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Relation of Dietary Calorie Intake to Protein Utilization in Man

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Summary of Major Accomplishments during the period July 1970-April 1972

Interrelationships between energy intake and level and quality of dietary protein on N utilization have been studied in young men. Studies have also been conducted on the effects of the pattern of distribution of protein in the daily meals on N utilization in young men consuming a diet providing an adequate or restricted caloric intake. Wheat gluten provided the sole dietary protein source in the first series of studies and skim milk protein was used as the dietary protein source in the series concerned with the effects of protein-meal pattern.

Caloric restriction reduced the efficiency of dietary N utilization and resulted in a loss of body weight throughout the 30 day experimental periods. Lysine supplementation of wheat gluten protein improved overall dietary N utilization at both the adequate and restricted levels of caloric intake. Therefore, with initially well nourished subjects, a 20% caloric restriction for a period of 2-4 weeks does not reduce the beneficial effects on N utilization which follow lysine supplementation of wheat protein. It is not yet known whether the beneficial response to amino acid supplementation under these conditions also applies to proteins limiting in other essential amino acids.

Studies on the effects of distribution of protein within the three major meals of the day (breakfast, lunch and dinner) in young adult men

given 0.4-0.5g protein per kg per day show that the omission of protein from the breakfast meal does not result in impaired N utilization at either adequate or inadequate levels of caloric intake. Protein consumed in the form of a breakfast consisting only of a glass of skim milk is utilized as well as when protein intake is delayed until the complete lunch and dinner meals. The results were similar at both adequate and restricted levels of total daily caloric intake.

The significance of these findings in relation to amino acid fortification and supplementary protein feeding programs for malnourished population groups is briefly discussed.

A. Introduction and Objectives:

The importance of calories in improving the utilization of dietary protein has been accepted for many years. There is extensive documentation on the qualitative aspects of dietary protein-calorie interrelationships, and this topic has been the subject of a number of reviews (1-3).

Sukhatme (4) has emphasized the importance of comparing both protein and calorie intakes in assessing the nature of the nutritional situation of populations. In countries such as India dietary surveys suggest that both protein and caloric intakes are inadequate (4). Under these circumstances it is often assumed that the supplementation of these diets with protein or amino acids would be essentially worthless because they would be used to meet the energy needs of the individuals.

There are two uncertainties to the above arguments; firstly, individuals would not survive with the chronic consumption of a diet which was markedly deficient in energy supply and they adjust to them by reducing physical activity and perhaps in part by metabolic changes (5, 6). Thus, although the diets are deficient by reference standards they must still approach the total energy needs of the individual. In essence, there should be a beneficial effect of supplementing the diet with protein or amino acids. Secondly, it is apparent from studies in experimental animals that protein is not entirely used for meeting energy needs when the diet supplies a grossly deficient calorie intake. Morrison et al (7) have shown with growing rats that under conditions of severe

caloric restriction protein is utilized for anabolic purposes. Furthermore, proteins still differed in their NPU values under these restricted dietary conditions although the differences in protein quality were less than those found with ad libitum feeding. Allison et al (8) showed with adult dogs that nitrogen was retained to the same extent in animals receiving either adequate calories or 50% of their caloric requirements, but when caloric intake was reduced to 25% there was a marked reduction in N utilization.

There are few data available from experiments with human subjects which allow evaluation of the extent to which the quantitative relationships observed in experimental animals can be applied to human feeding.

The studies completed so far were conducted with the following objectives:

(a) to determine the effects of increasing protein quantity and quality on N utilization in young men given adequate or inadequate energy intakes. For this purpose experiments were conducted in which wheat gluten was the sole dietary protein source so that adjustments could be made easily in the total energy intake as well as in the level and quality of protein.

(b) to examine the time relationships between caloric intake and the nutritional value of supplementary protein when subjects are given a low-protein, low-calorie basal diet.

B. Materials and Methods

1. Subjects

Young adult male MIT students participated in the studies. They were all in good health as determined by medical history and physical examination. Throughout the entire study each subject received close medical supervision. They continued their usual, everyday activities but recorded their daily events and the number of hours of sleep in a diary. They were also required to wear pedometers so that an objective, although approximate, measure could be made of physical activity during the various dietary periods.

2. Samples and methods of analysis

Complete 24-hour urine and fecal collections were made throughout each study. The daily urines were analyzed for total nitrogen, urea, creatinine as previously described (9-11). Pooled fecal samples were

prepared for total N analyses. Fasting and 3-hour post prandial blood samples were taken at intervals during the experiments and blood or serum analyzed for hemoglobin, total protein, albumin urea, tryptophan, free amino acids, all as previously described (9-11). Basal metabolic rate was measured at the end of each diet period in the experiments, described below, concerned with lysine supplementation of wheat gluten.

Complete blood counts and urinalysis were performed at the beginning and end of each study.

Data were analyzed by paired "t" test and analysis of variance, utilizing the facilities of the MIT Information Processing Center and the BMD (UCLA) Programs.

3. Experimental Designs and Results

(a) Effects of caloric intake and lysine supplementation on the utilization of wheat gluten protein by young adult men:

(i) design of study

The design of the experiments conducted is shown in Fig. 1. Each study lasted 60 days. During one 30-day period the subjects received an adequate intake of calories, designed to closely maintain body weight throughout the 30-day experimental period. For the other 30-day period caloric intake was restricted to 80% of the energy intake provided by the diet during the period

of adequate caloric intake. Lysine supplementation (2.25% of total protein) was included during a15-day period within each 30-day period at the two dietary caloric levels.

