Adipose tissue development, growth, and food consumption in protein-malnourished rats

O. Tulp, S. Gambert, and E. S. Horton

University of Vermont College of Medicine, Burlington, VT 05401

Abstract Effects of protein malnutrition on adipose tissue development were studied in weanling male Sprague-Dawley rats fed isocaloric diets ad libitum containing either 22% (controls) or 8% (protein-malnourished rats) casein, and in rats pair-fed to the protein-malnourished rats with the 22% casein diet. After 32 days on the diet, protein-malnourished rats were 37% and pair-fed 67% the weight of the controls, while torso length was 37% and 73% of controls, respectively. Food consumption relative to body weight was greatest in protein-malnourished rats. Compared to control rats, the distal epididymal adipocyte number in the protein-malnourished rats was decreased in proportion to the decrease in body size and was more closely related to the protein intake than to the total calories consumed. After 32 days on diet, mean adipocyte number per 2 distal pads was 11.7×10^6 in controls and 4.3×10^6 in protein-malnourished rats. In pair-fed rats, cell number lagged behind controls at 4 and 11 days, but was normal at 32 days (11.4×10^6 cells). The distal epididymal pad adipocyte size and percent lipid were similar in all groups during the first 25 days of dietary treatment. Adipocyte size was increased significantly in controls at day 32 compared to the other two groups. At each time studied through day 25 on diet, epididymal pad weight was related to the adipose cell number rather than the cell size. It is concluded that severe restriction of dietary protein during the postweaning period of growth in rats results in decreased epididymal adipocyte proliferation and/or differentiation concomitant with generalized growth retardation, whereas isocaloric feeding of a diet of normal protein content is associated with only a transient delay in adipose tissue development.

Supplementary key words adipocyte size and number · thermogenesis · diet composition and growth

Studies relating dietary manipulation to adipose tissue development suggest that dietary protein may play a critical role in the growth of this tissue, as it does in other organs (1). In clinical and experimental studies, protein deficiency is often associated with retarded growth, preservation of subcutaneous fat, fatty liver, edema, dermatitis, changes in pigmentation, and aberrant behavior (1–3). The specific effects of protein malnutrition on adipose tissue development have not been fully defined, but may include changes in both the proliferation and lipid filling of adipocytes (4).

In normally growing rats, the most rapid period of epididymal adipose cell proliferation occurs during the first 4–6 weeks of life, that is, prior to weaning and in the immediate postweaning period. Further adipose cell proliferation, at a slower rate, combined with lipid filling of preexisting cells, occurs up to 10–14 weeks of age, beyond which the total epididymal adipose cell number becomes stabilized and only changes in size occur (5).

In rats, both the epididymal adipocyte number and size can be altered when nutritional or other stresses are imposed during the preweaning period of growth. Overnutrition (6) or a constant 5°C environmental temperature (7) during growth increases the cell number, while undernutrition (4, 6, 8, 9) or increased exercise levels (8) result in decreased adipocyte proliferation. Food restriction (9), exercise (8, 10), and constant 5°C environmental temperature from weaning (7) reduce cell size, while cell size is increased after periods of overnutrition (4) or prolonged insulin administration from the time of weaning (11). Similar manipulations in adult animals produce changes only in adipocyte size without changes in cell number.

The purpose of the present study was to determine the effects of feeding ad libitum a low-protein, high-carbohydrate diet and equivalent caloric restriction of a normal diet on the development of adipose tissue in rats during the postweaning period of growth. Thus the effect of decreased protein consumption on adipocyte size and number has been separated from that of simple caloric restriction.

METHODS

Twenty-one-day-old weanling male Sprague-Dawley rats were obtained from the Charles River Co. and were housed six animals to a cage in temperature-controlled rooms with a 12-hr light–dark cycle.

TABLE 1. Composition of diets

Constituents	Grams/100 grams	
	Control	PD[a]
Casein, vitamin-free	22.00	8.00
Cornstarch	14.75	18.25
Dextrin	14.75	18.25
Glucose	14.75	18.25
Sucrose	14.75	18.25
Lard, Armour	5.00	5.00
Corn oil, Mazola	5.00	5.00
Cellulose	2.50	2.50
Mineral mix, Rogers-Harper	5.00	5.00
Mineral mix supplement[b]	0.50	0.50
Vitamin mix	1.00	1.00
	100.00	100.00
Caloric value, Kcal/gm	4.14	4.14

[a] PD, protein-depleted rat diet.

[b] This mixture contains the following elements in a final dietary concentration as follows: aluminum, 1 ppm; cobalt 1 ppm; nickel, 1 ppm; fluorine, 1.0 ppm; bromine, 1 ppm; boron, 2.5 ppm; vanadium, 1 ppm; chromium, 1 ppm; molybdenum, 0.3 ppm; tin, 1 ppm; zinc, 7 ppm; and manganese, 30 ppm.

