Effects of Types and Levels of Carbohydrates and Proteins on Carcass Composition of Adult Rats (34185)

MARY W. MARSHALL, MADELYN WOMACK, HAZEL E. HILDEBRAND, AND ARVID W. MUNSON[1]

Human Nutrition Research Division and Biometrical Services, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705

For many years studies have been reported on the effect of level of dietary protein on rat growth and carcass composition (1–6). In general, the studies on carcass composition showed that if protein intake was inadequate to meet the requirements of the animal, a higher proportion of fat was deposited in the body tissue gained, and the effect of high protein diets—which resulted in leaner animals—was a reflection of a decrease in food intake.

Other studies have implicated type of dietary carbohydrate (7–12) and type of protein (13) as influencing factors on body fat gain in rats and dogs. Much of the information on body composition in rats is reported on young growing animals in which the storage of protein and minerals, not fat, chiefly occurs. In view of the needs for calories as well as protein in some parts of the world and problems of overabundance in others, it seemed desirable to reinvestigate the influence on intakes and body gains of two major dietary components. These investigations were conducted with the view of studying interrelationships among the nutrients in adult male rats.

Methods. Rats and diets. Weanling male, BHE rats (14) were housed individually and fed the same "pre-experimental" diet until they were 100 days old. A purified diet rather than a stock diet was fed to all rats as the "pre-experimental" diet because it was previously observed that when adult animals are removed from a stock diet to a purified diet they tend to overeat. The "pre-experimental" diet, hereafter referred to as the control diet, contained 23% lactalbumin (Borden Co.) providing in the diet 3% N; 57.65% cornstarch; 15% corn oil (Mazola); 4% salt mixture (15); 0.05% vitamin A and D concentrate (Squibb's Navitol, containing 65,000 USP units of vitamin A and 13,000 units of vitamin D/g at the beginning of the study and later, Mead Johnson's Percomorph oil, containing 60,000 USP units of vitamin A and 8,500 units of vitamin D/g); 0.1% inositol; and 0.2% choline chloride. The following vitamins were added per kilogram of ration: 5 mg each, thiamine·HCl, pyridoxine·HCl, and nicotinic acid; 10 mg of riboflavin; 25 mg each, Ca pantothenate (*d*) and α-tocopherol acetate; 300 mg *p*-aminobenzoic acid; 2 mg each, 2-methyl-1, 4-naphthoquinone and folic acid; 100 μg of biotin; and 30 μg of vitamin B_{12}.

When the animals were 100 $\pm$ 5 days old, those weighing between 320 and 498 g were divided into 13 groups, of 16–22 animals each, with similar average body weights and weight ranges. One of the 13 groups (22 rats) was fasted overnight, killed and analyzed; and their data were used to construct regession lines (fat and protein vs. body wt) from which the initial composition of the 12 remaining groups was calculated. The 12 groups were fed for 14 weeks, *ad libitum*, purified diets with 1, 3, or 6% nitrogen from lactalbumin or from wheat gluten (Vicrum from Hercules Powder Co., containing 12.0% nitrogen) with either sucrose or cornstarch as the carbohydrate. The diets contained for the three protein levels either 7.65 or 8.28, 23.00 or 24.89, 46.00 or 49.79% lactalbumin or wheat gluten, respectively. Fat, salt mix, and vitamins were the same as in the control

[1] Present address: Ralston Purina Company, St. Louis, Missouri.

diet and carbohydrate was changed as required to make 100%.

Procedures. The animals were weighed weekly and individual food consumption was measured; scattered food was carefully recovered and weighed. During the seventh week of feeding, feces were collected from 10–14 animals fed each diet. The feces were dried under infrared lamps, ground in a Wiley mill and analyzed for nitrogen by the macro-Kjeldahl method with mercury as a catalyst. Caloric values of pooled samples of food and feces were determined in the Parr Bomb Adiabatic calorimeter. (Gross calories, kcal per gram of pooled samples of the diets were, for the rats fed 1% nitrogen with either protein or carbohydrate, 4.56–4.60; for those fed 3% nitrogen, 4.78–4.90; and for those fed 6% nitrogen, 5.13–5.23.) Digestibilities of nitrogen and calories were calculated from these data.

