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9. ABSTRACT

This study was undertaken to determine the optimum level of lysine which must be added to white wheat flour to make it effective as the main or only source of protein in the diets of growing infants and preschool children. In each of the four recovering malnourished infants studied for their isonitrogenous and isocaloric levels, the performance of unsupplemented flour was compared with casein and with the same flour enriched with lysine at three different levels. All diets were well accepted and tolerated. The percentage absorption of nitrogen was remarkably high for the lysine-added diets, with values almost identical to casein diets. As for nitrogen retention, the results for unenriched wheat were almost invariably lower than those of the preceding and following casein-diet periods. The results show that lysine has a notable supplementary effect in children receiving wheat as the main source of protein. The equivalent of 0.11% lysine enrichment of ordinary wheat flour produced striking improvements in weight gain and in apparent nitrogen retention, while maintaining normal serum protein and plasma amino acid levels. Further enrichment to the equivalent of 0.18% lysine is apparently resulting in further improvement, but additional observations are under way to confirm this preliminary finding.

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FACTORS INFLUENCING ADEQUACY OF DIETARY PROTEINS FOR YOUNG CHILDREN: LYSINE ENRICHMENT OF WHEAT FLOUR AS AN EXAMPLE[1]

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In the formulation and development of protein foods for consumption by infants and children, reliable estimates of potential nutritional value can be obtained from their biochemical composition and performance in experimental animals. Prior to their introduction for large-scale consumption, it is essential to determine acceptability and biological value in children of the age groups for whom they are intended. If these latter studies are made only at favorable levels of intake, i.e., high protein-to-calorie ratios, deceivingly good results are usually obtained and statements of "equivalence" to cow's milk protein are glibly made. It is commonly assumed that since these foods are meant to be consumed at favorable protein-to-calorie ratios, such testing is adequate. Worldwide experience tells us that this is usually not the case and that the main item of the diet is commonly diluted by the addition of low-protein foods. For this reason, and in order to make strict comparisons with milk protein, we carry out testing in infants at critical levels, designed to bring out in a measurable fashion the relative biological value, in accord with the recommendations of the Protein Advisory Group of the United Nations agencies (1).

In the human infant the rate of weight gain, and very probably of cell multiplication, is governed in great part by the total caloric intake. The amount of protein in the diet, and its quality, by making available the amino acids essential for protein synthesis, determine whether cells will have their normal complement of proteins and free amino acids, and also will determine the levels of certain labile proteins and of free amino acids in body fluids. In evaluating the adequacy of dietary protein, we must take into account a number of important factors. Rates of weight gain, particularly over relatively short periods of time, are a useful but crude indicator and are best interpreted in conjunction with nitrogen retention and serum proteins, particularly the albumin fraction. Thomas and Combs (2) have provided excellent evidence of the relation between serum proteins and body composition in experimental animals.

In infants and children, when steady gain in weight is accompanied by a measurably adequate nitrogen retention and the level of serum albumin remains normal, we can usually be quite confident that body composition has remained normal and that the amounts of all the available essential amino acids and of total nitrogen in the diet were adequate for the total caloric intake and the rate of growth. When one or more of the essential amino acids is present in inadequate amounts or if total nitrogen is inadequate, we commonly see one of two alterations: a decreased rate of weight gain or a fall in serum albumin, occasionally both. Ideally, we would like to see the first, indicating that body composition is being maintained, probably by an increase in the specific dynamic action of food, as recently suggested (3). The second alternative, a fall in serum albumin level despite continued weight gain, indicates an unfavorable alteration in body composition. On milk diets, when the percentage of protein calories has been reduced below 6.4, we have seen both changes, most commonly the second. On vegetable proteins, when lysine is the first-

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limiting amino acid, we usually but not invariably see a decrease in the rate of weight gain, and when methionine is first-limiting, we usually see a fall in serum albumin, again with exceptions. If the same diet is prolonged, however, the rate of weight gain and serum albumin both decrease and appetite eventually falls off. Differences in the amount of nitrogen retained, on diets which are isocaloric and isonitrogenous between milk and other protein sources, are more readily apparent at low protein-to-calorie ratios. At high ratios these differences can be obliterated by the fact that the supply of essential amino acids has exceeded the maximal rate of protein synthesis possible.

The above observations apply to children who are in a satisfactory physiologic state. In the seriously malnourished state, other more poorly understood differences in protein quality become apparent.

Atrophy of the intestinal mucosa (4) and a much decreased output of pancreatic enzymes (5) can bring out otherwise unimportant differences in digestibility. At the same time, by decreasing significantly the endogenous amino acid contribution to the intestinal pool, these alterations alter the pattern of amino acids absorbed. This, along with deficiencies in the necessary liver enzymes (6), might help explain the otherwise perplexing inferiority of many proteins in their ability to regenerate serum albumin and liver proteins, despite a simultaneous satisfactory support of growth (7,8).

