A SIGNIFICANT STRAIN DIFFERENCE IN "BIOCHEMICAL AGING" OF RATS

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Summary

We have measured the time course of induction of glucokinase in response to glucose refeeding following a fast. Old Charles River males (Sprague-Dawley) show a significantly greater lag before initiation of enzyme induction than do Wistar males of the same age. Female Wistar rats show no age dependent decrement in responsiveness between 4 and 14 months of age.

Adelman has recently reported the results of several studies using the adaptation of liver enzymes as a biochemical probe of aging (1). He cites 20 different enzymes for which a relative decrease in adaptation occurs as a function of age. Detailed analysis of several of these indicates a lag period proportional to age before response but no decrement in ultimate response level.

While studying the effects of lithium administration on carbohydrate metabolism we examined the response to refeeding of glucokinase in young and old rats of the Wistar and the Sprague-Dawley strains (2). We have found major differences between our strain (Wistar) and that used by Adelman (Sprague-Dawley). The apparent aging in our strain is much less than he has reported (3). The implications of these findings for research in "biochemical aging" are discussed.

Methods

Wistar animals were bred and maintained in our own colony as previously described (4). All animals for these studies were group housed from weaning until use. Sprague-Dawley males were obtained as weanlings from Charles River Laboratories and group housed. All animals received Wayne Lab-Blox *ad libitum* and tap water. Lights were on a controlled light cycle, 6 a.m. to 6 p.m. Room temperature varied from 20-27° C.

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For the feeding experiments, animals were housed in individual stainless steel or galvanized cages for at least one week before refeeding. After either a two or ten day fast, as indicated, the animals were refed by gavage of 50% glucose at a dosage of 5 mmoles/200 g (3). For 8, 24 or 48 hr time points refeedings were begun at 8 a.m. while for the 12 hr point they were begun at 8 p.m. They then received *ad libitum*, a diet of ground Lab-Blox and dextrose in equal parts. All animals were killed by decapitation, exsanguinated and livers removed quickly to ice-cold 10% sucrose. Livers were wiped dry, weighed, minced and homogenized with two volumes of buffer (150 mM KCL, 5 mM EDTA, 5 mM MSH pH 7.4) in a cold glass homogenizing vessel using a teflon pestle. Following centrifugation for 1 hr at 100,000xg a portion of the clear supernatant was used for enzyme determination. Blood was allowed to clot 30 min and then centrifuged 10 min at 3,000xg. Serum was removed for analysis of glucose.

Glucokinase was assayed as described by Sharma *et al.* (5). Serum glucose was determined using hexokinase and glucose-6-P dehydrogenase. All chemicals were reagent grade or better. Further details of procedures may be found in Grier *et al.* (2). All results are reported as mean ± standard error of the mean. A two-tailed Student's t test was used to determine significance of differences.

Results and Discussion

The observed glucokinase activity (Table 1) was significantly lower than that reported by Adelman (3) for animals of comparable ages, possibly as a function of the stock diet used. Glucokinase activity was also nearly twice as great in the late summer and autumn as in the winter and spring when compared in animals of the same age. To compare refeeding curves all values are expressed relative to the fed level for the particular class of animals studied.

Four month old Wistar males showed a nearly total return to fed levels of enzyme within eight hours of refeeding after a 2 day fast (Experiment 3 of Table 1). Females of the same age showed a somewhat smaller relative response but a greater absolute increase (refed minus fasted) in the same time (Experi-

TABLE I

Experiment Number	Fed	Fasted	Refed 8 hr	Refed 12 hr	Refed 24 hr	Refed 48 hr
1. W ♀ 4 mo[1]	.99±.10	.33±.08	.70±.08**	-	1.45±.10[a]	
Relative Activity[2]	100	33	70		145	
2. W ♀ 14 mo	.63±.09	.20±.01	.35±.04**	-	.91±.10[a]	
Relative Activity	100	32	56		145	
3. W o→ 4 mo	.80±.05	.50±.07	.75±.04**	-	-	-
Relative Activity	100	63	94			
4. W o→ 12 mo	.67±.06	.32±.05	-	.75±.01**	.88±.08[a]	.76±.20
Relative Activity	100	48	-	112	131	113
5. CR o→ 12 mo 2 Day Fast	.94±.06	.46±.09	.60±.09	.58±.11	.87±.14**	1.26±.13[a]
Relative Activity	100	49	64	62	93	133
6. CR o→ 12 mo 10 Day Fast	-	.21±.04*	-	.46±.08**,[b]	.64±.09[b]	1.14±.06[a,b]
Relative Activity	100	22	-	49	63	121

[1]Animals were fasted and refed as indicated in Methods. N = 3-11 for all groups. W = Wistar, CR = Charles River (Sprague Dawley derived). Young females refed late Dec. 1972, Wistar males in Feb. 1973, old females in March 1973, Charles River males April-May 1973.