Daily protein intake was 0.27g/kg body weight in Exp. 1. In Exp. 2 daily protein intake was 0.73g/kg body weight. The order of the 30-day periods of adequate and low caloric intakes were randomized as were also the periods of lysine supplementation within each caloric level. In the first study (Exp. 1) a seven-day break on a free choice diet was included between the two 30-day periods.

The characteristics of the subjects studied in the two experiments are summarized in Table 1.

The experimental diet was based on a liquid formula preparation as previously described (9). Wheat gluten (International Milling Company) was the sole source of dietary protein. Protein-free cornstarch cookies, cornstarch desert and carbonated beverages were used to meet the caloric needs of the individual. Four equal meals were consumed at 8-9 am, 12-1 pm, 4:30-5:30 pm and 9-10 pm.

(ii) Results:

A summary of the results of these two experiments is shown in the following tables. The details pertaining to each

subject in Exps. 1 and 2 are provided in the tables of Appendix 1.

The reduced caloric intake resulted in a significant loss of body weight during the 30-day period (table 2). Significant weight changes were not observed for subjects consuming an adequate caloric intake except in one subject who gained 0.053kg/day and 0.045kg/day respectively in the two 15-day periods (with and without lysine supplementation) at the adequate caloric intake.

No significant changes in basal metabolic rate were detected during the 30-day periods at each level of caloric intake (table 2). Nitrogen balance data were computed from the last 10-days of each 15-day diet period. Adaptation to the low-protein intake occurred within 5-days as indicated by the lack of a significant slope for the regression of nitrogen excretion on time during the final 10 days of each 15-day period.

Table 3 summarizes the nitrogen balance data for each diet group and table 4 summarizes the changes in nitrogen balance following lysine supplementation of the wheat gluten, when given at the lower (0.27g) and higher (0.73g) levels of protein intake

and at either the adequate or restricted levels of total caloric intake. The N balance results are also shown in Figs. 2 and 3.

At the lower protein intake the mean N balance for 6 subjects was improved by 0.24g N per day following lysine supplementation where the caloric level was adequate (table 4). The corresponding improvement at the reduced caloric intake was 0.33g N per day. This change in N balance was not statistically different ($P > 0.05$) from the former value of 0.24g N.

At the higher protein intake lysine supplementation also improved overall dietary N utilization and the effect of lysine supplementation did not differ significantly between the two levels of total energy intake.

The derived estimates of biological value (BV) and net protein utilization (NPU) are summarized in table 5. At the lower level of protein intake NPU was increased from 72 to a mean of 78 through lysine supplementation at the adequate level of caloric intake. In the second experiment, in which the subjects consumed wheat gluten at a higher level of protein intake, NPU was increased from a value of 30 to a mean of 37 following lysine supplementation of the adequate calorie diet. These changes were statistically significant. Estimates for NPU and BV have not been made for the reduced levels of

calorie intake because of the uncertainty of the appropriate values for obligatory urinary and fecal N losses under these conditions.

Table 6 summarizes the effects on N balance of increasing caloric intake from the inadequate or adequate levels of energy intake. N retention was improved by approximately lmg N per kcal increase in total energy intake and there were no significant differences among the diets at the two levels of protein intake, with or without lysine supplementation. However, a tendency was observed, as shown in this table, for the effect of raising caloric intake on N balance to be greatest at the higher level of protein intake when combined with lysine supplementation.

The findings for albumin and urea concentrations in serum obtained from blood samples taken after an overnight fast at the end of each diet period are summarized in table 7. Serum albumin was increased ($P < 0.05$) by lysine supplementation at the low level of protein intake but this effect was not observed at the higher level of wheat gluten protein intake. However, the physiological significance, if any, of the change in serum albumin level with lysine supplementation cannot be readily assessed from these data. Blood urea values were not affected by lysine

supplementation but were increased by dietary caloric restrictions. A highly significant negative correlation ($r = {}^{-}0.99$) was found between mean NPU and mean serum urea levels for the various diet groups.

Summary and Conclusions from studies in Section 3(a):

The two experiments described above demonstrate that lysine supplementation of wheat gluten improves overall N utilization in young adult man. In view of the large body of available data on the nutritional quality of this protein, our findings were anticipated and they are neither new nor surprising.

However, our experiments have provided new data on the influence of dietary caloric restriction in this context. Under the present experimental conditions a 20% restriction in caloric intake was without effect on the improvements of the protein quality of wheat gluten achieved through lysine supplementation.

The effects of caloric restriction on dietary protein utilization depend upon a number of factors. These include the physiological state of the individual, the level and quality of protein intake and the degree and duration of the dietary caloric inadequacy.

The present studies involved initially well-nourished subjects. At the inadequate level of caloric intake, fatty acids were undoubtedly being mobilized from adipose tissue to meet the deficit in the dietary

energy supply. However, under these conditions amino acids were also being oxidized at a relatively greater rate as evidenced by the reduced N balance and increased urinary urea output. Nevertheless the status of body protein metabolism and the mechanisms of body protein synthesis were responsive to changes in the level and pattern of dietary intake of amino acids.

The present results do show, therefore, at an inadequate caloric intake in well-nourished subjects who are losing body weight that the utilization of wheat protein can be improved through dietary supplementation with the limiting essential amino acid.