These principles have been applied in a study to determine the optimum level of lysine which must be added to white wheat flour to make it effective as the main or only source of protein in the diets of growing infants and preschool children. In each of the four recovering malnourished infants studied, at isonitrogenous and isocaloric levels, the performance of unsupplemented flour has been compared with casein and with the same flour enriched with lysine at three different levels.

The white flour used in these studies was processed by air classification to contain approximately 21% protein, making it possible to prepare diets which were very similar in all their components to the control casein diets. The amino acid composition of this flour is quite typical of those in common use throughout the world. Ten essential amino acids (the usual eight plus tyrosine and cystine) made up 33.77% of its protein, compared with 48.8% for human breast milk protein. In the latter, lysine provides 129 mg. of every gram of essential amino acids, while in flour, it supplies only 79 mg. Threonine, the reported next-limiting amino acid in wheat flour, supplies 94 mg. of every gram of essential amino acids in human breast milk protein and 83 mg. in wheat flour.

The first level of supplementation with lysine was calculated to make lysine equally limiting with threonine, so that their respective contributions to the total essential amino acid (EAA) content of the wheat flour, expressed as a percentage of that in human breast milk, would be equal. This was achieved by adding 215 mg. of lysine (as L-lysine-HCl) for every 100 g. of high-protein wheat flour. This corresponds approximately to 0.11% enrichment of a wheat flour with a 10% protein content. In this report the unenriched wheat flour is identified as (W), while the flour enriched to this first level is identified as (WL-1).

The second level of lysine supplementation (WL-2) was calculated to make lysine supply the same proportion of the EAA's as in human breast milk protein, 129 mg./g. EAA. This level should theoretically make threonine first-limiting in the diet and was achieved by adding 359 mg. lysine/100 g. flour, the approximate equivalent of 0.18% enrichment of ordinary flour.

The third level (WL-3) was chosen so that the lysine content of the wheat flour protein would be present in the same amount as that of human breast milk protein, 6.3 g. lysine/100 g. protein instead of the 2.67 g. found in wheat flour. This was achieved by adding 725 mg. lysine/100 g. wheat flour, the equivalent of 0.36% supplementation of ordinary flour. This should represent a definite excess of lysine.

Methods

Four infants suffering from severe malnutrition were first admitted to the Pediatric Service of the British American Hospital for control of infection, rehydration, and correction of acute electrolyte disturbances. Once their acute symptoms and signs were corrected, they were given a modified cow's milk preparation and transferred to our Metabolic Unit for study. After a steady rate of weight gain had been established, when serum proteins returned to normal and hapatic steatosis was corrected, they then became the subjects of these studies. For each child the minimum safe level of calories and protein which maintained steady growth was determined and the source of protein was changed to calcium caseinate (Casec). Cottonseed oil, cane sugar, and starch were added to achieve the desired caloric intake, maintaining the same proportion of fat and carbohydrate as in the modified cow's milk. Appropriate vitamin and mineral supplements were provided daily and the diet was recalculated daily to yield a predetermined protein and calorie intake per unit of body weight. Rates of weight gain were measured and nitrogen balances determined during three consecutive 3-day periods. Serum proteins and plasma amino acids were measured at the beginning and end of each diet period, as were hematologic values. Following the first 9-day period on casein, the diet was changed to the selected wheat diet, at isonitrogenous and isocaloric levels for 21 days. Each child received all four diets, W, WL-1, WL-2, and WL-3, in random sequence for 21 days each, with intervening 9-day periods on casein. Nitrogen balances on each wheat diet were determined for the first 9 and the last 6 days. For the casein diets, nitrogen balances were determined during the entire 9-day period. With some frequency, because of intercurrent infections, diet periods had to be prolonged. In two children, near the end of their studies, it was necessary to reduce the caloric intake because of obvious satiety.

In the wheat diets, as with casein, cottonseed oil and cane sugar were used to make the fat and carbohydrate contents similar to those of milk.

Results

All diets were well accepted and tolerated. The percentage absorption of nitrogen was remarkably high and in each instance was as high or even higher than that for the preceding and following casein diet. Most values were between 85 and 90% of intake, which is considerably higher than that of all cereal diets we have studied and moderately higher than the usual values for modified cow's milk in our unit. Because of the consistently good results, with values almost identical to casein in each instance, no attempt will be made to further analyze these results.

In regard to apparent nitrogen retention, the results for unenriched wheat were almost invariably lower than those of the preceding and following casein periods. In Table I we have expressed the rates of weight gain and the nitrogen retentions (lower half of table) for each child on each diet as a percentage of the related casein periods. In Fig. 1 the means of these values have been presented graphically. There is obviously an advantage to the first level of supplementation over unenriched wheat. There is also an apparent advantage to the second level over the first, and possibly a slight advantage to the third level over the second. These observations are being extended in further studies.

