"Metabolic Memory":[1] Effects of Diets, Fed During the First Refeeding, on Hepatic Enzyme Responses, During a Second Starvation–Refeeding[2] (37170)

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The activities of hepatic glucose-6-phosphate dehydrogenase [(G6PD) (D-glucose-6-phosphate:NADP oxidoreductase (EC 1.1.1.49)], and malic enzyme [(ME) (malic decarboxylating oxidoreductase (EC 1.1.1.40)] undergo a striking increase when starved rats are fed a high-carbohydrate, adequate-protein, and low-fat diet (1–4). The increase in enzyme activity requires adequate dietary protein (3, 5–7) and relatively high levels of dietary carbohydrate (8), and it is inhibited by high levels of dietary fat (9). It was found at this laboratory that a second episode of starvation–refeeding leads to an even greater enzyme response than is observed after one starve-refeed episode (10, 11). In the studies reported here the increased inducibility of enzyme activity observed during the second refeeding was affected by the diet refed during the first refeeding.

Procedure. Specific pathogen-free, male Wistar rats were purchased from Purina Laboratory Animals of Vincentown, NJ. The animals were shipped in such a manner as to avoid more than a slight drop in food and water consumption and more than a minimal change in temperature during shipment. These precautions were taken because we found that stress during shipment altered the animals' response to subsequent dietary manipulations. Rats were housed individually in screen-bottom, stainless steel cages and had free access to distilled water.

One group of rats was fed 4 days a diet containing 65% glucose, 25% casein, 5% corn oil, 4% minerals[3] and 1% vitamins.[4] These rats were designated as control.[5] Enzyme levels in excess of the control value would be referred to as an enzyme overshoot.

Two starve–refeed procedures were used. In one procedure (one cycle starve–refeed regimen, referred to as group 1), rats were starved 2 days and refed 3 days the 65% glucose diet. In the other procedure (two cycles of starvation–refeeding, groups 2, 3, 4, and 5), rats were starved 2 days, refed 2 days one of four diets, starved 2 days again and refed the 65% glucose diet for 3 days. The diets refed after the first starvation were as follows: group 2 was refed the 65% glucose diet; group 3 was refed a high-fat diet containing 20% glucose, 25% casein, 22.5% beef tallow, 22.5% lard, 5% corn oil, 4% mineral salts,[3] and 1% vitamins;[4] group 4 was refed a diet in which all the carbohydrate was replaced by casein; and group 5 was refed a diet in which all the protein was replaced by glucose. The latter two diets contained 5% corn oil, 4% minerals,[3] and 1% vitamins,[4] in addition to the glucose or casein. The difference between

[1] The term "metabolic memory" is used here in a limited sense to denote an effect by a diet during one dietary manipulation on the effect of another diet during a subsequent dietary manipulation.

[2] Mention of a proprietary product does not necessarily imply endorsement by the U.S. Department of Agriculture.

[3] Jones and Foster Salt Mix, Nutritional Biochemical Co., Cleveland, OH.

[4] Vitamin Diet Fortification Mixture, Nutritional Biochemical Co., Cleveland, OH.

[5] The rationale for the selection of the control value is as follows: Feeding the 65% glucose diet *ad libitum* to chow-fed rats will increase G6PD and ME activities. Comparison of the starved–refed rats to those fed the 65% glucose diet *ad libitum,* therefore, removes the effect due to the diet.

the enzyme levels in groups 1 and 2 is the measure of the increased inducibility of enzyme activity due to a second starve–refeed episode. The requirement for carbohydrate for the increased inducibility can be tested by comparing groups 1, 2, and 4: if group 4 did not differ from group 2, then carbohydrate was not required during the first refeeding for the observation of increased inducibility during the second refeeding. On the other hand, if carbohydrate were required then the enzyme levels in group 4 would be different (less) than in group 2; group 4 would be like group 1 if the carbohydrate requirement were absolute. By a similar process the requirement for protein can be tested by comparing groups 1, 2, and 5. The effect of feeding a high-fat diet during the first refeeding on the response to a subsequent starve–refeed episode can be tested by comparing groups 1, 2, and 3. No difference between groups 2 and 3 would indicate no effect due to the feeding of the high-fat diet during the first refeeding on the subsequent starve–refeed response. On the other hand, if groups 1 and 3 were the same then this would indicate that the high-fat diet can prevent the increased inducibility.

Rats were killed in the early morning by decapitation. The livers were removed, blotted, chilled, and weighed. The preparation of liver homogenates and enzyme assays have been previously described by Freedland (12). Enzyme activity is expressed as units per 100 g body wt. The data can be converted to units per gram of wet liver by dividing each value by the appropriate value of RLS (relative liver size) given in Table II. One unit of enzyme was defined as that amount of enzyme which can produce 1 μmole measured product (NADPH)/min under the conditions of the assay. Differences were tested by Student's t test, and differences with p values smaller than 0.05 are referred to as statistically significant.

