CARCASS NITROGEN AS A PREDICTOR OF LYSINE REQUIREMENT

IN THE ADULT FEMALE RAT

by

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(ABSTRACT)

Adult female Sprague-Dawley rats, age 10 months, were used to estimate the minimum dietary lysine requirement for tissue maintenance. Ten animals were assigned to one of eight treatment groups by weight. The dietary lysine levels ranged from 0.06 to 0.36 % of diet and the feeding period lasted 56 days. Carcass and liver nitrogen and total serum proteins were determined, and a dietary lysine requirement was estimated from the data obtained.

Carcass and liver analysis included weight, total nitrogen, percent protein, percent water and percent fat. Using a one-way analysis of variance, results showed no significant differences in carcass or liver composition between the treatment groups.

The data indicated that the mature female rat has a dietary lysine requirement lower than 0.06 % of diet, or less than 20.1 mg/day/kg$^{0.75}$. Since previous investigators used a protein based diet, a possible cause for the insignificant differences between values seen in this study may be a consequence of using a nonprotein, amino acid mix base

supplemented with lysine.

In future studies for determining the dietary lysine requirement in the adult female rat, dietary lysine levels below 0.06 % of diet must be included when using a nonprotein based diet.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Minimum attention has been given to the protein require-
ment of the aging organism. Physiological and possibly psy-
chological factors may contribute to the altered protein
needs in senescence. With increasing age of the human and
mature rat, for example, a decrease in lean body mass occurs
(1). A decrease in protein synthesis in muscle tissue and
in certain organs such as the brain, liver, and pancreas
also takes place (1,2). This decrease could be a major rea-
son for a lower need of an exogenous source of nitrogen and
dietary essential amino acids required for tissue mainte-
nance in the aged rat. A study by Richardson and Birche-
nall-Sparks (3) further explains the decrease in aggregation
and activity of polyribosomes, and changes in the rates of
the initiation and elongation phases of protein synthesis.
The lack of aggregation of the polyribosomes does lead to a
drop in the efficiency of protein anabolism within the cell.

Early studies by Barnes et al. (4), Goettsch (5), Hamil-
ton (6), Hartsook and Mitchell (7), Hoagland et al. (8),
Mitchell and Beadles (9), and Rose et al. (10) determined
the protein requirement of the weanling rat to be a combined
average of 29 mg of protein per kilocalorie intake. The
diets used in these studies contained whole egg protein. The
National Research Council (11) has set the protein require-
ment for maintenance in the adult rat at 4.20 % of diet from
past studies using ideal protein. The results of these

studies indicate a 65 % decrease in protein requirement for the adult rat as compared to the growing, pregnant, or lactating rat.

In a more recent study conducted by Sheehan et al. (12), a method based on carcass nitrogen concentration was used to assess the protein requirement for mature female rats. Sheehan and coworkers (12) reported a diet containing 4.07 % dietary protein was sufficient to maintain nitrogen equilibrium. The study concluded that when the active growing phase is completed, there is an inverse relationship between age and protein requirement.

The National Research Council (11) has estimated the amino acid requirements for the rat from the studies by Bendit et al., Smith and Johnson, and Said and Hegsted (13-15). Currently, the National Research Council (11) lists two separate amino acid requirements. One set of requirements for the adult rat and a second for the growing, pregnant, and or lactating rat. Values for the adult rat range 38 to 86 % lower than those for the growing, pregnant, or lactating rat. The amino acid lysine is indicated as having the largest decrease in requirement necessary for tissue maintenance in the adult rat.

The growing rat is estimated to have a dietary lysine requirement of 0.70 % of diet , while the adult rat has been determined to have a lysine requirement of 0.11 % of diet (11). The interpretation of the data supporting a lysine requirement in the adult rat, set at 0.11 %, is controver-

sial and the amount of supporting data are insufficient. Researchers agree that lysine is a dietary essential amino acid for both the human and animal; however, recent studies concerning lysine metabolism and the ability of the adult rat to conserve and adapt to very low levels of dietary lysine bring a new perspective into the area of aging and lysine requirement (16,17).

The purpose of this study is to determine the lysine requirement for ten month old female rats based on initial and final weights, carcass and liver nitrogen analysis, carcass and liver fat analysis, and plasma protein concentrations.

LITERATURE REVIEW

Protein Requirement of The Adult Rat

The protein requirement needed for tissue maintenance in the adult rat has been studied by many researchers. Protein metabolism in the aged organism, such as the rat and human, has been shown to be altered as a result of senescence (18). The aging rat undergoes physiological changes which ultimately decreases the amount of exogenous nitrogen and dietary essential amino acids needed for tissue maintenance (1). Ordy (18) mentions that Young estimates a 30 % decrease of dietary protein requirement between 20 and 75 years of age in the human. A decrease in protein synthesis and a decline in lean body mass with age are believed to be two major reasons for a lower protein requirement.

An early study by Bricker and Mitchell (19) shows a diet containing 3.84 % whole egg protein is sufficient for nitrogen equilibrium in the adult rat. A diet containing 3.28 % whole egg protein was also used in their study; however, this level caused an average negative nitrogen balance of 3.1 mg per day. Bricker and Mitchell further explain that whole egg protein is utilized in the mature rat with little or no wastage.

