

Protein deficiency and energy restriction in young cebus monkeys

(protein-calorie malnutrition/albumin/anemia/protein requirements)

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ABSTRACT Infant cebus monkeys (*Cebus albifrons*) were fed liquid formulas that were limited in protein, energy, or a combination of the two restrictions. Weight gain, food intake, hematological development, and plasma protein and cholesterol levels were monitored over a 20-week period. The animals restricted in protein developed the classical signs of protein deficiency—reductions in plasma albumin, a mild anemia, accumulation of fat in the liver, and, in a few cases, facial edema. These animals maintained a relatively high energy intake, and apparently wasted energy when compared to similarly non-growing energy-restricted animals. Energy-restricted animals did not exhibit these symptoms, even when their daily protein intake was reduced to match that of protein-restricted monkeys. It is concluded that an energy restriction superimposed upon a limited protein intake did not increase protein requirements or precipitate protein deficiency.

The two extremes of protein-calorie malnutrition—marasmus and kwashiorkor—have generally been defined as severe partial starvation, characterized by stunting and muscle wasting, on the one hand, and protein deficiency with resulting hypoproteinemia, edema, and fatty liver on the other. Many children show components of both and are categorized as “marasmic-kwashiorkor.” Gopalan (1) was unable to show in a prospective study of the food intakes of Indian children, however, that the usual diet of children who subsequently developed kwashiorkor was lower in protein than that of those who subsequently developed marasmus. Compared to estimated requirements, their intakes were judged to be marginal with respect to protein and deficient in energy. Sukhatme (2) found that 89% of the diets in Marashtra State households that were categorized as protein deficient were also calorie deficient. Both Gopalan and Sukhatme conclude that protein is generally not the limiting factor in the diets of the populations studied and that if total food intake had been adequate, sufficient protein would have been consumed. Gopalan *et al.* (3) also found that a low-protein calorie supplement improved the growth of village children. These kinds of evidence plus more recent considerations of protein needs (4, 5) have led to the growing opinion that primary protein deficiency is an unlikely cause of malnutrition in populations consuming cereal-based diets and that lack of food is the major problem.

The analyses of Gopalan and Sukhatme both assume that the energy and protein standards are equally valid. This seems unlikely since protein standards are, in theory, set relatively high compared to average needs, whereas energy standards are thought to more nearly approximate average needs. Many questions may, of course, be raised about the appropriateness of either standard for various populations as well as the accuracy of dietary data collected in the field.

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The standard teaching in nutrition, however, is that if insufficient energy is consumed, protein is burned as a source of energy. If this is true, it is not readily apparent how the marasmic child, whose diet is rarely high in protein, escapes protein deficiency. This tenet also leads to diametrically opposed conclusions—that additional protein will be of no utility since it will be only an expensive source of energy, or that the protein needs of deprived populations would actually be higher than those with adequate amounts of food. If the latter is true, it would appear to be a remarkably unfavorable adaptation, making it impossible to meet protein needs in exactly those conditions when protein deficiency is most likely to occur—when the availability of food of moderate or low protein content is restricted, limiting both protein and energy intake.

It appears unlikely that the relationship between limited energy and protein intake can be adequately explored with human subjects, particularly with young children who have the additional requirements for protein and energy to permit growth. Long-term dietary restriction of human subjects, and particularly of infants or young children, is not ethical. The nutritional antecedents of malnourished children are never known and are often complicated by infections. We have studied the problem in infant cebus monkeys (*Cebus albifrons*). This appears to be a particularly useful animal model since the protein concentration required in the diet for maximal growth is low and approximates that of the human infant, about 6–7% of the total calories (6). The experiment was designed to monitor the effects of energy and protein restriction over a relatively long period and to compare the effects of energy restriction at various levels of protein intake.