At the end of the 14-week experimental period, food was removed overnight (approx 17 hr). All animals, as well as the group killed at the end of 100 days were anesthetized with sodium amytal solution, 2 ml blood were removed from the heart for serum cholesterol determination, and livers and kidneys were removed, trimmed, blotted, and weighed immediately. Then the entire carcass, including the liver and kidneys, was placed in a jar and autoclaved for 15 min at 15 lb pressure. Subsequently each carcass was homogenized with a known quantity of water in a blender and aliquots were analyzed for nitrogen and moisture. Fat was estimated by difference, assuming 3% ash in the animal. Previous estimations of fat by this technique were found to be accurate to within 1% (9).

Statistical analysis. The data were analyzed by the method of least squares, by computer, employing models which evaluated the effects of type of carbohydrate, type and level of protein, and the differential effects of these factors on gross and digestible calorie intakes, nitrogen intakes, body weights, and gains of fat and protein. Because most of these measurements could be affected by initial and final body weight, calorie and nitrogen intakes, or combinations of these factors, the analysis of variance was conducted without and with adjustment of appropriate data for initial weight, gross calorie intake, and both of these variables, final body weight alone and with gross calorie intake. In addition, protein gains were adjusted for differences in digestible nitrogen intakes. The rationale for this type of statistical control of variables is discussed by Snedecor, p. 406 (16). Although this is a departure from the standard use of covariance, this analysis takes advantage of the fact that the treatments *did* affect variables such as calorie intake and body weight. The analysis enables us to determine whether there is any effect on body composition over and above that contributed by weight or food intake. The correction for initial body weight was made to test the assumption that animals varying in weight by more than 100 g at the beginning of the experiment might be expected to utilize the nutrients differently. The method of analysis is described in detail by Harvey (17). It can be stated without deviating too far from fact that indeed there is no control other than statistical that can be imposed in the study of the interrelationships among nutrients since they are not fed singly nor do they act in the body as single entities.

Linear regression lines and equations were computed to provide information on the relationships of changes in body fat and protein to changes in body weight and digestible calorie intake but the data are not presented.

Results. General. The significance of differences for the results obtained after adjustment of the data for initial body weight was similar to that for results of the analysis of variance for unadjusted data. Moreover, the significance of differences obtained after adjustment for gross calorie intake were, in most instances, similar to results when both gross calorie intake and initial weight were considered. Therefore, results of the statistical analyses where adjustments were made for initial weight alone or gross calorie intake alone are not included. In Tables I and II are means for body weight, fat, and protein gains unadjusted and adjusted for initial weight *and* gross calorie intake of rats fed the 12 diets for 14 weeks.

TABLE I. Unadjusted Means of Weights, Intakes and Body Gains of Young Adult Rats Fed for 14 Weeks Varying Kinds and Proportions of Carbohydrate and Protein.