All animals were weighed upon receipt and at 2- to 3-day intervals throughout the study. Control animals received a 22% casein diet and protein-malnourished rats an 8% casein diet, ad libitum (**Table 1**). In pair-fed animals, food consumption was estimated for the first day and thereafter the animals were fed a quantity of the control diet equal to the amount consumed by the protein-malnourished rats during the previous 24 hrs. All animals were fed at 7:00 PM daily and water was freely available throughout the study. The cumulative food consumption for each cage was recorded daily and casual observations were made of animal development and behavior. The animals were killed by decapitation, and the torso length was measured as the distance from the base of the cervical spine to the anus.

The distal epididymal fat pads, taken as that segment of adipose tissue just distal to the major blood vessels as described by Hirsch and Han (6), were removed and weighed immediately. The tissues were then cut into several smaller pieces, and randomly selected fragments were weighed and processed for determination of cell number, lipid content, or histological examination. The cell number in the weighed tissue fragments was determined using Method III of Hirsch and Gallian (12). From each animal 50–100 mg of adipose tissue was rinsed in saline, weighed, and rapidly plunged into a plastic counting vial containing osmium tetroxide (Engineered Materials) prepared in 0.05 M collidine buffer (Eastman Kodak Co.). Cells were fixed for 24–48 hr at 37°C, after which they were washed free of excess osmium with tap water, and the cells between 25 and 202 μm in diameter were collected on nylon sieves. The final washing was completed with saline, and the cell number per sample was determined in an electronic particle counter (Coulter Electronics, Hialeah, Fla.).

Cell lipid content was determined gravimetrically after extraction of 75–100 mg of minced adipose tissue fragments by the procedure of Dole and Meinertz (13). The residues were evaporated with the aid of a gentle flow of warm, dry room air in a hood and dried to a constant weight in a vacuum desiccator at room temperature for 2–3 days. Cell size was determined by dividing tissue lipid content by cell number per unit weight, and expressed as μg lipid per cell.

Data were analyzed by Student's t test, by analysis of variance, or by Kruskall–Wallis analysis (14).

RESULTS

Food consumption, growth and development

Animals were fed either the control, protein-depleted or pair-fed diet after weaning, and data on daily food consumption and weight gain were obtained throughout 32 days of the dietary regimen. Thinning of the body hair occurred in most protein-malnourished animals, and a mild dermatitis of the tail was often present. During the 32-day treatment period, protein-malnourished animals did not develop the usual secondary sex characteristics, whereas the control and pair-fed animals developed normally. In addition, the behavior pattern of the protein-malnourished animals appeared to be altered after a short time. They were often observed to eat during the day, while the control animals followed the usual nocturnal feeding pattern. Although the protein-malnourished animals were quieter than controls when undisturbed, they became more excitable during handling and, after 2–3 weeks on the diet, unsteady gait and athetoid movements of the head and torso were frequently observed.

The increases in weight and torso length of the three groups of animals are shown in **Fig. 1.** After 32 days of the dietary treatments, weights and torso lengths of protein-malnourished rats averaged only 37% and 73% of controls, respectively. Statistically significant differences in rat weight and linear growth in the three groups were present after only 3–4 days of the respective dietary treatments ($P = <0.001$ on day 3). The differences remained significant thereafter (analysis of variance).

Food consumption data from the three groups are illustrated in **Fig. 2.** The ad libitum daily food con-

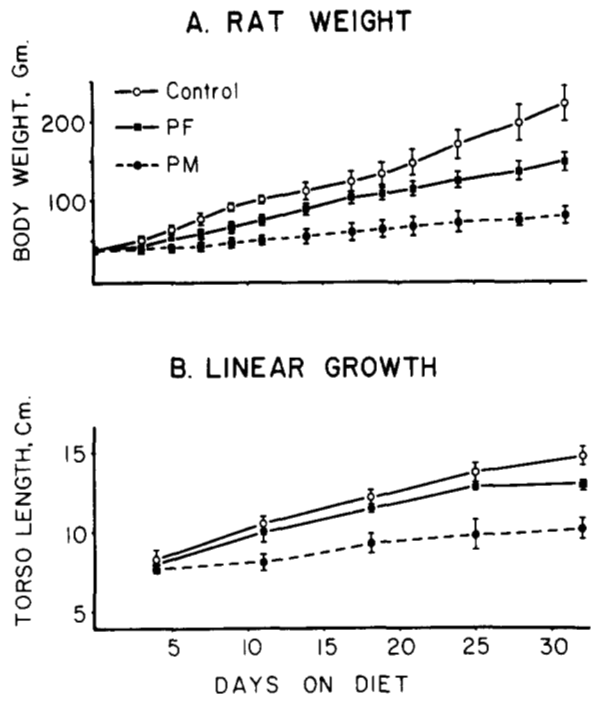

Fig. 1. Growth of rats fed isocaloric diets of different protein content. Panel *A*, rat weight in g, and Panel *B*, torso length, from base of neck to anus, in cm. Data are mean ± 1 SEM.

