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THE EFFECT OF LOW PROTEIN-HIGH DEXTRIN DIET AND SUBSEQUENT FOOD RESTRICTION UPON LIFE PROLONGATION IN FISCHER 344 MALE RATS

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SUMMARY

Fischer 344 rats fed low protein-high dextrin diet exhibit a higher median (but not 10th percentile) survival as compared to controls. The effect of this diet appears already if the diet is administered between 6 weeks and 6 months of age; after this treatment median survival of experimental animals is increased by 96 days while the 10th percentile is not different from standard diet-fed controls. Further treatment of animals with the same diet has minimum effect as animals that lived on this regimen throughout the whole life exhibited a median lifespan increase by 120 days and increase in the 10th percentile by 41 days. However, if such animals at the age of 6 months are transferred to a restricted (60%) food intake regimen (control diet, not carbohydrate enriched) a further increase in median and 10th percentile lifespan prolongation can be observed reaching + 328 and + 396 days respectively as compared to controls. The effects of this early feeding (6 weeks to 6 months) with a low protein-high carbohydrate diet available *ad libitum* and the food restricted regimen (standard diet 60% controls) fed from the age 6 months onwards are additive, the final results being identical to those obtained if the animals were kept on the 60% food restricted intake throughout the whole life. The fact that animals fed the low protein-high carbohydrate diet and those kept on 60% standard diet food restriction had different survival though they were equal in daily (identical) protein intake is emphasized.

Key words: Carbohydrate diet; Prolonged lifespan; Food restriction

INTRODUCTION

The early experiments of McCay *et al.* [1] represented until now the most effective way of life prolongation of laboratory rodents [2]. The food-restricted regimen

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can be started as early as 6 weeks [3], or at 6 [4], 12 [5] or 18 [6] months of age. The effect of the onset of the food-restricted regimen upon longevity is difficult to assess as different authors have used different animal strains (mostly Wistar Kyoto and Fischer 344). Nevertheless it appears that in order to get a reasonable increase in animals' lifespan the restricted regimen has to start at least at the age of 1 year. Severe dietary restrictions, however, imposed after growth cessation, have been shown to have no effect on life prolongation [7] or even to decrease it [14].

There are several proposals for mechanisms that may underlie extended lifespan of animals under the conditions of restricted food intake. Sacher [8] proposed that food restriction slows down the aging process by reducing the metabolic rate per gram body mass. However data published by Masoro *et al.* [4] disagreed with this. Another suggestion says that increased growth duration is responsible for life extension in food-restricted animals [9] and last but not least life prolonging effect of reduced diets was compared to body composition; though it has been traditionally believed that increased body fat contents accompanies aging, so far not all studies support this conclusion [5,9,10].

To what extent the lifespan may be influenced by the composition of food ingested remains equally unanswered. To our knowledge, the sole report of Yu *et al.* [3] emphasizes the possibility of obtaining a small but distinct increase in median and 10th percentile survival in Fischer 344 rats that since 6 weeks of age were kept on a diet in which polysaccharides constituted a considerable portion of its energy content.

It was the aim of this study to reveal whether the life prolonging effect of the restricted dietary regimen can be dissociated from the effect of increased carbohydrate proportion in the diet.

MATERIALS AND METHODS

Five groups of male Fischer 344 rats, 60 animals per group, were used in the present study. The animals (obtained from Portage, MI plant, Charles River Laboratories) were kept under specific pathogen-free conditions and housed one per cage in plastic containers on a wire mesh floor. Monitoring of the barrier facility was identical to the scheme described by Yu *et al.* [11].