MATERIALS AND METHODS

Infant cebus monkeys from the laboratory breeding colony were removed from their mothers within hours after birth and reared in a primate nursery (7). For the first 8 weeks of life the infants were fed either a commercially prepared human infant formula (Similac, Ross Laboratories) or a laboratory-prepared diet supplying 13% of the calories as casein protein. At 8 weeks of age the animals weighed approximately 500 g and were assigned to one of the following dietary regimens for a 20-week experimental period: a control diet (control), to which the animals had free access, supplying 13% of the calories as lactalbumin protein, which is approximately double the concentration required by these animals for the attainment of their

Abbreviations: CAL, calorie-restricted diet; PROT, protein-restricted diet; PC-1, protein- and calorie-restricted diet (6.5% of calories as protein); PC-2, protein- and calorie-restricted diet (4.5% calories as protein).

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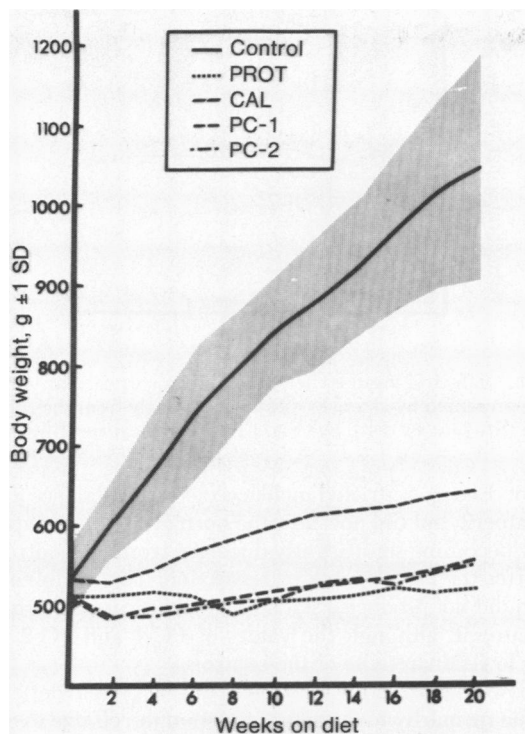


FIG. 1. Change in body weight. Shaded area represents ± 1 SD for control animals.

maximum growth rate (6); a protein-restricted diet (PROT), to which the animals also had free access, providing lactalbumin protein as 2.8% of the calories, a level that does not permit gain in weight (6); a calorie-restricted diet (CAL) of the same composition as the control diet but fed at only 67% (90 kcal/day) of the normal intake of animals of the same weight; a protein- and calorie-restricted diet (PC-1) supplying lactalbumin as 6.5% of the calories and fed at only 67% of the normal intake, providing sufficient protein to meet previously defined requirements for maximum growth rate when caloric intake was insufficient; and a second protein- and calorie-restricted diet (PC-2) supplying 4.5% of the calories as protein and fed at 67% of the normal intake, providing approximately the same daily protein intake (1.0 g/day) as the average ingested by the protein-restricted animals but with a concomitant energy restriction of the same degree as the two other energy-restricted groups. These experimental diets provided 21% of the calories as fat (corn oil), 28% as sucrose, and 50.5% from protein plus dextrin. The dry ingredients contained 4.7% salt mixture (8), 0.5% vitamin mixture (6), 0.1% inositol, 0.28% choline chloride, 4.6% cellulose, and appropriate amounts of solubilizers, stabilizers, and flavoring. These diets were made with water to a concentration of 1 kcal/ml.

Eight animals were assigned to each dietary group, except for PC-2, which contained only four animals. Three monkeys in the protein-restricted group (PROT) that died were replaced by additional animals. For animals that had free access to food, liquid diets were available continuously from 9:00 a.m. to 11:30 p.m., administered in three bottles during the day and three during the evening, each containing 100 ml. The daily food allotment for the calorie-restricted animals was divided into six portions; the animals were fed at approximately 3-hr intervals throughout the day and evening. Daily food intake and biweekly body weights were recorded throughout the experiment.