Hartsook and Mitchell (7) report nitrogen balance with a diet containing 3.18 % protein as casein in 300 day old male rats. The diet, however was supplemented with the sulfur

containing amino acid L-methionine. Sheehan et al. (12) report values similar to Bricker and Mitchell's findings in the amount of 3.91-4.19 % ideal protein needed to maintain nitrogen equilibrium in the adult rat. Sheehan used a methodology consisting of carcass and liver nitrogen analysis, change in body weight, percent body fat and percent body water to determine the protein requirement of the adult female rat. Bricker and Mitchell (19) used urinary and fecal nitrogen, and change in body weight to derive their conclusions. The diets that were used in the above experiments contained a high biological value protein, such as lactalbumin or casein. A treatment containing a source of low biological value protein would not be expected to maintain nitrogen equilibrium at 3.84 %.

Goettsch (5) determined the protein requirement of adult male rats using a diet in which the protein was supplied by rice, beans, and casein. The biological value of this protein mixture was determined to be 72.3 %, whereas the biological value of casein alone is approximately 95 %. Because of the lower biological value of the protein in the diets that Goettsch (5) used, a higher percentage of protein was needed for tissue maintenance in the adult rat (7.25 %). Many similar studies have been performed which support these findings.

Dietary Essential Amino Acids in the Adult Rat

The nutritive value of a dietary protein is generally assumed to be dependent on the amounts of the amino acids it contains relative to the amounts of these amino acids required by the animal consuming the protein (20). Burroughs et al. (21) concluded in 1940 that the following amino acids were not needed for the replacement of endogenous losses of nitrogen in the adult rat: lysine, leucine, histidine, arginine, and phenylalanine. In contrast, a study by Wissler et al., as explained by Benditt et al. (13), demonstrated that nine dietary amino acids are indispensable for nitrogen equilibrium in the mature rat. The so-called dietary essential amino acids were tryptophan, lysine, histidine, phenylalanine, theonine, methionine, valine, leucine, and isoleucine. The discrepancy between the two studies was the result of a limited feeding time in the study performed by Burroughs et al. (21). The length of the feeding period within the experiment was an average of six days.

Benditt et al. (13) supported the findings by Wissler et al. with a follow-up study using 84 adult male albino rats. Since these two early studies, researchers have proven that the adult rat requires a dietary source of tryptophan, lysine, histidine, phenylalanine, threonine, methonine, valine, leucine, and isoleucine for nitrogen equilibrium. The estimated amount of each of the above dietary essential

amino acids needed for maintenance in the adult rat can be found in reference number 11. Since the objective of this study is to determine the amount of lysine needed for maintenance in the adult rat, the remainder of this section will be devoted to studies which relate to lysine requirement and metabolism, and the methodology used.

Lysine Requirement in the Adult Rat

In 1916, Rose (22) stated that Osborne and Mendel were the first to provide conclusive evidence of lysine as a dietary essential amino acid for the function of growth. Burroughs et al. (21), however, reported lysine as a dietary nonessential amino acid for the adult female albino rat in 1940 on the basis of a six-day feeding period. Studies thereafter proved that lysine is a dietary essential amino acid for maintenance in the adult rat.

Said and Hegsted (15) used adult female albino rats to determine the minimum lysine HCl requirement for maintenance. The weights of the rats ranged from 200 to 220 g. The experimental design consisted of six treatments with variable amounts of all the dietary essential amino acids. A lysine deficient diet, compared to the other dietary essential free amino acid diets, was shown to cause the least amount of decrease in body weight. Said and Hegsted concluded that the adaptability of the adult rat to low levels of lysine is great and estimated the lysine HCl requirement

at 0.89 % of diet, or 34 mg/day/kg$^{0.75}$. These results were based on body weight, total body water, and total body protein analysis. The estimate of 34 mg/day/kg$^{0.75}$ was calculated relative to metabolic body size.

Smith and Johnson (14) determined the lysine requirement of adult rats using a nitrogen balance assay. Their results were shown to be similar to Said and Hegsted's (15) finding, estimated at 27 mg/day/kg$^{0.75}$. Nasset and Ely (23) also used a nitrogen balance assay to determine the lysine requirement of adult albino rats. In contrast to the findings of Said and Hegsted (15) and Smith and Johnson (14), Nasset and Ely estimated the lysine requirement of the adult rat at 3.6 mg/day/kg$^{0.75}$. The variability seen in these studies may be attributed to the length of feeding time, methodology and/or the biological value of the proteins used within the diets. A summary of the results of these studies, and others, concerning the lysine requirement of adult rats can be found in Table 1.

Adaptability of the Rat to Low Dietary Lysine Levels

Recent studies involving the adaptability to low levels of lysine by the rat has been studied by Yamashita and Ashida (17), Shu-Heh and Hegsted (16), and Mittal (24).

Yamashita and Ashida used young male rats weighing 60 to 70 grams to study the effect of a lysine-free diet and a threonine-free diet on lysine metabolism. Free lysine and

Table 1

Maintenance Requirement of Lysine in the Adult Rat

Source	Lysine in $mg/day/kg^{0.75}$	Method used	Reference
Benditt et al.	37.0	N Balance	13 [1]
Nasset	4.0	N Balance	15
Smith and Johnson	27.0	N Balance	14
NRC	32.2	---------	11 [2]
Said and Hegsted	34.0	Carcass water	15
Nasset and Ely	3.6	N Balance	26
Wang et al.	<40.0	Carcass N	30

[1] Estimate reported by Said and Hegsted (15).

