Effect of the Level of Dietary Protein Intake on Fat Absorption in Children

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Summary

The quantity and quality of protein in the diet affect both nitrogen balance and energy utilization in man. One possible mechanism is by a direct effect on the process of fat digestion and absorption. The effect on fat absorption of feeding diets providing 0%, 6.4%, or 12.5% of energy as protein was assessed in nine children. Liquid diets were prepared by high speed blending of calcium caseinate, a soy-cottonseed oil blend (80:20), sucrose, water, vitamins, and a mineral mixture and fed by bottle five times per day. Diets varied in the amount of protein and were fed to maintain constant the intakes of fat and carbohydrate. All children began by consuming the diet providing 6.4% of energy as protein for a period of 6 days: 3 days of adaptation, 3 days of metabolic collection of urine and stool. Successive 3-day dietary periods and metabolic collections followed in one of two sequences: 12.5%, 0%, 6.4% energy as protein in five children; 0%, 12.5%, 6.4% energy as protein in four. Mean fat excretion during the two 6.4% periods did not differ significantly (initial $14.5 \pm 6\%$ of intake; final, $11.7 \pm 5.0\%$ of intake). Fat excretion was significantly greater during the period when no protein was consumed ($23.9 \pm 7.7\%$ of intake) than during either the period in which protein provided 6.4% of energy ($P < 0.01$) or during that in which protein provided 12.5% of energy ($10.0 \pm 2.6\%$ of intake, $P < 0.001$). The difference between the 6.4% and 12.5% periods did not attain significance ($P = 0.10$). Fecal fat expressed as a percentage of fecal dry weight was significantly greater during periods when the diet contained no protein than during periods in which protein provided either 6.4% ($P < 0.05$) or 12.5% ($P < 0.01$) of energy.

Additional studies were carried out in a similar fashion in six children consuming diets in which protein provided 5%, 9.6%, and 15% of energy. All six possible dietary sequences were used. At these intakes fat excretion did not vary significantly with protein intake.

Speculation

The low protein intake of children in much of the developing world may be detrimental to maximal fat absorption and consequently may increase their energy requirements.

The quantity and quality of protein in the diet affect both nitrogen balance and energy utilization in man. Consumption of inadequate amounts of protein may result in decreased nitrogen retention and poor weight gain despite sufficient energy intake (5). Under such conditions, energy output may be regulated by increasing the amount of energy dissipated from the organism as heat (10). A second possible mechanism by which protein intake may affect energy balance is by a direct effect on the process of fat digestion and/or absorption.

In studies designed to assess the effect on nitrogen retention of consuming a diet in which all of the day's protein was fed to children in a single meal, with energy distributed evenly in five

feedings throughout the day, we noted a marked increase in fecal fat excretion relative to control periods of even protein/energy distribution (7). We speculated at that time that the presence of protein in the diet (and in the digestive tract) might facilitate the digestion or absorption of dietary fat.

Recently reported studies *in vitro* and in bile-deficient rats support the role of protein in fat absorption and suggest that this may in part be the result of protein stabilization of the fat emulsion, thereby enhancing lipolysis (8).

The studies reported here were designed to assess further the importance of dietary protein for normal fat absorption in the human. Using the balance technique the effect of no protein and of two levels of intake of high quality dietary protein (6.4% or 12.5% of energy provided by protein) were studied in nine children. Subsequently six additional children were studied using diets providing 5%, 9.6%, or 15% of energy as protein.

MATERIALS AND METHODS

STUDIES IN WHICH PROTEIN PROVIDED 0%, 6.4%, OR 12.5% OF ENERGY

Subjects. Nine children in the Instituto de Investigacion Nutricional, Lima, Peru, participated in the study (15). Five of these were 4-6-month-old infants who had resided in the unit since the age of 3-5 days; they had not been previously malnourished and were in good health at the time of study. Four children, previously malnourished, who were remaining for long term convalescence also took part. Their ages ranged from 11-18 months; they were well advanced in their convalescence and were maintaining a constant rate of weight gain.

Diets. A diet which provided 6.4% of energy as protein ($N \times 6.25$) and 50% as fat was designed using calcium caseinate, a blend (80:20) of soy-cottonseed oil, sucrose, water, a mineral mixture, and vitamins. The composition of this diet per 100 Kcal is shown in Table 1. Two additional diets (not shown in Table 1) were prepared, one devoid of protein (protein-free) and one in which the amount of protein was doubled (12.5% of energy as protein). Except for the removal or addition of protein, these diets were identical to the diet providing 6.4% of energy as protein in their major constituents. All three diets were prepared daily in liquid form by high speed blending and fed by bottle in five feedings (0800, 1200, 1500, 1800, 2200).

