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Effect of Age on the Responses to Starvation–Refeeding in the Rat (38310)

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Aging in animals appears to be accompanied by a decrease in the activities of a number of enzymes (1–16). Some of the changes associated with aging include an impaired intestinal absorption of carbohydrates (17, 18), a decrease in glucose tolerance (19) and decreased responsiveness of tissue to insulin (20), testosterone (21) and lypolytic stimuli (22, 23). The deteriorations in the responses to dietary and hormonal stimuli, however, are not confined to the pathways of glycolysis and lipid metabolism alone, as evidenced by the impaired response of glucose 6-phosphatase and fructose 1,6-diphosphatase to hormonal stimulation (24) and the impairment in the response of tryptophan pyrrolase to trytophan injection (9–11).

Previously, Adelman observed that the recovery of glucokinase activity to prestarvation levels was impaired in starved–refed, aged rats (14). This recovery was improved by the administration of large doses of insulin. The question, whether the failure of glucokinase recovery in the aged rat was due to a lack of insulin response to refeeding or to a lack of sensitivity by the tissue to insulin, was left unanswered. We have investigated the possible role of insulin on the response of glucose 6-phosphate dehydrogenase and malic enzyme to starvation–refeeding (25). Both enzymes reached higher activities than found in rats fed *ad libitum*. This "enzyme overshoot" was inhibited by 8-azaguanine. The effect of 8-azaguanine on malic enzyme activity was partially reversed (*i.e.*, malic enzyme activity was increased) by the administration of exogenous insulin. In the experiments reported herein the starve–refeed response was used to test whether the impairment in enzyme response with age is due to a lack of insulin response or a decrease in tissue sensitivity to insulin.

Materials and Methods. Specific pathogen-free male Wistar rats weighing 125–150 g (henceforth referred to as young rats) or 275–400 g (henceforth referred to as older rats) were provided during shipment with a food packet containing 70% water and 30% ground chow. This precaution was taken in order to minimize the possibility of starvation during shipment, since subsequent starvation has been reported to cause a larger enzyme overshoot (26). Following receipt, the animals were equilibrated to laboratory conditions for at least three days. The animals were housed individually in screen-bottom cages and had unlimited access to water.

Rats fed *ad libitum* were given either a commercial chow preparation or a semisynthetic diet containing 65% glucose,[1] 25% casein, 5% corn oil, 1% vitamin mix (27) and 4% Jones–Foster salt mix (28). Starved–refed rats were starved two days, and then were refed for two days the 65% glucose diet or a 90% casein diet; the latter group was then refed the 65% glucose diet for two more days. The 90% casein diet contained the same percentage and components as the 65% glucose diet, except that the glucose was replaced with casein. Two groups of older rats were refed a 65% sucrose diet immediately after starvation instead of the 65% glucose diet.

When administered, 8-azaguanine[2] (7.5 mg/0.5 ml, in dil NaOH, pH 10) was injected at 12 hr intervals beginning at the time when the

[1] Food components were purchased from Nutritional Biochemical Company of Cleveland, OH. Mention of a trademark or proprietary product does not constitute a gurarantee or warranty of the product by the U. S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

[2] 8-Azaguanine was purchased from the K and K Company of Plainview, NY.

rats were refed. Insulin (Iletin[3], protamine zinc insulin) was injected subcutaneously in a volume of 0.1 ml. Insulin injections contained 0.5 or 1.0 IU/dose, and were given once a day during the evening hours.

Rats were killed by decapitation or heart puncture. Blood was collected by heart puncture for the assay of serum immunoreactive insulin (29). The liver was quickly removed, blotted, chilled and weighed. A 1.0 g sample was used for the assay of glucose 6-phosphate dehydrogenase and malic enzyme activities by procedures previously described (30). The remaining liver was used for the determination of total liver lipid by a modification[4] of a previously described acid extraction method (31).

