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Body Weight and Composition in Laboratory Rats: Effects of Diets with High or Low Protein Concentrations

Abstract. *Adult rats fed high concentrations of dietary protein for 9 weeks gained more weight than rats fed isoenergetic diets containing less protein. There were no significant differences in tail and body lengths among several groups of rats on diets containing different amounts of protein; however, total body fat was significantly greater in the rats fed on diets containing 25 percent protein compared to the rats fed 5 percent protein diets. These findings suggest that the role of dietary protein in obesity and other conditions deserves further scrutiny.*

According to conservative estimates, 30 million adults in the United States are 20 percent or more over their ideal weights (*1*, *2*). Although nutrition is an etiologic factor, at least to the extent that the obese must consume energy in excess of their caloric expenditures (*3*), the role of specific nutrients is unclear. In 1965, the typical American diet was found to contain 16.1 percent of kilocalories as protein from mixed animal and vegetable sources (*4*). This amount exceeds both the daily protein requirement (7.0 percent) estimated by the Food and Agricultural Organization (*5*, p. 74) and the National Academy of Sciences-National Research Council (*6*) recommended dietary allowance for men (7.6 percent). Although both excess and inadequate intakes of energy are potentially harmful, an abundance of protein is generally not considered to be an undesirable feature of the American diet, and harmful effects have not been demonstrated in humans consuming well above the probable requirement.

The aging laboratory rat is a useful model of human obesity and has been used extensively for studies of metabolic changes associated with obesity at the cellular level (*7*). In a study by Edozien and Switzer (*8*) that was confirmed by Donald (*9*) the body weights of rats increased with increasing dietary protein when the rats were fed specially prepared diets from the time they were weaned. However, under the conditions of these experiments, growth was affected (*8*, *9*). In the experiments described here we tested the possibility that body weight and adiposity are influenced by dietary protein in adult animals. We fed a specially prepared diet with varying concentrations of protein to rats that had already achieved full growth.

As shown in Fig. 1, mature rats fed a diet with a high protein content gained weight more rapidly than those fed a low protein diet; linear growth was not affected. Although we did not measure adiposity directly, we speculated that rats fed higher protein diets are more obese than rats fed lower protein diets, because of their resemblance to the spontaneously obese old rat whose adipocytes are large compared to those from young lean rats (*7*). It is noteworthy that commercial laboratory rat food contains 23 percent of protein from mixed animal and vegetable sources, which is equivalent to 18.4 percent as lactalbumin (*10*).

We also fed two groups of rats diets containing either 5 or 25 percent protein for 8 weeks and measured food consumption, body weight, total body fat, and fat-free body mass (FFBM) (Fig. 2). The group that received the 25 percent protein diet had a mean 23.9 percent of its total body mass as fat, whereas the group that received the 5 percent protein diet had a mean of 15.8 percent of total body mass as fat. Final body mass (after removal of the hair and gastrointestinal tract contents) of the rats fed the 5 percent protein diet was 397.3 ± 8.3 g (mean ± standard error) and of the rats fed the 25 percent protein diet was 486.6 ± 9.0 g (t = 7.3, α = .0001). Fat-free body mass was 334.1 ± 12.2 g and 371.0 ± 28.2 g in the 5 and 25 percent protein groups, respectively; this difference was not significant (t = 1.22, α = .1). Total body fat was significantly different between the two groups (t = 2.01, α = .05). The mean value was 63.2 ± 11.7 g in the group fed 5 percent protein and 115.1 ± 23.3 g in the group fed 25 percent protein. These data do not exclude the possibility that the rats fed the 25 percent protein diet have a greater

