PN-AAS-579

PN 67 ~~APPENDIX V-y~~

EVALUATION OF THRIPOSHA IN HUMAN INFANTS

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SUBJECT:

Progress Report - June 7, 1976
Research Contract AID/Ta-C-1286
Clinical Assay of High Protein Foods

INTRODUCTION

Thriposha is a food blend designed for use in preschool feeding programs. The mixture is made from 80% wheat-soy blend and 20% dehulled sorghum. The sorghum used in production of the blend was imported from Sri Lanka, the country for which the product is intended. The development, biochemical, microbiological, and animal evaluations of Thriposha were supervised by Colorado State University.

To complete the evaluation of Thriposha, studies have been carried out in human infants in the Nutrition Research Institute, Lima, Peru. The protein quality of the mixture was assessed using short-term comparative nitrogen balance studies. Long-term consumption studies using diets based on Thriposha were carried out to ascertain if linear growth would be supported by such a diet. In addition, plasma free amino acids both fasting and postprandial, were sampled to determine the first limiting essential amino acid of the mixture. The present report contains the results of the comparative nitrogen balance and long-term consumption studies. Results of plasma amino acid determinations will be reported separately when these have been completed.

MATERIALS AND METHODS

Methods used in the present study were similar to those used in previous studies (1, 2) and will be described in detail only as necessary.

Short-term studies: Subjects: Seven male Mestizo infants ages 6 to 13 months, weight 4.7 to 6.8 kg participated in the

study with the informed consent of their parents. All infants had been admitted to our unit for the treatment of malnutrition and were remaining for long-term convalescence. All were in good health at the time of study.

Diets: A test diet based on Thriposha (Table 1) was prepared as follows: Thriposha was blended with sucrose and soy-cottonseed (80:20) oil to produce a diet in which protein (N x 6.25) provided 6.7% of energy, 52% of non-protein energy being provided by fat, 48% by carbohydrate. A control diet was prepared in a similar fashion so that casein provided 6.7% of energy, non-protein energy being similarly distributed between sucrose and the soy-cottonseed oil blend. Vitamins and minerals were supplemented to both diets to meet Recommended Daily Allowances. Diets were prepared in liquid form and fed by bottle five times per day.

Plan of Study: The casein-based control diet was fed for an initial period of nine days. After a three-day adaptation period, each child was placed in a metabolic bed and two consecutive three-day collections of urine and stool were made. The diet was then changed to the Thriposha-based test diet for nine days (three days adaptation, two three-day metabolic collections). A final similar nine-day casein control period then followed.

Serum total protein and albumin concentrations were measured at the beginning and end of each dietary period.

Nitrogen in urine and stool was determined by the micro-Kjeldahl method. Fecal fat was determined by the method of

Van de Kamer (3). Serum total protein was determined by the Biuret method and albumin concentration by starch gel electrophoresis.

No attempt to measure insensible losses was made and consequently apparent nitrogen absorption and retention are reported. Each child served as his own control and statistics were carried out using a "t" test for paired comparisons.

Long-term studies: Subjects: Four male Mestizo infants ages 8½ to 13 months participated in the long-term studies. Two of these children had also participated in the short-term studies. All children were in good health, free of infection and gaining weight when the studies were carried out.

Diets: Thriposha was altered by the addition of sucrose and soy-cottonseed oil blend to produce a diet in which protein (N x 6.25) provided 8% of total energy. Non-protein energy was distributed between fat and carbohydrate 52%-48%. Vitamins and minerals were supplemented to meet Recommended Daily Allowances.

Plan of Study: Using a casein-based or modified cow's milk diet the energy intake required to maintain a steady weight gain of approximately 3 to 5 g/kg body weight/day was ascertained for each child. The child was then placed on the diet of Thriposha and this diet was continued for a period of three months. Changes of height, weight, serum albumin concentration and serum triglycerides and cholesterol were monitored throughout the study. Daily physical examination was carried

out looking for signs of dietary intolerance or specific nutrient deficiency.

These studies were analyzed by comparing changes in height and weight expressed as height age, height quotient[1], and weight age in comparison with the advancement in chronological age. The ability of the diet to maintain or improve serum albumin concentration was also used as a criterion.