[2] An average estimate by the National Research Council (11) calculated from various studies.

threonine concentrations were determined in plasma, liver, and muscle of the rats in both treatments. The level of threonine declined markedly in all three tissues as a result of the threonine-free treatment. The lysine-free diet, however, had little effect on the levels of lysine in plasma, liver, and muscle tissues. Yamashita and Ashida summarized that a mechanism to maintain the level of lysine in the body is apparent.

Mittal (24) studied the effect of low lysine levels on rats of various ages: 3, 13, 26, and 52 weeks old. This study indicated a greater affect on the younger rat as opposed to the adult rat. The three week old rat was unable to adapt to a lysine deficient diet as did the adult rat. The 52 week old rats, however, had lower liver protein levels after prolonged feeding of a low protein quality-lysine deficient diet. Mittal further explains the adaptation in the adult rat may be a consequence of reduced turnover of protein or increased reutilization of amino acids in the mature rat.

Liver enzyme activity was measured in adult female rats fed a lysine-free and a threonine-free diet by SHU-Heh and Hegsted (16). This interesting study directly shows the ability of the rat to adapt to low levels of dietary lysine. Body weights were measured throughout the experiment, and livers were removed and analyzed for enzyme activity after termination of the feeding period. The threonine dehydratase activity of rats fed either a protein- free diet or a 5

% lactalbumin diet was shown to have no significant difference, although a marked decrease in body weight was observed. The rats fed a lysine-free diet lost significantly less body weight than rats fed a protein-free diet or the threonine-free diet. A significant reduction in liver lysine -ketoglutarate reductase activity was seen in rats fed the lysine-free diet. The liver lysine- ketoglutarate reductase activity was shown to be 10.81 umoles/hr/g of liver with a complete amino acid diet. The activity of this enzyme decreased to 3.29 umoles/hr/g when a lysine-free diet was consumed by the adult rat. This is nearly a three-fold decrease in activity. Therefore, conser- vation of lysine occurs significantly when dietary lysine is depleted.

Carcass Analysis

In the well nourished adult rat and human maintaining approximately the same weight over long periods of time, body composition should remain constant (25). Protein requirements for animals and humans for the most part have been estimated using nitrogen balance studies (26). Balance studies to determine the protein requirement, the biological value of a protein, and or the dietary amino acid requirement relates to the amount of nitrogen consumed compared to the amount of nitrogen lost via urine, feces, skin, hair, and nails. Wallace (27) states that balance studies will always yield falsely high retentions because of a consistent

bias in these data. Excretion will usually be underestimated since some loss is inevitable.

Because an adequate level of exogenous nitrogen and dietary essential amino acids are needed for nitrogen equilibrium and maintenance of body weight in the adult rat, direct carcass nitrogen analysis and body weight changes have recently been used to determine the protein and amino acid requirements of the adult rat (12, 26). Sheehan et al. (12) used carcass nitrogen analysis to determine the protein requirement of adult female rats. Diets consisting of protein (casein) ranging from 0.84 to 9.90 % of diet supplemented with L-methionine were used as the treatments. Comparison of treatment means were made using Duncans' multiple-range test and an inverse regression was used to predict dietary protein requirements. The values reported by Sheehan et al. indicate a protein requirement which is both higher and lower than estimates reported in the literature. A requirement of 3.18 % protein as casein was found by Hartsook and Mitchell (7) to support maintenance for adult male rats, whereas Bricker and Mitchell (19) reported a requirement of 4.33 % protein of diet. Currently, the National Research Council (11) has set the protein requirement for adult rats at 4 % of diet, a level very close to the range indicated by Sheehan et al. (12).

Carcass nitrogen analysis also has been used to determine the requirement of dietary essential amino acids (26). Direct carcass nitrogen analysis, which was performed by

Sheehan et al. and Wang et al. (26), can be assumed to be valid since a diet which is not adequate in a single dietary essential amino acid causes decreased nitrogen retention. Therefore, determination of total carcass nitrogen, was used in this study to measure carcass nitrogen concentration. The variable manipulated in this study was the dietary essential amino acid lysine, ranging from 0.06 to 0.36 % of diet.

Liver Analysis

The rate of protein synthesis in liver cells has been shown to decline in senescence (28-30). Cook and Beutow (28) compared the rate and extent of protein synthesis in 10-13 month old adult rats with 24 to 30 month old senescent rats. The results indicated a lower activity level of liver polysomes, and tRNA, and a lower activity level of tRNA synthetases of senescent rats as compared to the younger rats. Coniglio et al. (29) in a similar study found a 44 % decrease in liver protein synthesis between 2.5 and 18 month old rats. A substantial number of studies have shown liver protein synthesis to be decreased in senescence. However, studies which report the effects of various levels of lysine on liver protein synthesis in aging are limited.

Wang et al. (26) studied the effects of dietary lysine, ranging from 0.097 to 0.317 % of diet, on liver protein, total liver nitrogen, and liver weights of 50 twelve-month

old rats. The results showed a lower liver percent protein concentration in rats fed the highest level of lysine, 0.317 %. The value was found to be significantly different at the p <= 0.05 level from all other treatments. Total liver nitrogen, however, was not found to be significantly different between groups.

Serum Proteins and Amino Acids

A deficient level of a dietary essential amino acid in the diet has been shown to reduce the concentration of that amino acid in the plasma of man (31-33). In experimental animals, the pattern of amino acids in plasma, as well as the level of a specific dietary essential amino acid in plasma, correlate with the ability of the dietary protein to support growth (26). McLaughlan and Illman (34) reported that an almost linear relationship between free lysine in plasma and the dietary level of lysine occurs in the growing rat. The growing rat, however, also has been shown to have a less adaptive response to low levels of dietary lysine (24). Mittal (24) reports that the serum protein level decreased significantly in three and 13 week old rats fed a low lysine diet; whereas, the serum protein level was not significantly altered in 52 week old rats fed a low lysine diet. The source of protein was supplied by wheat, which is very low in lysine.

