# The Effect of Diet Protein on Late Burn Mortality (34996)

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Ever since Davidson and Cuthbertson first showed an increased urinary nitrogen excretion in patients with burns (1) and fractures (2, 3), respectively, the increased catabolism of protein observed after many kinds of injury is considered by many to be an undesirable effect of trauma. For this reason therapy of injured patients has been aimed at replenishing the lost protein by administering diets high in protein and/or calories (3, 5–9), since it has been shown that high-protein feedings reduced urinary nitrogen excretion (3–6). To date, however, there has been no study designed to test the importance of protein in the diet on mortality after injury.

In this report, mice were fed diets, *ad libitum*, containing various amounts of casein protein supplemented with carbohydrate and fat to make the caloric contents equivalent. After severe thermal injury, the effects of the diets were noted on late mortality, food intake, and weight change.

*Materials and Methods. Animals.* Conventional, Swiss–Webster, albino, female mice (NIH stock) were used in all experiments.

*Diets.* The standard 20% casein diet contained the following ingredients in 1 kg: 200 g vitamin-free casein (Labco), 40 g salt mix W (Wesson), 50 g fat (Crisco), 710 g corn starch (Argo), 0.4 ml Natola (Parke Davis Co., containing 55,000 USP units vitamin A and 11,000 USP units vitamin D per gram), 50 mg $\alpha$ tocopherol acetate (NBC), and 6.25 ml vitamin mixture.[1]

The 6% casein diet differed only in the quantity of casein (60 g) and corn starch (850 g); the 40% casein diet in the amount of casein (400 g) and corn starch (510 g). Since methionine was reported to be beneficial in experimental burn shock (10) and wound healing (11), methionine was added to the 6% casein diet, which was deficient in methionine compared to the adequate protein diet (2.04 g/kg versus 6.8 g/kg). The following quantities of D, L methionine (Eastman Organic Chemicals) were added to the 6% casein diet: 0.5 g (0.05%), 2 g (0.2%), or 4.8 g (0.48%) per kg of diet. In several food intake experiments, 2 g methionine (0.2%) per kg were added to the 6, 20, and 40% casein diets.

All these diets have an approximate caloric value of 4000 Cal/kg.

*Diet regimens.* Three regimens were used: (a) 18- to 20-g mice reared on standard pellets (Purina Lab Chow) were started on the diets 1 day prior to thermal injury and continued on the same diet afterward;[2] (b) weanling mice were placed on the diets for 17–19 days prior to thermal trauma and continued on the same diet for 21 days postburn; (c) 18- to 20-g mice reared on the standard pellets were placed on partially restricted amounts of the diets for 3 weeks prior to burning and continued on the same diet *ad libitum* after burning.

*Thermal trauma.* Mice were given a 2/3 body surface burn by immersing them to the axilla in water at 70° for 8 sec. After removal from water, the mice were dabbed dry, and from 2–8 animals were placed in shoe box-type cages. The mice were injected subcutaneously with 3 ml 0.85% NaCl 5–10 min after burning, because the 21-day mortality

[1] Contained 10 mg menadione, 60 mg thiamine HCl, 20 mg pyridoxine HCl, 150 mg calcium pentothenate, 100 mg niacinamide, 1 g ascorbic acid, 1 mg biotin, 0.1 mg vitamin $B_{12}$, 1 g choline chloride, 50 mg para-aminobenzoic acid, 10 mg folic acid, and 30 mg riboflavin.

[2] If the diets were not started 1 day prior to burning, the animals did not eat the diet for several days postburn.