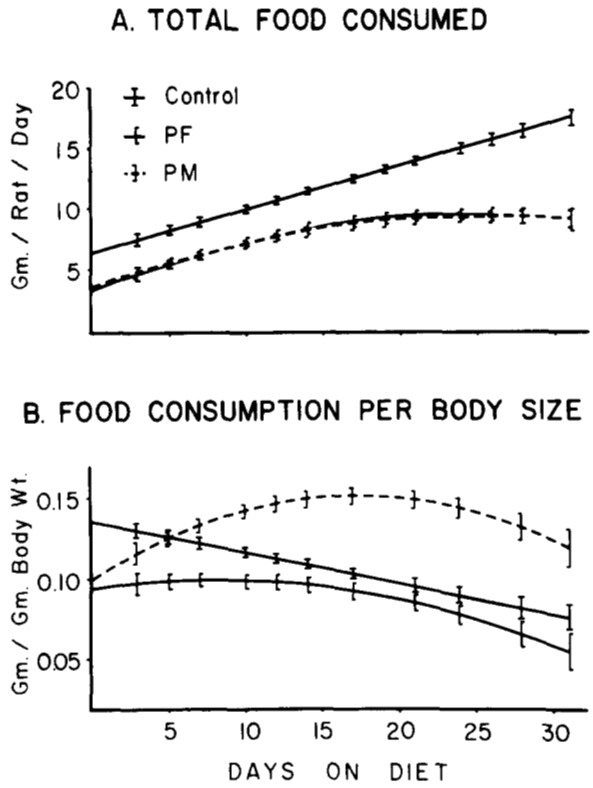

Fig. 2. Food consumption of rats fed isocaloric diets of different protein content. *A*, Total food consumed, in g per rat per day, and *B*, food consumption per body weight, expressed as g eaten per day per g of body weight. Data are regression lines of best fit ±95 confidence intervals. Equations for the lines presented are: Panel *A*: Control = $Y_x = 6.466 + 0.3596$; PM = $Y_x = 3.539 + 0.435 - 0.00818X^2$; PF = $Y_x = 3.166 + 0.5236 - 0.01075X^2$. Panel *B*: Control = $Y_x = 0.1352113 - 0.001915$; PM = $Y_x = 0.099405 + 0.005899 - 0.0001697X^2$; PF = $Y_x = 0.093468 + 0.001271 - 0.0000816X^2$. N: Control = 12; PM = 12; PF = 6 animals at each observation.

sumption per animal was consistently less in protein-malnourished animals than it was in controls ($P = <0.05$). However, food consumption relative to body weight was greater in control animals only initially, and after 7–10 days of the dietary regimen the protein-malnourished animals consumed a significantly larger quantity of food for their body weight than did either the pair-fed or control animals ($P = <0.01-<0.005$ in simultaneous comparison by Kruskall–Wallis analysis).

The average gain in weight, consumption of food, calories, and casein and the net efficiencies of gain in weight of animals fed each of the three dietary regimens were calculated for the entire 32-day study period (**Table 2**). For each gram of food eaten, control and pair-fed animals gained an average of 0.469 and 0.448 g, respectively, whereas the protein-malnourished animals gained only 0.179 g. Expressing the data in terms of caloric intake, animals fed the control diet gained 113 g per 1000 kcal consumed, pair-fed animals gained 108 g, and the protein-malnourished animals gained only 43 g/1000 kcal. The protein-malnourished animals, therefore, had a net efficiency of weight gain which was only 38–40% that of the control or pair-fed animals receiving a 22% casein diet. If gain in weight is related to casein intake, the efficiency of growth is similar in all three groups of animals (2.04–2.24 g/g casein). This suggests that restricted protein intake, rather than total caloric intake, was the major limiting factor for growth in the protein-malnourished and pair-fed animals in this study.

TABLE 2. Weight gain, food intake, and calculated 32-day efficiency of weight gain[a]

Group	Weight Gain	Food Eaten	Gain/g of Food	Total Kcal	Gain/1000 Kcal	Casein Eaten	Gain/g of Casein
	g	*g*	*g*		*g*	*g*	*g*
Control	185	395	0.469	1636	113	87	2.13
Pair-fed	110	246	0.448	1019	108	54	2.04
Protein-malnourished	45	254	0.179	1050	43	20	2.24

[a] Data are calculated from the means of g of weight gain and g of total food consumption for all animals represented in this study.