Sheehan et al. (12) reported significant differences in

serum proteins between adult rats fed 2.14 % dietary protein and 4.17-9.90 % dietary protein as casein. Serum lysine concentrations in the study by Sheehan et al. were not analyzed. The results in the above studies indicate a need for further serum protein analysis relating to dietary protein and lysine intakes.

METHODS AND MATERIAL

Animals

The choice of methods used for this experiment have been accepted and used by many researchers in the area of protein and fat analysis in the experimental animal. The rats were treated throughout the study in a manner which was within the rules and regulations involving animal ethics.

Ten month old female rats of the Harlan Sprague-Dawley strain, obtained from Madison, Wisconson, were used in this study. The rats were housed in suspended wire bottom cages; a twelve hour light and a twelve hour dark cycle was used via automated controlled lighting system. The temperature of the feeding room was held at a constant 72 ^{o}F, or 21 ^{o}C. The animals were weighed once a week and food and water were allowed ad libitum throughout the entire feeding period.

Experimental Design

The animals were first taken off rat chow and put on a powdered casein diet to acclimate the subjects to the powder form of the experimental diets. Following a period of 12 days, the animals were randomly assigned by weight to one of eight treatments. A total of ten animals were allotted to each treatment group. The percent concentration of lysine,

16

ranging from 0.06-0.36 % of diet, for the eight diets are noted in Table 2.

All diets were isocaloric and isonitrogenous and supplied 4.0 kcal/g of feed. The varying levels of lysine were made at the expense of the amino acid mix. A supplement of L-methionine was added to each diet to assure an adequate supply of sulfur amino acids in a proportion recommended by the National Research Council (11). A summary of the composition of each experimental diet can be found in Table 3. The duration of the feeding was 56 days.

Sacrifice of Animals

At the end of the experiment, each animal was anethesized using carbon dioxide. The unconscious rat was then exsanguinated by heart puncture and the liver was removed, rinsed with saline, blotted dry, and weighed. After weighing, the livers were put in a polycarbonate specimen cup with a snap-on lid. Each carcass also was weighed, put into a ziploc storage bag, and frozen until analysis. The whole blood drawn from each carcass was centrifuged at approximately 3000 rpms at a temperature of 4 ºC. After centifuging, the serum was removed (top layer), placed in disposable test tubes, and stored frozen until further analysis.

TABLE 2

Lysine Levels in Experimental Diets

Group	% Lysine in Diet[1]
1	0.298
2	0.298
3	0.060
4	0.120
5	0.180
6	0.240
7	0.300
8	0.360

[1] Lysine levels include amount in 4.31 % casein (group 1) and the amount of lysine added to lysine-free amino acid mix (groups 2-8).

TABLE 3

DIET COMPOSITION[1,2]

	Ingredients (expressed as grams/100 grams)		
Diet (% lysine)	casein	custom amino acid mix	Added L-lysine
0.298	4.31	----	0
0.298	----	4.69	0.298
0.060	----	5.00	0.060
0.120	----	4.92	0.120
0.180	----	4.84	0.180
0.240	----	4.76	0.240
0.300	----	4.69	0.300
0.360	----	4.61	0.360

[1] Ingredients which were held constant in each diet were as follows: L-methionine, 0.10; corn starch, 37.50; dextrose, 37.50; corn oil, 13.00; AIN mineral mix, 4.00; AIN vitamin mix, 2.00; alphacel, 2.00.

[2] Corn oil was purchased from Proctor and Gamble, Cincinnati, Ohio. All other ingredients were purchased at ICN, Cleveland, Ohio.

Carcass Analysis

The term carcass in this study is defined as the total animal body minus the liver. Five animals per group were used for carcass analysis. A meat cleaver and a rubber mallet were used to chop each frozen carcass in pieces approximately one inch square. The chopped carcasses were placed in aluminum loaf pans, covered with cheesecloth, and placed in a freezer to prevent thawing. After freezing, the carcasses were then put into a freeze-drier for 72 hours. Immediately after freeze-drying, the samples were placed in an oven overnight at a temperatue of 55 oC to expell any moisture during handling. Upon removal from the oven, the carcasses were re-weighed. Carcass water content was determined as the weight lost during freeze-drying (appendix A).

Each carcass was ground with a 4:1 ratio of sodium sulfate to carcass weight using a Hobart grinder. Sodium sulfate was added to the grinding to promote a homogenized mixture. During the grinding process, much effort was given to retain all of the carcass content. Approximately 30 minutes were needed per carcass to complete the grinding. When the grinding was finished, the carcass- sodium sulfate mixture was sealed in a mason jar until further analysis.

The nitrogen content of the carcass was determined using the Kjeldahl method (appendix B). Duplicate 2 g samples per carcass were used for the nitrogen assay. Carcass fat content was determined using a Soxhlet fat extraction method.

A duplicate was also used for this analysis with each sample weighing approximately 10 g (appendix C).

