Geriatric Nephrology and Urology **7:** 81–86, 1997.

Effects of diets containing different proportions of macronutrients on longevity of normotensive Wistar rats

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Accepted 7 February 1997

Key words: high blood pressure, insulin, lifespan, macronutrients, nutrition, sugar-induced hypertension

Abstract

The present investigation examined effects of diets containing different proportions of macronutrients on longevity in two substrains of normotensive Wistar rats – Wistar Kyoto (WKY), the most widely accepted normotensive control for spontaneously hypertensive rats (SHR) and Munich Wistar rats (WAM as designated here). Each substrain was divided into five dietary groups composed of 15 rats each. Compared to a baseline diet composed of near equal calories of sucrose, fat, and protein, the remaining four diets were high sucrose-low protein, high sucrose-low fat, low sucrose-high protein, and low sucrose-high fat. Significantly higher systolic blood pressures were found in the two groups of WKY and WAM ingesting the high sucrose diets compared to the other three groups. The high sucrose groups were also hyperinsulinemic. Although only the group of WKY consuming the high sucrose-low fat diet showed a significantly shortened lifespan, the lifespan of WKY positively correlated with systolic blood pressure when data from all dietary groups were combined. WKY and WAM with an average systolic blood pressure exceeding 150 mm Hg had a significantly shorter lifespan than the rats with lower average blood pressure. Accordingly, elevated systolic blood pressure, especially when the blood pressure exceeds 150 mm Hg, significantly shortens lifespan.

Introduction

In 1993, data were published showing that spontaneously hypertensive rats (SHR) had a shortened lifespan while ingesting high sucrose levels in the diet, i.e., 52% of calories [1]. In this substrain of Wistar rat, high sucrose ingestion led to shortened lifespan whether the sugar replaced proteins or fats. Due to a significant correlation between lifespan and systolic blood pressure (SBP), we concluded that the abbreviated lifespan associated with congestive heart failure was brought on mainly by the high blood pressure (BP) emanating from the excess sucrose consumption [2–4]. The purpose of the present investigation was to investigate longevity under conditions similar to those experienced by the hypertensive rats [1] in two substrains of normotensive Wistar rats – Wistar Kyoto (WKY), the most widely accepted control for SHR [5], and a local strain of Munich Wistar rats (designated here as WAM) [2].

Material and methods

One hundred-fifty male rats of two substrains of Wistar rats, 75 rats in each group, were obtained at six weeks of age from Taconic Farms, Germantown, NY. These included Wistar Kyoto rats (WKY) and normotensive Wistar rats which we previously designated WAM [2]. All rats ingested ordinary laboratory rat chow until three months of age. The Wistar rats were then fed one of five diets differing in the concentrations of macronutrients [6]. Accordingly, the two substrains contained five dietary groups consisting of 15 rats over their lifetime.

The experimental diets have been described as diets I through V in previous communications (Table 1) [6]: I = Baseline, where virtually equal calories were derived from sucrose, fats and proteins, II = High Sugar-Low Protein (HS-LP), III = High Sugar-Low Fat (HS-LF), IV = Low Sugar-High Protein (LS-HP), and V = Low

Table 1.

	Energy source of diets (% of total)		
	Sucrose	Protein	Fat
Control	36	31	33
HS-LP	50	17	33
HS-LF	50	40	10
LS-HP	11	56	33
LS-HF	10	32	58

	Mineral content (% W/W)				
	Na	K	Mg	CA	Cu*
Control	0.14	0.41	0.60	0.62	7.2
HS-LP	0.13	0.42	0.59	0.61	7.0
HS-LF	0.13	0.38	0.53	0.55	6.3
LS-HP	0.14	0.46	0.63	0.66	7.5
LS-HF	0.17	0.57	0.78	0.82	9.4

* mg/kg

Sugar-High Fat (LS-HF). For ease, the diets will be referred to as: baseline, HS-LP, HS-LF, LS-HP, and LS-HF. The percentage of energy contributed by sugar (sucrose), protein, and fats were the variables of interest. The largest percentage of energy was contributed by sucrose (50%) in the HS-LP and HS-LF diets, while protein and fats contributed the smallest percentage of calories respectively. The sucrose fraction provided the lowest percentage of calories in the LS-HP and LS-HF diets and protein and fats the highest respectively. Minerals, electrolytes, and vitamins were virtually the same in all diets. These were relatively low salt diets, because sodium content was 0.13–0.17% w/w instead of the usual 0.30–0.40% w/w in most commercial feed.

SBP and body weight (BW) measurements were made every three months. SBP was measured in unanesthetized, slightly warmed rats by tail plethysmography which correlates well with direct SBP readings [7]. Data concerning SBP displayed in tables 2 and 3 were the average of the four dietary group readings taken at 6, 9, 12, and 15 months.