At 2-week intervals, 1 ml of whole blood with EDTA as an

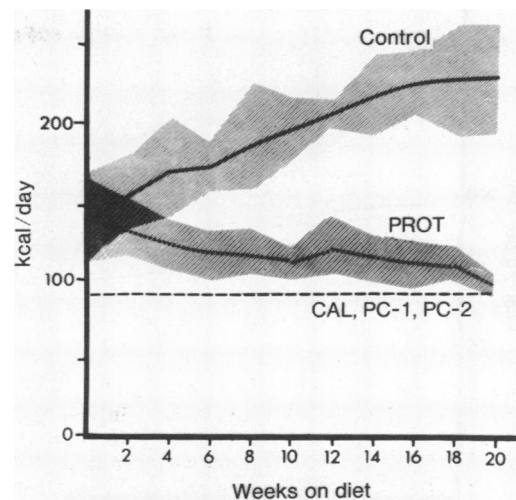


FIG. 2. Total daily energy intake for the various groups of animals. Shaded areas represent ± 1 SD for animals that had free access to food.

anticoagulant was drawn by femoral venipuncture from conscious animals. Packed cell volume was determined by the microhematocrit technique, hemoglobin by the cyanomethemoglobin method, and red and white cell counts by a Coulter counter. Plasma was frozen for subsequent analysis of total protein by the biuret method, albumin and globulin by polyacetate electrophoresis, and plasma cholesterol by the method of Carpenter *et al.* (9). Aliquots were also frozen for subsequent deproteinization with sulfosalicylic acid and analysis for free amino acids by ion exchange chromatography on a Technicon TSM amino acid analyzer.

Postmortem examinations were performed on animals that died during experiment. Organs were removed and weighed, and samples of some tissues were taken for compositional analysis. The small intestine was removed, rinsed free of waste with water, and blotted to remove excess moisture; a section was removed from the center for analysis. Organs and carcass were dried to a constant weight for water determination, ground to a fine powder, and analyzed for protein (nitrogen $\times 6.25$) by the micro-Kjeldahl method, for fat by ether extraction, and for ash by muffle furnace combustion.

RESULTS

Growth, Energy Intake, and Body Composition. Earlier papers have reported the weight gain and skeletal elongation of the control, protein-restricted, and calorie-restricted animals (10), and the effects of these dietary treatments on skeletal maturation (11) and behavioral development (12). The average weight gain for the five groups of animals is shown in Fig. 1. The dietary restrictions were sufficient to severely limit weight gain. Protein-restricted animals that had free access to food gained no weight over the entire 20-week period. Calorie-restricted animals, after an initial 2-week period of no gain, gained slowly for the remainder of the experiment. Protein- and calorie-restricted animals lost weight during the initial 2-week period, but gained very slowly thereafter. All restricted animals were approximately half the body weight of the well-fed control animals at the completion of the restriction period.

The total energy intake of protein-restricted monkeys fell gradually as the experiment progressed (Fig. 2). Calculated on a kcal/kg basis, their intakes were not significantly different from growing control monkeys, but were substantially higher than those of calorie-restricted animals of approximately the

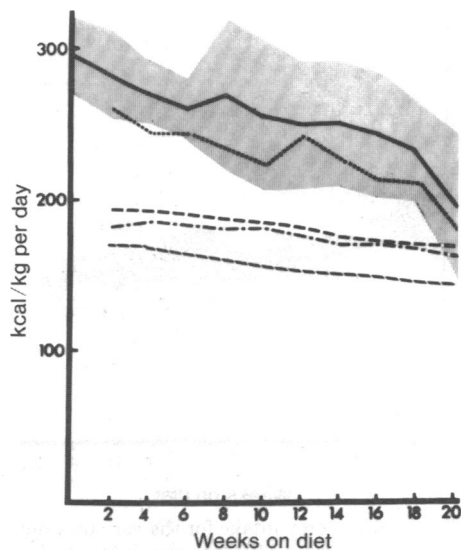


FIG. 3. Total daily energy intake per unit body weight for the various groups of animals. Shaded areas represent ± 1 SD for animals that had free access to food. See Fig. 1 for identification of lines.

same body weight, which were gaining at a very slow rate (Fig. 3). The average cumulative energy intake for the control, protein-restricted, and energy-restricted groups were 27,034, 18,409, and 12,600 kcal, respectively, over the 20-week period.