Protocol. The energy intake needed to maintain adequate weight gain in each child was determined prior to starting the study. In eight of the children consumption of 125 kcal and 2 g protein/kg body weight/day was sufficient to sustain a rate of weight gain of 3-5 g/kg/day. In the remaining child, 150 kcal and 2.5 g protein/kg/day were required.

All children began by consuming the diet providing 6.4% of energy as protein at the appropriate level for a period of 6 days. The initial 3 days served as a period of adaptation. During the second 3 days the child was placed in a metabolic bed; urine and stool were collected. Successive 3-day dietary periods and metabolic collections then followed in one of two sequences: 12.5%

Table 1. Composition per 100 kcal of diet providing 6.4% of energy as protein¹

	Energy, kcal	Protein, g	Fat, g	CHO, g	Na, mEq	K, mEq	Ca, mg	P, mg
Ca-caseinate, ² 1.875 g	6.938	1.65	0.038		0.072	0.011	32.96	13.62
Oil, ³ 6.22 ml	49.26		5.47		0.012	0.008		
Sucrose, 10.95 g	43.8			10.95	0.002	0.001		
Mineral mixture, ⁴ 0.68 g					2.04	3.03	60.62	46.99
Total	99.99	1.65	5.508	10.95	2.126	3.050	93.58	60.61

¹ Diet providing 0% energy as protein was prepared in the same way except that Ca-caseinate was not added. Diet providing 12.5% protein energy differed only in that 3.750 g Ca-caseinate were used. Polyvisol, Mead Johnson, Evansville, Ind., 0.6–1.2 ml depending on the child's age, was added to all diets. One milliliter of Polyvisol contains the following: vitamin A, 1500 IU; vitamin D, 400 IU; vitamin E, 5 IU; ascorbic acid, 35 mg; thiamin, 0.5 mg; riboflavin, 0.6 mg; niacin, 8 mg; vitamin B₆, 0.4 mg; vitamin B₁₂, 2 µg. Folic acid, 250 µg/day was also added to all diets.

² Cascec, Mead Johnson Company, Evansville, Ind.

³ Soy-cottonseed blend (80:20).

⁴ Composition of mineral mixture: NaCl, 17.300%; MgSO₄, 12.850%; CaHPO₄, 23.450%; KHCO₃, 41.000%; KCl, 2.650%; ferric citrate, 2.000%; CuSO₄·5H₂O, 0.352%; ZnSO₄·7H₂O, 0.200%; MnSO₄·H₂O, 0.156%; KI, 0.014%; NaF, 0.028%. The mineral mixture was prepared and assayed by Pfizer Pharmaceuticals, Pfizer Inc., Groton, Conn.

Table 2. Actual intakes of energy, protein, fat, and carbohydrate from the 0%, 6.4%, and 12.5% protein energy diets as consumed by eight children who required 125 kcal and 2 g protein/kg/day¹

Diet	Energy, kcal/kg/day	Protein, g/kg/day	Fat, g/kg/day	CHO, g/kg/day
0% Protein energy	117	0.0	6.89	10.95
6.4% Protein energy	125	2.0	6.89	10.95
12.5% Protein energy	133	4.0	6.89	10.95

¹ Intakes of the ninth child were 20% higher.

of energy as protein, protein-free, 6.4% of energy as protein in five children; protein-free, 12.5% of energy as protein, 6.4% of energy as protein in four children. Total daily intakes of fat, carbohydrate, minerals, and vitamins were kept constant for the entire study. The addition or removal of protein altered total nitrogen and energy intakes. The resulting intakes are shown in Table 2. Diets were analyzed weekly during the studies for N, Na, K, and Cl to verify that actual intakes were as calculated.

Balance studies were carried out by procedures standard for our unit (6). Stool and urine were collected and analyzed separately in the eight boys and together in the only girl in the study. Daily fecal weights, wet and dry, were measured. Fecal nitrogen was measured in 3-day pooled collections by the micro-Kjeldahl method, fecal fat by the method of van de Kamer *et al.* (13).

Statistical analysis of the data was carried out by a two-way analysis of variance with orthogonal comparisons and using a *t*-test for paired comparisons (12).

STUDIES IN WHICH PROTEIN PROVIDED 5%, 9.6%, OR 15% OF ENERGY

Subjects. Six additional children, 5.5–12 months of age, were studied with diets which provided more usual amounts of protein. Two of the infants were normal; four had been previously malnourished. All were in good health at the time of study and gaining weight steadily.

Diets. As in the first studies, diets were prepared in liquid form by high speed blending of calcium caseinate, soy-cottonseed oil blend, sucrose, water, vitamins, and a mineral mixture. Energy supplied by protein was varied at three levels: 5%, 9.6%, and 15%. The rationale for choosing these levels is discussed below.