Results and Discussion. Ad libitum-fed rats consumed 7.5 to 8.5 g/100 g body wt/day of the 65% glucose diet and increased their body size by 3 to 5%/day (data not shown). Earlier experiments gave similar results (13). The body weight changes and food intakes of rats subjected to the various starve–refeed regimens are summarized in Table I. The actual body weights in grams and food intakes in grams per rat per day are tabulated. However, we consider the percentage of original body weight and the grams of food eaten per day per 100 g body weight more meaningful for the purpose of comparison. The reasons for this should be apparent: food intakes (expressed as g/rat or body weight changes expressed as g/rat) are affected by two things—body size and treatment (or type of food). By expressing the results on the basis of a standard body weight, the effect of body weight on these parameters is at least minimized.

Rats lost from 15 to 20% of their original body weight during the first 2-day starvation period. The average weight loss was 18%. After refeeding for 2 days, rats in groups 2 and 3 (refed the 65% glucose or the 50% fat diet) regained approximately two-thirds of the weight lost during starvation. Rats refed the 90% casein diet (group 4) gained back approximately one-third of the weight lost during starvation, while rats refed the 90% glucose diet (group 5) did not appreciably increase their weight. A second starve–refeed cycle led to a weight loss which averaged 11 to 15% of the body weight after the first refeeding. For example (see Table I), in group 2 the average body weight after the second starvation period was 159 g. This corresponds to 82.9% of the original, prestarvation weight (191 g) and 86.4% of body weight measured after the 2-day refeeding (184 g). For groups 3, 4, and 5 the corresponding values (% of the body weight measured after the 2-day refeeding) were 89.2, 85.4, and 86.1%, respectively. It appears, therefore, that while groups 4 and 5 recovered less body weight during the first refeeding, the weight losses during the second starvation, as percentage of the body weight immediately preceding starvation, were similar in all the groups studied. The experiments indicate that the effect of starvation is not influenced by the diet fed before starvation but may be influenced (decreased) by a previous starve–refeed regimen. Conversely, the weight gained during the second refeeding (as % of the body weight immediately preceding refeed-

TABLE I. Body Weights and Food Intakes of Rats Subjected to Repeated Starvation–Refeeding.

Group[a]	No. of rats	(0) Body wt at start (g)	Body wt: First starve–refeed cycle (St. 1) Body wt after 2 days starvation, g	(St. 1) % of (0)	(Fed 1) Body wt after refeeding for 2 days, g	(Fed 1) % of (0)	Second starve–refeed cycle (St. 2) Body wt after second 2-day starvation period, g	(St. 2) % of (0)	(Fed 2) Body wt after refeeding for 3 days, g	(Fed 2) % of (0)
2[a]	(5)	191 ± 9[b]	160 ± 9	83.3 ± 1.0	184 ± 9	96.0 ± 0.6	159 ± 9	82.9 ± 1.1	196 ± 8	102.5 ± 0.7
3	(5)	173 ± 2	141 ± 2	81.8 ± 0.7	167 ± 2	96.8 ± 1.1	149 ± 2	86.3 ± 0.7[c]	181 ± 3	105.0 ± 1.8
4	(3)	177 ± 4	145 ± 3	81.8 ± 1.0	160 ± 2	89.9 ± 1.5[c]	136 ± 4	76.8 ± 0.4[c]	174 ± 3	98.2 ± 1.9[c]
5	(3)	174 ± 4	141 ± 5	81.2 ± 0.8	145 ± 3	83.5 ± 0.3[c]	125 ± 7	71.9 ± 4.1[c]	155 ± 8	89.1 ± 2.5[c]

Group[a]	No. of rats	Food intake: First refeeding, Food (g/rat/day)	First refeeding, Food (g/100 g body wt/day[d])	Second refeeding, Food (g/rat/day)	Second refeeding, Food (g/100 g body wt/day[d])
2[a]	(5)	16.7 ± 0.5	10.6 ± 0.4	19.9 ± 0.2	12.7 ± 0.7
3	(5)	15.2 ± 0.7	10.8 ± 0.4	17.1 ± 0.8	11.5 ± 0.5
4	(3)	14.7 ± 0.7	10.1 ± 0.5	18.7 ± 0.7	13.7 ± 0.7[e]
5	(3)	12.5 ± 1.3	8.8 ± 0.7[c]	17.8 ± 1.0	14.3 ± 1.0[e]

[a] Groups 2 to 5 were starved 2 days, refed 2 days one of 4 different diets, starved for 2 days again and refed for 3 days the 65% glucose diet. The diet refed during the first refeeding episode was as follows: group 2 was fed the 65% glucose diet, group 3 was fed the 50% fat diet, group 4 was fed the 90% protein diet, and group 5 was fed the 90% glucose diet.

[b] SEM.

[c] Differs significantly ($p < .05$) from group 2.

[d] The food intake in this column was calculated by taking the average daily food intake in grams × 100 and dividing by the body weight at the beginning of the period during which the food intake was measured.