[2]Fed level set equal to 100%, absolute enzyme levels are I.E.U. (μmoles/min) per gram liver.

*p<.05 10 day fast lowers activity more than 2 day fast.
**p<.05 activity greater than fasting level.
a - p<.05 (one tail) activity greater than fed level.
b - p>.2 10 day fast differs from 2 day fasted.

ment 1). Among older animals the difference in response was less. Old females gave essentially the same response as young females (compare Experiments 1 and 2), old males showed a greater relative decrease of enzyme level on fasting and a refeeding response quite similar to that of females (Experiment 2 vs. 4).

One year old Charles River males (contrast Experiments 4 and 5) showed a long lag before a significant increase in glucokinase activity was observed. The

time lag can be estimated to be approximately 6-8 hr, as reported by Adelman (3).

The long lag in response might have been due to an insufficient depletion of body weight in these old animals (1, 6). We therefore fasted another group of same age males for 10 days and refed them in the same way (Experiment 6). The prolonged fast resulted in a much greater reduction in the enzyme level, and a loss of body weight of approximately 15 g/day. Within 12 hours after refeeding there was a doubling of enzyme activity, although animals fasted two days still had a higher absolute activity level. At 24 and 48 hours the animals fasted 10 days did not differ significantly from those fasted for only 2 days (compare Experiments 5 and 6). Following a 10 day fast there was no apparent lag before the response to refeeding and at 12 hr the enzyme level was significantly elevated above the fasted level. The increment of total response (48 hr minus 12 hr values) was identical for 2 day fasted and 10 day fasted animals.

We also measured a number of variables other than glucokinase activity. The liver weights of fed Charles River animals were similar to those reported by Adelman for 2 year old animals while those of Wistar animals were much less ($\sim$20 g for Charles River, $\sim$12 g for Wistar). After fasting there was only a small difference (11.5 g Charles River, 9 g Wistar).

The marked difference in liver weight and enzyme adaptation between Wistar and Charles River males may be simply a function of the relatively large difference in their fat reserves (6). The Wistar males attain a nearly stable body weight at 4 months, increasing from 450-500 g at that age to 500-600 g at 1 yr (550±15 g, n = 24 untreated old males). Approximately one fifth weigh between 600-700 g. The Charles River animals show a larger variation in weight among the 1 yr old animals with about one fourth weighing more than 800 gm prior to fasting. This difference in fat reserves may in turn be a result of genetic difference in metabolic regulation, or a response to the stress of early weaning and shipping (7).

Serum glucose levels were determined during the time course of fasting and refeeding (8). Young and old females showed essentially identical patterns of

response to fasting and refeeding. There was no significant difference in response of old males of either strain when compared to each other or to the response of females, although Wistar males had lower serum glucose at 24 hr than either Wistar females or Charles River males.

Our ability to reverse, to some extent, the lagging response of the Charles River animals suggests that a significant portion of their apparent unresponsiveness during refeeding is not an *irreversible* aging phenomenon (6). The much larger fat stores and livers of these animals are indicative of a profound difference in homeostasis between Charles River and Wistar animals.

We have recently found an impact of handling in infancy on the responsiveness of tyrosine aminotransferase and tryptophan oxygenase in mature female animals during glucose refeeding (8). Both the induction of tyrosine hydroxylase (9), a rate limiting enzyme in catecholamine biosynthesis, and the secretion of glucocorticoids (10) in response to stress have been previously shown to be modified by infant handling. Similar modifications of response have been obtained using footshock in mature animals (9). We have not, however, been able to increase significantly the responsiveness of glucokinase in Wistar males by: 1) limiting food consumption to minimal maintenance levels (18 g/day) for up to three weeks; 2) subjecting animals to total fasting for 1 week followed by 1 week refeeding, and then the usual fasting-refeeding regimen (7); or, 3) giving three 10' sessions of 0.5 mA footshock one week prior to fasting and refeeding, which will reverse the age dependent decrement in responsiveness of tyrosine hydroxylase (9). Food limitation produces a slightly increased (10%) responsiveness in old males, though this effect has not been examined in detail. The one week fast and refeed results in an elevated fed level of glucokinase but a reduced response to the second fast-refeed cycle.

The results reported here are consistent with Adelman's observations on aging and the involvement of the neuroendocrine system in the aging process (1, 11). They also indicate that "biochemical aging" may be amenable to environmental modification in some cases. We would like to suggest that the

marked difference between strains, and between animals of the same strain receiving different infant experience (8,9,10) produce an ideal system in which to examine the relative impact of genetic and environmental components in aging.

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