Body composition data are available from three protein-deficient animals, two of which died during the tenth and eleventh week of restriction and the third during the final week (Table 1). The table also includes the average body composition of eight well-fed animals, ranging in body weight from 400 to 1000 g, that died for various reasons in other studies and that presumably qualify as reasonable controls for body composition comparisons. At autopsy some excess accumulation of intraperitoneal fat was apparent in the protein-restricted animals, particularly in the mesentery proper and the greater omentum. Fatty accumulation in the gut and liver was also apparent, and analysis of these organs revealed a 2- to 3-fold increase in fat content compared to control animals.

Hematological Development. The normal developmental change in hematocrit is a drop during the first few weeks of life followed by a steady rise from approximately 42.5% to 50% during the 8- to 28-week period covered by this experiment (13). Protein-deficient animals showed a steady drop in hematocrit for the first 8 weeks of restriction and were significantly different from the control group at this time. Only a small rise occurred after 8 weeks, and the average hematocrit remained significantly depressed for the remainder of the ex-

Table 1. Carcass composition of protein-deficient animals that died during the experiment

Animal	Age at death, wk	Weight, g	Organ-free carcass composition, %			
			Water	Fat	Protein	Ash
Control (n = 8)		591 (241)*	65.3 (3.6)	8.5 (2.5)	20.3 (1.1)	4.7 (0.5)
CB-42	18	510	58.3	20.0	15.6	4.2
CB-32	18	525	62.9	11.6	19.6	5.1
CB-271	28	453	60.2	15.3	18.3	5.2

* Values in parentheses represent ± 1 SD.

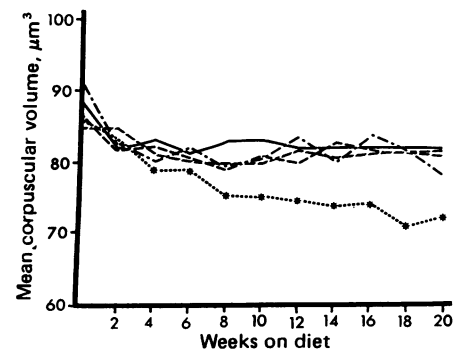


FIG. 4. Effect of various diets upon mean corpuscular volume. Means represented by asterisks differ significantly from the control, $P < 0.05$ (Student's *t* test). See Fig. 1 for identification of lines.

periment. Energy-restricted monkeys did not experience a drop in hematocrit, but did not show the normal rise in hematocrit, thereby becoming significantly different from the control animals after the eighth week of restriction. The combined restriction did not produce a statistically significant abnormality in hematocrit, although the values of PC-1 and PC-2 were slightly lower than those of the controls.

The lower hematocrit values found during protein deficiency were due primarily to a steady reduction in cell size over the duration of the experiment; the number of cells was not significantly lower than control values (Fig. 4). Calorie-restricted animals, on the other hand, had cells of normal size but the increase in cell number that normally occurs at this age was somewhat retarded. Animals subjected to the combined restrictions showed no abnormality in either cell size or cell number. Hemoglobin levels, like hematocrit, followed the changes in cell number and size.