Epididymal fat pad growth

Epididymal fat pad weight, lipid content, and adipocyte size and number were determined in the three groups at intervals over the 32-day period (**Table 3**). The combined weight of the two distal epididymal fat pads increased progressively throughout the study in all dietary groups, with the largest increase occurring in the control animals. After 32 days, distal pad weight in the pair-fed animals was approximately twice that of the protein-malnourished rats, despite the fact that both groups had the same caloric intake. Highly significant differences between epididymal fat pad weights in control and protein-malnourished rats were observed on day 18 and between all groups on days 25 and 32 ($P = <0.001$).

The lipid content of distal epididymal adipose tissue, expressed as g lipid per 100 g wet weight, increased in all treatment groups during the study and there was no significant difference in lipid content between the protein-malnourished and control rats at any time. The lipid content of the epididymal pads from the pair-fed rats was significantly less than controls on days 18 and 32 ($P < 0.005$) but not at any other time. When compared to day 25 values, all groups exhibited a rapid and significant increase ($P < 0.001$) in lipid content on day 32, with the greatest increase occurring in the control animals.

The data on size and number of adipocytes in the distal epididymal fat pads are illustrated in **Fig. 3.** Cell size increased progressively in all experimental groups throughout the period of dietary treatment and there were no significant differences among the groups during the first 18 days of the study. By day 25, the control animals exhibited a more rapid increase in adipocyte size than occurred in the other two groups and between days 25 and 32 a phase of rapid lipid filling occurred in control animals but not in the pair-fed or protein-malnourished groups. As a result, by day 32 adipocyte size was significantly greater in the controls than in the other two groups ($P < 0.0005$).

A progressive increase in epididymal adipocyte number also occurred in the control and pair-fed animals throughout the study. In controls, the cell number/2 distal pads was $11.7 \pm 0.6 \times 10^6$ after 32 days. Adipocyte number in the pair-fed animals lagged behind controls at 4 and 11 days, but reached control levels on day 32 ($11.4 \pm 1.1 \times 10^6$). In contrast, adipocyte number in the protein-malnourished rats increased only moderately to a peak of $4.5 \pm 0.4 \times 10^6$ cells at day 25 with no further increase on day 32. Kruskall–Wallis analysis of the cell number data indicated that effects of diet on epididymal cell proliferation were highly significant at all points measured ($P = <0.01$ to <0.005), and that these differences were due entirely to the consistently lower cell numbers found in the protein-malnourished rats.

After 32 days of dietary treatment, epididymal adipocyte number was 37% of controls, the same percentage by which body weight and torso length were decreased. Thus, the decreased adipocyte number in the protein-malnourished rats was proportional

TABLE 3. Distal epididymal fat pad weight, lipid content, cell size and cell number[a]

Days on Diet	4	11	18	25	32
A. Weight/2 distal pads[b]					
Control	93 ± 10	388 ± 31	559 ± 51	1143 ± 129	1697 ± 108
PF	56 ± 7	193 ± 21	489 ± 38	761 ± 69	943 ± 83
PM	60 ± 10	107 ± 22	259 ± 36	389 ± 53	417 ± 37
B. Distal pad lipid content, g/100 g[c]					
Control	51.2 ± 3.1	53.3 ± 1.7	55.8 ± 1.8	57.2 ± 0.8	80.8 ± 2.2
PF	46.8 ± 2.5	50.4 ± 2.2	41.6 ± 5.0	53.9 ± 2.5	68.5 ± 2.2
PM	53.4 ± 2.0	53.4 ± 3.3	55.6 ± 2.2	60.1 ± 1.1	75.3 ± 2.4
C. Adipocyte size, g lipid/cell[d]					
Control	0.0250 ± 0.0027	0.0357 ± 0.0028	0.0435 ± 0.0028	0.0624 ± 0.0040	0.1181 ± 0.0073
PF	0.0226 ± 0.0022	0.0294 ± 0.0035	0.0360 ± 0.0040	0.0483 ± 0.0057	0.0582 ± 0.0053
PM	0.0270 ± 0.0022	0.0298 ± 0.0037	0.0458 ± 0.0070	0.0505 ± 0.0027	0.0729 ± 0.0040
D. Adipocyte number $\times 10^6$/2 distal pads[e]					
Control	1.92 ± 0.17	5.93 ± 0.43	7.12 ± 0.42	9.78 ± 0.63	11.70 ± 0.56
PF	1.08 ± 0.18	3.55 ± 0.21	5.74 ± 0.51	8.77 ± 0.52	11.41 ± 1.14
PM	1.19 ± 0.18	1.78 ± 0.26	3.28 ± 0.28	4.48 ± 0.39	4.32 ± 0.35