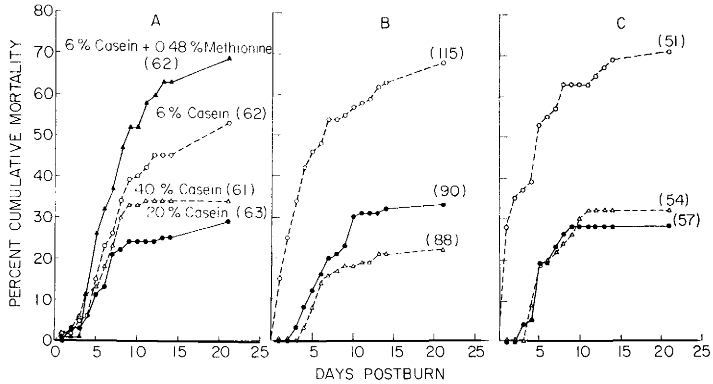


FIG. 1. The effect of varying amounts of protein in the diet on late mortality of burned mice. In A, mice were started on diets 1 day prior to thermal injury. In B, weanling mice were placed on diets for 17–19 days prior to thermal trauma; and in C, 18- to 20-g mice reared on pellets were placed on diets for 3 weeks prior to burning. All mice were given a ⅔ body surface area burn at 70° for 8 sec and received 3 ml 0.85% NaCl subcutaneously postburn. After burning, mice were placed on the same diet they had prior to injury. Numbers in parentheses represent the number of animals in each experiment.

of burned mice was approximately 85% if no fluid therapy was given. Water and diet were allowed *ad libitum*.

*Parameters studied.* Mortality was recorded daily for 21 days postburn. Diet intake in a separate group of animals was measured daily by the change of weight of a food container especially constructed to prevent spillage. Mice were weighed daily or at weekly intervals, depending upon the experiment.

*Statistical analysis.* The probability of 0.05 ($p = 0.05$) was chosen as the level of statistical significance. Computations using the chi-square method for mortality and the Student $t$ test for weights were made.

*Effect of protein in diet fed to mice after thermal trauma.* Figure 1A shows the cumulative mortality of burned mice fed 40, 20, and 6% casein diets 1 day prior to burning and during the 21-day postburn period. The group receiving the adequate-protein diet (20%) had a 21, 25, and 29% mortality at 7, 14, and 21 days, respectively. The group receiving the high-protein diet (40%) showed no significant decrease in 21-day mortality; in fact, the mortality was slightly higher (34% vs 29% at 21 days ($p > .5$) ) than those receiving an adequate-protein diet. On the other hand, the group receiving the low-protein diet (6%) showed a significantly higher mortality ($p < .01$) than the 20% casein group with mortalities of 26, 45, and 53% at 7, 14, and 21 days postburn.

When methionine was added to the 6% casein diet in 0.05, 0.2, or 0.48% concentrations, 21-day mortality increased above the basic 6% casein diet in all cases (53% vs 62, 67, and 69%, respectively). Since the 6% casein with 0.48% methionine diet contained an amount of methionine equivalent to that of the 20% casein diet, only that diet is illustrated in Fig. 1A. Mortality was 37, 63, and 69% at 7, 14, and 21 days postburn. The difference between this diet and the adequate-protein diet was, consequently, even more significant ($p < .001$) than that with the 6% casein diet alone.

The high late mortality of the 6% casein with 0.48% methionine group could be blamed on the toxicity of methionine by causing an imbalance in the relative proportion of amino acids in the diet. This explanation seems unlikely, since half that amount of methionine added to the 20 and 40% casein diets caused no significant change in late mortality between the groups with or without added methionine.

In Fig. 2A the weight changes of burned mice on these diets are illustrated. All groups showed a marked drop in weight 7 days postburn. The average weight continued to decline in the 6% casein group, whereas the

TABLE I. Food Intake on Diet Regimen.