Enzyme activity is expressed as units per 100 g body wt. One unit of enzyme was defined as that amount of enzyme which can produce 1 μmole of measured product/min under the conditions of the assay. The justifications for reporting enzyme activity on the basis of standard body weight have been previously presented (32). Total liver lipid is expressed as mg/100 g body wt. Insulin values are reported as μunits/ml serum. Differences were tested by Student's t test.

Results and Discussion. Young rats lost an average of 15.4% body wt during starvation, whereas the weight loss in the older rats was 8.2% (Table I). During refeeding, young rats ate 11.0 g food/day/100 g body wt, whereas older rats ate 7.3 g food/100 g body wt. Both the young and the older, heavier rats returned to the same percentage of prestarvation weight after two days of refeeding.

Feeding the 65% glucose diet *ad libitum* caused a small increase in glucose 6-phosphate dehydrogenase and malic enzyme activities in young rats, above that observed when the chow diet was fed, as shown in Table II. Only glucose 6-phosphate dehydrogenase was increased by the 65% glucose diet in the older rats. None of these differences were statistically significant ($P > 0.05$). Starvation of rats prefed the chow diet reduced enzyme activities, and relative liver size and total liver lipid (Table II, III) by approximately the same amount in both the young rats as well as in the older rats. Refeeding the 65% glucose diet led to a glucose 6-phosphate dehydrogenase overshoot the extent of which was the same in both the young and older rats. The malic enzyme overshoot and the elevation in total liver lipid were, however, much less in older rats than in young rats. Treatment with azaguanine during refeeding prevented the overshoot in enzyme activities and total liver lipid but allowed recovery up to the amounts found in *ad libitum*-fed rats. Treatment of young rats with insulin in addition to azaguanine, caused a partial restoration of the malic enzyme overshoot but did not alter glucose 6-phosphate dehydrogenase activity. In older rats insulin actually caused a decrease in both enzyme levels and total liver lipid.

It may be reasoned that, since the food intake of older rats during refeeding was considerably less than that of the young rats (Table I), the reduction of the malic enzyme and total liver lipid response in the older rats may have been due to the reduction in food intake. However, in previous experiments (33, 34) it was found that reducing the food intake of young rats to a level comparable to that of the older rats used in the experiments reported herein, failed to diminish the enzyme overshoot. The results seem to indicate, therefore, that the malic enzyme response to dietary glucose or exogenous insulin is less in older rats than in young rats and that this difference is not due to reduced food intake.

It has been reported that in starved–refed rats treated with azaguanine, the absence of enzyme overshoot is accompanied by an inhibition of increased insulin release during the evening (25). The extent of insulin release was, therefore, studied in the young and older rats refed the 65% glucose diet with or without azaguanine treatment (Fig. 1). In young rats elevated insulin levels were noted 12 hr after refeeding. This elevation was abolished if the young rats were treated with azaguanine ($P < 0.05$). In older rats the response in serum insulin following refeeding was even higher than in the young rats but the 12 hr refed values were not decreased by azaguanine. The higher level of serum insulin in older Wistar rats is in agreement with earlier findings (20) and also with the report of increased insulin storage capacity, increased

[3] Protamine Zinc Insulin (Iletin) was purchased from the Eli Lilli Company of Indianapolis, IN.

[4] The procedure to determine total lipids has been modified by E. S. Conway and D. M. Adams (Agricultural Research Service, Human Nutrition Research Division, Beltsville, MD) to make it applicable for the extraction of wet tissue. A detailed description of the modifications has been submitted to the Association of Official Analytical Chemists, Washington, DC.

TABLE I. Comparison of Body Weight Changes and Food Intake of Young and Older Rats.

Size	Young (125–200 g)	Older (275 and larger)
Percent body wt lost during 48 hr starvation	15.4[a] ± 1.1	8.2 ± 0.7 ($P < 0.05$)
Percent of original body wt after 48 hr refeeding	95.8 ± 7.8	95.9 ± 5.0
Food intake, g food/100 g body wt/day (rats were fed the 65% glucose diet)	11.0 ± 0.8	7.3 ± 0.5 ($P < 0.05$)

[a] Body wt changes and food intakes were measured in 12 rats per group.

serum insulin levels in the blood and decreased sensitivity to insulin in mature Wistar males[5]. The lack of effect of azaguanine on serum insulin levels of older rats indicates that insulin release in these rats, or perhaps even insulin production, is independent of *de novo* RNA synthesis, at least during the 12 hr period following refeeding.

