Dawley rats diets containing 2, 3, or 4% casein at three levels of intake. These levels of intake were ad libitum, 80, or 60% ad libitum. At each casein concentration and level of intake, the 25-month-old animals had lower mean maintenance requirements than the 15-month-old animals.

Requirement levels in this study were determined by regression equations relating change in energy of total

carcass and carcass protein to metabolizable energy intakes. Both 15- and 25-month-old rats gained weight at a 2.16% casein plus methionine level. These results disagree with Sheehan et al. (1981) who report that 12-month-old female rats lost weight at casein plus methionine concentrations of 2.14% or below. Fischer and Canolty suggest that these findings are indicative of a decreasing protein requirement with increasing age in rats.

Baldwin and Griminger (1985) conducted nitrogen balance studies to compare the protein requirements of mature (12-month-old) and aged (24-month-old) rats. Male Fischer 344 rats were fed diets containing 4.5 or 6.0% protein supplemented with methionine for balance periods of seven days. The nitrogen balance of mature rats fed 4.5% protein was 9.5 mg/rat/day indicating that these animals were in nitrogen equilibrium at or near the requirement level. Aged rats, however, were in negative nitrogen balance of -14.4 mg/rat/day. Food consumption, and therefore nitrogen intake, of the older animals was much lower than that of the mature rats at 79 versus 102 grams per day. Urinary nitrogen excretions of the aged animals were also greater than those of the mature rats.

By regression, the daily amount of nitrogen needed

to maintain N equilibrium was determined to be 90.0 mg for mature and 98.0 mg for aged rats. While these differences were not significant, they do suggest that aged rats require a higher level of dietary protein than mature rats to maintain nitrogen balance equilibrium. It must be noted that the short balance periods used in this study may not have allowed for adjustment to a decreased level of dietary protein. It is possible that longer feeding periods would have resulted in a moderation of the age-related differences observed in this study.

The inconsistency of results in the literature cited warrants an examination of the effectiveness of methods used to determine protein requirements. The nitrogen balance method has frequently been used to estimate dietary protein requirements of rats and humans. Nitrogen balance is generally calculated as nitrogen intake minus nitrogen excreted in urine and feces plus integumental losses (Baldwin and Griminger, 1985). The maintenance of nitrogen equilibrium as an indicator of protein requirements is dependent upon accurate measurement of nitrogen intake and excretion. Nitrogen may be lost from the body in the form of urine, feces, sweat, hair, and nails.

Several criticisms of the balance study technique

have been offered (Wallace, 1959; Forbes, 1973). Wallace (1959) argues that nitrogen intake will generally be overestimated and nitrogen excretion underestimated. This consistent bias in the data will lead to falsely high nitrogen retention values. Forbes (1973) suggests there is a slow modification of body composition after a change in dietary nitrogen level. Commonly used short balance periods would be inadequate to account for these changes.

Body weight maintenance (Goettsch, 1951), carcass water (Said and Hegsted, 1969), and carcass nitrogen (Sheehan et al., 1981) have also been used to predict protein requirements of rats. Body weight maintenance does not appear to be an appropriate method for estimating protein needs since body weight changes may result from an alteration in body constituents other than nitrogen. Said and Hegsted (1969) advocate the use of carcass water, which is highly correlated with carcass nitrogen, as a measure of the protein requirement level. These researchers state that, while body nitrogen may be the best parameter of response to protein utilization, difficulty in measuring this body component can result in substantial error.

Sheehan et al. (1981), on the other hand, propose that carcass nitrogen is a more reliable method for

estimating protein needs. In their rat study, when carcass nitrogen, rather than carcass water, was used as the dependent variable in linear regression, more consistent point estimates and narrower confidence intervals were obtained.

Adaptation by Rats to Restricted Levels of Dietary Protein

There is an extensive amount of evidence that animals are able to adapt to a wide range of protein intakes. Adaptation is accomplished through an adjustment in food consumption (Meyer and Hargus, 1959), alteration in the activities of specific tissue enzymes, and changes in organ size (Harper, 1965). The response of rats to a restricted intake of protein and energy has been investigated by Khan and Bender (1979). Adult male Sprague-Dawley rats, 6- to 7-months-old, were fed 5% casein diets ad libitum. This level of casein was adequate to maintain body weight and nitrogen equilibrium. After three weeks, half of the rats were switched to diets restricted to 70% of the amount consumed ad libitum. The immediate response to protein and energy restriction was an increase in urinary N excretion with N balances becoming negative. Animals then gradually

adapted and, over a period of 30 days, N balances became progressively less negative. The extent of urinary N loss, which is indicative of protein catabolism, also decreased with adaptation.

Administration of labelled methionine resulted in greater radioactivity in the livers of rats fed restricted diets compared to those fed ad libitum. The opposite effect was observed in muscles. With restricted protein intake, protein synthesis appears to be concentrated in internal organs at the expense of muscle and skin. This response may be mediated through the hormones cortisone and insulin. Cortisone may increase liver protein synthesis while decreasing protein synthesis in the peripheral tissues. Insulin functions to promote the uptake of amino acids by muscle, but not by liver.

Adaptation may also be achieved through enzymic alterations and a decrease in metabolic rate. Khan and Bender (1979) found that glutamic-pyruvic transaminase increased after ten days but returned to normal after 31 days. The metabolic rate of rats fed restricted diets fell by 34% within 20 days.

Harper (1965) has reported on the effect of low protein intake on tissue enzyme activity. During protein depletion, there is an increased need for efficient

amino acid trapping for tissue protein synthesis. A decline in the rate of amino acid catabolism is accomplished through a decrease in the activity of enzymes which function in amino acid degradative pathways. These enzymes include glutamate-alanine transaminase and glutamate-aspartate transaminase which convert amino acids into easily oxidizable forms. The activity of urea cycle enzymes also declines during protein depletion.

Meyer (1958) has hypothesized that rats placed on low protein diets are unable to increase food intake to obtain more protein because of a limited capacity to store or dissipate energy. In order to confirm this hypothesis, Meyer and Hargus (1959) conducted a study in which rats fed a low protein diet were forced to expend energy. Weanling male Sprague-Dawley rats were fed diets containing 10 or 25% casein for 21 days with some rats subjected to a cold environmental temperature (2°C) or exercise (swimming). Carcass composition was determined at the end of the experimental period.

Weight gain was greater for animals on the low protein diet that were forced to expend energy. This weight gain consisted of an increase in fat and lean body mass. A greater proportion of body fat gain was correlated with a greater food intake by rats on the low protein ration which were subjected to exercise or cold

temperatures. The researchers conclude that the ability to gain more body fat allowed rats to increase intake of a low protein diet. These findings support the hypothesis that animals placed on low protein diets have their food intake limited by an excess consumption of energy in relation to protein.

Peng et al. (1974) fed male weanling Holtzman rats diets containing 2, 10, 20, 40, or 60% casein ad libitum for nine days. Food intake, body weights, and alterations in amino acid metabolism were determined. Rats fed 2, 20, 40, or 60% casein diets consumed less food than rats fed the 10% casein (control) diet. At 2, 40, and 60% casein, rats gained significantly less weight than controls. Rats fed 2% casein conserved more labelled methionine, while rats fed the higher levels of casein converted a larger portion of methionine to CO_2 . These findings demonstrate the animals' ability to shift the balance of protein metabolism towards anabolism or catabolism when fed low or high levels, respectively, of dietary protein.

