A STUDY OF WHOLE BODY PROTEIN KINETICS IN MALNOURISHED CHILDREN WITH PERSISTENT DIARRHEA: A DOUBLE BLIND TRIAL OF ZINC SUPPLEMENTATION

[1]Z. A. BHUTTA, [1]S. Q. NIZAMI, [2]Z. ISANI, [3]S. HARDY, [3]K. HENDRICKS, [4]V. YOUNG

[1]Departments of Pediatrics, The Aga Khan University (AKU), Karachi, Pakistan
[2]National Institute of Child Health, Karachi, Pakistan
[3]Combined Program in Paediatric Gastroenterology & Nutrition, Harvard Medical School, Boston, USA
[4]Massachusetts Institute of Technology, Boston (USA)

Abstract

Persistent diarrhoea (PD) is globally recognized as a major cause of childhood morbidity and mortality. PD is closely associated with malnutrition and nutrition rehabilitation especially domiciliary dietary therapy posses a therapeutic challenge. While there has been success in managing such children with locally home available traditional diets, there has been concern with the potential of associated micronutrient especially zinc deficiency. We are evaluating the impact of zinc supplementation of a traditional rice-lentil (khitchri) and yogurt diet in malnourished children with PD in randomized double blind study. In addition to the impact on weight gain, stool output and body composition, we will evaluate whole body protein kinetics using the modified CRP protocol [employing ^{15}N-glycine, $H^{13}CO_3$ and 1-^{13}C leucine]. We will also estimate the effect of coexisting illnesses, especially febrile episodes on nutritional recovery and protein metabolism.

1. IMPORTANCE OF PERSISTENT DIARRHOEA AND NUTRITIONAL REHABILITATION

Persistent Diarrhea (PD) has been recognized in recent years as a major cause of morbidity and mortality in the developing world [1]. While improved management of acute diarrhea with oral rehydration has led to improved survival, PD is still considered to account for up to 50% of all mortality associated with diarrheal disorders, with case fatality rates as high as 14% [2]. The close association between PD and malnutrition is well recognized and PD has been rightfully considered a nutritional disorder [3]. Nutritional management is therefore considered a cornerstone for its optimal management.

Although a variety of different dietary formulations have been tried in PD, the optimal management of PD remains controversial [4]. In general, enteral nutrition is considered superior to parenteral forms of therapy, with better weight gain and reduction in diarrheal severity [5]. The types of dietary formulations are of particular importance for much of the developing world where cost-effectiveness is a crucial factor in diet selection and compliance of therapy. In addition, it is important to consider factors such as home-availability, efficacy and cultural acceptability of the dietary formulations.

In a series of studies, we have evaluated the efficacy of a traditional rice-lentil-yogurt combination (Table I) in children with PD and demonstrated satisfactory tolerance and nutritional impact in comparison with standard soy formulation [6]. In a randomized comparison of the traditional rice-lentil (Khitchri) and yogurt diet with soy formula in children with PD, a significantly better increment in weight gain and reduction of stool output was demonstrated (Figure 1). Although accumulation of fibre and fluid in the body was a possibility (Figure 2), the weight gain was considered to be due to increased lean tissue deposition as demonstrated by a corresponding increment in the mid-arm muscle

circumference and comparable dynamic skin fold thicknesses in both groups. In subsequent studies the diet has also been shown to be effective in children with severe protein energy malnutrition (PEM), although the addition of dilute buffalo milk was associated with higher rates of treatment failure [7].