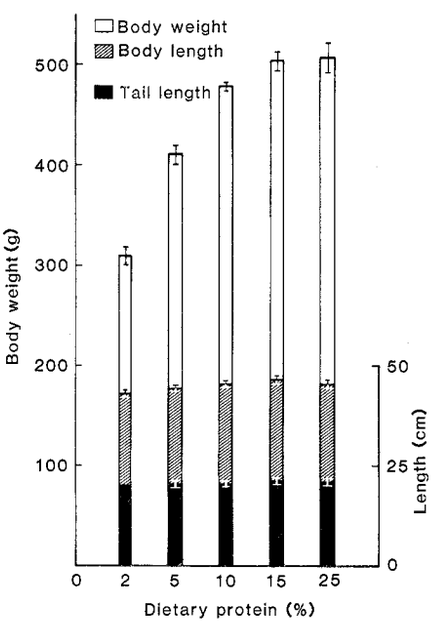

Fig. 1. Body weight, body length, and tail length in mature rats fed diets containing high or low concentrations of protein. Male Sprague-Dawley rats (Hilltop) aged 10 weeks and weighing 300 g at arrival were given free access for 9.5 weeks to isoenergetic (4.1 kcal/g) pelleted diets (Teklad) containing 2, 5, 10, 15, or 25 percent protein (as lactalbumin) with correspondingly decreasing amounts of carbohydrates (as sucrose, cornstarch, and cellulose) and 10 percent fat (as cottonseed oil). There were three rats, housed together, in each dietary group. Body length did not change significantly during the experiment. All animals were fasted for 16 hours and then killed in the morning with an overdose of sodium pentobarbital. We then measured body weight, body length (nose to tail), and tail length. To analyze the differences between the mean variables from the five dietary groups we used the one-way analysis of variance (ANOVA) (*16*). Differences between mean initial and final body weights of each group were determined by the t-test (*5*, p. 71). There were no significant differences in mean body weights among the five groups at the beginning of the experiment (F = 0.21). However, by the end of the feeding period, body weights of the five groups varied significantly (F = 855.5, α = .01). Throughout the study, animals fed the 5, 10, 15, and 25 percent protein diets appeared healthy and well-nourished. The rats in each of these four groups had gained significant amounts of weight since the initiation of the feeding period (t = 4.9 to 22.6, α = .01), whereas the rats in the group fed the 2 percent protein diet had lost an average of 13.8 g (t = 0.8, not significant). However, there were no significant effects of dietary protein on tail (F = 1.89) or body (F = 1.44) lengths.

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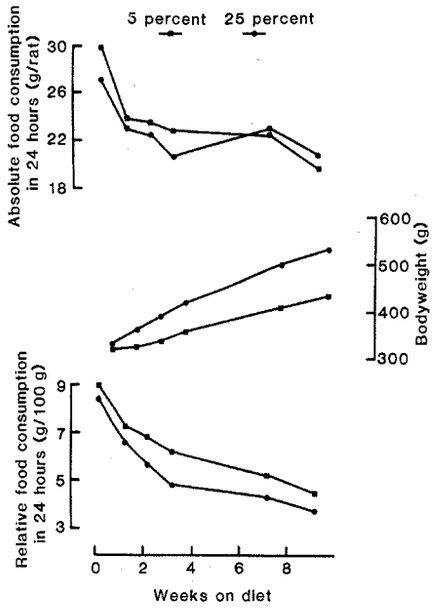

Fig. 2. Body weight and food consumption during feeding and body composition after feeding with 5 or 25 percent protein. Six rats in each group were fed on special diets for 9 weeks as described in the legend to Fig. 1. Body weight was measured at the times indicated. Food consumption during intervals of 24 hours was measured by weighing the food available at the beginning and end of the period. Fragments of dietary pellets were collected from the cages and included in the final weight. At the end of the feeding period the rats were killed by decapitation, bled, and sheared as closely as possible with animal clippers. The contents of the gastrointestinal tract was then removed and weighed. Body mass after death, plus mass of shed blood, minus mass of fur and gut content and minus the mass of extracted lipid yielded FFBM. The carcass was ground and equal portions were taken for determination of water content (by freeze-drying) and fat content (by extraction with petroleum ether, boiling point 30° to 60°C) by the Soxhlet apparatus (*10, 17*) as previously described. Midweekly records of food consumption showed that the group on 5 percent protein diet consumed more food, when expressed per 100 g of body weight, than the group fed the 25 percent protein diet. When food consumption was expressed as an absolute amount per rat, these differences were not apparent. The animals fed the 5 percent protein diet gained a mean of approximately 100 g, while the mean weight gain of the group fed the 25 percent protein diet was approximately 200 g by the end of the feeding period.

muscle mass than rats fed 5 percent protein. We did not measure muscle mass directly, and a study with larger numbers of rats might detect a statistically significant difference in FFBM. Rats fed the higher protein diet, however, had significantly more body fat. Lesser *et al.* (*11*) reported that rat colonies contain long-lived subgroups characterized by lower body weight and smaller FFBM throughout life. It will be interesting to see whether rats fed low protein diets live longer than rats fed high protein diets.

The mechanisms responsible for the increased body weights of rats fed high protein diets are not known. The food consumption data (Fig. 2) weigh against differences in energy intake as an explanation. Rats fed low protein diets may not absorb ingested nutrients as well as rats fed high protein diets; however, there is no reason why this should be the case, and we observed no gross differences in quantity or appearance of feces among the groups. The remaining possibility is that rats fed low protein diets deposit calories less efficiently in adipose tissue than do rats fed high protein diets. During the past 2 years there has been renewed interest in the long-debated concept of luxus consumption or "burning off" of excess calories. Rothwell and Stock (*12*) have shown that diet-induced thermogenesis occurs in laboratory rats and have provided evidence that this phenomenon is due to increased activities of the sympathetic nervous system and brown adipose tissue. Rats fed different amounts of dietary protein may also differ in physical activity. Thus our rats fed low protein diets may have expended more calories in brown adipose tissue or through increased activity and gained less weight. This possibility is supported by Tulp *et al.* (*13*) who demonstrated increased diet-induced thermogenesis in weanling rats fed 8 percent protein compared to 22 percent protein diets.

In studies of protein allowances attempts have been made to estimate nitrogen equilibrium, efficiency of utilization, and amount of tissue protein deposited during growth (*6*). Scrimshaw (*14*) suggests that the role of protein in health and disease must be examined with the use of other indices of metabolism and performance. A role for excess dietary protein in the pathogenesis of atherosclerosis has been postulated (*15*). Our data support the notion that the role of dietary protein in obesity and other conditions associated with degenerative diseases must be examined further in experimental animals and man.

PATILLO DONALD
Department of Internal Medicine, University of Virginia, Charlottesville 22908
GROVER C. PITTS
Department of Physiology, University of Virginia, Charlottesville
STEPHEN L. POHL
Department of Internal Medicine, University of Virginia, Charlottesville

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