| Experiment | Regimen | Diet group | No. of mice | 21-Day average total food intake (g) | Average weight at 21 days (g) |
|---|---|---|---|---|---|
| A | Diet started 1 day prior to burning | 6% casein | 15 | 43.4 | 11.6 |
| | | 6% casein + 0.48% methionine | 10 | 41.3 | 13.5 |
| | | 20% casein | 15 | 44.8 | 16.6 |
| | | 40% casein | 15 | 50.6 | 17.8 |
| B | Burned mice on diet since weaning[a] | 6% casein | 7 | 54.3 | 14.2 |
| | | 6% casein + 0.2% methionine | 8 | 58.5 | — |
| | | 20% casein + 0.2% methionine | 18 | 55.5 | 17.4 |
| | | 40% casein + 0.2% methionine | 16 | 52.5 | 17.1 |
| C | 18- to 20-g Burned mice started on diet 3 weeks prior to burn | 6% casein | 5 | 41.4 | 15.7 |
| | | 20% casein | 5 | 42.9 | 19.0 |
| | | 40% casein | 5 | 47.3 | 19.2 |
| D | Normal weanling mice prior to burning | 6% casein | 5 | 63.8 | 12.2 |
| | | 20% casein + 0.2% methionine | 5 | 63.8 | 18.0 |
| | | 40% casein + 0.2% methionine | 5 | 63.1 | 18.8 |

[a] Mice 18–20 g at time of thermal trauma.

weight of the other groups increased with time. The best recovery of weight occurred in the 40% casein diet group.

Table I, Expt. A, lists the average 21-day food intake and average weight at 21 days of mice in this experiment. In general, the average food intake of all groups was in the same range with no significant difference between them; yet, as previously described, there was a marked difference in weight in mice receiving the 6% casein diet.

*Effect of protein in diet on burn mortality of mice reared since weaning on varied protein diets.* Mice reared on varied quantities of protein in the diet for 17–19 days after weaning showed marked weight differences at the time of thermal trauma, even though food intake was remarkably alike in a separate, but similar experiment (see Table I, Expt. D). For example, 79% of the mice on the 6% casein diet weighed below 18 g, whereas only 6% of the 20% casein group and 2% of the 40% casein group weighed less than 18 g. Furthermore, none of the mice in the 6% casein group weighed more than 20 g, whereas 50% of the 20% casein group and 44% of the 40% casein group weighed over 20 g.

To avoid selection of animals, however, the mortality of all mice in each group, regardless of weight at the time of burning, is plotted in Fig. 1B. The group that received the low-protein diet throughout showed a markedly elevated mortality of 54, 63, and 68% at 7, 14, and 21 days postburn, compared with 20, 32, and 33% mortality at the same time intervals for mice on an adequate protein diet ($p < .001$). The mortality of the mice on the high-protein diet was slightly lower than that of the adequate-protein diet with 16, 21, and 22% at the same time intervals; however, the difference between these two groups was not significant ($0.1 > p > .05$).

If the 21-day mortalities of burned mice on different diets, but in the same 18- to 20-g weight range, are examined, the 6, 20, and 40% casein diets produced 58, 32, and 24% mortalities. Even though the mortality of the low-protein diet was much higher than the adequate-protein diet, the difference was not quite significant at the 5% level, due probably to the small number of animals in the 6% casein group (24 vs 38). In the mortality comparison of the 20 and 40% protein groups, the differences were not significant with sufficiently large numbers of animals for evaluation.

These results demonstrate that mice reared on a deficient-protein diet have a high late mortality. The question then arises: how deficient must the diet be in protein in order to get this effect? In another set of experiments not illustrated here, burned mice fed 10, 20, and 40% casein diets since weaning had 21-day mortalities of 25, 29, and 41%, respectively. Since there was no significant difference between any of these diets, the answer, therefore, lies somewhere between 6 and 10% casein protein.

Figure 2B shows the postburn weight changes of the mice burned in Fig. 1B. As before, all groups had a marked drop in weight at 7 days postburn and with a gradual recovery of weight in the 20 and 40% casein groups at 14 and 21 days. The 6% casein group maintained its weight at the low level during this period.

In a separate, but similar, experiment, the food intakes of the diet groups resembled each other closely (see Table I, Expt. B).