Urine was collected overnight in steel metabolic cages. The urine was assayed for proteins and creatinine by routine clinical procedures. Creatinine clearance was estimated from an overnight urinary specimen, and the serum creatinine analyzed on a blood specimen obtained within days of the urine collection.

Blood was collected from tails. Blood chemistries were performed by routine clinical procedures. PRA, catecholamines, and insulin were measured by RIA [8, 9]. Values for comparison were obtained at different times close to term as indicated in Tables 1 and 2.

Statistical evaluations were assessed by either the Student's T test or one-way analysis of variance (ANOVA). Where a significant effect of diet was detected by ANOVA, Dunnett's t-test [10] was used to establish which differences between means reached statistical significance. Statistical significance was set at $p < 0.05$. Linear regression lines were calculated by least squares analyses.

Results

While the average lifespans of hypertensive SHR were reported to be significantly shorter in the two dietary groups consuming the higher sucrose intake (HS-LP, HS-LF) [1], this was not generally the case for the WKY and WAM substrains. WKY consuming diet HS-LF had a significantly shortened lifespan compared to the Wistar rats consuming lower sugar diets, but this was not found in dietary group HS-LP (Table 2). In addition, there were no significant differences in average lifespan among the five dietary groups of WAM (Table 3). Tables 2 and 3 also show the mean ± SEM of many blood and urinary parameters measured toward the end of the lifespan of WKY and WAM. These will be presented in more detail in another communication [11]. Basically, the major differences between the high (HS-LP, HS-LF) and low sugar (Baseline, LS-HP, LS-HF) consumers were higher SBP and insulin concentrations. Plasma renin activity (PRA) was significantly lower in WKY. No WKY or WAM showed a SBP above 200 mm Hg at any time, but 15 WKY and 4 WAM were found to have a mean SBP of 150 mm Hg or above, with the highest mean value being 188 mm Hg. Of the 19 WKY and WAM with SBP averaging 150 mm Hg or above, 17 were in either dietary groups HS-LF or HS-LP. The average lifespan of 100 weeks ± 5.8 (SEM) among these 19 WKY and WAM with elevated SBP was significantly less than the 109 weeks ± 1.0 (SEM) of the rest of WKY and WAM combined ($p < 0.01$).

Because the data suggested involvement of SBP in longevity and previous experience was consistent with this as well [1], we correlated individual SBP with longevity in the two substrains (Figure 1A & 1B). There was a significant correlation between SBP and lifespan in WKY ($p < 0.02$), but not in WAM.

Table 2. Correlation of life span in WKY with various parameters in the five different dietary groups.

Parameter	Time-Weeks	Base	HS-LP	HS-LF	LS-HP	LS-HF
Life Span (Wks)	–	106 ± 4.6	107 ± 5.9	98 ± 3.5*	114 ± 4.1	110 ± 3.3
SBP (mm Hg)	6–18	135 ± 0.6	150 ± 0.4*	159 ± 1.4*	140 ± 0.8	136 ± 0.8
BUN (mg/dL)	18	18 ± 1	11 ± 1*	20 ± 1	27 ± 2	19 ± 1
Creatinine (mg/dL)	18	0.6 ± 0.02	0.7 ± 0.04	0.6 ± 0.02	0.6 ± 0.05	0.7 ± 0.02
Cr Clearance(mL/min)	15	0.42 ± 0.02	0.26 ± 0.05	0.30 ± 0.04	0.28 ± 0.05	0.40 ± 0.05
Proteinuria (mg/mg Cr)	15	1.8 ± 0.1	1.8 ± 0.1	3.8 ± 1.3*	2.3 ± 0.2	2.6 ± 0.6
PRA (ng/mL/h)	21	14.1 ± 2.0	9.5 ± 2.9*	10.8 ± 0.9*	21.6 ± 3.6	18.0 ± 1.4
NE (ng/mg Cr)	12	176 ± 33	167 ± 37	186 ± 49	143 ± 10	225 ± 17
Cholesterol (mg/dL)	18	217 ± 4	146 ± 15	124 ± 7	126 ± 14	210 ± 2
Triglycerides (mg/dL)	18	196 ± 18	180 ± 32	123 ± 6	126 ± 14	182 ± 24
Glucose (mg/dL)	18	116 ± 2	122 ± 14	143 ± 38	139 ± 4	170 ± 6
Insulin (uIU/mL)	21	33.8 ± 3.9	51.1 ± 13.2*	47.9 ± 7.4*	34.0 ± 3.1	28.8 ± 1.6

mean ± SEM
* Significantly different from control, LS-HP, and LS-HF.
5–15 rats studied in each group.

Table 3. Correlation of life span in WAM with various parameters in the five different dietary groups.