MATERIALS AND METHODS

Animals

The animals used in this study were 50, 11-month-old and 50, 18-month-old male rats. Animals were purchased from Harlan Sprague Dawley, Madison, Wisconsin. Throughout the experiment, rats were housed in suspended wire bottom cages in a room with a controlled temperature (21 to 22 °C) and a 12 hour light/dark cycle. One third of the animals were started on experimental diets on each of three consecutive days. In this way, staggered weekly weighing and sacrifice of the animals over a period of three days was possible. Food and water were provided ad libitum throughout the experimental feeding period. Food spillage was estimated by weigh-back.

Experimental Design

Table 2 shows the experimental design of this study. At the beginning of the experiment, ten rats from each age group were randomly assigned by weight to one of five experimental diets. In this way, the average weight of animals in each treatment group was initially

Table 2. Experimental Design
Percent Dietary Protein (N x 6.54)

Age (months)	1.53	3.41	4.98	6.52	8.05
11	10 ¹	10	10	10	10
18	10	10	9 ²	10	10

- 1 Number of animals per treatment
- 2 One rat died before the end of the experimental period.

the same. Diets contained either 1.53, 3.41, 4.98, 6.52, or 8.05 percent protein by weight, with casein as the source of dietary protein. Diets were isocaloric, providing 4 kilocalories per gram of diet, with percent protein increased at the expense of equal amounts of cornstarch and sucrose. Dietary casein was supplemented with d-l-methionine at 2.3 grams of methionine per 100 grams of casein. This is the amount of methionine recommended by the National Research Council (1978) to ensure an adequate supply of dietary sulfur amino acids. Composition of experimental diets is given in Table 3.

All animals were fed the 8.05% protein diet for two weeks prior to the experimental period in order to allow physiological adjustment to this level of dietary protein and to the powdered form of diet. Animals fed the 8.05% protein diet throughout the experimental period were used as a concurrent control group since reports in the literature indicate that this level of protein is greater than the maintenance protein requirement of both age groups (National Academy of Sciences, 1978; Sheehan et al., 1981; Fischer and Canolty, 1983). Rats were placed on experimental diets for a five week feeding period.

Procedures

At the end of the feeding period, each rat was

Table 3. Composition of Experimental Diets (g/100g)

Dietary constituent	Diet (expressed as percent protein)				
	1.53	3.41	4.98	6.52	8.05
cornstarch ¹	39.93	39.08	38.23	37.38	36.52
sucrose ²	39.93	39.08	38.23	37.38	36.52
vegetable shortening ³	11.0	11.0	11.0	11.0	11.0
casein ⁴	1.11	2.78	4.44	6.11	7.78
d-l-methionine ⁵	0.03	0.06	0.10	0.14	0.18
vitamins ⁶	2.0	2.0	2.0	2.0	2.0
minerals ⁷	4.0	4.0	4.0	4.0	4.0
Non-nutritive fiber ⁸	2.0	2.0	2.0	2.0	2.0

1 ICN Pharmaceuticals, Cleveland, Ohio

2 d-(+)-sucrose ICN Pharmaceuticals, Cleveland, Ohio

3 Crisco

4 Vitamin-free casein, ICN Pharmaceuticals, Cleveland, Ohio;
Kjeldahl analysis determined that small amounts of protein were
contributed to diets by cornstarch, vitamin mix, and non-nutritive
fiber

5 ICN Pharmaceuticals, Cleveland, Ohio

6 AIN Vitamin Mixture 76, ICN Pharmaceuticals, Cleveland, Ohio

7 AIN Mineral Mixture 76, ICN Pharmaceuticals, Cleveland, Ohio

8 Alphacel Non-nutritive Bulk, ICN Pharmaceuticals, Cleveland, Ohio

anaesthetized with carbon dioxide and blood was drawn by heart puncture from unconscious rats. Ten ml Vacutainers (Becton-Dickinson Co., Rutherford, New Jersey) equipped with 21 gauge 1 1/2 inch needles were used to draw blood. After collection, each tube was allowed to stand at room temperature for 30 minutes to allow clotting. Blood was centrifuged in the vacutainer tubes in an IEC Refrigerated Centrifuge Model DPR-6000 (International Equipment Corp., Needham Heights, Massachusetts) at 4 °C for 30 minutes at 3000 rpm. Serum was removed from the red cells with Pasteur pipets and stored frozen in polypropylene vials until further analysis. Rats still alive after heart puncture were placed back into the carbon dioxide chamber until death occurred.

Immediately after sacrifice, the entire liver was removed, rinsed with saline, blotted dry, and weighed. Livers were quickly frozen in polycarbonate specimen cups with snap-on lids. Carcasses (minus livers and drawn blood) were weighed and quickly frozen in plastic Ziploc bags until further analysis.

Carcass Analysis

Frozen carcasses, defined as the total animal minus the liver and the blood drawn at sacrifice, were chopped into approximately one inch square pieces using a meat cleaver and rubber mallet. The pieces of each carcass were placed in an aluminum loaf pan covered with cheesecloth and freeze-dried for 120 hours to remove all moisture. After freeze-drying, carcasses were placed in an 80 °C oven overnight for further removal of moisture. Dried carcasses were weighed and carcass water content was determined as the weight lost during freeze-drying.

Each dry carcass was ground in a Hobart grinder with a ratio of sodium sulfate (Na_2SO_4) to carcass weight of 4:1. Sodium sulfate was added to promote mixing and thorough grinding of each carcass. During grinding, care was taken to retain all carcass pieces and sodium sulfate. Thorough grinding took approximately 20 to 30 minutes per animal. Ground carcasses plus Na_2SO_4 were stored in Mason canning jars until further analysis. Eight to ten gram samples of each ground carcass were analyzed in duplicate for fat content by petroleum ether extraction in a Soxhlet apparatus (Reynolds, 1982). Carcass nitrogen was determined from 1.0 to 1.5 gram duplicate samples by the Kjeldahl method

(AOAC, 1980) using copper sulfate as the catalyst.

Liver Analysis

Liver tissue was freeze-dried for 72 hours, oven-dried (80 °C) overnight, and weighed. Moisture content was determined as the weight lost during freeze-drying. Liver tissue was ground to a fine powder using a mortar and pestle. Approximately 0.6 to 0.7 gram samples were analyzed in duplicate for fat content by petroleum ether extraction in a Soxhlet apparatus (Reynolds, 1982). Approximately 0.18 to 0.22 gram samples of fat-free dry liver tissue were analyzed in duplicate for nitrogen content by the Kjeldahl method (AOAC, 1980) with copper sulfate used as the catalyst.

Blood Analysis

Serum samples were thawed overnight in a refrigerator. Total serum protein concentration was determined by a biuret method using the Stanbio Total Protein Kit (Stanbio Laboratories, San Antonio, Texas). Standard curves were prepared using Fraction V Bovine Albumin and used to determine the protein concentrations of the samples. Absorbance was read on a Bausch and Lomb Spec-

trophotometer 2000 at 550 nm.

Diet Analysis

Samples of experimental diets were analyzed in duplicate for nitrogen content by the Kjeldahl method (AOAC, 1980) using copper sulfate as the catalyst. Protein content of experimental diets was determined as total nitrogen times 6.54, the conversion factor for casein (Jennes, 1970). Other dietary constituents were also analyzed for nitrogen content.

Statistical Analysis

Two-way analysis of variance was used to determine significant interaction effects of diet and age. An alpha level of 0.05 was considered significant. Pairwise comparisons were made using Student's t-tests to determine significant differences between treatment and age groups. An alpha level of 0.01 was considered significant for pair-wise comparisons.