			Intake			Carcass fat		Body gains (g)[b]		
Groups[a]	No. of rats	Init. wt (g)	Food (g)	DC (kcal)	DN (g)	(%)	Total (g)	Wt	Fat	Protein
1% Nitrogen										
S-Lact.	17	401	1649	7175	14.3	26.3	129.5	96	58.0	9.4
CS-Lact.	14	403	1633	7195	13.4	25.8	131.2	111	59.4	13.6
S-WG[c]	18	405	1576	6843	14.2	24.4	103.6	29	31.3	0.6
CS-WG	18	397	1592	7090	14.1	24.9	108.0	41	37.4	1.5
3% Nitrogen										
S-Lact.	17	400	1626	7665	44.9	26.8	139.7	134	68.6	17.3
CS-Lact.[d]	15	395	1505	6956	40.7	20.8	98.3	90	28.3	16.5
S-WG[c]	18	400	1692	7885	49.0	28.1	154.3	158	83.1	19.7
CS-WG	18	401	1563	7233	43.9	24.7	128.0	120	56.5	17.8
6% Nitrogen										
S-Lact.	19	400	1518	7651	81.7	25.2	125.9	115	54.6	15.5
CS-Lact.	18	399	1452	7220	78.4	21.5	104.1	98	33.2	16.9
S-WG[c]	17	403	1601	7948	95.0	26.7	142.1	135	70.2	16.9
CS-WG	17	396	1457	7183	83.9	23.6	115.0	101	44.6	15.0
SE[e]								±7.8	±7.5	±1.4

[a] S, sucrose; CS, cornstarch; Lact., lactalbumin; WG, wheat gluten; DC, digestible calories; DN, digestible nitrogen.

[b] Mean initial fat and protein of 100-day-old rats calculated from regression line (fat or protein, Y vs. weight, X) varied for fat, 70.1 to 72.2 g; and for protein, 72.0 to 73.7 g among the 12 groups. Average composition of control rats, 59.5% water, 19.1% protein, and 18.3% fat.

[c] Some of the data from these rats were used to compare age changes in another report (19).

[d] Fed same diet as control group.

[e] Standard error of experiment, [error mean square/(av number/group)]$^{1/2}$.

Effect of type of carbohydrate. Overall, gross calorie and digestible nitrogen intake, and weight and fat gains were higher for rats fed diets containing sucrose than for rats fed diets containing cornstarch (Table II). No significant differences due to type of carbohydrate were found in *unadjusted* protein gains. Adjusting the body weight gains removed the difference due to type of carbohydrate; but adjusting body fat gains to equal calorie intake did not remove the significance of the difference due to type of carbohydrate (Tables II and III). These results can be interpreted to mean that had the animals weighed the same at the start of the experiment and had had the same gross calorie intakes, both sucrose- and cornstarch-fed rats would have weighed the same but sucrose-fed rats would still have contained more fat than cornstarch-fed rats. Further, under these conditions, cornstarch-fed rats would have gained significantly more protein than sucrose-fed rats.

The results showed that rats fed wheat gluten with cornstarch were fatter than those fed lactalbumin with cornstarch. But rats fed lactalbumin with cornstarch gained significantly more protein than those fed lactalbumin with sucrose.

A significant differential response was obtained when the two types of carbohydrates were evaluated with the different levels of protein. Cornstarch-fed rats had higher gains in protein only at the lowest protein level and sucrose-fed rats had significantly higher fat gains only at the two higher protein levels.

Effect of type and level of protein. Significant differential responses occurred when type and level of protein were considered. The smaller digestible nitrogen intakes of lactalbumin-fed rats were due in part to the

TABLE II. Adjusted Gains in Weight, Fat, and Protein.

Groups	Body gain (g)		
	Wt[a]	Fat[a]	Protein[a]
1% Nitrogen			
S-Lact.	99 ± 5	58.7 ± 6.6	9.8 ± 1.2
CS-Lact.	119 ± 6	61.6 ± 7.3	14.7 ± 1.3
S-WG	46 ± 5	37.4 ± 6.7	3.2 ± 1.2
CS-WG	52 ± 5	42.3 ± 6.5	2.7 ± 1.2
3% Nitrogen			
S-Lact.	122 ± 5	63.9 ± 6.7	15.7 ± 1.2
CS-Lact.	106 ± 6	35.3 ± 7.1	18.3 ± 1.3
S-WG	137 ± 5	75.0 ± 6.7	17.0 ± 1.2
CS-WG	125 ± 5	58.2 ± 6.4	18.5 ± 1.2
6% Nitrogen			
S-Lact.	104 ± 5	50.4 ± 6.3	14.1 ± 1.1
CS-Lact.	103 ± 5	35.6 ± 6.4	17.6 ± 1.2
S-WG	113 ± 6	60.6 ± 6.9	14.1 ± 1.3
CS-WG	106 ± 5	47.5 ± 6.6	15.6 ± 1.2