[e] Differs significantly ($p < .05$) from group 3, but not from group 2.

ing) was different: 23.2% in group 2; 20.8% in group 3; 27.9% in group 4; and 24.0% in group 5. It appears, therefore, that rats which regained less weight during the first refeeding, because of the diet refed, gained relatively more during the second refeeding when all groups were refed the same diet.

Food intakes are summarized in Table I. The values obtained and tabulated as grams of food eaten per 100 g body weight/day agree with the expectations deducible from the changes in body weight. For example, during the first refeeding rats refed the 90% glucose diet (group 5) ate significantly less than the other groups and, therefore, gained less weight. However, group 4, which was refed the 90% protein diet, had eaten about as much as groups 2 and 3 but gained significantly less weight. The tendency of a very high-protein diet to reduce body weight gain has already been documented (14). We compared the food intakes during the second refeeding to each other and to the food intake of group 2 during the first refeeding. It was found that group 2 ate significantly more during the second refeeding than during the first refeeding. This indicates that the starve–refeed regimen alters (magnifies) the response to a second starve–refeed regimen. The food intake of group 3 during the second refeeding was not significantly different from the food intake of group 2 during the first refeeding. This would indicate that the "metabolic memory" caused by the first cycle of starvation–refeeding as affecting food intake during a second cycle of starvation–refeeding is abolished by interposing a high-fat diet between the 2 starvation episodes. This is again borne out by the fact that the food intakes of groups 4 and 5 during the second refeeding were larger than the food intake in group 3 during the same period.

The enzyme data are summarized in Table II. One cycle of starvation–refeeding (group 1) caused a large and significant increase in G6PD and ME activities. A second cycle of starvation–refeeding (group 2) led to even higher levels of G6PD and ME. This increased inducibility was abolished by feeding the 50% fat diet during the first refeeding (group 3) but not by the 90% casein (group 4) or 90% glucose diet (group 5).

There are interesting parallels between food intake, body weight changes, and liver enzyme activities. For example, starvation–refeeding increased food intake, weight gain, and enzyme activity. A second starve–refeed episode increases food intakes and enzyme levels even further. If the diet refed during refeeding contains large amounts of fat, then

TABLE II. Enzyme Responses to a Second Starve–Refeed Episode as Influenced by the Diet Refed in the First Starve–Refeed Episode.

Treatments[a]	No. of rats	Body wt at killing (g)	RLS[b]	units[c]/100 g body wt G6PD[b]	ME[b]
Control	(5)	246 ± 7[d]	5.05 ± 0.01	49.9 ± 2.6	21.2 ± 1.5
Group 1[a]	(5)	198 ± 6	5.62 ± 0.22[e]	113 ± 7[e]	56.7 ± 4.9[e]
Group 2	(5)	196 ± 8	6.40 ± 0.17[ef]	160 ± 7[ef]	81.3 ± 4.7[ef]
Group 3	(5)	181 ± 3	5.80 ± 0.22[e]	122 ± 5[e]	64.1 ± 3.2[e]
Group 4	(3)	174 ± 1	7.11 ± 0.22[ef]	183 ± 9[ef]	93.6 ± 7.9[ef]
Group 5	(3)	155 ± 8	7.37 ± 0.17[ef]	184 ± 14[ef]	115 ± 13[ef]

[a] Treatments: control rats were fed ad lib. for 4 days the 65% glucose diet, group one was starved 2 days and refed 3 days the 65% glucose diet. The treatments of groups 2 to 4 were described in footnote a of Table I.

[b] Abbreviations: RLS = relative liver size = (liver wt × 100)/(body wt); G6PD = glucose-6-phosphate dehydrogenase; ME = malic enzyme.

[c] For the definition of enzyme activity see "Procedure."

[d] SEM.

[e] Greater than control value ($p < .05$).

[f] Greater than corresponding value in group 1 ($p < .05$).

the increased enzyme inducibility during a second starve–refeed episode is inhibited, and the food intake is no longer larger during the second refeeding than during the first refeeding. These parallels between food intake, body weight gain, and enzyme levels may be a reflection of the underlying adaptive processes.

In examining the effects of diet during the first cycle of starvation–refeeding, we conclude that neither dietary protein nor dietary carbohydrate is required during the first refeeding for the increased enzyme inducibility during a second cycle of starvation–refeeding. On the other hand, the increased inducibility during the second starve–refeed cycle can be abolished by feeding a high-fat diet during the first starve–refeed cycle.

Summary. A second starvation–refeeding episode increases food intake and G6PD and ME activities compared with one cycle of starvation–refeeding. The increases can be obtained even if dietary protein or carbohydrate is omitted during the first refeeding, but not if a high-fat diet is fed during the first refeeding. The parallel changes in food intake, body weight changes, and enzyme responses suggest a basic, underlying adaptive response to caloric restriction which may have important nutritional implications.

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