It is not possible to extrapolate the quantitative aspects of the results obtained in the present studies to cover dietary protein-calorie relationships as they affect the malnourished individual. In this case the individual would be expected to have a limited energy reserve in the form of stored adipose tissue triglyceride and thus a caloric restriction of the degree imposed in the present studies would lead to a relatively greater increase in body protein catabolism and amino acid oxidation. Nevertheless, under these conditions protein synthesis continues to occur at a significant rate and without direct study it is not possible to predict whether an improvement in the amino acid pattern of the dietary protein would be without any beneficial affect on body and organ protein metabolism

From a practical point of view we are led to the following conclusion; in the light of the above discussion and the arguments raised in the introduction to this report the present findings suggest that nitrogen retention can be improved through lysine supplementation of wheat gluten during short-term periods at inadequate caloric intakes.

(b) Effect of time of ingestion of protein on dietary N utilization.

The purpose of these experiments was to provide a basis for assessing the effects of time of consumption of a protein supplement under conditions of marginal intakes of total daily protein and energy. Leverton and Gram (12) observed impaired N balance in adult women when animal protein was distributed in 2 meals, lunch and dinner, in comparison with a meal pattern providing animal protein at breakfast, lunch and dinner. In this study protein intake was about 62g daily. In a second study by Leverton et al (13) they did not observe an impaired utilization dietary N when protein was excluded from lunch and at a daily protein intake of approximately 63g. However, the utilization of dietary N was reduced when the daily protein intake was about 43g. These results suggested that exclusion of protein from the morning meal was of greater significance in overall N utilization than its exclusion from the mid-day meal.

Exp. 1: The first experiment in the present series was designed to determine, at about a maintenance intake of total protein, whether

the utilization of N was affected by the presence or absence of protein in the morning meal, particularly when overall energy intake is inadequate.

Seven, male MIT students participated in this study. The diet periods were as follows: (a) a 1 day protein-free period to aid adaptation to the "low-protein" diet; (b) a 15-day period at an adequate-caloric intake and at a protein intake of 0.5g/kg body weight per day. The protein was skim milk protein and was distributed equally among the three major meals, breakfast, lunch and dinner; (c) a consecutive isonitrogenuous, isocaloric 15-day period with the protein provided at lunch and dinner only; (d) as in (b) above except that the daily caloric intake was restricted to 80% of the adequate level; (e) as in (c) above except that the caloric intake was restricted to the 80% level.

The order of the periods at adequate and restricted caloric intake was randomized among the subjects as was also the two meal patterns within each level of energy intake.

Two isocaloric meals were given at noon and 5 pm and a protein-free snack (300kcals) was given at 10 pm. Breakfast and the snack provided the same total caloric intake as provided by the lunch and dinner (5 pm) meals.

Results: Body weight changes during the four diet periods are summarized in table 8. Restriction of caloric intake resulted in a loss

of body weight whereas the adequate diet resulted in a mean gain in body weight for the seven subjects studied. The gain in body weight was significantly higher ($P<0.01$) when protein was distributed among the three major meals of the day compared with protein given only at the lunch and dinner meals. Nitrogen balance data are summarized in table 8 and the derived values of NPU and BV are also summarized in this table.

Nitrogen balance was reduced by caloric restriction. Nitrogen balance did not differ significantly when protein was provided at each of the three major meals of the day versus the pattern providing a protein-free breakfast, with the daily protein supply (0.5g/kg/day) distributed equally among the lunch and dinner meals. The NPU and and BV values did not differ according to the protein-meal pattern but were lower at the restricted levels of dietary caloric intake.

Results for various blood constituents are summarized in table 9 and they show little consistent change with the altered distribution of protein among the three meals.

From these results it is concluded that the absence of protein in the breakfast meal does not have a significant effect on overall N utilization. Further, these results also show that even under an inadequate daily caloric intake protein N utilization is not affected significantly by the withdrawal of protein from the breakfast meal and its subsequent consumption during the lunch and dinner meals.

Exp. 2: On the basis of the above findings we next designed the second experiment. The purpose was to determine whether, at an inadequate daily caloric intake, the protein consumed during a breakfast meal consisting only of a glass of skim milk protein and providing one-third of the daily protein intake was relatively well utilized for purposes of meeting protein needs. The experimental comparison in this case was performed against a meal pattern in which no breakfast was provided and the total daily protein and caloric intakes provided in two essentially equal meals taken at lunch- (noon-1 pm) and dinner-time (5-6 pm).

Seven male MIT students participated in this study. The dietary periods, each of 15-days duration, were as follows;

Period 1	Adequate daily calories, with no breakfast
Period 2	Adequate daily calories, with skim milk breakfast
Period 3	Restricted daily calories, with no breakfast
Period 4	Restricted daily calories, with skim milk breakfast.

The order which the periods at adequate and restricted intakes were presented to the subjects was randomized as was also the two meal patterns within each 30-day period at the adequate and restricted levels of energy intake.

Total protein intake (skim milk protein) was approximately 0.4g/kg body weight per day. During periods 2 and 4 a glass of skim milk was consumed at breakfast time. This supplied 8.8g protein and 105kcals. During periods 1 and 3 this amount of protein and energy were equally divided between the lunch (noon) and dinner (5-6 pm) meals. As in Exp. 1 a protein-free snack (300kcals) was given at 10 pm.