*Effect of diet on burn mortality of 18-g mice reared on pellets and fed varied protein diets for 3 weeks prior to burn.* In this experiment, 18-g mice reared on pellets were fed daily an average of 4–6 g of 6% casein protein, 3 g of 20% casein protein, or 2–3 g of 40% casein protein diet per mouse for 3 weeks prior to burning. This quantity of diet was used in order to maintain adequate nutrition without allowing a great gain in weight. Figure 2C shows the average weight changes of these mice at intervals prior to burning. At 14 days preburn there was usually a slight to marked drop in weight with a gradual increase above the initial weight at the time of burn injury. On the day of burn, the percentage of mice with weights below, at or above 18–20 g was fairly evenly distributed.

Again, in order to avoid selection of animals, the mortality of all mice in each diet group is plotted in Fig. 1C. The group that was fed the low-protein diet throughout showed a markedly increased mortality of 57, 69, and 71% at 7, 14, and 21 days postburn compared with a 23% mortality at 7 days and a 28% mortality at 14 and 21 days postburn ($p<.001$) in the adequate-protein diet group. The mortality of mice on the high-protein diet was slightly higher than that of the adequate-protein diet with a 24% mortality at 7 days and a 32% mortality at 14 and 21 days postburn; the difference, however, was not statistically significant ($p>.9$).

If the mortalities of diet groups are compared by equivalent weights, the 18- to 20-g mice fed the 6% casein diet had a significantly higher ($p<.05$) 21-day mortality than the 20% casein diet (71% vs 30%). There was no significant difference between the mortality of the 40 and 20% casein diets (27% vs 30%). In the over-20-g mice groups, again the 21-day mortality of the 6% casein group was significantly higher ($p<.05$) than the

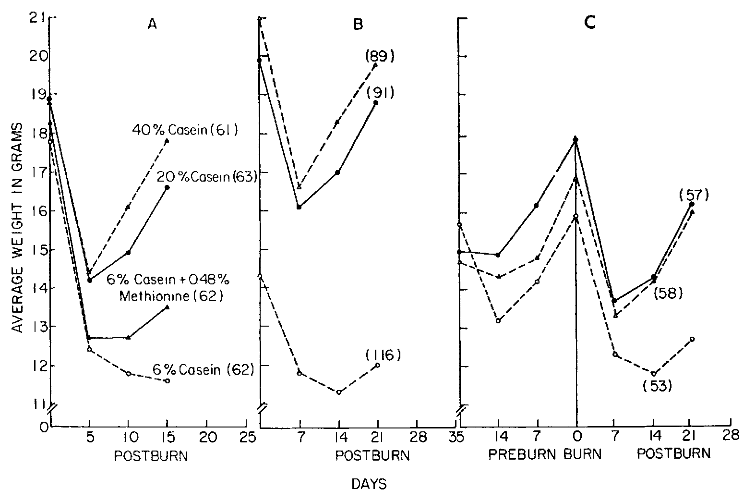


FIG. 2. The effect of varying amounts of protein in the diet on average weight of the mice prior to and/or after burning. A, B, and C as described in legend of Fig. 1.

20% casein group (60% vs 27%), whereas no such difference existed between the 40 and 20% casein groups.

The weight changes of this experimental group after the burn are also illustrated in Fig. 2C. As before, there is a marked drop in weight 7 days postburn with gradual recovery of the 20 and 40% casein groups and with a maintenance of a low average weight of the 6% casein group. These changes occurred in spite of no marked changes in the average food intake of all groups (see Table I, Expt. C).

*Discussion.* The results of these experiments clearly demonstrate that a low-protein diet fed *ad libitum* to mice prior to or after thermal injury significantly increased late mortality. The converse of this situation, however, does not appear to be true, since a high-protein diet did not significantly decrease burn mortality below that of mice fed an adequate protein diet. In fact, when mice were fed a high-protein diet after thermal trauma or for 3 weeks prior to burning, late mortality was slightly higher than the burned mice fed an adequate protein diet. Only when mice were fed since weaning on a high-protein diet was the mortality slightly, but not significantly, below the adequate-protein control group.