RESULTS AND DISCUSSION

Body Weight and Food Intake

Average body weight changes for the different age and treatment groups are shown in Figure 1. Comparison of body weight changes between age groups and varying levels of dietary protein for weeks 1, 3, and 5 (final) of the experimental period are shown in Table 4. Initially, the average body weights of 18-month-old rats were significantly greater ($p < 0.01$) than those of the 11-month-old animals. Body weight of rats is related to chronologic age. Yu et al. (1982) have reported that body weight of male SPF Fischer rats peaks at 18 months and is maintained through 25 to 30 months of age. Coniglio et al. (1979) found that body weight of male Fischer 344 rats increased until 12 months, then steadily declined. In male Wistar rats, body weight reached a maximum at approximately 600 days, and decreased thereafter (Fujita and Ichikawa, 1986).

After one week on experimental diets, 18-month-old rats fed the 1.53% protein diet weighed significantly less ($p < 0.01$) than rats fed 6.52 or 8.05% protein diets. While 11-month-old rats fed 1.53% protein lost weight during the first week, average body weight was

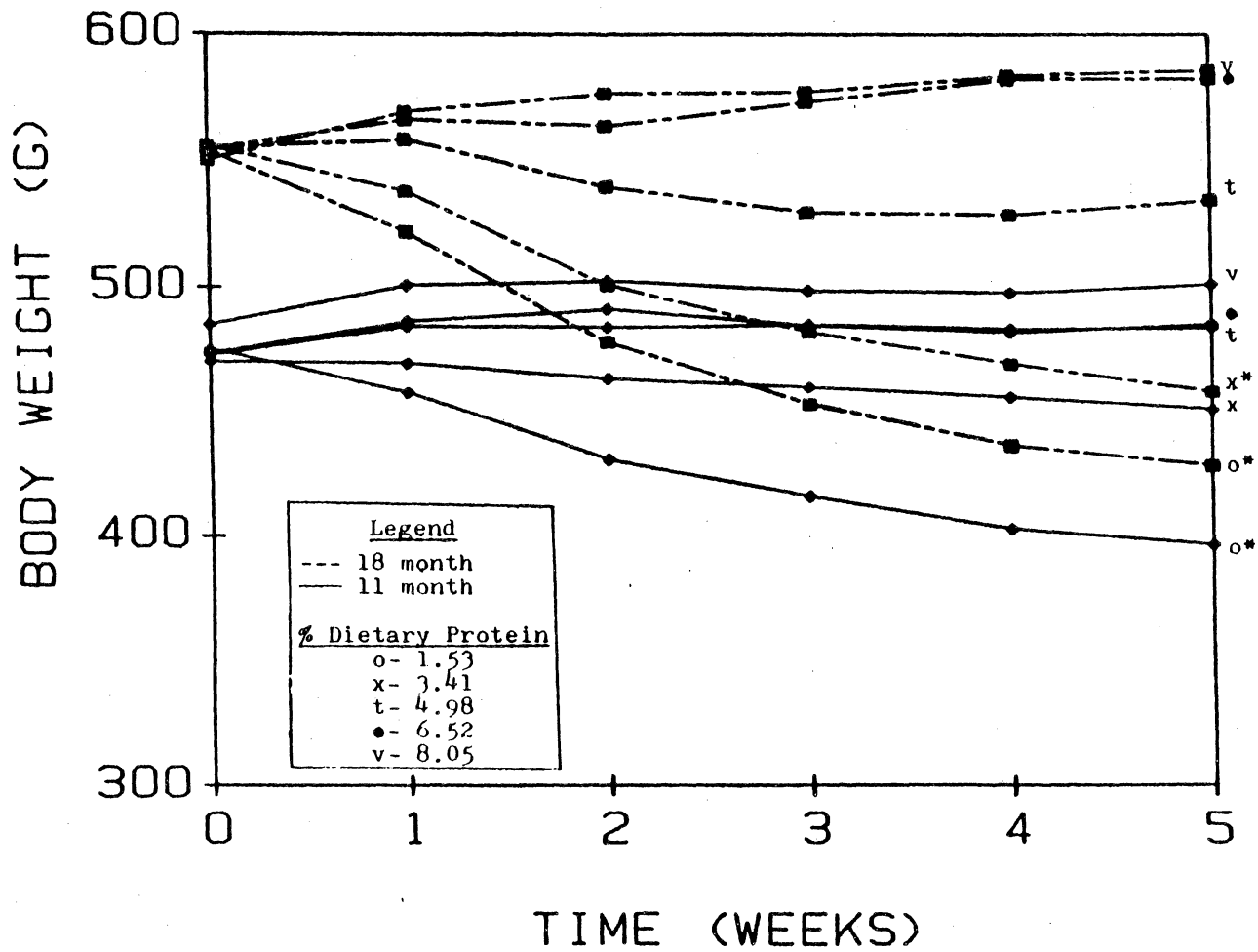


Figure 1. Body Weight Changes of 11- and 18-month-old Male Rats Fed Varying Levels of Protein. All points represent group means. Asterisks indicate that final weight is significantly different from initial weight ($p < 0.0001$).

Table 4. Body Weights of 11- and 18-month-old Male Rats at weeks 1, 3, and 5 of the Experimental Feeding Period.¹

Group (percent dietary protein)	Week 1		Week 3		Week 5 (final)	
	11	18	11	18	11	18
1.53	456.9±10.5 ^a	521.6±10.5 ^a	415.4±11.1 ^a	452.0±11.1 ^a	396.1±11.7 ^a	427.7±11.7 ^a
3.41	469.0±10.5 ^a	537.6±10.5 ^{ab}	459.0±11.1 ^b	481.9±11.1 ^a	450.1±11.7 ^b	457.2±11.7 ^b
4.98	484.5±10.5 ^a	557.7±11.1 ^{ab}	485.2±11.1 ^b	529.2±11.7 ^b	484.0±11.7 ^{bc}	534.3±12.3 ^c
6.52	486.6±10.5 ^a	565.4±10.5 ^b	484.7±11.1 ^b	572.4±11.1 ^c	485.6±11.7 ^{bo}	581.7±11.7 ^d
8.05	500.6±10.5 ^a	568.7±10.5 ^b	498.4±11.1 ^b	576.5±11.1 ^c	501.1±11.7 ^c	585.3±11.7 ^d

¹ All values represent group means ± SE

^a Indicate values which are significantly different between age groups (p<0.01)
Different superscripts indicate values which are significantly different within age groups (p<0.01)

not significantly lower than that of other diet groups. By week three of the experimental period, body weights of the older animals receiving the two lowest levels of protein were similar to those of the younger rats fed these levels of protein. These weights continued to be similar throughout the remainder of the feeding period.

By week five, body weights of 18-month-old rats fed 1.53, 3.41, and 4.98% protein were significantly different from each other ($p < 0.01$) and significantly lower than those of rats fed the two highest levels of protein. Average final body weight of 11-month-old rats fed 1.53% protein was significantly lower than that of the other protein groups, but body weights of rats fed 3.41, 4.98, and 6.52% protein were not significantly different from each other. It appears that the older rats, compared to the 11-month-old animals, require a greater level of dietary protein for the maintenance of body weight.

The weight loss in animals fed low protein diets is presumed to be a loss of body fat, water, and protein, with more protein lost at lower levels of dietary protein. At the end of the five week period, 11-month-old rats fed 1.53% and 18-month-old rats fed 1.53 and 3.41% protein diets had mean body weights which were significantly different from their initial body weights

(Figure 1). This finding disagrees with Fischer and Canolty (1983) who report that 15- and 25-month-old male Sprague-Dawley rats gained weight at a 2.16% casein plus methionine concentration.