Results for N balance are summarized in table 10. At the adequate level of caloric intake, overall N utilization was not different for the two meal patterns. Caloric restriction reduced overall N utilization. Furthermore, the results show that there were no differences in overall N utilization between the two patterns of protein-distribution when the subjects were consuming a diet which was inadequate in total energy supply.

Thus, the results of this experiment lead to the conslusion that protein provided by a glass of skim milk is well utilized for purposes of protein synthesis if consumed without further calories at breakfast time even when the daily intake of calories in inadequate for maintenance of body weight.

Conclusions drawn from studies in Section 3(b):

From the results of two experiments discussed above for initially well-nourished subjects who are lossing body weight during a period

of dietary caloric restriction it can be concluded that (a) N utilization is not impaired by omitting protein from the breakfast meal and (b) protein provided by a glass of skim milk is as well utilized when consumed alone at breakfast time as it is if consumption of the equivalent amount of protein is delayed until the more complete lunch and dinner meals.

Extending these findings to a practical situation, bearing in mind the experimental restrictions discussed under 3(a), consumption of a protein-snack (provided in the form of a glass of skim milk) in the morning would result in improved N balance even when the snack followed a previous period of an inadequate daily energy intake. These results further suggest that a protein supplement would be effective if consumed at mid-morning by an individual who had not eaten breakfast and whose previous daily caloric intake was inadequate and daily protein intake marginal or deficient.

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Fig 1

Experimental Design for Study of Lysine Supplementation of Wheatgluten at Two Levels of Caloric Intake