TABLE I. MACRONUTRIENT COMPARISON OF STUDY DIET [CONTENTS / 100 g (after preparation)]

	KHITCHIRI	YOGURT
Protein g (% kcal)	3.3 (11)	3.1 (20)
Fat g (% kcal)	3.1 (24)	4.0 (60)
Carbohydrate g (% kcal)	19.0 (56)	4.0 (20)
Energy k. cal	118	60

2. FACTORS ASSOCIATED WITH OUTCOME OF NUTRITIONAL THERAPY OF PD AND THE IMPORTANCE OF COINCIDENTAL INFECTIONS

Of the various prognostic factors in malnourished children receiving enteral nutritional therapy, young age, severity of diarrhea and coincidental infections were considered of paramount importance [8,9]. In particular coincidental pyrexia, even in the absence of systemic bacteremia, was found to be an important determinant of the rate of nutritional recovery and reduction of diarrheal severity (Figures 3, 4). While the effect of clinical and subclinical infections on nutritional recovery can be multifactorial, it is likely that anorexia, reduced caloric intake as well as increased catabolism all play a significant role. In particular, there has been major interest in the potential role of subclinical infections and immunostimulation in such children [10]. It is possible that associated systemic infections may be important determinants of nutritional recovery in children with PD because of the 'diversion' of important nutrients to synthesis of acute phase proteins [11].

3. IMPORTANCE OF MICRONUTRIENTS ESP. ZINC IN NUTRITIONAL REHABILITATION

In addition to protein-energy-malnutrition, most children with PD have coincidental micronutrient deficiencies. Of these there has been considerable interest in the role of zinc deficiency in such children [12]. Zinc has been recognized as an essential nutrient and component of several metalloenzymes [13] as well as in DNA synthesis [14]. While the optimal laboratory parameter of zinc status in a population remains unknown, several zinc supplementation studies have demonstrated a positive impact on intestinal recovery [15], growth [16] and neurodevelopment [17].

Many studies of feeding traditional diets rich in fiber and phytate to malnourished children suggest that these may be associated with coincidental micronutrient deficiencies due to decreased bioavailability. Indeed, our past studies of feeding a traditional K-Y diet in malnourished children with PD indicated that the weight gain slowed down during the second week of therapy suggesting the possibility of concomitant micronutrient deficiency (Figure 5). Recognition of such micronutrient deficiencies may allow for better and effective nutritional interventions. Studies of zinc supplementation in malnourished children have also indicated that deficient children may deposit more fat compared to lean body mass, as the

energy cost of tissue deposition may be high in deficient children [18]. In contrast, preliminary studies of whole body protein synthesis in malnourished children receiving supplemental zinc indicate a preferential deposition of protein [19].

4. HYPOTHESIS

It is hypothesized that malnourished children with PD have protein energy malnutrition as well as micronutrient (especially zinc) deficiency. Such zinc deficiency may be an important factor in limiting the rate of nutritional recovery in such children on standard dietary therapy. It is further hypothesized that in addition to macronutrients, dietary zinc supplementation in such children may lead to an improved nutritional outcome and rate of recovery. It is also expected that zinc supplementation may have an immunomodulatory effect with reduction in the rates of coincidental infections and their subsequent negative impact on nutritional recovery.

5. OBJECTIVES

Our proposed study will have the following study objectives:

1. To study the zinc status, rates of coincidental infections and acute phase reactants in children with PD and malnutrition;

2. To study the impact of zinc supplementation of a traditional K-Y diet in malnourished children with PD on rates of nutritional and diarrheal recovery in a double blind randomized clinical trial;

3. To study the impact of dietary zinc supplementation in such children on body composition, protein accretion rates and whole body protein synthesis using stable isotopes (^{15}N-glycine, $H^{13}CO_3$ and [1-^{13}C] leucine).

6. STUDY DESIGN

6.1. Patient selection and randomization

Children aged 6-36 months with PD (diarrhea lasting 14 or more days with growth faltering) will be eligible for inclusion in the study. After informed consent and baseline evaluation, these children will be admitted to a metabolic research ward at the National Institute of Child Health with a research medical officer and nurse in constant attendance. After an initial period of stabilization and rehydration for $\leq$ 24 hours, the children will be randomized in a double blind fashion to receive a zinc supplement (4 mg/kg/day) or a placebo in addition to the K-Y diet. The diet will be provided in graded increments to achieve a caloric intake of at least 100 kcal/kg/day by day 3.