Average daily food and protein intakes of experimental animals are shown in Table 5. The older rats, compared to the younger age group, consumed significantly more of the control diet ($p < 0.01$). Food intake on this diet averaged 16.2 and 18.9 g/day for the 11- and 18-month-old rats, respectively. There were no significant age differences for other dietary treatment groups. For both ages, food consumption was lowest at 1.53% dietary protein and highest at 4.98% dietary protein. Food consumption by rats fed the lowest level of protein may have been limited by an excess energy consumption in relation to protein consumption (Meyer and Hargus, 1959).

Average daily protein intakes of 11-month-old rats ranged from 241.3mg for the group fed 1.53% protein to 1307.3mg for the group fed 8.05% protein. For the 18-month-old animals, protein intake ranged from 204.4 to 1521.5 mg/day for groups receiving 1.53 and 8.05% protein, respectively. Said and Hegsted (1969) have reported a maintenance protein requirement of 387mg casein per day for 200g rats. A requirement level of

Table 5. Average Daily Food and Protein Intakes of Experimental Animals¹

Group (percent dietary protein)	Food Intake (g/day)		Protein Intake (mg/day)	
	11	18	11	18
1.53	15.8 ± 0.69 ^a	13.4 ± 0.69 ^a	241.3 ± 32.0 ^a	204.4 ± 32.0 ^a
3.41	16.5 ± 0.69 ^{ab}	14.5 ± 0.69 ^a	561.3 ± 32.0 ^b	495.5 ± 32.0 ^b
4.98	18.7 ± 0.69 ^b	19.6 ± 0.73 ^b	929.3 ± 32.0 ^c	975.0 ± 33.8 ^c
6.52	16.9 ± 0.69 ^{ab}	19.4 ± 0.69 ^b	1100.6 ± 32.0 ^{*d}	1267.5 ± 32.0 ^{*d}
8.05	16.2 ± 0.69 ^{*ab}	18.9 ± 0.69 ^{*b}	1307.3 ± 32.0 ^{*e}	1521.5 ± 32.0 ^{*e}

¹ All values are expressed as group means ± SE

* Indicate values which are significantly different between age groups (p<0.01)

Different superscripts indicate values which are significantly different within age groups (p<0.01)

872mg protein per day as casein for 300g rats was found by Hartsook and Mitchell (1956). Rats used in the current study had initial body weights averaging 475.1g for 11-month-old and 553.4g for 18-month-old animals. The greater body weights of these animals may indicate a greater maintenance requirement for protein than the values reported by Said and Hegsted (1969) and Hartsook and Mitchell (1956). Goettsch (1951) reported a protein requirement for rats of 217mg protein per 100g body weight.

Based on the initial weights of rats used in the present study, this requirement level would correspond to a daily intake of 1030.8mg protein for 11-month-old and 1200.0mg for 18-month-old rats. These levels fall between the actual daily amounts of protein consumed by both age groups fed 4.98 or 6.52% protein diets. Goettsch (1951) used body weight maintenance as the criteria for determining the protein requirement of rats. This method may have resulted in an estimated protein requirement which was above the actual minimum requirement level.

Total Serum Protein

Table 6 shows total serum protein values for each

Table 6. Total Serum Protein Values of 11- and 18-month-old Male Rats¹

Group (percent dietary protein)	<u>Serum Protein (g/ 100 ml)</u>	
	11	18
1.53	5.69±0.13 ^a	5.30±0.13 ^a
3.41	5.89±0.13 ^{ab}	5.58±0.13 ^a
4.98	6.21±0.13 ^b	6.59±0.13 ^b
6.52	6.83±0.13 ^c	6.76±0.13 ^b
8.05	6.96±0.13 ^c	7.06±0.13 ^b

¹ All values are expressed as group means ± SE.

Different superscripts indicate values which are significantly different within age groups (p<0.01)
 There were no significant differences between age groups for total serum protein (p<0.01)

age and treatment group. For 11-month-old rats, when dietary treatment groups are compared to the control group for this age, it can be seen that the groups fed 1.53, 3.41, and 4.98% protein had significantly lower total serum protein values ($p < 0.01$). These values were 5.69, 5.89, and 6.21 g/100ml, respectively, compared to 6.96 g/100ml for the control group. Compared to the 18-month-old control group, older rats fed 1.53 or 3.41% protein had significantly lower total serum protein values ($p < 0.01$). These values were 5.30 and 5.58 g/100ml, respectively, versus 7.06 g/100ml for the 8.05% protein control group. These findings indicate that older rats could maintain normal serum protein values at lower dietary protein levels than 11-month-old rats.

Sheehan et al. (1981), in studying the protein requirement of mature female rats found that diets containing 0.84 and 1.86% protein as casein fed to rats for four weeks were unable to support normal serum protein values. In their study, Sheehan et al. (1981) compared total serum protein values to values of a baseline group sacrificed at the beginning of the experimental period. Low serum protein values indicated that normal and adequate liver protein synthesis was prevented by an insufficient dietary protein intake. In another experiment, Sheehan et al. (1981) used a concurrent control group

fed 9.90% dietary protein as casein for comparative purposes. Rats fed 3.62, 4.17, or 5.18% protein diets for 8 to 12 weeks had total serum protein values which were not significantly different from the 9.90% control group. The findings indicate that these levels of dietary protein were adequate to support normal serum protein synthesis by the liver of 12-month-old female Sprague-Dawley rats.

In the study by Sheehan et al. (1981), total serum protein values for 12-month-old rats ranged from 6.1 to 6.5 g/100ml for rats fed diets containing 2.14 to 9.90% protein, respectively. The total serum protein values for 11- and 18-month-old male Sprague-Dawley rats were slightly lower for groups fed 1.53 and 3.41% protein diets and slightly higher for groups fed the 6.52 and 8.05% protein diets. These differences may be related to the different age and sex of the rats used.

No significant differences in total serum protein between age groups were observed at any of the dietary treatment levels ($p < 0.01$). Rodgers and Gass (1982) have investigated the effect of age on total serum proteins in mice. These researchers found that the pattern of total serum protein was curvilinear as a function of age. Total serum protein concentration decreased during the rapid growth phase from 42 to 136 days, then

increased to a peak at 467 days of age. Total serum protein then began a downward trend at more advanced ages. If total serum protein concentration is altered with age in rats, it is possible that the age differences of rats used in this study were not sufficient for any such changes to be evident.

Liver Composition

Liver fat, protein (N x 6.25), and water composition for each age and treatment group are shown in Table 7. Total liver nitrogen and liver wet weight are also included in this table. Two-way analysis of variance showed no significant interaction effect of diet and age on liver wet weight, percent water, percent fat, percent protein, or total nitrogen ($p < 0.05$). Livers of 11-month-old rats fed 1.53 or 3.41% protein had a significantly higher percentage of liver fat than the control group ($p < 0.01$). Eighteen-month-old rats fed 1.53, 3.41, or 4.98% protein had a significantly higher percentage of liver fat compared to the control animals for this age group ($p < 0.01$). Protein deficiency has been reported as a cause of fatty livers in laboratory rats (Harper, 1958).