Liver Analysis

The frozen livers were freeze-dried for 48 hours. After freeze-drying, the livers were placed in an oven overnight at 55 ºC to expell any remaining moisture. The livers were placed in a dessicator after drying in the oven until cooled, then weighed, and the moisture content was determined as the weight lost during freeze-drying (appendix A). Using a mortar and pestle, each liver was ground up into a fine powder. A 0.5 g sample was weighed out and used for determining the fat content utilizing the Soxhlet fat extraction method (appendix C).

Total Serum Proteins

Total serum protein concentration was determined using a biuret method (Stanbio Total Protein Procedure No. 0251). The frozen serum samples were placed in the refrigerator overnight to allow for thawing. Once thawed, total serum protein analysis was achieved using a Bausch and Lomb Spectrophotometer.

Statistics

A one way analysis of variance was used to analyze the data. A probability value of .05 was considered to be significantly different.

RESULTS AND DISCUSSION

Body Weight

The average initial and final body weights of each
treatment group (Table 4) indicate no significant differ-
ences in final body weight between the groups. The 4 %
casein group, containing 0.298 % lysine, was the only group
which gained weight after eight weeks of feeding. All other
groups lost weight. The rats consuming 0.06 % lysine showed
the greatest amount of weight lost at -11.8 ± 3.2 g. The
weight loss was expected to occur in this group since the
estimated requirement of lysine for maintenance in the adult
rat is 0.11 % of diet (11).

When a dietary essential amino acid is lacking in any
protein, or amino acid mixture, the animal is expected to
show the same results as if given a protein-free diet, which
is a marked decrease in body weight. Said and Hegsted (15)
found this to be true as a result of feeding adult female
rats a threonine-, isoleucine-, methionine- and cystine-free
diet. However, these researchers did not find a significant
decrease in weight when fed a lysine-free diet. In addi-
tion, Wang et al. (26) observed body weight change in adult
female albino rats fed a wheat-based diet ranging in lysine
levels of 0.097 to 0.317 % of diet. There were no signifi-
cant differences observed relating to weight gain or loss
between the experimental groups. The limited body weight

23

TABLE 4

AVERAGE BODY WEIGHT[1]

Percent Lysine in Diet	Body Weight (g)		
	Initial	Final	Difference
0.298 (4.31 % casein)	267.6±6.4	268.4±6.1	0.8
0.298 (4.31 % amino acid mix)	263.7±6.4	259.6±6.1	−4.1
0.060	265.5±6.4	253.7±6.5	−11.8
0.120	266.8±6.4	260.7±6.1	−6.1
0.180	267.5±6.4	266.7±7.9	−0.8
0.240	259.2±6.4	258.5±6.1	−0.7
0.300	264.1±6.4	259.4±7.3	−4.7
0.360	266.8±6.4	265.6±6.1	−1.2

[1] All values are expressed as group means + SE.

loss found in this study and in other studies relating to lysine metabolism in the adult rat, support the findings by Said and Hegsted (13) and Wang et al. (26).

Food Intake

The average food intake for each treatment group can be found in Table 5. Throughout the feeding period, food intake was not shown to be significantly different between the groups. The average consumption of the treatment groups ranged from 11.8 ± 0.4 to 12.6 ± 0.4 g/day. In addition, the average consumption of lysine of the groups ranged from 7.2 ± 1.1 mg/day in the 0.06 % lysine treatment to 45.5 ± 1.0 mg/day in the 0.36 % treatment.

Shu-Heh and Hegsted (16) observed the level of food intake in adult rats using a lysine-free diet, a threonine-free diet and a complete amino acid mix diet. The amount of food consumed in g/day in the threonine-free group was reported to be significantly lower than the lysine-free and complete amino acid mix treatment groups. However, there were no differences found between the lysine-free group and the complete amino acid mix treatment group.

Wang et al. (26) fed adult female albino rats diets containing various lysine levels ranging from 0.097 to 0.317 % of diet. Wang et al. also reports no significant differences between groups concerning the amount of diet consumed in g/day. The results found in the studies by Shu-Heh and

Table 5

Average Daily Food and Lysine Intake[1]

Percent Lysine in Diet	Feed g/day	Lysine mg/day
0.298 (4.31 % casein)	12.2 ±0.4	36.3±1.0
0.298 (4.31 % amino acid mix)	12.2 ±0.4	36.4±1.0
0.060	12.0 ±0.4	7.2±1.1
0.120	11.8 ±0.4	14.1±1.0
0.180	12.1 ±0.4	21.8±1.0
0.240	12.0 ±0.4	28.7±1.0
0.300	12.2 ±0.4	36.6±1.0
0.360	12.6 ±0.4	45.5±1.0

[1]All values are expressed as group means ± SE.

Hegsted (16), Wang et al. (26) and the present study, all support the assumption that lysine levels of 0.06 to 0.36 % of diet do not affect the amount of food consumed per day ad libitum.

Carcass Analysis

There were no significant differences in total carcass nitrogen, percent body protein, percent body water, and percent body fat between the treatment groups in this study (Table 6). The ability of the adult female rat to adapt to low levels of lysine is quite apparent, as indicated in Table 6. After eight weeks of consuming a diet with 0.06 % lysine, the adult female rat was able to maintain "equal" levels of carcass nitrogen, percent body protein, percent body water, and percent body fat when compared to the adult female rat consuming a 0.12, 0.18, 0.24, 0.298, 0.30, or 0.36 % lysine diet. This estimate is lower than the suggested requirement set by the National Research Council (11).