There may be some argument as to the interpretation of the results of mortality of mice fed low-protein diets since weaning, since mortality may have been influenced by the small size of a greater percentage of animals in the low-protein group. In rebuttal, when mortalities of the mice of the same age and weight (18–20 g) were compared, the low-protein group also showed an elevated mortality over the adequate-protein group. Consequently, since the data of the groups as a whole parallelled those of the group with the same weight, the results of whole groups were calculated in order to avoid selection of animals.

Methionine was added to the methionine-deficient 6% casein diet in order to determine whether the addition of one essential amino acid could prevent the increased mortality due to lack of protein. Others have shown previously that methionine significantly increased survival of rats subjected to thermal trauma (10) and promoted wound healing in rats (11). The results of our experiments demonstrate that when methionine was added to the low-protein diet, mortality increased, rather than decreased, after thermal trauma. Perhaps our results are not directly comparable with the previous studies mentioned because the diets and dosages were different.

If our experiments in mice can be applied to human burned patients, the results would give at least partial evidence for the clinical impression that severely burned patients need adequate protein replacements to combat the delayed wound healing, the increased local infection, and the increased morbidity and mortality in inadequately nourished patients. However, our results do not confirm the clinical impression that burned patients given a greater than normal intake of protein would get significant benefit from such therapy (5–7, 9). It must be emphasized, however, that our experiments studied only the *ad libitum* ingestion of diets high in one type of protein (casein) and did not examine the effects of force-fed high-protein diets.

*Summary.* The effect of protein in the diet on late mortality, food intake, and weight changes of severely burned mice was studied. Mice were fed *ad libitum* diets containing low (6%), adequate (20%) or high (40%) casein protein diets supplemented with carbohydrate and fat to make caloric contents equivalent. Three diet regimens were used: (a) adult mice fed the diets after thermal injury; (b) weanling mice reared on the diets which were continued after burning; and (c) adult mice fed the diet for 3 weeks prior to thermal trauma and continued on the same diet afterward. The results show that on all diet regimens a low-protein diet produced a significantly higher late mortality in burned mice and an increased weight loss when compared with burned mice fed an adequate protein diet. In contrast, a high-protein diet did not prove more beneficial by the same criteria than an adequate-protein diet. In all groups compared, food intake was not significantly different. When various amounts of methionine (also reported to be beneficial in

experimental burn shock and wound healing) were added to the low-protein diet, survival did not improve.

We wish to thank Dr. John G. Bieri, NIH, for his helpful suggestions.

1. Davidson, E. C., Arch. Surg. **13**, 262 (1926).

2. Cuthbertson, D. P., Biochem. J. **25**, 236 (1931).

3. Cuthbertson, D. P., Brit. J. Surg. **23**, 505 (1936).

4. Calloway, D. H., Grossman, M. I., Bowman, J., and Calhoun, W K., Surgery **37**, 935 (1955).

5. Hirshfield, J. W., Abbott, W. E., Pieling, M. A., Heller, C. G., Meyer, F., Williams, H. H., Richards, A. J., and Obi, R., Arch. Surg. **50**, 194 (1945).

6. Holden, W. D., Krieger, H., Levey, S., and Abbott, W. E., Ann. Surg. **146**, 563 (1957).

7. Blocker, T. G., Jr., Levin, W. C., Nowinski, W. W., Lewis, S. R., and Blocker, V., Ann. Surg. **141**, 589 (1955).

8. Levenson, S. M., Pulaski, E. J., and del Guercio, L. R. M., *in* "Physiologic Principles of Surgery" (Zimmerman and Levine, eds.), chap. 1, pp. 1–43. Saunders, Philadelphia (1964).

9. Taylor, F. H. L., Levenson, S. M., Davidson, C. S., Browder, W. C., and Lund, C. C., Ann. Surg. **118**, 215 (1943).

10. McCarthy, M. D., and Bodkin, R. E., Proc. Soc. Exp. Biol. Med. **63**, 377 (1946).

11. Williamson, M. B., McCarthy, T. H., and Fromm, H. J., Proc. Soc. Exp. Biol. Med. **77**, 302 (1951).

Received May 2, 1970. P.S.E.B.M., 1970, Vol. 135.