RESULTS

The results of the balance studies carried out in seven children consuming the diet in which Thriposha supplied 6.7% protein energy are shown in Table 2. The age, height, height age, weight and weight age of each child are shown and give an approximation of the stage of recovery. Most children completed the protocol without incident. Patient #485 had a fall in serum albumin concentration noted following the Thriposha period and consequently the study was terminated after the first two nine-day periods. The Thriposha period and the second control period for patient #491 had to be prolonged slightly because of problems with an upper respiratory infection encountered during the adaptation period.

Mean apparent nitrogen absorption from Thriposha was 75.9±4.8% of intake compared with corresponding values for the first and second control periods of 84.4±2.7% and 86.2± 3.1%. Apparent nitrogen absorption during the Thriposha

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[1] 100 x HA/CA

period was significantly less than during either control period ($t=7.071$, $P<0.001$).

Mean apparent nitrogen retention was 35.7±12.2% of intake during the first control period, 38.3±9.6% during Thriposha consumption, and 42.3±8.6% during the second casein control period. Apparent nitrogen retention while consuming Thriposha did not differ significantly from that during either control period. Apparent nitrogen retention during the second control period was, however, significantly greater than that during the first control period ($t=-3.544$, $P<0.01$).

Fecal wet weight tended to increase during periods of Thriposha consumption. This was significantly different only when the second control and Thriposha periods were compared (control 70.5±28.5 g/day, Thriposha 109.8±60.9 g/day, $t=-2.72($ $P<0.05$).

Fecal fat excretion was generally lower while consuming Thriposha. This difference was significant when fat excretio (expressed as a percent of fat intake) during the first control period was compared to that during the Thriposha period (control mean 19.7±7.6% of intake, Thriposha mean 10.5: 7.3% of intake, $t=2.833$, $P<.05$). Although the effect of diet on fecal fat excretion was in the same direction when the second control period and the Thriposha period were compared, the difference was not statistically significant.

The rates of weight gain while consuming the two diets were similar. There was marked patient-to-patient and diet-to-diet variation in this parameter, as well as in the changes in serum albumin concentration. There was no statistical differences between diets when either change in serum albumin concentration or final serum albumin concentration was analyzed.

Long-term studies: The advancement of height age, height quotient and weight age in the four children in the long-term studies are shown in Table 3. In general, the diet was well tolerated and both linear growth and rate of weight gain were adequate during the three-month period of study. Only patient #469 suffered any untoward effects during the study. Two weeks after beginning the long-term study, this patient was found to have a precipitous decrease in serum albumin concentration as well as blood loss in the stool. For this reason, the study was terminated. No cause other than possible protein and blood losing enteropathy secondary to diet could be found after a complete clinical evaluation and consequently it was decided to challenge the child with the Thriposha-based diet a second time. As shown in Table 3, the diet was subsequently tolerated for a period of three months.

One child (#481) had an increase in height age greater than the corresponding increase in chronologic age. All children except #469 either maintained or increased their height quotient. Increase in weight age was greater than increase in chronological age for three of the four children studied, the exception being #469. Serum albumin concentration,

also shown in Table 3, remained stable or increased during the three-month period of the study in all but #469. In this child, serum albumin concentration decreased rapidly during the initial two weeks of the second study and then remained stable.

Serum triglyceride and cholesterol values in the children consuming the Thriposha-based diet are shown in Table 4. All children had normal triglycerides and cholesterols at the beginning of the study; both values tended to decrease during the study.

DISCUSSION

Based on the short-term nitrogen balance studies, the protein quality of Thriposha is quite satisfactory for human consumption. Although Thriposha normally provides 20% of energy as protein, the comparative balance studies with casein were carried out with protein providing 6.7% protein energy. This was done for two reasons, to minimize errors in the balance technique by keeping protein intakes as low as safely possible, and to increase the chance of uncovering differences in protein quality between Thriposha and the casein. Under these conditions no statistically significant difference was found between the apparent nitrogen retentions while consuming the two diets. This was so despite the significantly inferior apparent nitrogen absorption from the Thriposha-based diet.