Table 1 summarizes the estimates of past studies relating to the minimum dietary lysine requirement of the adult rat. Benditt et al. (13), Smith and Johnson (14), the National Research Council (11), and Said and Hegsted (15) all report lysine levels much higher than the level estimated in this study (estimated at < 20.1 mg/day/Kg$^{0.75}$). Nasset, as reported by Said and Hegsted (15), and Nasset and

TABLE 6

Carcass (Minus the Liver) Composition of Adult Rats Fed
Different Levels of Dietary Lysine[1]

Percent Lysine in Diet	Total Nitrogen (g)	Percent of Body Weight		
		Protein	Water	Fat
0.298 (4.31 % casein)	8.8±0.2	19.8±0.4	60.6±0.8	16.4±1.1
0.298 (4.31 % amino acid mix)	8.5±0.2	19.9±0.4	60.6±0.8	16.6±1.1
0.060	8.5±0.2	20.9±0.4	60.7±0.8	17.4±1.1
0.120	8.8±0.2	20.6±0.4	60.0±0.6	16.9±1.1
0.180	8.7±0.2	19.8±0.4	59.3±0.8	17.7±1.1
0.240	8.3±0.2	20.5±0.4	61.3±0.8	15.5±1.1
0.300	8.4±0.2	20.1±0.4	59.7±0.8	16.7±1.1
0.360	8.7±0.2	20.8±0.4	59.6±0.8	18.7±1.1

[1]All values are expressed as means ± SE.

Ely (23), however, report dietary lysine levels markedly lower. The great variability between the values can possibly be related to many aspects involved in determining the dietary lysine requirement of the adult rat. Nasset and Ely (23) for example used a feeding pattern of the following: maintenance diet (9.6 % whole egg protein), two weeks; nitrogen-free diet, one week; amino acid diet, contained approximately half of the maintenance requirement of total nitrogen, one week. This five week feeding cycle is drastically different than the feeding pattern used in this study. Also, the age, weight, and sex of the rats vary from study to study. An important point to consider is the differences in the ability of the young adult rat compared to the ability of the senesent rat to adapt to the levels of dietary lysine used in these studies. At this time, no known study has been completed to indicate these differences in lysine adaptation ability between young adult rats and senescent rats in lysine adaptation ability. The results of this study, indicate that the mature rat (10 months of age) has the ability to adapt to a dietary lysine level markedly lower than the requirement set by the National Research Council (11).

Liver Analysis

The averages for percent liver protein, water, and fat for each treatment group are shown in Table 7. Total liver

TABLE 7

Liver Composition of Rats Fed Different Levels of Dietary
[1] Lysine

Percent [2] Lysine in Diet	Nitrogen (mg/g)	Percent of Liver Weight		Liver weight (g)
		Water	Fat	
0.298 (4.31 % casein)	38.9±0.6	70.3±0.3	7.8±0.9	6.77±0.2
0.298 (4.31 % amino acid mix)	38.7±0.6	70.0±0.3	7.4±0.9	6.61±0.2
0.060	39.2±0.6	70.3±0.3	8.8±0.9	6.26±0.2
0.120	39.1±0.6	70.3±0.3	9.9±0.9	6.35±0.2
0.180	40.3±0.6	69.3±0.3	9.3±0.9	6.35±0.2
0.240	38.9±0.6	69.8±0.3	8.9±0.9	6.50±0.2
0.300	39.2±0.6	70.0±0.3	8.6±0.9	6.46±0.2
0.360	38.1±0.6	70.6±0.3	7.3±0.9	7.02±0.2

[1]
 All values are expressed as means ± SE.

[2]
 Percent of lysine includes amount in casein (group 1).

nitrogen and liver weights also are presented in this table. There were no significant differences between the groups in all categories listed in Table 7.

The results on liver analysis both disagree and agree with findings in past studies. Wang et al. (26) found significant differences in percent liver protein and fat in adult rats consuming 0.317 % lysine of diet compared to adult rats fed a 0.097, 0.147, 0.217, and a 0.267 % lysine diet. The percent liver fat was found to be higher while the percent liver protein was observed to be lower in the 0.317 group. In 52 week old rats, Mittal (24) also found a significantly higher liver protein content in rats consuming a wheat diet supplemented with lysine compared to rats on an unsupplemented lysine wheat diet. These results are somewhat confusing because the dietary lysine levels utilized in this study are both higher and lower than the dietary lysine levels used in the studies by Wang et al. (26) and Mittal (24). Therefore, one would expect to see a significantly higher percent liver fat in the 0.36 % dietary lysine treatment. A possible cause for these differences may reside in the composition of the diets used. As explained earlier, an amino acid mix with various levels of supplemented dietary lysine and a 4.31 % casein diet was used in the present study. Wang et al. (26) and Mittal (24), however, used a wheat based diet supplemented and unsupplemented with dietary lysine. The amount and source of total dietary nitrogen, therefore, may have played a key role in the val-

ues found within the studies. In addition, Brinegar et al. (35) observed a lysine requirement in the pig of 0.6 %, using a ration containing 10.6 % of protein and a require-ment of 1.2 % using a ration containing 22 % of protein. The point indicated here is that a nonprotein diet was uti-lized in seven of the eight treatments in this study. The use of the amino acid mixture could have resulted in the insignificant differences found in liver composition between the treatment groups.