6.2. Evaluation

Apart from a detailed clinical examination and nutritional history, the following clinical observations will be made at admission and subsequently as per protocol.

Vital signs (temp, pulse, resp rate)	twice daily
Hydration status	twice daily
Nude body weight (pre-feed)	daily
Quantitative stool output	daily

Quantitative urine output	daily
Skin fold thickness	day 0,7,14
Mid arm circumference	day 0,7,14
Complete blood count	day 0,7,14
Serum albumin and prealbumin	day 0,7,14
Plasma zinc	day 0,7,14
Bioimpedance analysis	day 0,7,14
Serum retinol & retinol binding protein	fortnightly

The bulk of the laboratory investigations will be performed in the Nutrition Research Laboratory at the Aga Khan University using standard techniques. The estimation of zinc on plasma specimens will be performed on an atomic absorption spectrophotometer (Perkin Elmer model 303). This equipment will be available to the investigators though the courtesy of the Dept. of Pharmacology at AKU.

Following stabilization, the stable isotopes will be administered on Day 3 & 14 as follows with timed urine and breath sample collections.

In order to estimate protein metabolism, namely, breakdown and oxidation, the following protocol of stable isotope administration will be followed:

		Time	Dose
^{15}N-Glycine			
	Primary dose	0 h	2 mg/kg
$H^{13}CO_3$			
	Priming dose	0 h	0.4 mg/kg
	Continuous	0.1-3 h, q 20 min	0.40mg/kg/h
1-^{13C}-Leucine			
	Priming dose	3 h	2 mg/kg
	Continuous	3.1-9 h	2 mg/kg/h
	Breath Collection		
	2-3 h		q 20 min
	6-9 h		q 20 min
	Blood samples x 3	9 h	
	Urine Collection		
	0-24 h		Complete

A subgroup of 10 children (5 in each group) will undergo two consecutive metabolic balance studies between days 3-5 & 12-14 to estimate energy, protein, fat and zinc accretion.

6.3. Definitions

Persistent Diarrhoea (PD): Since pre-admission weight may not be available, PD will be defined as three or more watery stool per day for ≥ 14 days following an acute episode of diarrhoea with no more than 2 diarrhoea free days which do not occur consecutively.

Cessation of diarrhoea: Achievement of semisolid stool consistency with a stool output of ≤ 30 g/kg/day.

Nutritional Recovery: Weight gain for a consecutive ≥ 3 days after achievement of a caloric intake of 100 cal/kg/day.

Clinical Failure: Persistence or development of severe vomiting or diarrhoea necessitating intravenous fluids for ≥ 12 hours.

6.4. Outcome variables

The following outcome variables will be studied in both groups and compared:

- Stool output (consistency, volume & frequency)
- Weight gain
- Anthropometric parameters
- Recovery rate: Cessation of diarrhoea and nutritional recovery
- Plasma zinc levels
- Serum retinol binding protein
- Serum pre-albumin
- Whole body protein synthesis rate

7. FUTURE STUDIES

Future studies planned by the Department include the following:

1. An evaluation of metabolic and immune status of growth retarded newborn infants with serial follow-up and monitoring. Local epidemiological data indicates that these low birth weight infants are at major risk for the development of morbidity and diarrhoeal illness [20]. There is little data on protein kinetics and immunostimulation in these children who form almost 25% of all births in Pakistan. We propose to serially follow a birth cohort of such infants for the first six months with periodic evaluation of clinical morbidity data, nutritional status, body composition and protein kinetics. Such information may provide important clues as to potential early and appropriate nutritional interventions.

2. An evaluation of multimixes i.e. locally available dietary combinations providing the optimal macronutrient and micronutrient intake in immunostimulated malnourished infants and children.