Plasma Proteins. In the control animals plasma albumin was relatively constant, with a value of 3.5 g/dl, and globulin concentration rose from approximately 2.0 to 2.8 g/dl during the period covered by the experiment (13). Total plasma protein rose from approximately 5.5 to 6.3 g/dl (Fig. 5). Protein-deficient monkeys experienced a marked fall in total plasma protein during the first 4 weeks and protein concentration was significantly depressed during the first 8 weeks. Although levels remained quite low for the duration of the experiment, the large

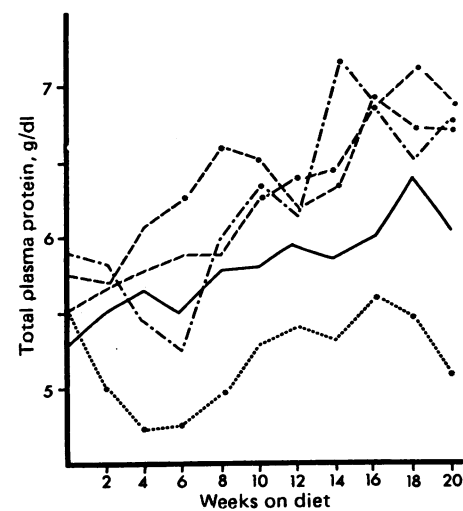


FIG. 5. Effect of various diets upon total plasma protein. Means represented by asterisks differ significantly from the control, $P < 0.05$ (Student's *t* test). See Fig. 1 for identification of lines.

Table 2. Total protein, albumin, and globulin concentrations (g/dl) in control and malnourished monkeys

	Total protein	Albumin	Globulin
Protein-restricted (n = 9)	5.12 ± 0.64* (76)	2.65 ± 0.50* (54)	2.54 ± 0.48* (54)
Calorie-restricted (n = 8)	6.27 ± 0.80* (78)	3.61 ± 0.52 (44)	2.77 ± 0.73* (44)
PC-1 (n = 8)	6.39 ± 0.68* (75)	3.68 ± 0.45* (42)	2.81 ± 0.46* (42)
PC-2 (n = 4)	6.19 ± 0.62* (38)	3.41 ± 0.33 (37)	2.76 ± 0.37* (37)
Control† (n = 39)	5.61 ± 0.43 (95)	3.45 ± 0.35 (48)	2.33 ± 0.29 (48)

Data for each dietary group have been pooled over the entire 20-week experimental period. Values are expressed as mean ± SD; numbers in parentheses represent number of observations.

* Significantly different from control ($P < 0.01$, Student's t test).

† Data taken from Samonds *et al.* (13).

interanimal variability in both the control and protein-deficient groups made these differences insignificant. Calorie-restricted animals exhibited an elevation of total protein concentration, as did both protein- and calorie-restricted groups after an initial reduction in the PC-2 group.

Pooled data on plasma albumin and globulin levels for the dietary groups over the period of the experiment are shown in Table 2. The reduction in total protein during protein deficiency resulted from a considerable reduction in the albumin concentration, with a modest increase in globulin concentration. No change in albumin concentration occurred during energy restriction, and the increase in total protein was due to an elevation of globulin concentration. Both albumin and globulin were significantly increased in group PC-1, whereas the more severe combined restriction (PC-2) resulted in no change in albumin but an elevation of globulin concentration.

Edema developed in two of the protein-restricted animals (PROT). In both instances transitory puffiness under the eyes and marked swelling in the submandibular region was evident in the morning. A small increase in the protein content of the animal's diet for a few days eliminated the edema. The animals in which edema developed did not show albumin levels lower than those of other animals in this group in the sample obtained prior to the onset of edema.

Plasma Amino Acid Levels. Amino acid levels in the plasma were determined somewhat irregularly during the study, and no specific time trends in the groups could be distinguished. The values obtained are not presented in order to conserve space. The levels of essential amino acids, with the exception of methionine, were significantly depressed in the group with free access to the low-protein diet. Group PC-2, which was severely restricted in both protein and energy, exhibited nearly normal values. Energy restriction alone resulted in depressed levels of histidine and phenylalanine but an elevated level of valine. The levels of the nonessential amino acids were almost uniformly depressed by energy restriction, with the exception of glutamic acid.