Parameter	Time-Weeks	Base	HS-LP	HS-LF	LS-HP	LS-HF
Life Span (Wks)	–	100 ± 3.9	104 ± 4.2	106 ± 3.8	101 ± 4.6	105 ± 3.3
SBP (mm Hg)	6–18	134 ± 0.7	147 ± 1.1*	148 ± 1.4*	133 ± 0.8	134 ± 1.0
BUN (mg/dL)	18	33 ± 3	18 ± 3	48 ± 13	30 ± 8	37 ± 19
Creatinine (mg/dL)	18	0.9 ± 0.20	0.8 ± 0.04	0.8 ± 0.05	0.8 ± 0.08	0.8 ± 0.05
Cr Clearance(mL/min)	21	0.50 ± 0.07	0.31 ± 0.05	0.41 ± 0.06	0.69 ± 0.08	0.31 ± 0.04
Proteinuria (mg/mg Cr)	15	9.2 ± 1.3	3.2 ± 0.7	8.6 ± 2.1	14.4 ± 5.4	4.8 ± 0.6
PRA (ng/mL/h)	21	7.8 ± 1.6	10.1 ± 7.6	11.8 ± 4.1	14.0 ± 4.6	15.7 ± 1.6
NE (ng/mg Cr)	12	128 ± 15	231 ± 31	104 ± 17	206 ± 46	226 ± 56
Cholesterol (mg/dL)	18	184 ± 18	201 ± 34	230 ± 10	228 ± 48	210 ± 46
Triglycerides (mg/dL)	18	218 ± 21	386 ± 165	205 ± 41	201 ± 38	296 ± 81
Glucose (mg/dL)	18	134 ± 12	133 ± 8	165 ± 19	144 ± 37	137 ± 9
Insulin (uIU/mL)	21	33.6 ± 4.3	50.8 ± 10.8*	46.0 ± 6.7*	32.4 ± 3.4	30.6 ± 3.0

mean ± SEM
* Significantly different from control, LS-HP, and LS-HF.
5–15 rats studied in each group.

Discussion

A previous study showed that feeding diets high in sucrose content decreased longevity significantly in hypertensive Wistar rats (SHR) whether the sucrose replaced protein or fats [1]. In general, SHR died in congestive heart failure as exemplified by large hearts and pulmonary edema found at autopsy. It has long been recognized that SHR are more sensitive to sucrose-induced hypertension than WKY; and WKY, in turn, are more sensitive than WAM [2]. Similar type studies focusing on longevity and sugar-induced BP elevations had not been reported in normotensive rats. Although diets containing high content of sucrose steadily increased SBP over approximately 5 months in young and old Fischer 344 and hybrid Fischer 344/BN rats [12], two strains of rats most frequently used in aging research, the studies were not carried out long enough to discern effects on lifespan.

In the present study, WKY, the normotensive control for SHR [5], showed a significantly shortened lifespan only with the HS-LF diet. Even though heavy sucrose consumption increased SBP significantly in both groups, i.e., HS-LP and HS-LF, average SBP in

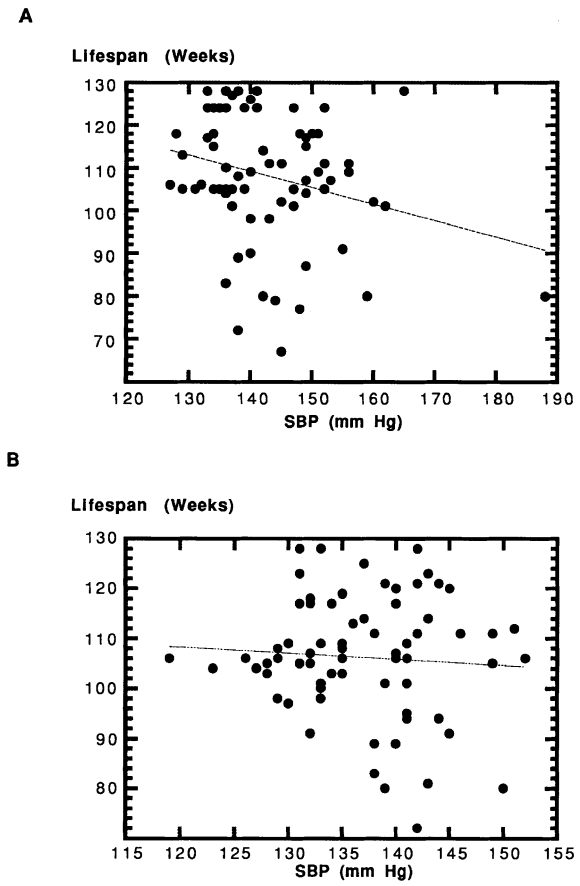

Figure 1. Linear regression by least squares method between SBP and lifespan of WKY (A) and WAM (B). The line is statistically significant for WKY ($p < 0.028$, $r = 0.259$, $DF = 70$), but not for WAM ($p < .563$, $r = 0.070$, $DF = 69$).