[a] Data are expressed as mean ± 1 SEM. N:C = 12; PF = 6; PM = 12.
[b] Pad weight C vs. PM $P = <0.025$ days 4–32; PM vs. PF $P = <0.025$ days 11–32; C vs. PM $P = <0.025$ days 4, 11, 32.
[c] Pad lipid content C vs. PF $P = <0.05$ days 18, 25; PM vs. PF $P = <0.05$ day 18, day 35 vs. day 32, $P = <0.001$ all groups.
[d] Adipocyte size C vs. PM, PF $P = <0.001$ day 32.
[e] Adipocyte number PM vs. C, PF $P = <0.001$ days 11–32.

to the overall stunting of growth that occurred in these animals. In contrast, identical caloric restriction in the pair-fed rats produced only a transient effect on adipocyte number and did not result in a decreased number compared to controls when measured after 32 days of treatment. Thus, restricted dietary protein appears to have been a limiting factor for increase in adipocyte number in the protein-malnourished animals, but not in the pair-fed group.

To gain insight into the problem of whether or not the decreased adipocyte number observed in the protein-malnourished animals might be the result of decreased lipid filling of preadipocytes and failure to measure these small cells (<25 μm in diameter) by the method used, the relationships among adipocyte size, body weight, and caloric intake were determined (**Fig. 4**). A close correlation was observed between mean adipocyte size and total caloric intake in all three experimental groups. Thus, the protein-malnourished animals did not have, on the average, smaller adipocytes than animals in the other two groups after comparable consumption of calories. In fact, when adipocyte size was related to body weight, protein-malnourished animals were found to have relatively larger cells than either the pair-fed or control rats.

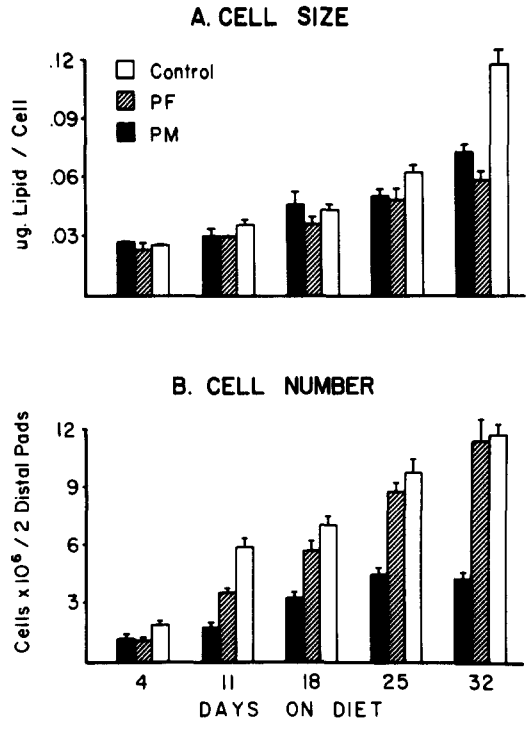

Fig. 3. Adipose cell size and number of rats fed isocaloric diets of different protein content. Data are expressed as the mean ± 1 SEM. In Panel *A*, Kruskall–Wallis analysis indicated that C vs. PM or PF = P = <0.05–0.001 on days 25, 32 and in Panel *B*, PM vs. C, P = <0.01–0.001 days 4–32; and PM vs. PF, P = <0.001 days 18–32. C vs. PF = N.S.

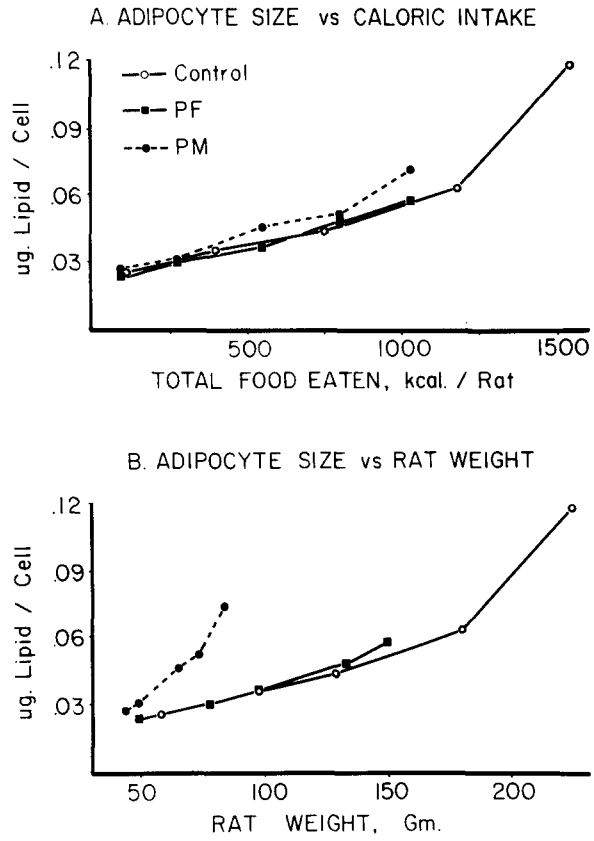

Fig. 4. Relationships among adipose cell size, total caloric intake, and animal body weight. Data points were obtained after 5, 11, 18, 25, and 32 days of treatment, and are plotted from left to right in that respective order for each dietary group. Figure 4*a*: Mean adipocyte size vs. cumulative caloric intake; Figure 4*b*: Mean epididymal adipocyte size vs. mean animal body weight.