REFERENCES

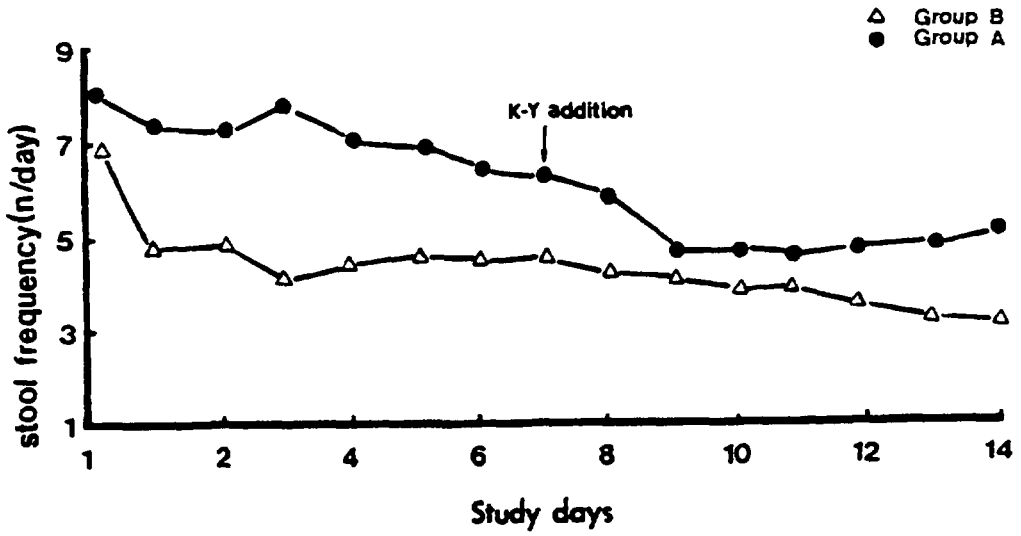
[1] "Anonymous". Persistent diarrhea in children in developing countries: Memorandum from a WHO Meeting. Bull WHO. **66** (1988) 709-717.

[2] VICTORIA, C. G., HUTTLY, S. R. A., FUCH, S. C., *et al.*, International differences in clinical patterns of diarrheal deaths. A comparison of children from Brazil, Senegal, Bangladesh and India, J Diarr Dis Res. **11** (1993) 25-29.

[3] LO, C.W., WALKER, W. A., Chronic protracted diarrhea of infancy: A nutritional disease, Pediatrics. **72** (1983) 786-800.

[4] BHUTTA, Z.A., HENDRICKS, K.H., Nutritional management of persistent diarrhoea in childhood: a perspective for the developing world, J Pediatr Gastroenterol Nutr. 1995 (in press).