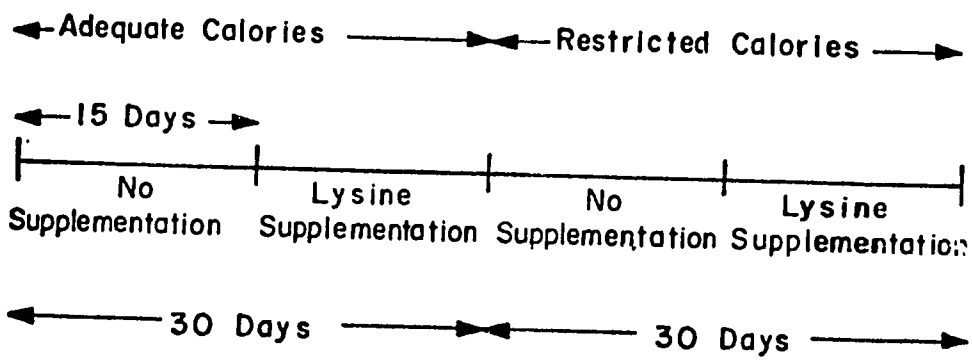

Fig. 2

Six Young Men Fed 0.27g
Wheat Gluten Protein per kg

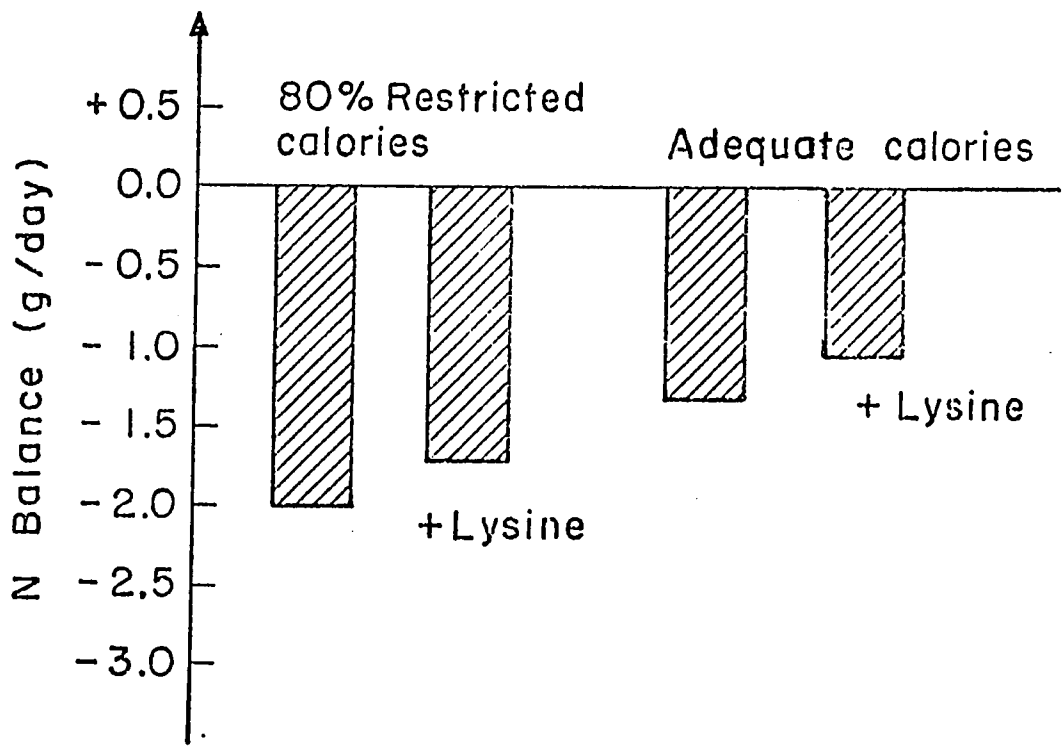

Fig 3

Eight Young Men Fed 0.73 g
Wheat Gluten Protein per kg

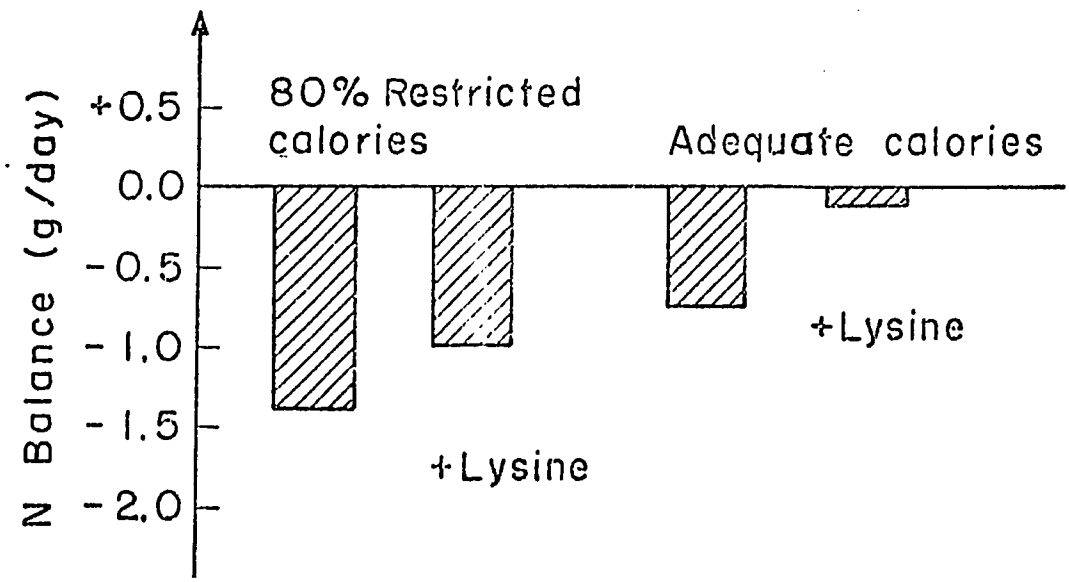

Table 1

Characteristics of Young Men Studied
for the Effects of Lysine Supplementation
on Wheat Gluten Utilization

Exp. No.	Protein Intake (g/kg/day)	No. of Subjects	Initial Weight (kg)	Height (cm)	N Intake (mg/kg)	Caloric Intake (Kcal/kg)	
						Adequate	Low
1	0.27	6	71.5±8.6[1]	181±11	39.2±0.9	53.3±2.6	41.2±2.0
2	0.73	8	74.2±8.0	177± 4	118 ±6.2	47.8±6.6	38.2±5.4

1. Mean ± std. dev.

Table 2

Basal Metabolic Rate and Weight Change
at Two Levels of Caloric and Protein Intake
in Young Men Receiving Wheat Gluten Protein

Protein Intake (g/kg/day)	Caloric Intake			
	Adequate		Restricted	
	B M R (Kcal/24 h)	Weight Change (g/day)	B M R (Kcal/24 h)	Weight Change (g/day)
0.27	1620±145[1]	5.2±23.0	1663±127	-65.3±56.4
0.73	1618±222	-14.6±23.2	1637±299	-73.1±44.3

1. Mean ± std. dev.

Table 3

Nitrogen Balance in Young Men Studied
for the Effects of Lysine Supplementation of Wheat Gluten
at Adequate and Restricted Caloric Intakes

Level of Protein (g/kg/day)	Caloric Level			
	Adequate		Restricted	
	No Lysine	Plus Lysine	No Lysine	Plus Lysine
	gN per day			
0.27	-1.29±0.33[1]	-1.05±0.48	-2.02±0.49	-1.70±0.96
0.73	-0.72±0.73	-0.09±0.69	-1.40±0.54	-0.97±0.61

1. Mean ± std. dev.

Table 4

Improvement of N Balance in Young Men with Lysine Supplementation of Wheat Gluten at 2 Levels of Caloric Intake

Level of Protein (g/kg/day)	No. of Subjects	Caloric Level	
		Adequate	Restricted
		gN per day	
0.27	6	0.24±0.38[1]	0.33±0.50
0.73	8	0.63±0.29	0.42±0.44

1. Mean ± std. dev.

Table 5

Derived Estimates for Biological Value (BV) and Net Protein Utilization (NPU) for Wheat Gluten when given to young men consuming adequate calories.

Level of Protein (g/kg/day)	Supplementation			
	None		Lysine	
	BV	NPU	BV	NPU
0.27	73±7	72±8	80±16	78±15
0.73	30±9	30±9	37± 8	37± 8

Table 6

Improvement of N Balance with Change from Restricted to Adequate Caloric Intake in Young Men given Wheat Gluten

Level of Protein (g/kg/day)	No. of Subjects	Supplementation	
		None	Lysine
		mg N per Kcal	
0.27	6	0.95±0.66[1]	0.74±0.77
0.73	8	0.98±1.07	1.30±1.40

1. Mean ± std. dev.

Table 7

Serum Albumin and Urea Concentrations
at the End of Each 15-Day Diet Period in Young Men
Receiving Wheat

Protein Intake (g/kg/day)		Caloric Intake			
		Adequate		Restricted	
		-Lysine	+Lysine	-Lysine	+Lysine
0.27	Albumin[1]	3.58±0.21[2]	3.67±0.19	3.55±0.23	3.70±0.24
	Urea[3]	5.5 ±1.8	5.8 ±1.7	8.3 ±2.0	7.7 ± 3.3
0.73	Albumin[1]	3.44±0.20	3.39±0.11	3.43±0.15	3.44±0.24
	Urea[3]	10.0 ± 1.9	9.7 ±1.9	11.4 ±1.9	11.7 ±1.8