This suggests that the smaller adipocyte number observed in the protein-malnourished group is the result of a decrease in adipocyte proliferation and not due to impaired lipid filling of preadipocytes.

DISCUSSION

The effects of chronic protein malnutrition on adipose tissue development have not been defined previously, in part because it has been difficult to separate the specific effects of protein deficiency from those of restriction of calories. In children with protein–calorie malnutrition, McLaren (1) observed that the subcutaneous adipose tissue appears to be relatively preserved, but no quantitative data are available. Meyer (15) observed that animals raised on diets of low protein content have a higher percentage of carcass fat than animals fed normal rations. These studies suggest that protein restriction results in a smaller lean body mass and a relative preservation of adipose tissue. This is consistent with the observa-

tion that rats attempt to compensate for an inadequate supply of protein in the diet by overeating (16–19), and that energy consumption relative to body weight is increased. The excess calories ingested may be stored as fat or dissipated by increased heat production (20, 21). This is in contrast to the changes observed in chronic starvation where both protein and total calories are restricted and adipose tissue stores become depleted (6).

The goals of the present study were to examine more closely the effects of undernutrition of calories and protein on adipose tissue development during the postweaning period of growth in the rat and to separate the effects of restricted protein intake from those of an equivalent restriction of calories fed as a normal diet.

The principal effect of caloric restriction on adipose tissue cellularity was to produce a transient delay in the rate of increase in epididymal adipocyte number during the first 18 days of treatment, with normal cell numbers appearing between 25 and 32 days. Protein restriction at the same level of total calories resulted in a much greater decrease in the rate of adipocyte hyperplasia and cell number remained subnormal during the entire 32-day period of protein restriction.

Cell size was similarly affected by both protein and caloric restriction. Cleary, Greenwood, and Brasel (22) have recently shown that a rapid phase of lipid filling in the epididymal adipocytes begins soon after the normally growing rat is 35 days old. This may be associated with a concomitant increase in adipocyte lipoprotein lipase activity (23). In the present study this phase of lipid filling occurred in the control animals soon after the 18th day of the experiment when the animals were 39 days old, but failed to occur in the protein-malnourished or pair-fed animals. The factors that initiate this accelerated phase of lipid filling are unknown, but these studies suggest that the level of caloric intake may be important in this process.

Our results indicate that both dietary protein and total caloric intake during the postweaning period of growth are important factors in the growth and development of epididymal adipose tissue, as they are in other tissues (2), and that protein restriction during that time may severely limit the rate of adipocyte proliferation and/or differentiation. Previous studies of the effects of reduced caloric intake on adipose tissue development have been conducted prior to weaning (4, 24) or after 6 weeks of age (6) and showed that only the preweaning treatment consistently led to a reduction in cell number in the adult animal. Acute starvation of one week duration at 6 weeks of age was without lasting effect on cell number (6) but, in another study, chronic underfeeding from this time resulted in marked decreases in the cell number of epididymal and retroperitoneal fat pads in the adult animal (25). Using pulse-labeling techniques, Greenwood and Hirsch (5) showed that [^{3}H]thymidine incorporation into epididymal adipocyte DNA occurred readily until the animals were 28–35 days old, but incorporation was minimal or absent thereafter. It is apparent, therefore, that in the normal rat adipocyte replication in the epididymal fat pads continues to occur for at least 2 weeks into the postweaning growth period.

A limitation of the Hirsch and Gallian (12) method of cell counting is its inability to measure preadipocytes or small adipocytes that might be lost during the sieving process. Hirsch and Han (6) observed an apparent counting artifact in their starved animals which they related to severe lipid depletion, resulting in adipocytes that were below the limit of detectability of the method. After their animals were refed, the cell numbers returned to control values, indicating that the adipocytes had regained their lipid stores. It is recognized that the lower cell numbers observed in the present study could possibly represent a counting artifact because of this methodologic limitation. It is unlikely that this occurred, however, because neither pad lipid content nor adipocyte size in the malnourished animals was significantly less than in controls through the 25th day of the experimental diet, when marked differences in cell number were present. Additionally, in the pair-fed animals both pad lipid content and adipocyte size were similar to those of the malnourished animals, whereas cell number in the malnourished animals differed markedly after only 4–11 days of dietary treatment. Because many preadipocytes are normally formed prior to weaning and may not have a sufficient quantity of lipid deposited to be recognizable as adipocytes for several weeks afterward (5), the possibility remains that feeding the protein-restricted diet resulted in impaired lipid filling of some cells, therefore delaying their appearance as adipocytes. Studies to determine the extent to which the observed decrease in cell number may become normalized with nutritional remediation are now in progress but data from other experiments suggest that full recovery may not occur. Winick and Noble (2) found that recovery of cell number in other tissues did not occur when refeeding of the undernourished animals was initiated after the normal time of hyperplasia for that tissue had passed, and Bertrand and Masoro (25) showed that chronic undernutrition from 6 weeks of age resulted in lower epididymal and