The composition of the three diets used is summarized in Table I. Control rats were fed diet A *ad libitum* (group 1). In group 2 the animals were restricted to 60% of the food consumed (modified diet A) by controls starting on the 6th week of their age. In the third group (group 3) the animals were fed diet B from 6 weeks of age onwards. In group 4 the animals were kept on diet B from their 6th week of age till 6 months old and then they were transferred to diet A which was available *ad libitum*. In the last group (group 5) animals were fed diet B from the age 6 weeks until they were 6 months old and thereafter they were transferred to diet A restricted to 60%. Restriction was related to the food consumption of controls and evaluated once per

TABLE I

COMPOSITION OF DIETS

Dietary component	*Diet A (%)*	*Diet B (%)*
Casein	21	12.6
D,L-methionine	0.15	0.09
Sucrose	15	15
Dextrin	43.65	52.11
Vegetable oil	10	10
Vitamin-mineral mix (Ralston Purina)	7	7
Choline chloride	0.2	0.2
Solca Floc	3	3

The details of the vitamin-mineral mix were reported in Ref. 9; this premix contains over 96% sucrose. In the case when diet A was used for feeding food-restricted animals the proportion of the vitamin-mineral mix was increased to represent 11% of the total; concomitantly the portion of dextrin was decreased to 39.65%. The reason for this alternation was to provide the food-restricted animals with the same daily intake of calcium, phosphorus and sodium as the main intake of the rats eating the unmodified diet A *ad libitum*.

week. With rats fed a restricted amount of food the animals were offered the respective amount 1 h before the start of the dark phase of a 12-h light/12-h dark cycle. The body weight of each rat was estimated once a fortnight.

The survival curves were estimated using product-limit estimates and curves compared using Wilcoxon test [12]. The median of the 10th percentile of survival times of the groups were compared using the quantile test as described by Conover [13].

RESULTS

The data summarized in Table III fully confirm the results of Yu *et al.* [3] regarding the survival of Fischer 344 male rats kept on a dietary regimen restricted to 60%

TABLE II

DIETARY PROTOCOL

Age (experimental period)	*Diet fed to group No.*				
	1	*2*	*3*	*4*	*5*
4—6 weeks	A	A	A	A	A
6 weeks—6 months	A	modified A, restricted to 60%	B	B	B
6 months to death	A	modified A, restricted to 60%	B	A	modified A, restricted to 60%

TABLE III

LONGEVITY DATA (n = 60)

Group		*Median*	*10th Percentile*
No. 1	(Control, diet A *ad libitum*)	715 (680—742)	834 (785—931)
No. 2	(Food-restricted from the 6th week onwards, modified diet A)	1092 (1030—1141)[a]	1278 (1210—1241)[a]
No. 3	(Kept on diet B from the 6th week onwards)	835 (755—868)[a]	875 (821—907)[c]
No. 4	(Kept on diet B from the 6th week till 6 months old; then transferred to diet A *ad libitum*)	811 (750—864)[a,d]	863 (830—896)[b,d]
No. 5	(Kept on diet B from the 6th week till 6 months old; then transferred to modified diet A and the food-restricted regimen)	1043 (996—1110)[a,c]	1230 (1198—1267)[a,c]

[a]Significant difference with respect to group No. 1 at the 1% level.
[b]Not significant with respect to group No. 1.
[c]Not significant with respect to group No. 2.
[d]Not significant with respect to group No. 3.
[e]On the border of significance.

of controls as well as the increase in median survival (but not in the 10th percentile) of animals kept in *ad libitum* available modified low protein-high dextrin diet (groups 1, 2 and 3). Moreover it is evident that the median survival increase caused by the latter dietary regimen is the same whether the low protein-high dextrin diet is applied to animals within 6 weeks—6 months of their age only or starting from the 6th week onwards. Further application of this diet beyond the age of 6 months has no additional effect on median survival as demonstrated by comparing groups 3 and 4; animals that were kept on a low protein-high dextrin diet from the 6th week of age onwards are practically indistinguishable in their survival from those that at the age of 6 months were from this regimen transferred to *ad libitum* feeding of the basic diet (diet A). In comparison to controls the median survival in groups 3 and 4 is increased by 120 and 96 days respectively. If, however, instead of letting these animals consume as much as they wanted they were restricted in feeding to 60% of controls (from the age of 6 months onwards), the result is indistinguishable from the survival of rats that were kept on a restricted food intake throughout the whole life (groups 2 and 5). The coincidence refers to both the magnitudes calculated, i.e. median and 10th percentile; moreover also the maximum observed lifespan is practically equal in these two groups. The increases in median and 10th percentile survival are summarized in Table IV.