Flores et al. (1970) have examined the effect of

Table 7. Liver Composition of 11- and 18-month old Rats Fed Varying Levels of Dietary Protein¹

Group (percent dietary protein)	Percent Water		Percent Fat		Percent Protein		Total Nitrogen (mg)		Liver Weight (g)	
	11	18	11	18	11	18	11	18	11	18
1.53	67.88±0.65 ^{*a}	64.65±0.65 ^{*a}	9.84±0.94 ^{*a}	14.11±0.94 ^{*a}	18.76±0.41 ^a	18.39±0.41 ^a	327.51±16.92 ^{*a}	465.82±16.92 ^{*a}	10.93±0.57 ^{*a}	15.89±0.57 ^{*a}
3.41	68.08±0.65 ^a	65.81±0.65 ^a	8.33±0.94 ^{*a}	12.28±0.94 ^{*a}	19.74±0.41 ^{ab}	18.93±0.41 ^{ab}	384.84±16.92 ^{*ab}	492.72±16.92 ^{*ab}	12.22±0.57 ^{*a}	16.23±0.57 ^{*a}
4.98	69.75±0.65 ^{*ab}	66.58±0.68 ^{*a}	6.42±0.94 ^{*ab}	10.91±0.99 ^{*a}	19.77±0.41 ^{ab}	19.30±0.43 ^{ab}	396.01±16.92 ^{*b}	574.02±17.84 ^{*c}	12.79±0.57 ^{*a}	18.57±0.60 ^{*b}
6.52	70.80±0.65 ^b	69.28±0.65 ^b	4.54±0.94 ^b	6.83±0.94 ^b	20.55±0.41 ^b	19.39±0.41 ^{ab}	402.74±16.92 ^{*b}	530.93±16.92 ^{*bc}	12.30±0.57 ^{*a}	17.14±0.57 ^{*ab}
8.05	70.90±0.65 ^b	70.19±0.65 ^b	4.18±0.94 ^b	5.25±0.94 ^b	21.05±0.41 ^b	20.02±0.41 ^b	417.45±16.92 ^{*b}	517.70±16.92 ^{*abc}	12.47±0.57 ^{*a}	16.22±0.57 ^{*a}

¹ All values are expressed as means ± SE.

* Indicate values which are significantly different between age groups ($p < 0.01$)
 Different superscripts indicate values which are significantly different within age groups ($p < 0.01$)

protein depletion on triglyceride transport in rats. Male weanling rats were fed protein-free diets for 15 to 17 days to induce protein malnutrition. Compared to control animals fed a normal, well-balanced diet, malnourished rats had serum protein and triglyceride values which were reduced by 40 to 50%. In addition, liver triglycerides of protein depleted rats were greater than those of controls.

When malnourished rats were injected with serum protein fraction (1 mg/g body weight) containing low density lipoprotein (LDL) apoprotein, a marked increase in fasting serum triglycerides was observed. As expected, injection of this protein fraction into control animals had no effect since these animals already had an adequate supply of apolipoprotein available for triglyceride transport. These researchers conclude that the availability of apolipoprotein is the rate-limiting step for LDL biosynthesis in protein malnutrition. A deficiency of protein would cause triglycerides to accumulate, thus resulting in a fatty liver.

Liver fat levels of 11-month-old rats fed 4.98 and 6.52% protein were not significantly different from the control value. For 18-month-old rats, only the 6.52% dietary protein group had a percent liver fat value that was not significantly different from the 8.05% control

group. In these groups, the lower percentages of liver fat indicate that sufficient dietary protein was available to support normal liver lipoprotein synthesis. The older rats fed 1.53, 3.41, and 4.98% protein had significantly higher liver fat values than 11-month-old rats fed these levels of protein ($p < 0.01$). For the 6.52% protein and control groups, the older rats also had a greater liver fat level, but these differences were not significant.

These findings suggest that the percentage of fat in liver increases with increasing age in rats. It is interesting that 18-month-old rats, compared to younger animals, were able to maintain normal total serum protein values at lower dietary protein concentrations, while an inverse response was observed for liver lipoprotein synthesis. It is possible that, as an adaptive response to protein depletion, older rats sacrificed liver lipoprotein synthesis in order to maintain synthesis of other serum proteins, such as albumin.

As the percentage of liver fat decreased, a corresponding increase in percent water was seen for all treatment groups of both ages. Percent liver water was lower for all treatment groups of 18-month-old rats compared to the younger animals. These differences reached significance for the 1.53 and 4.98% protein diet groups

($p < 0.01$). Percent liver protein was lowest in 11- and 18-month-old rats fed the lowest level of dietary protein and increased as the protein content of the diet increased. For both ages, only groups fed 1.53% protein had liver percent protein values which were significantly lower than the respective control groups ($p < 0.01$). These results support the findings of Haider and Tarver (1969) who reported that the protein content of liver changed proportionally with dietary protein content when 200g female Sprague-Dawley rats were fed normal (27%), high (64%), low (8%), or protein-free diets for 5 to 7 weeks.

Total liver nitrogen content (mg) was significantly greater for 18-month-old rats compared to the younger rats at all dietary treatment levels ($p < 0.01$). This greater total nitrogen content can be explained in part by the larger size of the older rat livers. Livers of 18-month-old rats weighed significantly more than livers of 11-month-old rats at all treatment levels ($p < 0.01$). Lesser et al. (1980) found that, in male Sprague-Dawley rats, the fat-free mass of livers increased from weaning until 579 days of age. At older ages, fat-free liver mass remained relatively stable. Findings in the present study indicate that an increase in liver fat content with increasing age may also have contributed to

the larger liver size in older animals.

Several researchers have reported that total liver nitrogen content increases as the amount of protein in the diet increases, even at dietary protein levels considered to be above the minimum requirement level (Allison et al., 1962; Allison et al., 1964; Piedad-Pascual et al., 1970). In 11-month-old animals, total liver nitrogen did progressively increase with greater percentages of dietary protein. The total liver nitrogen value of the 1.53% protein group was significantly lower than the other treatment groups and the control group ($p < 0.01$).

In the 18-month-old rats, however, total liver nitrogen increased with increasing levels of dietary protein from 1.53 to 4.98%, then decreased with further increases in dietary protein to 6.52 and 8.05%. Total liver nitrogen content of 18-month-old rats fed 1.53 and 3.41% protein were not significantly different from the value for the 8.05% control group ($p < 0.01$). These findings disagree with those of the previously cited researchers who reported that liver nitrogen content increases with increasing protein consumption, even above the requirement level. The values for liver nitrogen content of 18-month-old rats correspond to changes in liver wet weight for this group (Table 7).

Carcass Analysis

Two-way analysis of variance showed a significant interaction effect of age and diet on carcass percent water, fat, and protein ($p < 0.05$). No significant interaction effect of diet and age on total carcass nitrogen was observed ($p < 0.05$). Carcass analysis for all groups is shown in Table 8. For this analysis, carcass refers to the entire animal minus the liver and blood drawn at sacrifice. Percent carcass fat was significantly greater for 18-month-old rats compared to the younger rats at all dietary treatment levels except 1.53% protein ($p < 0.01$). Body fat mass in rats has been reported to increase with age (Bertrand et al., 1980; Lesser et al., 1973; Mazeo and Horvath, 1986). Bertrand et al. (1980) found that in male Fischer 344 rats, body fat mass increased until about 70% of the lifespan and declined thereafter. The median length of life of the rats used in this study was 23.5 months.