The effect of interposing a high-protein, carbohydrate-free diet between starvation and feeding the 65% glucose diet was tested in both young and older rats. The results were similar to those reported previously (25, 26, 35). Enzyme induction was similar whether or not the high-protein diet was interposed (data not shown).

The response of malic enzyme and total liver lipid to starvation–refeeding appeared less in the older than in the younger rats. To ascertain if the responses in the older rats represented a maximum response rats were refed a 65% sucrose diet. It was previously reported that the response of these enzymes to dietary sucrose is greater than the response to dietary glucose (35). We, too, found that in the older rats the response to sucrose was greater than the response to glucose (Table II). This would indicate that even though the ability to respond to starvation–refeeding was decreased in the older rats the system was capable of a greater response. Hence, it may be concluded that with aging the ability to respond to starvation–refeeding decreases possibly along with a decrease in sensitivity to a number of other stimuli (19–23).

A possible explanation of the decreased response of malic enzyme and total liver lipid to starvation–refeeding in the older rats may be that older rats are less responsive to hormones. The decreased response to insulin injection (Table II) and the adequate levels of insulin in the older rats (Fig. 1) would certainly indicate that if insulin is involved in the decreased response of the older rats to starvation–refeeding, the involvement is at the level of sensitivity to the hormone.

Summary. The responses of young (150–200 g) and older (275–400 g) rats to

[5] Berdanier, C. D., P. B. Moser and D. L. Trout, 1971. Insulin status of the carbohydrate sensitive BHE rat. Fed. Proc. **30**, 579.

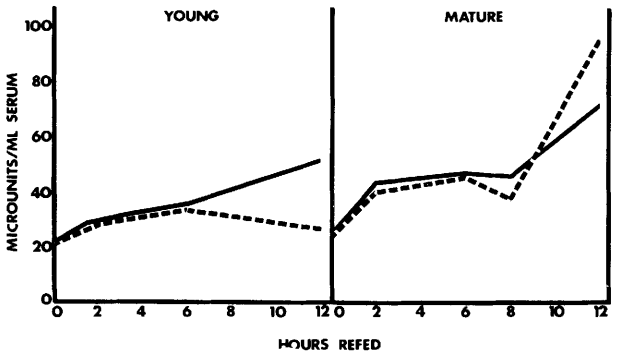

FIG. 1. Insulin response of young and older rats to refeeding the 65% glucose diet: ————, control; — — — — — —, azaguanine-treated. Each point represents the average of six animals. Vertical bars represent he SEM.

TABLE II. The Response of Young and Older Rats to Starvation and Refeeding: Changes in Glucose 6-Phosphate Dehydrogenase and Malic Enzyme Activities.

	Young rats (150–220 g)					Older rats (280 g and larger)				
Treatment	8AG[a]	Wt at killing (g)	RLS	G6PD	ME	8AG	Wt at killing (g)	RLS	G6PD	ME
Fed *ad libitum*										
chow	—	141 ± 5[b]	4.7 ± 0.1	23.5[c] ± 3.1	14.5 ± 2.1	—	291 ± 16	4.4 ± 0.1	22.2 ± 4.2	11.5 ± 1.0
65% glucose	—	212 ± 8	5.7 ± 0.2	32.1 ± 4.7	18.1 ± 3.7	—	296 ± 7	4.4 ± 0.1	28.4 ± 4.1	11.0 ± 1.0
Starved, 48 hr	—	163 ± 7	3.2 ± 0.1	13.1 ± 3.9	5.2 ± 1.0	—	267 ± 7	2.8 ± 0.1	18.4 ± 0.8	4.3 ± 0.7
Refed, 48 hr										
65% glucose	—	167 ± 7	5.8 ± 0.1	63.8[e] ± 7.1	37.1[e] ± 3.0	—	311 ± 5	5.1[e] ± 0.1	62.6[e] ± 7.5	20.1[e] ± 3.0
	+	169 ± 5	6.0 ± 0.1	23.7 ± 6.1	13.7 ± 3.5	+	295 ± 7	5.3[e] ± 0.1	25.2 ± 4.5	13.3 ± 3.4
insulin[d]	+	180 ± 3	6.7[f] ± 0.3	24.7 ± 2.1	25.5[f] ± 1.7	+	314 ± 10	4.9[e] ± 0.2	17.5 ± 3.1	8.6 ± 1.2
65% sucrose	—						317 ± 4	5.7[e] ± 0.1	86.9[e] ± 4.9	35.7[e] ± 2.0
65% sucrose	+						337 ± 7	5.6[e] ± 0.2	48.7[e] ± 7.1	24.6[e] ± 5.0