TABLE IV

INCREASES (IN DAYS) OF MEDIAN AND 10th PERCENTILE SURVIVAL IN ANIMALS KEPT ON DIFFERENT FEEDING REGIMENS

The increase in lifespan is calculated relatively to controls (median 715, 10th percentile 834)

Group		*Median (days)*	*10th Percentile (days)*
No. 2	(Food restricted from the 6th week onwards, modified diet A)	+ 377	+ 444
No. 3	(Kept on diet B from the 6th week onwards)	+ 120	+ 41
No. 4	(Kept on Diet B from the 6th week till 6 months old, then transferred to diet A *ad libitum*)	+ 96	+ 29
No. 5	(Kept on diet B from the 6th week till 6 months old; then transferred to modified diet A and the food-restricted regimen)	+ 328	+ 396

DISCUSSION

With the accumulated evidence of life prolongation of food-restricted animals [8] it is not surprising that any of the restricted regimens used in this study resulted in an increase of both median and 10th percentile survival; it was also confirmed that the composition of the food consumed by the experimental animals plays a considerable role in their survival, at least with regard to the protein and sugar (dextrin) components. Such an effect, namely that in two isocaloric diets in which one had a considerably higher proportion of sugars, the survival was increased in animals fed a sugar-enriched diet was published already by Yu *et al.* [3]. In our experiments feeding low protein-high dextrin diet to animals resulted in significant increase of the median survival but was without effect upon the 10th percentile. Further, the increase in the median survival was the same in animals who were fed this diet throughout the whole life or within 6 weeks and 6 months of their age only. This is, naturally, a rough estimate of the period in which such an effect occurs as the definition of this period coincides with our experimental protocol and no attempts were made to establish this period more precisely or to look for mechanisms by which such a life prolonging effect can be materialized. Nevertheless it was clearly demonstrated that beyond the 6th-month period further feeding of experimental animals with low protein-high dextrin diet has no effect upon median survival. The fact that

there are mechanisms capable of aging regulation which can be influenced during the first year of life in rodents was reported in our previous communication [5]. To our knowledge all the previous experiments were, however, directed to the evaluation of the amount of food consumed [3,7,14—18] and except in the report by Yu *et al.* [3] no attention was paid to the food composition.

The other observation we consider important is that after the initial period of feeding isocaloric low protein-high dextrin diet which results in increased median (but not 10th percentile) survival further life prolongation of the animals (involving both the 10th percentile and median survival) can be achieved by transferring them to a food-restricted regimen (60% of controls) at the age of 6 months. The final effect upon life prolongation achieved by such regimen is practically the same as if the animals were kept on 60% food intake throughout their whole life.

The question that can be posed here is to what extent the observed effects are caused by increased carbohydrate or by reduced protein intake in animals consuming the low protein-high dextrin diet. Taking both the diet composition (Table I) and the daily food intake (which is not different between *ad libitum*-fed animals with diet A and B) it emerges that the daily protein intake of animals fed 60% restricted diet A equals the daily protein intake of animals fed diet B *ad libitum*. (It is to be emphasized that in both cases the only protein involved is casein).

The results about reduced protein intake are controversial. While Goodrick [19] and Leto *et al.* [20] suggested that increased lifespan of food-restricted animals reflects a decreased proportion of protein intake (see also Yu *et al.*, Ref. 3), others were able to demonstrate that longevity correlates positively with the increased protein intake in animals fed a fixed level of protein [21—23]. Our data are basically in favor of the first alternative, i.e. correlation of life-span increase with the reduced protein intake. However since there is no difference in median and 10th percentile survival between animals kept in the low protein-high dextrin diet (diet B) from the 6th week onwards and those kept on this diet between 6 weeks and 6 months of age and since a further increase in the median and the 10th percentile survival can be introduced by restricting the food intake from the age of 6 months onwards it appears feasible to propose that aside to reduced protein intake there are other factors that may contribute to dietary lifespan increase. Changes in the balance between reduced protein intake and these other factors may explain the controversial observations about the effect of protein feeding upon longevity discussed above.

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