1. g per 100 ml
2. Mean ± std. dev.
3. mg/100 ml

Table 8

Body Weight changes, Nitrogen Balance and NPU for Young Men Studied for the effects of a protein-free breakfast when consuming adequate or restricted calories. (Exp. 1 - Section 3b)

	Caloric Intake			
	Adequate		Restricted	
	Protein Breakfast	Protein-free Breakfast	Protein Breakfast	Protein-free Breakfast
Body Weight change (g/day)	+34	+5	-37	-47
N Balance (g/day)	+0.21±0.28	+0.14±0.46	-0.52±0.45	-0.68±0.44
NPU[1]	64±6	62±9	49±10	45±10

1. Values at the restricted caloric intake are probably invalid in the strict sense but are given here for summary purposes.

Table 9

Summary of serum data for young men studied for the effects of a protein-free breakfast when consuming adequate or restricted calories. (Exp. 1 - Section 3b)

Parameter	Caloric Intake			
	Adequate		Restricted	
	Protein Breakfast	Protein-free Breakfast	Protein Breakfast	Protein-free Breakfast
Total Protein (g/100ml)	7.1±0.1	7.2±0.3	7.2±0.2	7.3±0.1
Albumin (g/100ml)	3.5±0.1	3.5±0.2	3.6±0.1	3.6±0.1
Insulin (μU/ml)	15.4±8.6	15.3±6.1	14.0±4.5	10.3±5.0
Uric acid (mg/100ml)	5.1±0.4	5.2±0.6	5.4±0.7	5.9±1.0
Urea Nitrogen (mg/100ml)	8.3±2.0	8.3±1.1	9.7±1.1	10.1±2.1

Table 10

Summary of Nitrogen Balance and BV and NPU
for young men studied for the effects of a skim-milk protein breakfast
when consuming adequate or restricted calories.
(Exp. 2 - Section 3b)

Parameter	Caloric Intake			
	Adequate		Restricted	
	No Breakfast	Skim-Milk Breakfast	No Breakfast	Skim Milk Breakfast
N Balance (g/day)	-0.38±0.30	-0.34±0.32	-1.40±0.77	-1.07±0.81
BV[1]	67±7	67±6	44±17	49±17
NPU[1]	63±7	63±8	41±16	47±19

1. Values for BV and NPU at the restricted caloric intake are probably invalid in the strict sense but are given here for summary purposes.

APPENDIX 1

Detailed Results for Studies Described in Sections 3(a) and 3(b) of the April 1972 Annual Report.

Table 1

Characteristics of Young Men Studied for the Effects of Calorie Intake and Lysine Supplementation on the Utilization of Wheat Gluten.

	Subject	Age	Height (cm)	Weight(kg) Initial	Urinary Creatinine (g/day)
0.27g prot/day	KD	19	180.0	65.70	1.49
	JG	20	180.0	62.40	1.68
	MH	23	183.5	79.75	1.90
	AP	21	170.5	68.40	1.57
	RR	20	200.0	84.30	2.04
	CS	19	172.0	68.35	1.67
	KS	18	178.5	80.95	
	NL	20	180.0	82.20	
0.73g prot/day	RB	19	173.5	75.10	1.66
	RR	24	176.0	83.40	1.53
	JE	20	179.0	78.80	2.05
	RH	19	175.0	58.85	1.49
	DL	18	178.0	67.75	1.77
	JN	20	175.0	71.10	1.76
	MS	19	172.0	78.40	1.79
	DW	18	184.0	80.20	1.92

Table 2

Body Weight Change per Day (kg) During Different Dietary Periods

	Subject	Adequate Calories		Reduced Calories	
		No Lysine	Lysine	No Lysine	Lysine
0.27 g prot/kg	KD	+0.053^{xx}	+0.045^{x}	-0.087^{xxx}	-
	JG	+0.016	-0.014	-0.047	-0.066^{xxx}
	MH	-0.015	-0.001	-0.052^{xxx}	-0.105^{xxx}
	AP	-0.049	+0.019	-0.094^{xxx}	-0.025
	RR	-0.039	-0.029	-0.033	-0.167^{xxx}
	CS	-0.036^{x}	+0.001	-0.130^{xxx}	-0.083^{xxx}
	KS	-	-	-0.107^{xxx}	-0.150^{xxx}
	NL	-0.021	-0.009	-0.107^{xxx}	-0.107^{xxx}
0.73 g prot/kg	RB	-0.028^{x}	-0.044^{x}	-0.089^{xxx}	-0.034^{x}
	RR	+0.031	-0.025	-0.053^{xxx}	-0.057^{xxx}
	JE	-0.049^{x}	+0.035	-0.103^{xxx}	-0.031^{x}
	RH	+0.002	-0.018	-0.052^{x}	-0.013
	DL	+0.001	-0.054^{xx}	-0.040^{x}	-0.044^{x}
	JN	-0.059^{x}	+0.012	-0.085^{xxx}	-0.102^{xxx}
	MS	-0.003	+0.013	-0.064^{xxx}	-0.106^{xxx}
	DW	+0.008	+0.006	-0.178^{xxx}	-0.132^{xxx}