[a] Adjusted for initial body weight and gross calorie intake.

lower digestibility of nitrogen of lactalbumin diets when compared with wheat gluten at all levels of protein feeding. As expected, lactalbumin was the superior protein for deposition of protein but this was due to differences only at the lowest level of feeding.

Mean gains in weight, fat, and protein were larger for lactalbumin-fed rats at the lowest protein level. Gains in weight and fat shifted to favor wheat gluten over lactalbumin at the two higher levels of protein.

The results of the linear regression analysis to predict storage of fat or protein calories from digestible calorie intake indicated that the relationships between storage of fat and protein calories vs. digestible calorie intakes depended on the particular combination of nutrients.

Discussion. As was shown from previous work in this and other laboratories, it is not sufficient to measure weight changes in experimental animals in relation to food intake or type of diet without considering that *composition* of the changes is different and important. That *source* of carbohydrate calories influences the *amount* of fat stored, in the presence of adequate protein, was pointed out previously (9) in 400-day-old rats; 200-day-old rats of that study, fed cornstarch, were not analyzed and comparisons could not be made. Young adult rats (200 days old) in the present study, fed the *lowest* protein level with sucrose or cornstarch showed small differences which were not statistically significant in average percentage fat or total gain in fat but showed consistent differences in favor of sucrose at the higher protein levels. As expected, lactalbumin was superior to wheat gluten for protein formation, but only at the lowest protein level. The observation that *adult* rats fed sucrose ate more and deposited more body fat than those fed cornstarch is similar to that of Feyder (7), and Allen and Leahy (12). Wiener *et al.* (10) and Romberg and Benton (11) found that *young* rapidly growing rats fed inadequate protein levels deposited more fat and less protein when the dietary carbohydrate was cooked starch than when sucrose was fed. Desai[2] (1967) showed that dextrin-fed young growing rats deposited more fat than sucrose-fed rats even when adequate levels of protein were fed.

It seems most likely that these apparently conflicting results can be attributed to many factors, among which are age, size, and strain of rat, as well as differences in dietary ingredients, e.g., raw vs. cooked cornstarch. Obviously, mechanisms involved in the control of food intake and fat storage are different in animals at different stages of development. That the food consumption of the adult animals in the present study varied with kind and level of protein as well as with the association of these factors indicates that there is no simple mechanism governing the interaction of these nutrients. Equal digestible calorie intakes did not always result in equal gains in fat or protein. In other words, the increase in fat storage in animals fed some combinations of nutrients was greater than could be accounted for by calorie intakes alone. An explanation for this apparent phenomenon has been advanced previously (12). Differences in metabolic pathways, when sucrose vs. starch are fed, may occur because of the more favorable environment

[2] Desai, K. S., unpublished thesis, Cornell University, 1967.

TABLE III. Level of Probability and Significance of Differences among Treatment and Interaction Means for Data from Analysis of Variance Unadjusted and Adjusted for Initial Weight and Gross Calorie Intake.