Plasma Cholesterol. The plasma cholesterol level was significantly elevated by protein restriction, whereas the plasma cholesterol level was significantly lowered by energy restriction. Cholesterol levels of the groups receiving the combined restriction were within normal levels. The elevation of plasma cholesterol by protein restriction appears to be a relatively

unique characteristic of this species and is not found in human beings.

DISCUSSION

Recent studies of protein needs of adult men have emphasized the interrelation and apparent dependence of protein needs upon the energy intake. In the studies of Calloway and Margen (14), Garza *et al.* (15), and Inoue *et al.* (16), adult subjects were fed the approximate amount of protein required for nitrogen balance while receiving an energy intake sufficient to maintain body weight. In all of these studies it was evident that a reduction in energy intake resulted in a negative nitrogen balance. The negative nitrogen balance could be overcome by the provision of more protein. The data appear to demonstrate that dietary protein is inefficiently utilized when energy intakes are restricted and support the common teaching that "protein is burned for energy" when energy intakes are inadequate.

We expected, therefore, that severe energy restriction in our animals—sufficient to nearly stop growth—would have a profound effect upon the protein needs. To our surprise, even when the protein intake was maintained at the bare maintenance level (group PC-2), which resulted in clear evidence of protein deficiency in animals with free access to food, a concomitant energy restriction not only failed to accentuate the development of protein deficiency, but appeared to have prevented the hypoproteinemia and anemia characteristic of protein deficiency. We suggest that the negative nitrogen balance that occurs in adult human subjects fed maintenance levels of protein with a modest energy restriction may represent a transitional phase in the adaptation to a lower energy level and probably cannot be extrapolated to chronic states of energy restriction.

The level of protein selected for these studies was just sufficient for the maintenance of body weight when the animals had free access to food. In these animals, the energy intake per unit weight was maintained in the normal range and much exceeded the energy requirement for maintenance of weight when energy was the limiting nutrient. These animals deposited somewhat more body fat, but the accumulated fat amounted to 200–600 more total calories in the body than in normal animals of the same size and in no way accounts for the excess energy consumed. We conclude that the animals fed the low protein diet are wasteful of energy compared to the normal animals and, particularly, when compared to the animals restricted in energy alone. Changes in physical activity might account for some of these differences in energy intake, but careful observation of the animals by a behavioral psychologist indicates this is not a major factor (12). The data point to substantial changes in the efficiency with which energy is utilized, as have other recent studies in human subjects (17–19). The inefficient use of energy during protein deficiency supports the observation of Miller and Payne (20) in swine.

The determination of plasma amino acid levels was less informative in these studies than we had hoped. In protein deficiency, the levels of essential amino acids tended to fall, while concentrations of nonessential amino acids were not markedly altered. The resulting increase in the ratio of nonessential to essential amino acids is similar to the results in children with kwashiorkor (21, 22). In group PC-2, the most severely restricted in both energy and protein, the amino acid levels of few animals could be shown to be significantly different from those in the normally fed animals. Those significant differences that did occur in the various groups suggest complex interactions, somewhat specific for the various amino acids, which do not appear to be interpretable.

The metabolic characteristics of kwashiorkor and marasmus are usually interpreted as the difference between protein deficiency and calorie deficiency. Gopalan's group (1, 23), however, suggest that the way different children respond to malnutrition may reflect differences in the ability of children to adapt to the stress of malnutrition. Thus, some of the metabolic differences may be causally related to the different syndromes rather than a result of the diet. Our data can be interpreted as supporting this concept and suggest that excessive energy intake may contribute to rather than protect against the development of kwashiorkor.

Much of the current discussion about the world food problem (24) focuses attention on low food or energy intakes with the assumption that this contributes to the development of protein deficiency. Our data suggest that this is not true as long as energy intakes are sufficient to maintain body weight as they generally are. Furthermore, the provision of energy alone, as fat, sugar, or starch, may be dangerous. This will dilute the protein content of the diet and may prevent whatever adaptive mechanisms the body has for coping with diets relatively low in protein.

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