In regard to rates of weight gain, the differences between the diets are similar in direction and degree to those for nitrogen retention, with the exception of the difference between the second and third levels, which is almost nil.

In three of the four children there were brief periods during which the serum albumin fell significantly below 4.0 g./100 ml., the lower limit of normal in our laboratory, for milk protein diets. All three episodes were in association with an evident infection, two of which occurred during wheat diet periods and one during a casein diet period, making it unlikely that the dietary protein was responsible for the fall. All recovered promptly when the infection cleared up. The occasional brief episodes of

TABLE I

Rates of Weight Gain and Apparent Nitrogen Retentions at Different Levels of Lysine Enrichment of Wheat Flour Expressed as a Percentage of those Attained during Casein Diets Immediately Before and Following Each Wheat Diet

	WHEAT DIET			
CASE NO.	W	WL-1	WL-2	WL-3
RATES OF WEIGHT GAIN				
167	58	84	80	88
192	57	115		120
182	48	96	93	105
188	50	65	119	82
Mean	53	90	97	99
APPARENT NITROGEN RETENTION				
167	64	77	116	111
192	43	80	98	100
182	62	101	94	105
188	78	93	83	103
Mean	62	88	98	105

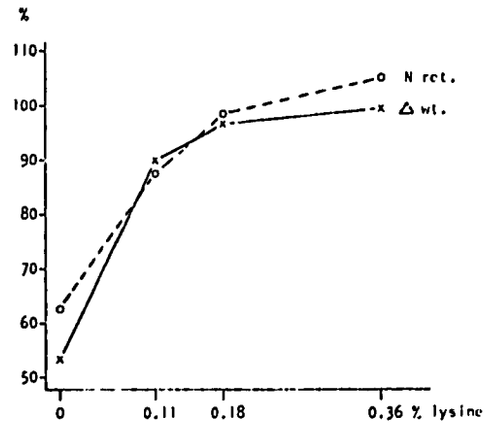

Fig. 1. Rates of weight gain (△wt.) and apparent nitrogen retentions at different levels of lysine enrichment of wheat flour. Rates of weight gain are expressed as a percentage of those attained with casein diets immediately preceding and following each wheat diet. Nitrogen retentions are expressed in the same manner. The levels of lysine enrichment have been converted to values which would apply to a wheat flour containing 10% protein although the studies were carried out with a white flour with a protein content of 21%.

diarrhea which were seen are common at this age and in all cases a continuation of the same diet was possible, again suggesting that they were not due to intolerance or allergy. It is impressive in our experience that, with the brief exceptions mentioned, all four children maintained normal serum albumin levels along with steady gain in weight on these

cereal diets, particularly when in three of the four, the percentage of calories as protein was less than 8.0. At these levels most vegetable protein diets, if they support steady weight gain, cause the serum albumin to be consistently below 4.0 g./100 ml.

An incomplete and preliminary analysis of the plasma aminograms taken at the end of each diet period does not reveal any striking deviation from the normal range. The mean value for plasma lysine at the end of the unenriched wheat diets is clearly lower than after the supplemented diets. The highest level of lysine enrichment failed to cause elevation of plasma lysine or fall of plasma threonine, which might have been expected if this amino acid had been made clearly first-limiting. In this respect, it is of importance to recall that Bressani et al. (9) were not able to demonstrate any supplementary effect of threonine alone on wheat diets with supplementary lysine. Four to five essential amino acids had to be added to these diets before any further supplementation could be demonstrated. This, as well as our experience with the plasma amino acids, suggests that the balance between the other EAA's in wheat is such that none is clearly next-limiting after lysine. It must be noted, however, that our plasma samples were obtained after a minimum of 3 weeks on each wheat diet and after an 8-hr. fast. The length of time on the diet might well have allowed for adaptation to the high lysine intake of diet WL-3, while the fasting state is not the ideal time to demonstrate that a particular amino acid is deficient in any protein (10).

Summary

These results reveal a notable supplementary effect of lysine in children receiving wheat as the main source of protein in the diet. The equivalent of 0.11% lysine enrichment of ordinary wheat flour produced striking improvements in weight gain and in apparent nitrogen retention, while maintaining normal serum protein and plasma amino acid levels. Further enrichment to the equivalent of 0.18% lysine enrichment of ordinary white wheat flour resulted in apparent further improvement but additional observations are under way to confirm this last impression. Additional long-term studies at these two levels are also in progress to see if the excellent results will continue over a period of many months. The amazing digestibility of wheat flour is clearly apparent in these studies.

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