In female Fischer 344 rats fed chow diets, percent body fat progressively increased in animals aged 3, 12, and 24 months. In their longitudinal and cross-sectional studies, Lesser et al. (1973) found that most male Sprague-Dawley rats continued to gain weight and fat throughout the life span. Median length of life of

Table 8. Carcass Composition of 11- and 18-month-old Male Rats Fed Varying Levels of Dietary Protein¹

Group (percent dietary protein)	Percent Water		Percent Fat		Percent Protein		Total Nitrogen (g)	
	11	18	11	18	11	18	11	18
1.53	60.59±0.66 ^a	58.45±0.66 ^a	14.93±0.89 ^a	17.80±0.89 ^a	20.10±0.42 ^a	20.00±0.42 ^a	11.78±0.36 ^a	12.48±0.36 ^a
3.41	59.22±0.66 ^{*a}	56.40±0.66 ^{*a}	17.56±0.89 ^{*ab}	21.01±0.89 ^{*a}	19.92±0.42 ^a	18.92±0.42 ^{ab}	13.31±0.36 ^b	12.83±0.36 ^a
4.98	58.43±0.66 ^{*a}	53.36±0.70 ^{*b}	18.73±0.89 ^{*b}	26.01±0.94 ^{*b}	19.45±0.42 ^a	18.26±0.44 ^b	13.90±0.36 ^{bc}	14.30±0.38 ^b
6.52	60.24±0.66 ^{*a}	52.57±0.66 ^{*b}	15.89±0.89 ^{*ab}	25.97±0.89 ^{*b}	20.59±0.42 ^{*a}	17.50±0.42 ^{*b}	14.87±0.36 ^c	15.11±0.36 ^b
8.05	60.30±0.66 ^{*a}	53.18±0.66 ^{*b}	15.92±0.89 ^{*ab}	25.40±0.89 ^{*b}	20.34±0.42 ^{*a}	17.54±0.42 ^{*b}	15.13±0.36 ^c	15.25±0.36 ^b

¹ All values represent group means ± SE.

* Indicate values which are significantly different between age groups (p<0.01)

Different superscripts indicate values which are significantly different within age groups (p<0.01)

these animals was 765 days. The longest-lived animals, however, were not representative of the rat population since these rats were characterized by a lower than average body weight.

In 11-month-old rats, percent carcass fat was highest for the group fed 4.98% protein. The tendency to lay down fat may have been influenced by the increased food consumption at this level of dietary protein (Table 5). The percent carcass fat value for the 4.98% protein group was significantly greater than that of the 1.53% protein group ($p < 0.01$). The 3.41, 4.98, 6.52, and 8.05% protein groups had percent carcass fat values which were not significantly different from each other ($p < 0.01$). For 18-month-old animals, groups fed 1.53 and 3.41% protein had significantly lower mean percent fat values than groups consuming higher levels of dietary protein.

Percent water, as expected, inversely followed the change in percent carcass fat. No significant differences in percent carcass water were found between dietary treatment groups for 11-month-old rats ($p < 0.01$). For older rats, percent carcass water was significantly higher in rats fed 1.53 and 3.41% protein compared to groups receiving greater amounts of dietary protein ($p < 0.01$). These differences reflect the decreased carcass

fat content at these low levels of dietary protein. In comparison to the younger animals, 18-month-old rats had significantly lower mean percent carcass water values at all dietary treatment levels except 1.53% protein ($p < 0.01$). Again, these differences reflect changes in carcass fat content.

Percent carcass protein of 11-month-old rats was not significantly different between treatment groups ($p < 0.01$). In the 18-month-old animals, however, mean percent carcass protein of the 1.53% protein group was significantly higher than the values for groups fed 4.98, 6.52, or 8.05% protein diets ($p < 0.01$). The higher percent protein value for this group is most likely due to the lower percent carcass fat content of rats fed this low level of protein. Percent protein values for other treatment groups were not significantly different from that of the control ($p < 0.01$). For the 6.52 and 8.05% protein groups, older rats had significantly lower carcass percent protein values than 11-month-old rats ($p < 0.01$). These differences probably result from a greater percentage of carcass fat relative to carcass protein with increasing age of the animal.

Total carcass nitrogen content for the two age groups is shown in Table 8. For 11-month-old rats, total carcass nitrogen of the group fed 1.53% protein

was significantly lower than other dietary treatment groups ($p < 0.01$). Total carcass nitrogen of groups fed 3.41 and 4.98% protein were not significantly different ($p < 0.01$). For groups fed 4.98, 6.52, and 8.05% protein, total carcass nitrogen values were also not significantly different ($p < 0.01$).

Eighteen-month-old animals fed 1.53 and 3.41% protein had total carcass nitrogen values which were not significantly different from each other, but were significantly lower than the values for groups fed higher levels of protein ($p < 0.01$). Carcass nitrogen values of groups consuming 4.98 or 6.52% protein were not significantly different from the control value ($p < 0.01$). Mean carcass nitrogen values which are lower than those of the respective control groups may indicate diets which did not supply adequate amounts of nitrogen for the maintenance of lean body tissue. It appears that 11-month-old rats were able to better adjust to a dietary protein level of 3.41% because the carcass nitrogen value for this group was not significantly different from the 4.98% protein group. Eighteen-month-old rats fed 3.41% protein, in comparison, had a carcass nitrogen value which was significantly lower than the value for the 4.98% protein group. These findings indicate that protein needs may increase with increasing age in rats.

An increasing protein requirement with increasing age in rats is supported by a study by Baldwin and Griminger (1985). These researchers reported that mature (12-month-old) rats could maintain nitrogen equilibrium when fed a 4.5% protein diet. This level of protein, however, was not sufficient to maintain positive nitrogen balance in aged (24-month-old) rats. A lower food consumption, and therefore nitrogen consumption, by the older rats probably influenced the results of this study. By regression, the amount of nitrogen needed to maintain nitrogen equilibrium was determined to be 90 mg/day for mature and 98 mg/day for aged rats. These differences, while not significant, are indicative of an increasing protein requirement with increasing age in rats.

In a conflicting study, Fischer and Canolty (1983) report that the protein requirement of rats appears to decrease with advancing age. In this study, 15- and 25-month-old male Sprague-Dawley rats were fed diets containing 2, 3, or 4% casein ad libitum, or at 80 or 60% ad libitum. At each casein concentration and level of intake, older animals had lower mean maintenance requirements than younger animals.

In the present study, it is interesting that no significant age differences in total carcass nitrogen

were observed at any dietary treatment level. Total carcass nitrogen of 11- and 18-month-old rats fed 4.98, 6.52, and 8.05% protein was comparable, despite significantly greater mean body weights of the older rats ($p < 0.01$). This finding indicates that the age-related increase in body weight primarily results from an increase in carcass fat content, rather than an addition of lean body tissue.

Figure 2 shows total carcass nitrogen in relation to final body weight of the experimental animals. This figure further indicates that weight gains were due primarily to increased body fat rather than nitrogen, and that weight losses resulted from a combined loss of nitrogen, fat, and water. An unexpected finding was that, for both ages, carcass nitrogen progressively increased with increasing levels of dietary protein. While differences were not significant at the three highest levels of protein ($p < 0.01$), this gain in carcass nitrogen continued even at levels of dietary protein thought to be above the maintenance protein requirement.