Protocol. An intake of 150 kcal and 2.5 g protein was determined to sustain an adequate rate of weight gain in all six children prior to starting the study. All six possible sequences of the three diets were used. Each child began by consuming the initial diet in his sequence for a period of 6 days. The initial days provided a period of adaptation from the previous diet. During the second 3 days, the child was placed in a metabolic bed and

Fig. 1. Fecal fat excretions of children consuming diets in which protein provided 0%, 6.4%, or 12.5% of energy. The numbers above each group of bars are the patient identification numbers.

collections of urine and stool were made. Two additional 3-day dietary periods and metabolic collections followed in succession. Fat intake remained constant for all three dietary periods. As consumed the diets provided the following intakes: the diet in which protein provided 5% of energy provided 1.87 g protein, 7.83 g fat, and 140 kcal/kg/day. That in which protein provided 9.6% of energy supplied 3.60 protein, 7.83 g fat, and 150 kcal, and that providing 15% of energy as protein supplied 6.0 g

protein, 7.83 g fat, and 160 kcal—all per kg body weight per day.

Biochemical and statistical analyses were the same as outlined above.

RESULTS

STUDIES IN WHICH PROTEIN PROVIDED 0%, 6.4%, AND 12.5% OF ENERGY

All children completed the protocol without incident. Consumption of the diets was complete in every instance. Because initial analysis of the data showed no difference in response of normal and recovered malnourished infants to the different diets, the data were combined for further analysis. Fecal fat excretions expressed as a percentage of intake for all nine children are shown in Figure 1. Mean values for fat excretion from the nine children are represented in Figure 2.

Five children had their lowest fecal fat excretion in association with highest protein intake. This effect was more striking in the four children in whom the protein-free period preceded the period in which protein provided 12.5% of energy. Seven children had their highest fecal fat excretion when no protein was consumed.

Mean fat excretion during the 6.4% periods did not differ significantly (initial, $14.5 \pm 6.0\%$; final $11.7 \pm 5.0\%$). Fat excretion was significantly greater during the protein-free periods ($23.9 \pm 7.7\%$) than during either of the periods with 6.4% of energy as protein ($P < 0.01$) or during the period in which protein supplied 12.5% of energy ($10.0 \pm 2.6\%$) ($P < 0.001$). The difference between the 6.4% and 12.5% periods did not attain significance ($P = 0.10$).

Fecal wet weight (Fig. 3) was significantly greater during the protein-free periods than during the 12.5% periods. Fecal dry weights tended to be higher when no protein was consumed but the difference was not significant. When fecal fat was expressed as a percentage of fecal dry weight this value was significantly greater during consumption of a protein-free diet than during

Fig. 2. Mean (+ SEM) values for fat excretion (percentage of intake) in nine children consuming diets in which protein provided 0%, 6.4%, or 12.5% of energy.

Fig. 3. Fecal weight, wet and dry, and fecal fat during consecutive 3-day dietary periods in which protein provided 0%, 6.4%, or 12.5% of energy ($n = 9$).

consumption of the diet in which protein provided either 6.4% ($P < 0.05$) or 12.5% ($P < 0.01$) of energy.

STUDIES IN WHICH PROTEIN PROVIDED 5%, 9.6%, OR 15% OF ENERGY

All studies were uneventful. An additional 3-day collection was made in *patient 493* duplicating the diet of the initial 3-day period. Fecal fat excretions (Fig. 4) for *patient 492* and *patient 494* were normal on all three diets. *Patient 483* had elevated fecal fat excretion on the diets providing 9.6% and 5% of energy as protein. This decreased markedly when the diet provided 15% of energy as protein. *Patient 491* and *patient 493* tended to excrete less fat with higher protein intake but the protein intake at which the changeover occurred was different for the two. *Patient 488* manifested a marked decrement of fecal fat on changing from intermediate to the high level of protein intake. No rebound in fat excretion occurred during the 3 days in which the diet providing 5% of energy as protein was consumed by this child.

The differences in fecal fat excretion among the three diets was not found to be statistically significant.

DISCUSSION

These studies suggest that dietary protein facilitates the digestion and/or absorption of fat. This was previously found to be so with diets containing similar amounts of nitrogen and energy in which the distribution of protein among five meals each day was altered relative to fat; fecal fat excretion during periods when protein and fat were consumed in parallel ($9.5 \pm 4.1\%$ of intake) nearly doubled during periods of nonparallel distribution ($18.3 \pm 7.7\%$ of intake) ($n = 12$, $t = 4.826$, $P < 0.001$) (7). The effect of protein on fat absorption is also apparent in the

Fig. 4. Fecal fat excretion expressed as a percentage of fat intake in six children consuming diets in which protein provided 5%, 9.6%, or 15% of energy. The numbers above each group of bars are the patient identification numbers.

current studies, in which fat and protein in all diets were fed in parallel but the level of protein intake was varied among the diets.