	Intake		Body gain (g)					
	GC[a]	DN[a]	Wt		Fat		Protein	
Source of variation	Unadjusted		Unadj.	Adj.	Unadj.	Adj.	Unadj.	Adj.
Carbohydrate (C)-type	.01	.01	.01	NS	.01	.01	NS	.01
a. Sucrose b. Cornstarch	a>b	a>b	a>b		a>b	a>b		a<b
Protein (P)-type	NS	.01	.05	.01	NS	NS	.01	.01
c. Lactalbumin d. Wheat gluten		c<d	c>d	c>d			c>d	c>d
Protein-level	.01	.01	.01	.01	NS	NS	.01	.01
e. 1% N f. 3% N g. 6% N	e<f,g f=g	e<f f<g	e<f,g f>g	e<f,g f>g			e<f,g f=g	e<f,g f=g
Type C × type P	NS	NS	NS	NS	NS	NS	NS	.05
Type C × level P	.05	.01	.01	.01	.01	.05	NS	NS
Type P × level P	NS	.01	.01	.01	.01	.01	.01	.01

[a] GC, gross calories; DN, digestible nitrogen.

for fat synthesis when the hexose monophosphate shunt is used to a larger extent than other glycolytic pathways. That oxidation of sucrose favors the hexose monophosphate pathway has been suggested (18). It is reasonable to assume that the young rapidly growing animal would differ from adult animals in the use of metabolic pathways. Energy demands for growth are well known to exceed those required for maintenance.

Summary. To study the interrelationships among dietary carbohydrates and proteins upon the production of body fat and protein, 12 groups of young adult male rats (200 days old) were fed, for 14 weeks, purified diets with sucrose and 1, 3, or 6% nitrogen from lactalbumin or wheat gluten, or corresponding diets containing cornstarch. Sucrose-fed rats ate more and gained significantly more weight and fat than cornstarch-fed rats only when fed the two higher levels of protein. After adjustment of the data to take into account differences in initial body weight and calorie intake, fat gains were still significantly higher in sucrose-fed animals. More efficient utilization of protein in cornstarch-fed rats was indicated but only at the 1% level of nitrogen. The data indicated that changes in body composition of adult rats are dependent to a larger extent upon specific combinations of nutrients than upon weight changes and calorie intakes.

1. Hamilton, T. S., J. Nutr. **17**, 565 (1939).

2. Forbes, E. B., Black, A., Thacker, E. J., and Swift, R. W., J. Nutr. **20**, 47 (1940).

3. MacKay, M., Barnes, R. H., and Carne, H. O., Am. J. Physiol. **135**, 193 (1941).

4. Bender, A. E. and Doell, B. H., Brit. J. Nutr. **11**, 138 (1957).

5. Meyer, J. H., Am. J. Physiol. **193**, 488 (1958).

6. Hegsted, D. M. and Chang, V.-O., J. Nutr. **85**, 159 (1965).

7. Feyder, S., J. Nutr. **9**, 457 (1935).

8. Harper, A. E. and Elvehjem, C. A., J. Agr. Food Chem. **5**, 754 (1957).

9. Marshall, M. W., Hildebrand, H. E., Dupont, J. L., and Womack, M., J. Nutr. **69**, 371 (1959).

10. Wiener, R. P., Yoshida, M., and Harper, A. E., J. Nutr. **80**, 279 (1963).

11. Romberg, B. and Benton, D. A., J. Nutr. **86**, 289 (1965).

12. Allen, R. J. L. and Leahy, J. S., Brit. J. Nutr. **20**, 339 (1966).

13. Allison, J. B., J. Am. Med. Assoc. **164**, 283 (1957).

14. Marshall, M. W. and Hildebrand, H. E., J. Nutr. **79**, 227 (1963).

15. Jones, J. H. and Foster, C., J. Nutr. **24**, 245 (1942).

16. Snedecor, G. W., "Statistical Methods," 5th ed., Iowa State Univ. Press, Ames, Iowa (1956).

17. Harvey, W. R., "Least-Square Analysis of Data", 157 pp., U. S. Dept. Agr. Publ. **20-8**, (1960).

18. Hodges, R. E. and Krehl, W. A., Am. J. Clin. Nutr. **17**, 334 (1965).

19. Womack, M., Marshall, M. W., and Hildebrand, H. E., J. Gerontol. **19**, 45 (1964).

Received March 18, 1969. P.S.E.B.M., 1969, Vol. 132.