The minimum protein requirement for adult rats has been estimated to range from 3.18 (Hartsook and Mitchell, 1956) to 4.07% protein as casein (Sheehan et al., 1981). Carcass nitrogen content was expected to

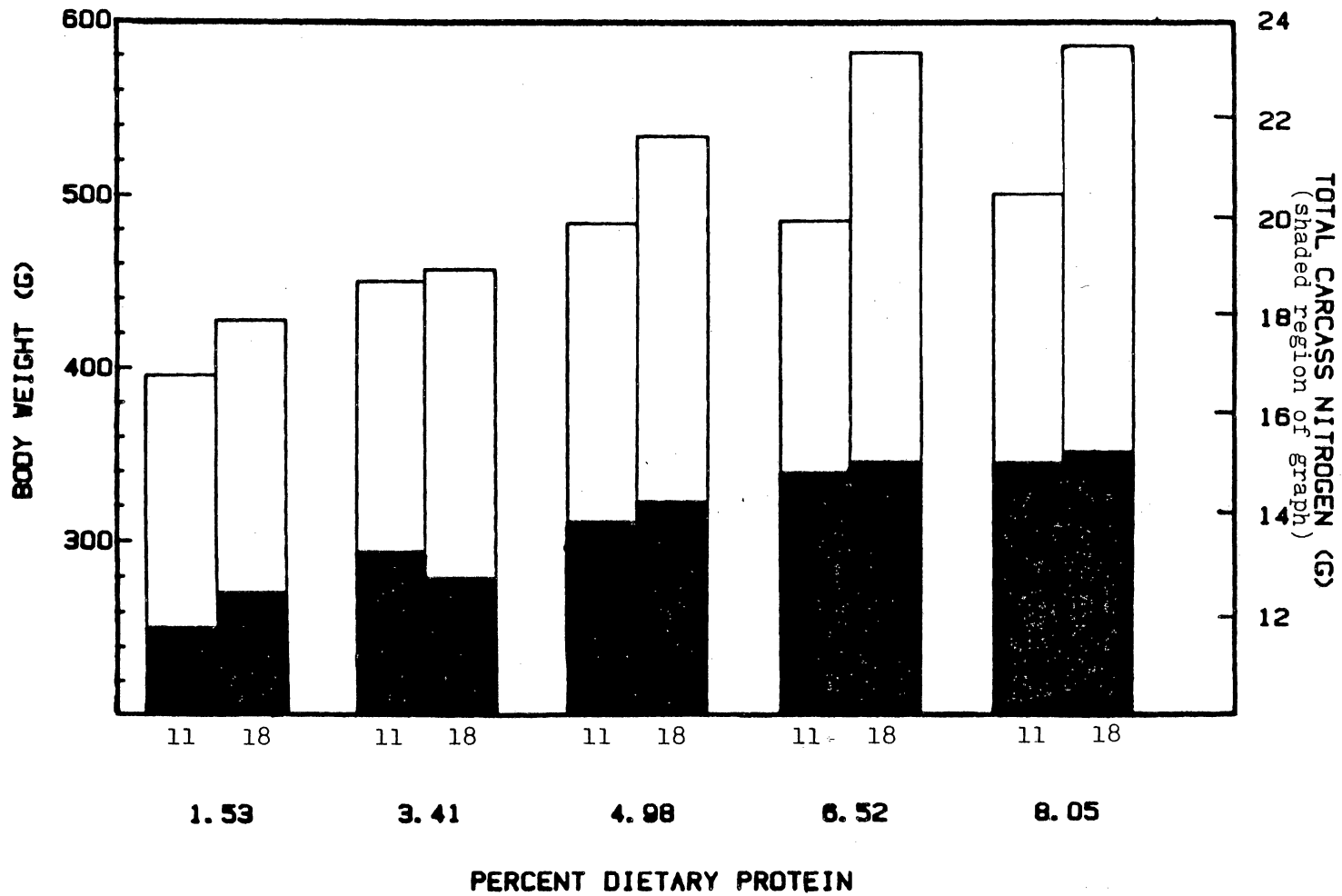


Figure 2. Final Body Weights and Total Carcass Nitrogen of 11- and 18-month-old Male Rats.

increase with higher levels of dietary protein until the maintenance requirement was met, and remain constant thereafter. Because this did not occur, inverse linear regression, using carcass nitrogen content of the 8.05% control groups to predict minimum protein requirements, could not be used.

Sheehan (1978) used carcass nitrogen as the dependent variable in inverse linear regression to predict the protein requirement of 12-month-old female rats. For this analysis, the minimum protein requirement was predicted at the point where change in carcass plus liver nitrogen was equal to that of a baseline group sacrificed at the beginning of the experimental period. By this method, the minimum protein requirement of these rats was estimated to be 4.07% of the diet. Treatment groups were fed diets containing either 0.84, 1.86, 2.81, 3.77, or 4.69% protein as casein supplemented with methionine.

The baseline group used by Sheehan (1978) for comparison was fed a laboratory chow diet prior to sacrifice. This chow diet contained approximately 23% protein supplied by animal and vegetable sources (Purina Rodent Laboratory Chow). In the study by Sheehan (1978), total carcass nitrogen values for groups fed varying levels of protein also increased with increasing

amounts of protein. If rats continue to accumulate carcass nitrogen at dietary levels above the maintenance requirement level, it is possible that comparison to a baseline group consuming 23% protein would result in a falsely high maintenance requirement estimate.

Said and Hegsted (1969) used inverse linear regression with carcass water as the dependent variable to predict the maintenance protein requirement of 200g female rats. Similar to Sheehan (1978), these researchers determined change in carcass water by comparison to initial values of a baseline group. Presumably, the baseline group used in this study was also fed a chow diet prior to sacrifice. The level of no change in carcass water was used as the point of prediction. By this method, the maintenance protein requirement was estimated to be 3.39% of the diet.

In the present study, total body water of both age groups, similar to carcass nitrogen, increased with increasing levels of dietary protein. This increase was expected since carcass water is known to be highly correlated with carcass nitrogen (Said and Hegsted, 1969). Using carcass water to determine protein requirements, as with total carcass nitrogen, may result in an overestimate of protein needs. In order for this method of regression analysis to be more accurate, a means of

determining an adequate minimum level of carcass nitrogen and water content are necessary. The determination of a precise minimum protein requirement is complicated by the rats' ability to adapt to a wide range of protein intakes by shifting the balance of body protein anabolism and catabolism (Peng, et al. 1974).

Relatively little information concerning the effect of varying levels of dietary protein on rat lean body mass content is available in the literature. Donald et al. (1981) found that the body weight of mature (10 week) male Sprague-Dawley rats increased with increasing levels of dietary protein above 5%. Rats in this study were fed isocaloric diets containing 2, 5, 10, 15, or 25% protein for 9.5 weeks. Unfortunately, these researchers did not measure muscle mass and, therefore, could not determine if body weight changes were partially attributable to increases in fat-free body mass. Body weight differences did not result from a greater food consumption by rats fed higher levels of protein. In view of the findings in the present study, it appears likely that body weight gain of rats consuming higher levels of protein could be explained, at least partially, by an increase in lean body mass as well as fat content.

Changes in lean body mass (LBM) of rats with

increasing age have been reported (Lesser et al., 1973; Lesser et al., 1980; Yu et al., 1982). In male Fischer 344 rats, LBM was found to increase for the first 75% of the life span, then remain stable until very late age. The decline in LBM observed in the longest-lived rats was attributed to the onset of the terminal disease process.

Lesser et al. (1980) found that fat-free body mass (FFB) of male Sprague-Dawley rats fed balanced diets increased 8% from 362 to 579 days of age. From 579 to 715 days, FFB remained stable, then declined at later ages. This decline in late age was attributed to selective longevity of smaller animals. Protein/FFB of rats used in this study increased until 421 days and remained relatively constant through 800 days of age. In agreement with Lesser et al. (1980), carcass nitrogen content of 11- and 18-month-old rats indicates that lean body mass increases very slightly during this age span in male Sprague-Dawley rats (Table 8). Masoro et al. (1980) propose that the rat differs from man since, in man, a progressive decline in lean body mass occurs after the third decade (Forbes and Reina, 1970).

LIMITATIONS

Several important limitations of this study should be discussed. Careful measurement of food spillage was needed to accurately determine food, and therefore nitrogen, intake. The powdered form of diets used, however, made estimation of food spillage difficult. In weighing back spillage collected from underneath cages, feces, urine, and rat hair mixed with food may have resulted in an overestimate of food spillage. An overestimate of spilled food could have resulted in an underestimate of food consumption. Consistency in the results obtained for food intake of both age groups of rats indicates that error in determining food intake was not substantial.