Examination of recent literature on lysine metabolism in the adult rat, reveals a great ability of the adult rat to adapt to low levels of dietary lysine (15, 16, 24). Shu-Heh and Hegsted (16) measured the liver enzyme activity in adult rats fed protein-free, threonine-free, lysine-free and com-plete amino acid mix diets. These authors found no differ-ence in the threonine dehydratase activity between rats fed a 5 % lactalbumin diet and a protein-free diet. Moreover, they did find a marked decrease in body weight of rats fed the protein-free diet. The threonine dehydatase enzyme in the dietary threonine deficient rat, therefore, was metabol-izing threonine at a rate similar to that of rats fed the 5 % lactalbumin. As the rate of threonine dehydratase acti-vity remains constant in a rat fed a threonine-free diet, the concentration of this essential amino acid decreases. Protein synthesis in the liver, therefore, would be inhi-bited. Degradation of muscle and liver tissue must occur in order to sustain an adequate level of threonine for protein

synthesis within the liver. This was definitely the cause of weight loss in the rats fed a threonine-free diet. In contrast, the lysine-ketoglutarate reductase activity is shown to be significantly lower in rats fed a lysine-free diet compared to rats fed a complete amino acid diet. Specifically, a threefold decrease in activity in the lysine-free treatment was measured. This decrease in the lysine-ketoglutarate reductase activity causes a conservation effect of endogenous lysine. A higher concentration of lysine results in a greater potential for protein synthesis activity. The lower inhibition in the rate of liver protein synthesis produces a "sparing effect" on tissue protein. Assuming the validity of the tissue protein sparing theory presented, the insignificant results found in Table 7 are a consequence of this phenomena. The present study indicates that a diet ranging from 0.06 to 0.36 % lysine is neither low enough nor high enough to affect liver composition in the adult female rat.

Serum Protein Analysis

The results of the effects of various levels of dietary lysine on total serum protein is shown in Table 8. Again, there were no significant differences observed between the treatment groups. Generally, investigators have measured the effect of diet on blood lysine concentrations. Few studies have been performed on the effect of various levels

Table 8

SERUM PROTEIN CONCENTRATION OF RATS FED DIFFERENT LEVELS OF

DIETARY LYSINE[1]

Percent Lysine in Diet	Serum Protein Concentration (g/dL)
0.298 (4.31 % casein)	7.2±0.16
0.298 (4.31 % amino acid mix)	6.9±0.16
0.060	7.1±0.17
0.120	7.0±0.16
0.180	6.8±0.16
0.240	6.9±0.16
0.300	7.1±0.16
0.360	6.8±0.16

[1] All values are expressed as group means ± SE.

of dietary lysine on total serum protein. Mittal (24), however, did observe the effects of dietary lysine on total serum proteins. The results found in his 1985 study indicate values similar to those observed in the present study. Mittal (24) reports no significant differences in serum protein levels in adult male rats fed a wheat based diet, which is known to be low in lysine, when compared to rats fed a wheat diet supplemented with lysine. Theoretically, if the conservation of lysine is great enough in the adult rat fed a diet with small amounts of lysine, then liver protein synthesis would only slightly be inhibited. The liver synthesizes proteins which are secreted into the blood. As long as the concentration of lysine, and all other amino acids are adequate then the synthesis of these blood proteins will continue. The results in Table 7 indicate that the lysine concentration in the liver was adequate to maintain the serum protein levels in the adult rat fed dietary lysine levels ranging from 0.06 to 0.36 % of diet.

SUMMARY AND CONCLUSION

This study was conducted to determine the minimum dietary lysine requirement for tissue maintenance in the adult female rat using carcass nitrogen analysis. There were a total of eight treatment groups in which the diets consisted of a lysine-free amino acid mix base and a 4.31 % casein diet. Various levels of L-lysine was added to the lysine-free amino acid mix ranging from 0.06 to 0.36 % of diet. The length of the feeding period lasted 56 days. The nitrogen, fat and water concentration of the carcass and liver were determined.

The amount of lysine added to the diets produced no apparent changes in body weight or the level of food intake. Weight loss was expected to occur in the 0.06 % dietary lysine group since the National Research Council (11) has set the dietary lysine requirement in the adult rat at 0.11 % of diet. The use of the amino acid mix base, instead of a protein based diet, may have had an affect on weight maintenance in the 0.06 % dietary lysine treatment.

There were no significant differences in total carcass nitrogen, percent body protein, percent body water and or percent body fat among the treatment groups. The similarity of the values can possibly be explained by the ability of the adult rat to conserve lysine in the body when fed low levels of dietary lysine. These results could indicate that the rat may have an even greater ability to adapt to low

dietary levels of lysine when fed a nonprotein based diet.

Further insignificant differences were observed after analyzing liver data. The same analysis for carcass were performed for livers. The various levels of dietary lysine used were neither high enough or low enough to produce changes in liver composition between the groups. The small affect from the dietary lysine levels are further supported by the insignificant differences found in total serum protein concentration between the the treatment groups. Since the liver produces the majority of blood proteins, the insignificant differences in total serum proteins suggests that protein synthesis was not altered enough in the liver to cause changes in blood protein levels.

Because of the consistant insignificant differences found between values, the exact amount of dietary lysine needed for tissue maintenance in the adult rat cannot be determined. However, from the present data, one can safely estimate the dietary lysine requirement to be less than 0.06 % of diet, or 20.1 mg/day/Kg$^{0.75}$ for the adult female rat. This value is lower than the amount estimated by the National Research Council (11) : 0.11 % of diet, or 32.2 mg/day/kg$^{0.75}$.