The studies with 0%, 6.4%, and 12.5% of energy as protein showed a progressive improvement of mean apparent fat absorption as dietary protein intake increased. The difference between the latter two levels was not statistically significant, probably due to the small *n*.

The levels of protein intake for the second studies were chosen as being more representative of diets which are actually consumed: the diet providing 5% of energy as protein approximates the current FAO/WHO recommendation for children of this age throughout the world (2), 9.6% of energy as protein is similar to the intakes of most developing countries, and 15% of energy as protein is in the range of intakes in more developed countries. Because the lowest level of protein in these studies approximated the middle level of intake in the previous studies, it is not surprising that the effect was more variable and less dramatic among the children studied in this more narrow range of intakes. As protein intake increases one would expect to reach a level beyond which apparent fat absorption would not continue to improve. Exactly where this level is reached may vary from individual to individual.

Similarly, the effect of increasing dietary protein intake was most pronounced in those children whose fat absorption was initially poorest. This was so in both sets of studies. Patients 460, 475, 467, and 483, for example, all had abnormally high fecal fat excretions which decreased markedly when dietary protein intake was maximal. In contrast, children whose fat excretion was initially low with lower protein intake (patients 469, 492, and 494, for example) did not have as marked a decrement in fecal fat when protein intake increased. With lower initial fat excretion the effect of any factor favoring improved fat absorption would tend to be minimized.

Although fecal dry weight did not vary significantly among diets, it tended to be higher with no protein in the diet. Fecal fat as a percentage of dry weight was significantly elevated in association with the protein-free diet.

Previous workers have suggested that steatorrhea may be

more accurately defined by expressing fecal fat as a percentage of fecal dry weight rather than as a percentage of fat intake (11). Our data agree. However, the previous suggestion that a fecal fat/fecal dry weight ratio of 0.2 be considered the upper limit of normal fat excretion for children consuming their habitual diet does not appear to be valid for those consuming a more digestible, low residue diet such as was used in the present studies.

Protein in the diet may affect fat absorption by one or all of several mechanisms. Proteins have been shown to stabilize fat emulsions *in vitro*, and effect which varied directly with protein concentration (8). When these emulsions were fed to bile-deficient rats, they were well hydrolyzed and absorbed (8).

The effect of dietary protein intake on pancreatic exocrine function may also play a role. In studies with five alcoholic patients at two levels of protein intake ("low" = 25 g protein, 5.6% of energy intake; "normal" = 100 g protein g protein, 15.4% of energy intake), pancreatic lipase output after pancreozymin secretion stimulation was significantly greater after consumption of the normal protein diet for 10 days than after periods of low protein intake (9). Two of the five patients initially manifested steatorrhea which disappeared with normal protein intake and did not recur when a low protein diet was reinstituted. This is similar to the course of patient 488 (Fig. 4), whose fat excretion decreased when protein intake increased from 9.6% to 15% of energy and did not deteriorate for the 3 days of low protein intake, 5% of energy. It has been suggested, however, that lipase activity must decrease to 10% of normal for fat malabsorption to occur on this basis (1); we have no direct measurement of lipase activity in our children.

Release of pancreatic lipase and liver bile, both critical elements in the luminal phase of fat digestion and absorption, is affected by pancreozymin-cholecystokinin (CCK-PZ). Although duodenal perfusion of fatty acids stimulates an increment in CCK-PZ secretion, a significantly greater and maximal response occurs with duodenal perfusion of amino acids in physiologic concentrations (4). In the absence of sufficient protein in the diet or in a single meal, CCK-PZ release could be expected to be submaximal, thereby affecting the availability of pancreatic lipases and bile.

Whether intracellular events of fat absorption are involved is speculative. Formation and release of chylomicrons from intestinal epithelial cells are dependent on protein synthesis (3). Changes in dietary protein level in our studies could have affected intestinal epithelial cell nutrition and, thereby, clearance of fat from the mucosa.

How critical a role dietary protein or its lack plays in fat absorption in most children is difficult to assess from these studies. In much of the developing world diets are low in protein. This appears to be detrimental to maximal fat absorption and conceivably helps explain the poor fat absorption documented in a large segment of so-called normal children in these countries.

CONCLUSION

The effect of the level of high quality dietary protein on fat absorption was studied in children. Fat excretion while on a protein-free diet was significantly greater than when dietary protein provided either 6.4% or 12.5% of energy intake (*n* = 9). Differences in fat excretion at the latter two levels of intake were suggestive but not statistically significant. In further studies (*n* = 6) using a more narrow range of fat intakes (protein providing 5%, 9.6%, and 15% of energy), no significant difference in fat excretion was found.

Dietary protein facilitates the digestion and/or absorption of fat. The effect appears to become maximal with diets providing between 6.4% and 15% of energy as protein, with marked variability among children in the level at which this occurs.

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