perirenal pad adipocyte numbers and decreased fat mass in the adult animal. Thus some impairment in achieving a normal adipocyte number is likely to remain after refeeding our protein-malnourished animals.

Similar to our results, DeCastro and Boyd (16) and Andik et al. (17) have reported increases in food consumption relative to body weight when weanling rats were fed protein-restricted diets, and Musten, Peace, and Anderson (26) observed that weanling rats offered diets varying only in protein content spontaneously regulated protein intake independently of total energy intake. Dilution of normal diets with noncaloric additives also results in voluntary increases in food consumption in animals, presumably in an attempt to maintain caloric balance (27). In the present study, the increased food intake relative to body weight that developed during the first 10 days of feeding the protein-restricted diet may represent an adaptive mechanism whereby the malnourished animals attempt to satisfy their protein requirements by overeating. This results in an increase in total protein intake, as well as an inappropriate increase in total energy intake from nonprotein sources because of the fixed high ratio of nonprotein to protein calories. Based on the efficiency of weight gain in the malnourished animals, the excess nonprotein energy consumed averaged approximately 600 kcal per animal. The fate of these apparently excess calories, presumed nonessential for growth, is unknown, but based on the adipocyte sizes and body weights found it is unlikely that lipid deposition in the adipose mass could account for a significant proportion of these calories. Casual observation of behavior indicates that the level of spontaneous activity in the malnourished rats is not markedly different from that of the control animals throughout much of the day, indicating that the excess calories are probably not expended through additional work. These observations are consistent, therefore, with the development of a diet-induced thermogenesis in the malnourished animals in which the excess calories acquired through relative overeating may be expended through thermogenesis or other energy-requiring metabolic processes. In related experiments we have found that serum triiodothyronine concentrations become elevated to twice the normal concentrations and resting oxygen consumption is increased in animals fed the same protein-restricted diet (20), lending further support to the proposal of increased thermogenesis. Regardless of the mechanism that produces the caloric inefficiency, the protein-malnourished animals were unable to overcome completely the effect of protein deprivation, resulting in severe growth retardation with proportionate effects on epididymal adipocyte number.

SUMMARY

The predominant effect of protein deprivation was a decrease in epididymal adipose cell number, which was proportional to the extent of overall growth retardation. Moderate restriction of a normal diet resulted only in a lag in the adipocyte hyperplasia, with normal epididymal adipocyte numbers attained after 25–32 days on diet. Adipocyte size was unaffected by diet through day 25, but the rapid phase of adipocyte lipid filling which normally occurs after that time was impaired or delayed in the malnourished and the pair-fed animals. Total food intake was less in the malnourished animals, but food intake relative to body weight became greater in the malnourished animals after 7–10 days of dietary treatment, and remained greater thereafter. The basis for this relative hyperphagia is unknown, but it may represent an element of an adaptive mechanism that enables the animals to partially compensate for the protein-poor diet by overeating. In this way the total intake of protein would be increased and the associated nonprotein calories not required for growth or other essential metabolic processes would be lost through thermogenesis or other energy-losing mechanisms.

We wish to acknowledge the assistance and advice of Dr. Robert Tyzbir, Department of Home Economics, in the preparation of the experimental diets; Drs. Lester Salans and Samuel Cushman, Dartmouth Medical School, in whose laboratory the adipocyte counting was done; Dr. Takamaru Ashikaga and Ms. Joyce Prescott, Biometry Facility, for the statistical analyses; and Mr. Fred Robley for technical support. This study was supported in part by USPHS Grant PHS R01 13307 and USPHS Grant PHS 5429-14-17. Dr. Tulp is the recipient of a USPHS postdoctoral fellowship (PHS F32 05034) and Dr. Gambert was supported by a Joseph A. Goldberger Fellowship from the American Medical Association.

Manuscript received 8 March 1976 and in revised form 27 June 1978; accepted 28 July 1978.