[a] Abbreviations: 8AG = 8-azaguanine; RLS = relative liver size = (liver wt × 100)/(body wt); G6PD = glucose 6-phosphate dehydrogenase; ME = malic enzyme.

[b] S.E. of mean. Numbers represent the average of 5 animals.

[c] Enzyme activity is expressed as units per 100 g body wt. One unit of enzyme activity was defined as that amount of enzyme which can produce 1 μmole measured product per min under the conditions of the assay.

[d] Insulin (0.5 U per dose) was injected subcutaneously at 12 and 36 hr after the rats were given food.

[e] Values greater than in rats fed the 65% glucose diet *ad libitum* ($P < 0.05$).

[f] Insulin effect statistically significant ($P < 0.05$).

TABLE III. Relationship Between Dietary Treatment and Total Liver Lipid.

Treatment	8AG[a]	wt at killing (g)	Total liver lipid[b]
		Young rats (150–200 g)	
Fed *ad libitum* (65% glucose diet)	—	180 ± 7[c]	181 ± 10
Starved 48 hr	—	163 ± 7	126[d] ± 9
Refed 48 hr			
65% glucose	—	167 ± 7	507[e] ± 34
65% glucose	+	169 ± 5	185 ± 15
		Older rats (275 g and larger)	
Fed *ad libitum* (65% glucose diet)	—	291 ± 16	173 ± 4
Starved 48 hr	—	267 ± 7	127[d] ± 8
Refed 48 hr			
65% glucose	—	311 ± 5	397[e] ± 30
65% glucose insulin	+	412 ± 10	237[e] ± 20
65% glucose	+	295 ± 7	163 ± 9
65% sucrose	—	317 ± 4	488[e,f] ± 28
65% sucrose	+	335 ± 7	273[e,f] ± 45

[a] Abbreviations: 8AG = 8-azaguanine.
[b] Mg/100 g body wt.
[c] SE of mean. Numbers represent the average of 5 animals.
[d] Values significantly less than in *ad libitum*-fed rats ($P < 0.05$).
[e] Values significantly greater than in *ad libitum*-fed rats ($P < 0.05$).
[f] Values significantly greater than corresponding values in *ad libitum*-fed rats or starved rats refed the 65% glucose diet ($P < 0.05$).

starvation–refeeding was studied. In younger rats 2 days of feeding a 65% glucose diet, following 2 days of starvation, caused glucose 6-phosphate dehydrogenase, total liver lipid to reach levels between 2–3 times higher than in rats fed the same diet *ad libitum*. In older rats the responses of malic enzyme and total liver lipid were less than in the younger rats. The enzyme responses and the responses in total liver lipid were inhibited by 8-azaguanine injections in both age groups. The malic enzyme response in azaguanine–treated rats could be partially restored by exogenous insulin in the young, but not in the older rats. Older rats responded more to dietary sucrose than to dietary glucose. Since older rats appeared to have at least as much serum immunoreactive insulin as did the younger rats, it was concluded that a probable cause of the lesser response in the older rats to starvation–refeeding may be a decreased sensitivity to hormones such as insulin.

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