[5] ORENSTEIN, S. R., Enteral versus parenteral therapy of intractable diarrhea of infancy: A prospective, randomized trial, J Pediatr. **109** (1986) 277-286.
[6] BHUTTA, Z.A., MOLLA, A.M., ISSANI, Z., BADRUDDIN, S., HENDRICKS, K., SNYDER, J. D., Dietary management of persistent diarrhea: Comparison of a traditional rice-lentil based diet with Soy formula, Pediatrics. **88** (1991) 1010-1018.
[7] BHUTTA, Z.A., MOLLA, A.M., ISSANI, Z., BADRUDDIN, S., HENDRICKS, K., SNYDER, J. D., Nutrient absorption and weight gain in persistent diarrhea: Comparison of a traditional rice-lentil-milk diet with soy formula, J Pediatr Gastroenterol Nutr. **18** (1994) 45-52.
[8] BHUTTA, Z.A., MOLLA, A.M., ISSANI, Z., BADRUDDIN, S., HENDRICKS, K., SNYDER, J.D., Nutritional management of persistent diarrhea: Factors predicting clinical outcome, Acta Paediatr Suppl. **381** (1992) 144-148.
[9] ROY, S.K., AKRAMUZZAMAN, S.M., HAIDER, R., MAJID, N., KHATUN, M., AKBAR, M.S., ALAM, A.N., Persistent diarrhoea: factors affecting absorption and clinical prognosis during management with a rice-based diet, Acta Paediatr Suppl. **381** (1992) 139-43.
[10] SOLOMONS, N., MAZARIEGOS, M., BROWN, K. H., KLASING, K., The underprivileged developing country child: environmental contamination and growth failure revisited, Nutr Rev. **57** (1993) 327-32.
[11] International Atomic Energy Agency "Coordinated Research Program on application of stable isotope tracer methods to studies of amino acid, protein and energy metabolism in malnourished populations of developing countries: Report on the First Research Coordination Meeting. IAEA-NAHRES-21, Vienna (1994).
[12] Hambidge, K.M., Zinc and diarrhoea, Acta Pediatr. Suppl **381** (1992) 82-86.
[13] CHESTERS, J.K., Metabolism and biochemistry of zinc. In. Prasad A S ed. Clinical biochemical and nutritional aspects of trace elements. New York. Alan R Liss. (1982) 221-238.
[14] WU, F. Y-H, WU, C-W., Zinc in DNA replication and transcription, Ann Rev Nutr. **7** (1987) 251-72.
[15] ROY, S.K., BEHRENS, R.H., HAIDER, R.A., AKRAMUZZAMAN, S.M., MAHALANABIS, D., WAHED, M.A., TOMKINS, A.M., Impact of zinc supplementation in Bangladeshi children with acute diarrhea and persistent diarrhea syndrome, J Pediatr Gastroenterol Nutr. 15 (1992) 289-96.
[16] WALRAVENS, P.A., KRELES, N.F., HAMBIDGE, K.M., Linear growth of low income school children receiving a zinc supplement, Am J Clin Nutr. **38** (1983) 195-210.
[17] FRIEL, J.K., ANDREUX, W.L., MATTHEW, J.D., LONG, D.R., CORNEL, A.M., *et al.*, Zinc supplementation in very-low-birth-weight infants. J Pediatr Gastroenterol Nutr. **17** (1993) 97-104.
[18] GOLDEN, B. E., GOLDEN, M.H.N., Plasma zinc, rate of weight gain, and the energy cost of tissue deposition in children recovering from severe malnutrition on a cow's milk or soya protein based diet, Am J Clin Nutr. **34** (1981) 892-899.
[19] GOLDEN, B. E., GOLDEN, M.H.N., Effect of zinc deficiency on lean tissue synthesis during recovery from malnutrition, Eur J Clin Nutr. **46** (1992) 697-706.
[20] MEARTEUS, T.E., CONGENS, S.N., FEACHEM, R.G., Evidence of a prolonged association between low birth weight and pediatric diarrhea in Sri Lanka, Trans Roy Soc Trop Med Hyg. **81** (1987) 81-196.