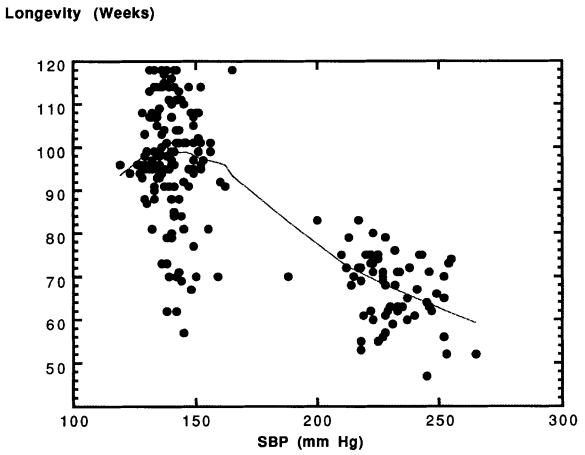

Figure 2. Line of best fit for all data (WKY and WAM) in this study and for SHR in previously reported study (ref 1). Line is weighted smooth curve with a smoothing factor of 50% using a Kaleidagraph software package.

the HS-LF group was 6 mm Hg higher than HS-LP. The actual mean SBP readings over the adult lifespan of WKY were HS-LF 156, HS-LP 150, and the other three dietary groups averaged between 135–140 mm Hg. In the case of WAM, SBP rose significantly on the two high-sucrose regimens compared to the other three dietary groups, but the differences were relatively small compared to WKY. The different effects of elevated BP on the two substrains were evident when linear regression analyses were made between lifespan and SBP. Similar to the correlation made in SHR [1], there was an overall statistically significant correlation in WKY, not in WAM. Although it is not certain why these different responses occurred, the most obvious explanation is that effects are more apparent when higher SBP levels are reached. In other words, although heavy sucrose feeding did increase SBP of WAM, that increase does not generally reach a deleterious level which would shorten lifespan markedly. The conclusion is strengthened when one combines data from the present study and the past one [1] (Figure 2). Perusal of Figure 2 suggests that lifespan shortening becomes more obvious when SBP exceeds 150 mm Hg.

Are any other causes apparent for the shortened lifespan seen in the WKY consuming the HS-LF diet? Heavy sugar consumption has been associated with renal perturbations [13–15]. However, in the present study, heavy sugar consumption did not produce any significant changes in renal parameters to suggest excess kidney damage in the sugar consumers. Although protein excretion was somewhat higher in WKY ingesting HS-LF, BUN, serum creatinine, and creatinine clearance were essentially not different compared to other dietary groups. Glucose, cholesterol, and triglycerides were not elevated to explain any organ damage from these perturbed metabolic parameters. What does stand out is that insulin concentrations were higher in the two groups of WKY consuming the high sucrose diets. However, one would have to explain why the HS-LP group of WKY did not show the same shortened lifespan as HS-LF in order to make these important pathogenic factors. Further, increased levels of circulating insulin were elevated in WAM rats consuming the high sugar diets, and these rats did not have a shortened lifespan.

The most popular laboratory model to study aging is caloric restriction [16–19]. One theory holds that

insulin resistance with hyperinsulinemia may play a prominent role in augmenting the rate of aging, and caloric restriction by enhancing insulin sensitivity can overcome this [20]. Heavy sugar consumption is known to cause insulin resistance in rats [21] and humans [22]. Perturbations in glucose/insulin metabolism could theoretically promote more rapid aging through enhanced glycosylation of proteins and nucleic acids [23–25] and/or enhanced free radical generation [26, 27]. This does not seem to be the case here. One plausible explanation for the difference between the present study and the caloric restriction studied, may lie in the shorter lifespan of the animals studies here, i.e., a two year lifespan compared to the 3.5 years of the Fischer 344 [17].

In the caloric restricted studies, where prolonged lifespan was found [16–19], the restriction of calories rather than presence or absence of any particular macronutrient was found to be important in determining lifespan [28]. In the present study, correlation of lifespan with SBP in WKY indicates that the SBP elevation caused by high sugar consumption, rather than a direct effect of sugar itself, was the major culprit in the pathology. In contrast to findings suggesting that insulin resistance with hyperinsulinemia is not important in the sugar-induced hypertension of SHR [8], increases in circulating insulin concentrations and lowering of PRA suggest that insulin resistance and volume expansion, at least in WKY, may be important in the SBP rise of these normotensive Wistar rats.

Acknowledgments

Supported by NRICGP/USDA 9201419 and NIH grant AG 06929.

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