REFERENCES

1. McLaren, D. S. 1970. Nutrition and its Disorders. Williams and Wilkins, Baltimore, Md. 99–110.
2. Winick, M., and A. Noble. 1966. Cellular response in rats during malnutrition at various ages. *J. Nutr.* **89:** 300–306.
3. Read, M. S. 1973. Malnutrition, hunger and behavior. *J. Am. Dietet. Assoc.* **63:** 379–385.

4. Knittle, J. L., and J. Hirsch. 1968. Effect of early nutrition on the development of rat epididymal fat pads: cellularity and metabolism. *J. Clin. Invest.* **47:** 2091–2098.
5. Greenwood, M. R. C., and J. Hirsch. 1974. Postnatal development of adipocyte cellularity in the normal rat. *J. Lipid Res.* **15:** 474–482.
6. Hirsch, J., and P. W. Han. 1969. Cellularity of rat adipose tissue: Effects of growth, starvation, and obesity. *J. Lipid Res.* **10:** 77–82.
7. Terriault, D. G., and D. B. Mellin. 1971. Cellularity of adipose tissue in cold-exposed rats and the calorigenic effect of norepinephrine. *Lipids.* **6:** 486–491.
8. Oscai, L. B., et al. 1974. Effect of exercise on adipose tissue cellularity. *Federation Proc.* **33:** 1956–1958.
9. Smith, J. E., and M. DiGirolamo. 1974. Effect of weight reduction in the rat on epididymal fat-cell size and relative dispersion. *Am. J. Physiol.* **227:** 420–424.
10. Booth, M. A., M. J. Booth, and A. W. Taylor. 1974. Rat fat cell size and number with exercise training, detraining and weight loss. *Federation Proc.* **33:** 1959–1963.
11. Salans, L. B., M. J. Zarnowski, and R. E. Weisman. 1972. Effect of insulin upon the cellular character of rat adipose tissue. *J. Lipid Res.* **13:** 616–623.
12. Hirsch, J., and E. Gallian. 1968. Methods for the determination of adipose cell size in man and animals. *J. Lipid Res.* **9:** 110–119.
13. Dole, V. P., and H. Meinertz. 1960. Microdetermination of long-chain fatty acids in plasma and tissues. *J. Biol. Chem.* **235:** 2595–2599.
14. Conover, W. J. Practical Non-parametric Statistics. John Wiley & Sons, London, England and New York, N.Y. 256–265, 393–395.
15. Mayer, J. H. 1958. Interactions of dietary fiber and protein on food intake and body composition in growing rats. *Am. J. Physiol.* **193:** 488–494.
16. DeCastro, E. S., and E. M. Boyd. 1968. Organ weights and water content of rats fed protein deficient diets. *Bull. WHO* **38:** 971–977.
17. Andik, I., S. Donhoffer, M. Farkas, and P. Schmidt. 1963. Ambient temperature and survival on a protein deficient diet. *Br. J. Nutr.* **17:** 257–261.
18. Menaker, L., and J. M. Navia. 1973. Appetite regulation in the rat under various physiological conditions: The role of dietary protein and calories. *J. Nutr.* **103:** 347–352.
19. Tulp, O., S. Gambert, and E. S. Horton. 1975. Effect of protein malnutrition on development of adipose tissue in rats. *Clin. Res.* **23:** 319A.
20. Tulp, O. L., E. S. Horton, E. D. Tyzbir, E. Danforth, Jr., and J. Bollinger. 1976. Increased plasma triiodothyronine and caloric inefficiency in experimental protein malnutrition. *Am. J. Clin. Nutr.* **29:** 471.
21. Stirling, J. L., and M. J. Stock. 1968. Metabolic origins of thermogenesis induced by diet. *Nature.* **220:** 801–802.
22. Cleary, M. P., M. R. C. Greenwood, and J. A. Brasel. 1977. A multifactor analysis of growth in the rat epididymal fat pad. *J. Nutr.* **107:** 1969–1974.
23. Hietanen, E., and M. R. C. Greenwood. 1977. A comparison of lipoprotein lipase activity and adipocyte differentiation in growing male rats. *J. Lipid Res.* **18:** 480–490.
24. Johnson, P. R., J. S. Stern, M. R. C. Greenwood, L. M. Zucker, and J. Hirsch. 1973. Effect of early nutrition on adipose cellularity and pancreatic insulin release in the Zucker rat. *J. Nutr.* **103:** 738–743.
25. Bertrand, H. A., and E. J. Masoro. 1977. Post-weaning food restriction reduces adipose cellularity. *Nature.* **266:** 62–63.
26. Musten, B., D. Peace, and G. H. Anderson. 1974. Food intake regulation in the weanling rat: Self-selection of protein and energy. *J. Nutr.* **104:** 563–572.
27. Wangness, P. J., and G. H. Soroka. 1978. Effect of energy concentration of milk on voluntary intake of lean and obese piglets. *J. Nutr.* **108:** 595–600.