Notes:

x	Significant at 5% level		
xx	"	1%	"
xxx	"	0.1%	"

Table 3

Basal Metabolic Rates at end of a 30-day Period at Adequate or Restricted Caloric Intake

	Subject	Adequate Calories Cal. /24 hrs.	Restricted Calories Cal. /24 hrs.
0.27g prot/kg	KD	1685	1858
	JG	1440	1656
	MH	1656	1670
	AP	1454	1469
	RR	1814	1714
	CS	1670	1613
	Mean	1620±59	1663±52
0.73 g prot/kg	RB	1541	1541
	RR	1627	2030
	JE	(1598)	-
	RH	1512	1310
	DL	(1958)	-
	JN	1224	1310
	MS	1627	1872
	DW	1858	1757
	Mean	1565±84	1637±122

Differences not significant by paired t-test

Table 4

Nitrogen Balance (g/day) in Young Men Studied for the Effects of Caloric Intake and Lysine Supplementation on Wheat Gluten Utilization

Protein Intake (g/kg/day)	Subject	Caloric Intake			
		Adequate		Restricted	
		No Lysine	Lysine	No Lysine	Lysine
0.27	KD	-1.19	-1.29	-1.69	-1.14
	JG	-0.88	-0.90	-1.86	-1.48
	MH	-1.69	-1.53	-1.84	-1.66
	AP	-1.32	-0.38	-1.60	-0.74
	RR	-1.64	-1.54	-2.93	-3.51
	CS	-1.01	-0.66	-2.17	-1.66
	KS	--	--	-2.33	-2.68
	NL	-1.73	-1.16	-2.54	-1.85
0.73	RB	-1.86	-0.68	-1.63	-1.17
	RR	-0.18	0.34	-0.61	-0.38
	JE	-0.84	-0.25	-1.63	-0.45
	RH	-0.07	0.72	-0.76	-0.42
	DL	-1.60	-1.29	-1.19	-0.52
	JN	-0.83	-0.04	-2.03	-1.29
	MS	0.21	0.72	-1.28	-1.48
	DW	-0.58	-0.26	-2.05	-2.02

Table 5

Urinary Urea: Total Nitrogen Ratio in Young Men

	Subject	Adequate Calories		Reduced Calories	
		No Lysine	Lysine	No Lysine	Lysine
0.27 g prot/kg	KD	0.66 ± 0.02	0.67 ± 0.02	0.74 ± 0.01	0.68 ± 0.03
	JG	0.59 ± 0.02	0.66	0.70 ± 0.04	0.67 ± 0.07
	MH	0.69 ± 0.05	0.68 ± 0.04	0.74 ± 0.05	0.70 ± 0.02
	AP	0.66 ± 0.09	0.50 ± 0.07	0.65 ± 0.09	0.63 ± 0.03
	RR	0.68 ± 0.05	0.65 ± 0.05	0.73 ± 0.09	0.76 ± 0.02
	CS	0.64 ± 0.04	0.59 ± 0.09	0.70 ± 0.04	0.72 ± 0.03
0.73 g prot/kg	RB	0.80 ± 0.03	0.82 ± 0.02	0.81 ± 0.08	0.81 ± 0.02
	RR	0.86 ± 0.02	0.81 ± 0.03	0.84 ± 0.01	0.84 ± 0.01
	JE	0.84 ± 0.03	0.75 ± 0.08	0.84 ± 0.02	0.81 ± 0.01
	RH	0.82 ± 0.02	0.78 ± 0.01	0.85 ± 0.02	0.81 ± 0.03
	DL	0.81 ± 0.03	0.81 ± 0.02	0.83 ± 0.01	0.83 ± 0.01
	JN	0.82 ± 0.02	0.80 ± 0.02	0.82 ± 0.04	0.85 ± 0.02
	MS	0.79 ± 0.01	0.79 ± 0.01	0.79 ± 0.04	0.84 ± 0.01
	DW	0.81 ± 0.01	0.81 ± 0.01	0.87 ± 0.04	0.81 ± 0.03

Table 6

Biological Value of Wheat Gluten in Young Men

	Subject	Adequate Calories		Reduced Calories	
		No Lysine	Lysine	No Lysine	Lysine
0.27 g prot/kg	KD	75	68	52	75
	JG	84	83	41	52
	MH	68	67	57	63
	AP	73	105	59	88
	RR	65	66	23	7
	CS	72	88	36	48
0.73 g prot/kg	RB	18	32	20	25
	RR	40	44	35	36
	JE	31	37	21	33
	RH	37	49	29	33
	DL	19	23	22	30
	JN	27	35	13	23
	MS	41	45	25	23
	DW	31	34	17	16

Table 7

Net Protein Utilization of Wheat Gluten in Young Men

	Subject	Adequate Calories		Reduced Calories	
		No Lysine	Lysine	Nc Lysine	Lysine
0.27 g prot/kg	KD	72	66	53	72
	JG	84	80	42	54
	MH	64	66	57	63
	AP	69	103	58	88
	RR	65	66	22	7
	CS	76	86	32	47
0.73 g prot/kg	RB	18	32	20	25
	RR	40	44	34	36
	JE	31	37	21	34
	RH	36	47	29	33
	DL	19	23	22	30
	JN	27	35	14	23
	MS	40	44	25	23
	DW	31	33	17	16

Table 8

Biochemical and Hematological Values at Beginning and End of Study and After Each 15-day Diet Period in Young Men Studied for the Effects of Caloric Intake and Lysine Supplementation on Wheat Gluten Utilization