● = Soy feeds
△ = K-Y Diet

Figure 1. Stool Frequency

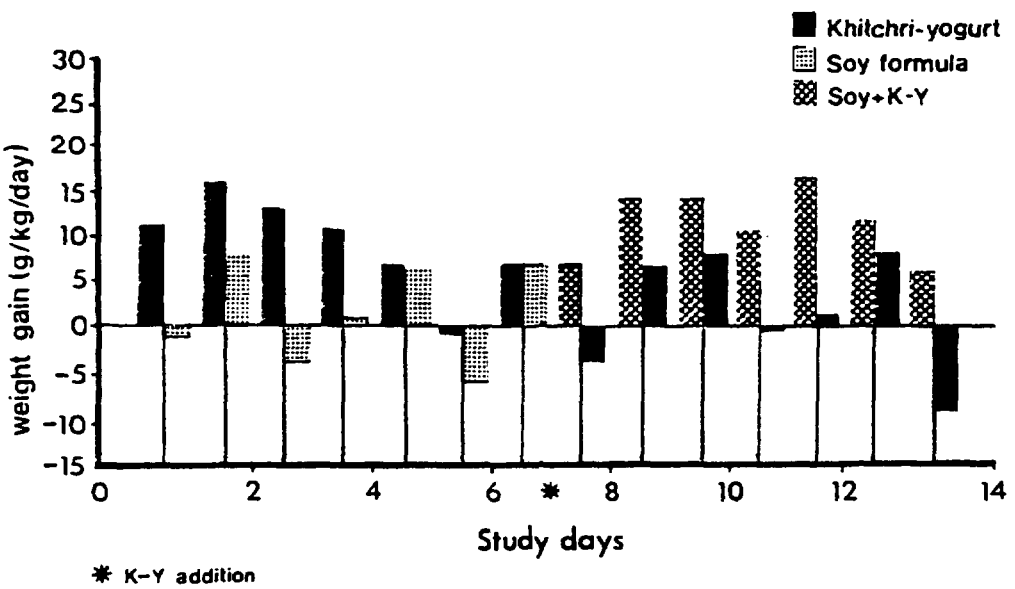


Figure 2. Trend of Weight Gain

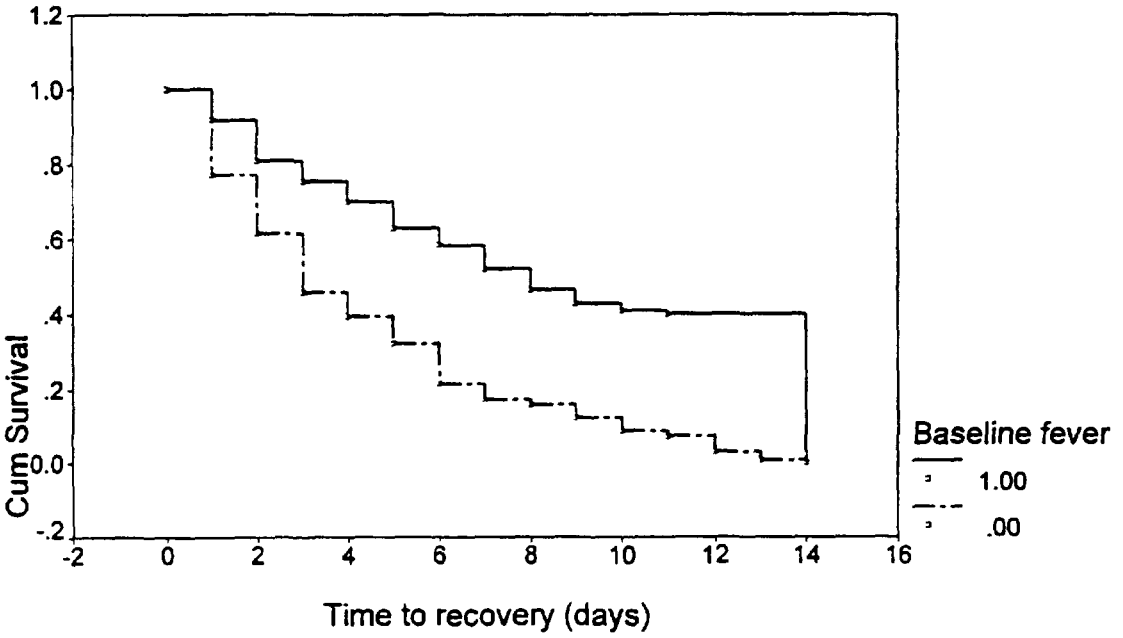