When the mean apparent nitrogen retentions for all three dietary periods were considered a picture of increasing nitrogen retention with time appeared to emerge. When the individual children were looked at, however, this did not appear to be the case. The mean apparent nitrogen retention for the second casein period was significantly greater than that for the first casein period. Why this was so was not clear. Although at first it appeared that this might have represented compensatory nitrogen retention in the second casein period following inadequate retention from the Thriposha diet, this was not borne out by either a statistical difference between the nitrogen retentions during the second casein and Thriposha periods nor by significant changes in the serum albumin concentrations during the same two periods.

Stool wet weights tended to be higher during the periods of Thriposha consumption. Stool wet weight has previously been shown to correlate well with fecal energy loss (4). Taken together with the inferior apparent nitrogen absorption from Thriposha, the product was adjudged to be slightly inferior in digestibility to the control diet. Because the control diet is a low residue diet and the difference between the two diets was not great, this should not be considered a drawback.

Fat absorption from the Thriposha-based diet tended to be superior than that from the casein control diet. (This difference was significant only when the first casein period was compared with the Thriposha period). The reason for this

tendency is unclear. Thriposha contains 5.4% fat (approximately 13% of energy) in the form of soy oil. This is not provided by the use of a full fat soy flour but rather by the addition of soy oil during the production of the mixture. In both the diets consumed for the comparative studies, the majority of fat was provided by the addition of soy-cottonseed oil blend used in preparation of the diets. Consequently no difference in fat absorption between the two diets was expected.

The three-month consumption studies confirmed that Thriposha was an adequate source of protein in the diets of growing infants over longer periods of time even when significantly diluted with additional non-protein energy. Three children maintained or improved their height quotient during the three-month period, indicating that they were growing steadily along a given growth percentile. Those children whose height age increased more than their chronologic age or who showed an improvement in height quotient were achieving catch-up growth during the three-month period. The relation of the protein and blood losing enteropathy in patient #469 to the Thriposha diet is unclear. Although this may have been diet related, the fact that the child was able to reinitiate consumption of the diet for a three-month period of time without a recurrence of symptoms militates against this interpretation. On the other hand, this child's linear growth was slower and serum albumin concentration lower than those of the other three children. A higher percentage of energy as

protein in this child might have improved these parameters. The lack of other untoward reactions or signs of specific nutrient deficiency confirm that the product is safe for prolonged consumption in significant quantities.

In summary, Thriposha appears to be a food mixture of significant potential. The slightly lower digestibility encountered was in keeping with the vegetable source of protein. The inferior digestibility appeared to be offset by the quality of the protein, resulting in excellent apparent nitrogen retention when compared to casein. Linear growth, weight gain and serum total protein and albumin concentrations were adequately maintained when Thriposha was the only source of protein for extended periods of time. Our results confirm the impression gained from pre-clinical evaluations that Thriposha is both nutritious and suitable for the feeding of growing infants and preschool children.

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Table 1

Diets used for comparative nitrogen balance studies (per 100 kcal). The protein in either calcium caseinate or thriposha provided 6.7% of energy

		Control Diet	Test Diet
Protein - g		1.67	1.67
Source:	Ca caseinate	1.67	--
	Thriposha	--	1.67
Fat - g		5.39	5.39
Source:	Ca caseinate	0.05	--
	Thriposha	--	0.47
	S-C (80:20) oil[1]	5.34	4.92
CHO - g		11.20	11.20
Source:	Thriposha	--	5.43
	Sucrose	11.20	5.77

[1] Soy-cottonseed (80:20) oil blend.

Table 2

Results of balance studies in 7 children consuming a diet in which protein (supplied by either thriposha or casein) supplied 6.7% of 150 kcal/kg/d