The use of carcass nitrogen analysis to predict the dietary lysine requirement in the adult rat is a very optimistic method. A direct measurement of tissue nitrogen is performed instead of, for example, measuring the amount of nitrogen excreted as in a nitrogen balance study. In future

studies involving the dietary lysine requirement in the adult rat, it is suggested that two separate groups of rats be used. One group would be fed a protein based diet with various levels of lysine, and a second group would be fed an amino acid based diet with the same dietary lysine levels used in group one. This method will enable the researcher to determine the affects of a protein based diet and an amino acid based diet on the lysine requirement in the adult rat. Also, two requirements can be estimated, one for each group. This procedure can possibly alleviate the effects from the different diets used which, in effect, may promote a standard diet for determining the dietary lysine requirement in the adult rat.

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APPENDIX A. MOISTURE DETERMINATION

1. Dry the samples 48-72 hours in freeze-dryer. Remove
    from freeze-dryer and place in an oven overnight at
    55 xC.

2. Cool in a dessicator and weigh. Report loss in weight
    as moisture.

Calculations:

$$\% \text{ Moisture} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100$$

APPENDIX B. MODIFIED KJELDAHL PROCEDURE FOR NITROGEN AND PROTEIN

Principle

The nitrogen is oxidized to $(NH_4)_2SO_4$ by digestion with concentrated H2So4 and copper as the catalyst. The digest is made alkaline with concentrated NaOH, and the NH3 is distilled into a saturated solution of Boric acid. The amount of nitrogen obtained is multiplied by the factor 6.25 to arrive at the crude protein content of the sample.

Procedure

Sample size: 0.5 - 2.0 g

Digestion:

Place weighed sample in Kjeldahl flask. Prepare two blanks, also. Add 10 g of Na_2SO_4-CuSo4 mixture and four glass beads to each flask. Pour 25 ml H_2SO_4 into the flasks, making sure to wash down any sample adhering to the side of the flask. Set rack containing the tubes into the preheated digestion block. The digestion block should be preheated at 440 xC for at least 30 minutes. Next, fit the exhaust caps over the tubes and turn on water supply to exhaust manifold. Adjust the water flow until fumes are just contained.

Let the samples digest until a clear light green color

is obtained. Heat for an additional 30 minutes. After 30 minutes, turn water supply to the exhaust manifold off and carefully remove exhaust manifold from the top of digestion tubes and place in holding rack. Remove the rack containing tubes from digestion block and allow samples to cool under the hood until the tubes can be handled by hand.

Once cooled, slowly add 250 ml of distilled water to each tube. Cover the tubes with parafilm until further analysis.

Distillation:

Measure 25 ml of Boric acid and 25 ml of distilled water into 500 ml Erlenmeyer flasks. Add approximately 5 drops Bromocresol green/ methyl red indicator. Place flasks under the distillation rack and insert the delivery tube under the surface of the liquid. Turn on water to the condenser and check to make sure cold water is flowing.

Turn on burners to number 2 to let the heating blocks warm-up. Pour the digestion products into properly labled Kjeldahl flasks. Add approximately 1/16 teaspoon of granulated zinc and then immediately add 85 ml of 50 % NaOH (add slowly down the side of the flask to layer the NaOH below the diluted sulfuric acid digest).

Connect the flask with the distillation rack. First swirl the flasks slowly and then vigorously to mix the contents of the flasks. Turn the heat up to 5 immediately and place the corresponding lable on the receiving flask. If

the mixture did not turn blue, the acid was not neutralized
and more NaOH should be added to the samples. Distill until
150 ml are in the collecting flask and the lower the col-
lecting flask so the tube is out of the liquid. Distill to
200 ml total and turn off heating unit.

Titration:

Titrate in order of coming off the distillation apar-
atus. Titrate to a slight yellow-green color. Titrate all
samples until the color matches the end-point of the blank.

Calculations:

One equivalent of HCl reacts quantitatively with one
equivalent of nitrogen as ammonium borate. Therefore:

Normality of acid x 14.00 = mg of N equivalent to 1 ml
of acid

Total N = (ml HCl - ml blank) x equivalent of N
            (normality of acid x 14.00)

$$\% \text{ protein} = \frac{\text{mg N}}{\text{g sample (wet)}} \times 6.25 \times 0.1$$

APPENDIX C. SOXHLET FAT EXTRACTION METHOD FOR TOTAL FAT

1. Label filter paper. Weigh paper plus 2 paper clips. Record this weight. Weigh and record sample weight (10 grams for carcass; 0.5 grams for liver). Fold sample in filter paper, fasten with paper clips, and place in large Whatman thimble.

2. Fill the Soxhlet flask over the 5000 ml mark with petroleum ether.

3. Assemble flask, put thimble in reservoir, and attach condenser.

4. Turn on cold water to circulate through condenser.

5. Turn on heat to approximately 87 xC.

6. Operate 48 hours checking the ether level several times during this period.

7. Turn off heat. Allow petroleum ether to stop boiling and then turn off cold water. Remove thimble and pour ether back into the 5000 ml flask. Place samples in a pipette drying rack and leave rack under fuming hood fo 1-2 hours until petroleum ether has evaporated.

8. Place rack containing the samples in drying oven over-
    night at a temperature of 45-50 xC.

9. After drying, place samples in dessicator to cool. Weigh
    when cool.

Calculations:

$$\% \text{ Fat Dry} = \frac{\text{sample weight (dry)} - \text{extracted sample weight (dry)}}{\text{sample weight (dry)}} \times 100$$

$$\% \text{ Fat Wet} = \% \text{ Fat Dry} \times \frac{\text{wet weight of sample}}{\text{dry weight of sample}} \times 100$$

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