	Protein	Albumin	BUN	SGOT	SGPT	Hgb.	Hct.
0.27g protein/kg (6 subjects)							
Initial values	7.13±0.41	3.88±0.34	15.0±1.7	12.3±3.5	9.5±1.8	16.2±0.9	43.8±1.3
Adequate cal. no lysine	6.78±0.34	3.58±0.21	5.5±				
Adequate cal. + lysine	6.87±0.26	3.67±0.19	5.8±1.7				
After 1 week break	7.04±0.19	3.98±0.15	18.2±4.0				
Reduced cal. no lysine	6.77±0.15	3.55±0.23	8.3±2.0				
Reduced cal. + lysine	7.04±0.18	3.7±0.24	7.7±3.3				
Final values				17.9±8.2	12.8±7.5	14.4±0.2	43.8±1.3
0.73g protein/kg (8 subjects)							
Initial values	7.16+0.21	3.76±0.18	11.7±2.8	20.5±25.4	22.9±42.0	15.2±0.7	44.9±2.
Adequate cal. no lysine	6.90±0.30	3.44±0.20	10.0±1.9				
Adequate cal. + lysine	6.81±0.34	3.39±0.11	9.7±1.9				
Reduced cal. no lysine	6.85±0.14	3.43±0.15	11.4±1.9				
Reduced cal. + lysine	6.86±0.33	3.44±0.24	11.7±1.8				
Final values						15.0±0.9	42.4±2.2

Table 9

Characteristics of Young Men Studied for the Effects of Caloric Intake and a Protein-free Breakfast on N Utilization

Subject	Age(years)	Height(cm)	Weight(kg)	Caloric Intake (Kcal/ day)	
				Adequate	Restricted
MB	19	179.0	61.95	3100	2500
GI	18	194.5	85.85	4000	3200
GS	19	163.0	58.10	3530	2820
DK	21	179.0	70.35	2900	2300
AP	21	170.5	72.10	3600	2900
MT	18	185.0	70.85	3600	2900
JZ	19	175.0	63.20	2970	2360

Table 10

Body Weight Change Per Day (kg) During Different Dietary Periods

Subject	Adequate Calories		Reduced Calories	
	Protein Breakfast	No Protein Breakfast	Protein Breakfast	No Protein Breakfast
MB	+0.081^{xxx}	+0.062^{xx}	-0.053^{xx}	-0.065^{x}
GI	+0.030^{xx}	+0.019	-0.008	-0.031^{x}
GS	+0.081^{xxx}	+0.025^{x}	-0.023^{x}	-0.008
DK	-0.043^{xx}	-0.039^{x}	-0.042^{x}	-0.038^{x}
AP	+0.100^{xx}	+0.056^{xx}	-0.029^{x}	-0.080^{xxx}
MT	+0.025	-0.070^{xx}	-0.029^{xx}	-0.069^{xxx}
JZ	-0.035	-0.019	-0.077^{xx}	-0.035^{x}
Mean	+0.034	+0.005	-0.037	-0.047

x, xx, xxx; $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

Note: At adequate caloric intake weight gain was significantly higher when protein was provided in 3 meals as opposed to 2 meals ($t=3.77$).

Table 11

Nitrogen Balance (g/day) Results for Young Men Studied for the Effects of Caloric Intake and a Protein-free Breakfast on N Utilization of Skim Milk Protein

Subject	Caloric Intake			
	Adequate		Restricted	
	Protein Breakfast	Protein-free Breakfast	Protein Breakfast	Protein-free Breakfast
MB	0.40	0.58	-0.83	-1.30
GI	0.09	-0.07	-0.17	-0.17
GS	0.45	0.31	-0.28	-0.18
DK	0.11	-0.20	-0.31	-0.64
AP	0.57	0.85	-0.07	-1.01
MT	0.09	-0.06	-0.68	-0.49
JZ	-0.24	-0.49	-1.32	-1.00

Table 12

Characteristics of Young Men Studied for the Effects of a Skim-Milk Protein Breakfast on N Utilization at Adequate and Restricted Calories

Subject	Age (yrs.)	Height (cm)	Initial Weight (kg)	N Intake (g/day)	Caloric Intake (Kcal/day)	
					Adequate	Restricted
MB	19	181	60.0	3.83	3050	2440
RG	19	176	69.5	4.47	3255	2600
CH	19	179	63.9	4.09	3587	2870
RM	19	186	80.5	5.17	4039	3231
RR	21	200	83.8	5.31	4000	3200
DR	20	184	72.8	4.68	3630	2904
MS	20	172	75.2	4.79	3375	2700

Table 13

Nitrogen Balance (g/day) Results for Young Men Studied for the Effects of a Skim Milk Protein Breakfast on N Utilization at Adequate and Restricted Calories

Subject	Caloric Intake			
	Adequate		Restricted	
	No Breakfast	Skim-Milk Breakfast	No Breakfast	Skim-Milk Breakfast
MB	-0.67	-0.07	-0.46	-0.61
RG	-0.44	-0.86	-2.33	-1.96
CH	-0.76	-0.57	-0.75	-1.44
RM	0.0	-0.36	-1.10	-0.19
RR	-0.37	+0.14	-2.53	-2.24
DR	-0.47	-0.42	-1.15	-0.71
MS	0.0	-0.24	-1.47	-0.29