Pt. No.[1]	Diet: Protein Source	Diet: No. Days	Nitrogen Balance: On Days	Nitrogen Balance: Int. mg	Nitrogen Balance: Ret. mg	Nitrogen Balance: % of Intake Abs.	Nitrogen Balance: % of Intake Ret.	Stool: Wt. g/d	Stool: Fat g/d	Stool: Fat % Int.	Δ Body Weight g/kg/d	Serum Albumin: Δ mg/dl	Serum Albumin: Final mg/dl
478	C	9	4- 9	2800	1232	87	44	46	6.9	12.0	5.7	+0.35	3.91
	T	9	4- 9	2840	1477	80	52	88	3.1	5.4	2.8	+0.25	4.16
	C	9	4- 9	2840	1374	88	51	58	6.3	10.8	6.3	-0.19	3.97
481	C	9	4- 9	2840	994	84	35	132	17.6	30.2	3.2	-0.19	3.57
	T	9	4- 9	2840	762	76	27	86	4.4	7.6	6.0	-0.13	3.44
	C	9	4- 9	2840	1151	90	41	46	6.1	10.3	6.0	+0.05	3.49
483	C	9	4- 9	2360	859	88	36	53	7.1	14.6	5.8	-0.68	3.50
	T	9	4- 9	2360	1138	77	48	118	10.2	21.1	2.8	-0.01	3.49
	C	9	4- 9	2360	904	86	38	77	8.6	17.7	5.4	+0.29	3.78
485	C	9	4- 9	2245	1075	83	48	103	6.5	16.0	4.8	--	--
	T	9	4- 9	2000	686	74	34	89	1.9	4.6	10.0	--	3.17
489	C	9	4- 9	2720	548	80	20	128	17.2	30.7	4.9	+0.16	4.13
	T	9	4- 9	2720	813	66	30	226	11.5	20.6	4.4	-0.43	3.70
	C	9	4- 9	2720	1051	81	39	125	12.9	23.0	5.1	+0.23	3.93
491	C	9	4- 9	2160	1032	85	48	44	7.1	16.0	5.0	+0.23	3.76
	T	14	9-14	2158	944	78	44	50	1.8	4.2	6.0	-0.61	3.15
	C	11	6-11	2320	1253	87	54	59	7.8	16.3	3.0	+0.74	3.89
494	C	8	3- 8	2640	507	84	19	89	10.0	18.4	0.8	-0.30	3.45
	T	9	4- 9	2640	878	80	33	91	5.3	9.8	9.4	+0.23	3.68
	C	9	4- 9	2631	816	85	31	58	7.7	14.3	1.5	-0.06	3.62

[1] Age in months, height in cm (height age, mos.), weight in grams (weight age, mos.), for these children at the onset of the study were as follows:

#478: 8.0 mos., 64.0 cm (4.6), 6860 g (5.0); #481: 12.0 mos., 67.0 cm (6.5), 6820 g (5.0);
#483: 6.0 mos., 62.6 cm (4.0), 5680 g (3.0); #485: 7.5 mos., 58.8 cm (2.5), 4750 g (1.5);
#489: 10.0 mos., 66.6 cm (6.3), 6800 g (5.0); #491: 7.0 mos., 59.0 cm (2.5), 5680 g (3.0);
#494: 13.0 mos., 66.6 cm (6.3), 6630 g (4.5)

Table 3

Chronologic age (CA) in months, height age (HA), height quotient (HQ)[1] and weight age (WA) at the onset and completion of long-term consumption of a diet in which the protein in Thriposha provided 8% of energy

Pt. No. and Sex	Av. Int. Protein[2] kcal	INITIAL VALUES					FINAL VALUES				
		CA	HA	HQ	WA	Serum Alb.[3]	CA	HA	HQ	WA	Serum Alb.[3]
466 M	2.74/137	9.5	4.1	43	4.2	3.66	12.5	6.0	48	8.3	3.80
469 M	2.5 /125[4]	8.4	4.2	50	4.1	3.85	9.0	4.7	52	5.3	3.05
	3.0 /150	9.8	6.3	64	5.7	4.19	12.8	7.8	61	7.3	3.48
478 M	2.86/143	8.8	6.1	69	6.0	3.58	12.0	8.1	68	9.25	4.02
481 M	2.5 /125	13.0	7.4	57	6.8	3.22	16.0	11.9	74	15.5	3.50

1 100 x HA/CA

2 Average intake of protein (grams) and energy (kcal) per kilogram body weight per day

3 Serum albumin concentration - g/dl

4 Terminated because of falling serum albumin concentrations

Table 4

Serum triglyceride and cholesterol concentrations in children before and after prolonged consumption of a diet with 8% of energy supplied by protein from Thriposha

Pt. No.	Days Consumption	INITIAL		FINAL	
		Triglyc.	Cholest.	Triglyc.	Cholest.
466	86	117	159	55	134
469	16	54	106	57	77
	90	46	77	39	72
478	90	100	39	110	135
481	90	79	111	55	95