Figure 3. Association of fever and nutritional recovery

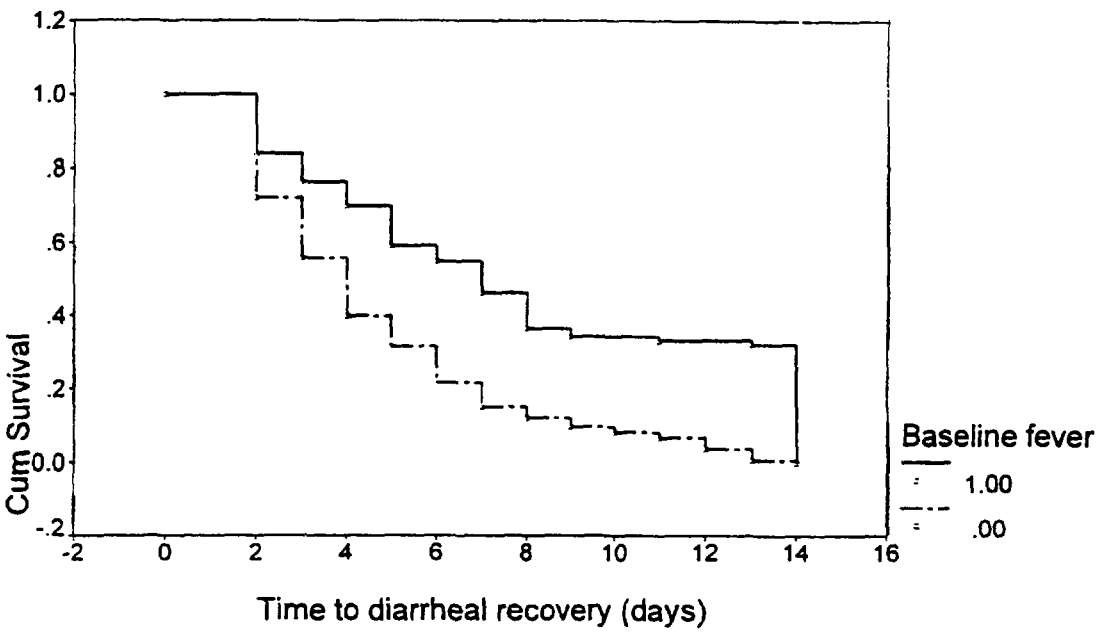


Figure 4. Association of fever and diarrheal recovery

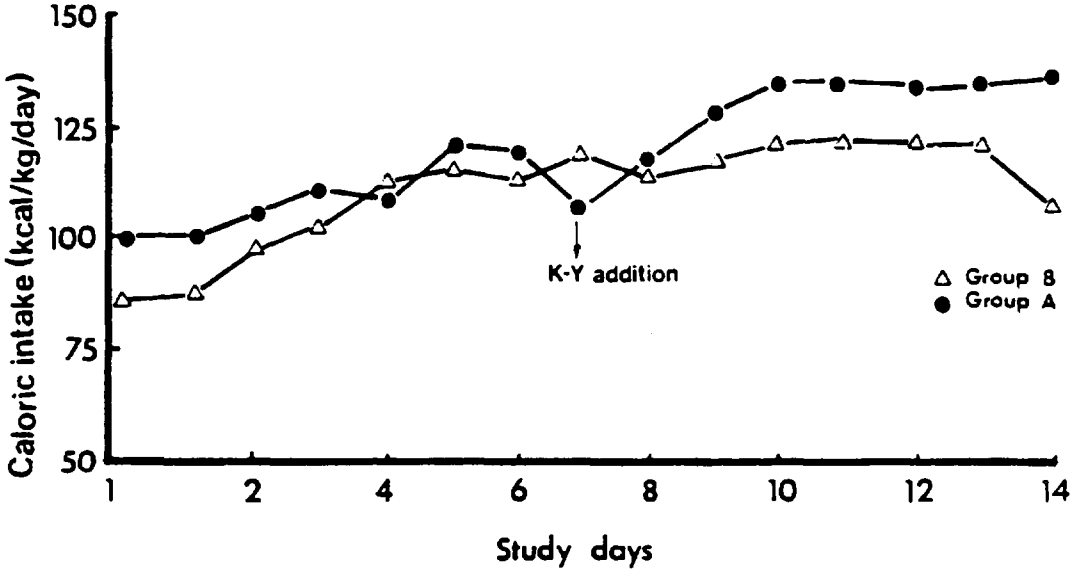


Figure 5. Caloric intake