The ages of rats used in this study may also have been a limiting factor. According to Lesser et al. (1980), the choice of age groups used in rat aging studies is generally based upon at least two assumptions. First, it is important for the young controls to have reached maturity and attained full growth. Rats approximately one year of age, such as the 11-month-old rats used in this study, are commonly chosen for the young control group. Secondly, the older animals used must be old enough to evidence the sought-for senescent

changes. The 18-month-old rats used in the present study may not have been of sufficient age to satisfy this assumption. While differences in protein requirement levels are suggested by the current findings, it is not possible to make definite conclusions about changing protein requirements during aging. Physiological differences between rats aged 11 and 18 months may not be sufficient to provide a precise indication of the extent to which protein needs are altered with age.

Another limitation of this study was the difficulty encountered in obtaining homogenous whole carcass samples. The Hobart grinder used to grind carcasses with Na_2SO_4 did not thoroughly grind some pieces of rat skin. Despite this difficulty, good duplication of samples, to within 10% error, was obtained.

Sacrifice of a baseline group, as well as a concurrent control group, would have provided an additional means for comparison of treatment groups. By the method of Sheehan et al. (1981), this baseline group could have been used to predict protein requirements for each age. Because carcass nitrogen increases with increasing levels of dietary protein to an undetermined level, the validity of this method of determining maintenance protein requirements may be questioned.

SUMMARY AND CONCLUSIONS

This study was conducted to determine the effect of varying levels of dietary protein on the carcass composition of 11- and 18-month-old male Sprague-Dawley rats. Fifty rats of each age were fed diets containing from 1.53 to 8.05 percent protein as casein supplemented with d-1-methionine. The experimental feeding period was continued for five weeks. Rats fed the 8.05% protein diet were used as a concurrent control group. The effects of the various dietary treatment levels on total serum protein, liver composition, and carcass composition were determined.

An important finding was that rats of both age groups continued to increase in carcass nitrogen content even at dietary protein concentrations above the estimated requirement level. Because of this, inverse linear regression, with carcass nitrogen as the dependent variable, could not be used to predict specific protein requirements of 11- and 18-month-old rats. Although it was not possible to estimate a specific minimum protein requirement for each age group, differences in the physiological responses of 11- and 18-month-old rats to the varying dietary treatment levels were apparent.

Eighteen-month-old rats, compared to the younger

animals, appear to require a higher level of protein for the maintenance of body weight. The 11-month-old rats were able to maintain body weight when fed a 3.41% protein diet. Older rats, however, lost a significant amount of weight at this level of dietary protein ($p < 0.01$). Food intakes of both age groups were similar, with the greatest amount of food consumed by the 4.98% protein groups. Food consumption was lowest for groups fed 1.53% protein. Decreased food consumption by animals fed the lowest level of dietary protein may have been limited by an excess consumption of energy in relation to protein (Meyer and Hargus, 1959).

Excessive liver fat values were observed for 11-month-old rats fed 1.53 and 3.41% protein. For 18-month-old rats, fatty livers were found in groups consuming 1.53, 3.41, and 4.98% protein diets. These results indicate that a higher level of dietary protein is required by the older animals to provide adequate protein for lipoprotein synthesis by the liver. Liver fat values were greater for 18-month-old rats compared to the younger animals at all dietary treatment levels. These differences were significant for the 1.53, 3.41, and 4.98% protein groups ($p < 0.01$). Liver fat content, therefore, appears to increase with age in rats.

For all groups, percent liver water varied

inversely with liver fat content. Percent liver protein was lowest in groups fed 1.53% protein and increased as the protein content of the diet increased. Total liver nitrogen of older rats, versus younger rats, was significantly greater at all dietary treatment levels ($p < 0.01$). The higher nitrogen content of 18-month-old rat livers corresponded to the larger liver size of this age group.

In 11-month-old rats, total liver nitrogen increased with increasing amounts of dietary protein. In older rats, total liver nitrogen progressively increased in groups fed 1.53 to 4.98% protein, then decreased at higher dietary protein levels. This decrease corresponded to changes in liver wet weights of 18-month-old rats fed 6.52 and 8.05% protein.

Compared to control values for each age group, older rats were able to maintain normal total serum protein values at lower levels of dietary protein than 11-month-old rats. Younger rats fed 1.53, 3.41, and 4.98% protein had total serum protein values which were significantly lower than that of the control ($p < 0.01$). For 18-month-old rats, only groups fed 1.53 or 3.41% protein could not maintain normal serum protein values ($p < 0.01$). This finding suggests that older rats may adapt to protein depletion by limiting liver lipoprotein

synthesis in order to maintain synthesis of other serum proteins.

Compared to the younger animals, 18-month-old rats had a significantly greater body fat mass at all but the 1.53% protein level ($p < 0.01$). It is well established that carcass fat increases with age in rats. Percent carcass water, as expected, inversely followed the change in carcass fat. Values for percent carcass water and percent protein were affected by fat content and body size differences of the two age groups of rats. The greater body weight and increased fat mass of 18-month-old rats was a complicating factor in this study.

Eighteen-month-old rats fed 3.41% protein had a total carcass nitrogen value which was significantly lower than that of the 4.98% protein group ($p < 0.01$). The younger rats consuming a 3.41% protein diet had a total carcass nitrogen value which was not significantly different than the value for the 4.98% protein group ($p < 0.01$). A level of 3.41% protein appears to be adequate to maintain carcass nitrogen of the 11-month-old, but not the 18-month-old rats. These results are suggestive of a higher protein requirement for older rats. In the literature, both increasing and decreasing protein requirements with advancing age in rats have been

reported (Baldwin and Griminger, 1985; Fischer and Canolty, 1983).

Body weight change, liver fat, and carcass nitrogen content of 11- and 18-month-old rats in response to varying levels of dietary protein indicate that older rats have greater protein needs than younger mature animals. However, since total carcass nitrogen values between age groups are not significantly different ($p < 0.01$), any increase in the protein requirement from 11 to 18 months appears to be very slight.

A general age-related decline in the protein synthetic rates of numerous tissues in rats has been reported (Pluskal et al., 1984; Cook and Buetow, 1981; Ricketts et al., 1985; Geary and Florini, 1972; Ekstrom et al., 1980). This decline in protein synthetic activity is suggestive of a decreasing protein requirement with advancing age. Other factors such as decreased metabolic rate and physical activity with age also indicate that protein requirements would decrease. The results of this study, however, do not support this hypothesis. Factors such as decreased food consumption and an increased susceptibility to disease may serve to increase protein needs at older ages.

Rapid rates of protein turnover (synthesis and degradation) are thought to provide an organism with the

ability to successfully adapt to changes in the internal and external environments (Young, 1984). Under conditions of nutritional deprivation, the ability to rapidly turnover protein may facilitate redistribution of body nitrogen and amino acids. If the ability for rapid protein turnover is diminished in older rats, a decreased efficiency of adaptive response to low dietary protein levels may result.

More studies are needed to further clarify the nature and extent of age-related changes in the protein needs of rats. Using rats as a model system can contribute to knowledge concerning changing protein requirements in the aging human population. In future research, it is suggested that rats of greater age differences be used. Three different ages of experimental animals would provide an even more effective means of determining age-related changes in protein needs. Sacrificing a baseline group in addition to having a concurrent control group would also be useful for comparative purposes. Finally, further study to investigate the extent to which carcass water and nitrogen content increase with increasing dietary protein levels seems warranted.

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