Effects of Chronic Kombucha Ingestion on Open-field Behaviors, Longevity, Appetitive Behaviors, and Organs in C57-BL/6 Mice: A Pilot Study

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Kombucha is a lightly fermented tea beverage popularly consumed as a self-prescribed folk-remedy for numerous ailments. Kombucha is claimed to enhance cognition, aid weight loss, and prolong life. This pilot study reports longevity, general health, and open-field exploratory behavioral outcomes from a 3-y longitudinal study of 64 C57-BL/6 mice (males and females), half of which chronically drank kombucha, and all of which experienced natural mortality. Compared by MANOVA to controls, mice that drank kombucha showed greater vertical exploration ($P = 0.001$) and a sex-interactive effect in novel object manipulation ($P = 0.049$). MANOVA of kombucha-drinking mice compared to controls detected differences in appetitive behaviors (food consumption, $P < 0.001$; beverage consumption, $P = 0.008$), and gross body weight ($P < 0.001$). Appetitive behaviors changed with the addition of voluntary exercise on a running wheel, with differing patterns of change noted for males and females. Both male and female mice who drank kombucha lived longer than controls ($P < 0.001$), with the greatest variability among the male mice (sex interactive effect, $P < 0.001$). Comparable effects and mechanisms in humans remain uncertain, as do health safety issues, because serious health problems and fatalities have been reported and attributed to drinking kombucha. *Nutrition* 2000;16:755–761. ©Elsevier Science Inc. 2000

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INTRODUCTION

Kombucha is the internationally used Germanized form of the Japanese name for a lightly fermented tea beverage, first used for its healing benefits in the Orient. Kombucha, known by many names, is traceable to as early as 220 BC in China ("Ling zhi") and 414 AD in Japan ("Kocha Kinoko," "Combucha"). As trade routes expanded, kombucha (former trade name, "Mo-Gu") found its way first into Russian ("Cainii grib," "Cainii kvass," "Japonski grib," "Kambuha," "Sakvasska") then into Eastern European countries, appearing in Germany ("Heldenpelz," "kombuchaschwamm") around the turn of the 20th century. The habit of drinking fermented tea became acceptable throughout Europe until World War II brought widespread shortages of the necessary tea and sugar ingredients. In the postwar years, Italian society's passion for the beverage ("Funko cinese") peaked in the 1950s. Then, in the 1960s, scientific research in Switzerland reported that drinking kombucha was similarly beneficial to eating yogurt, and kombucha's popularity increased.[1–3] Today, in the United States, kombucha is sold nationwide in retail food markets as part of an herbal tea blend, Sun Luck Green Tea with Kombucha (San Francisco, CA, USA), and *The Kombucha Journal* is electronically published worldwide in several languages.[2] Even as the testimonials attributing health benefits have accumulated, the peer-reviewed scientific literature lags far behind.

Currently, kombucha is alternately praised as "the ultimate health drink"[1–3] or damned as "unsafe medicinal tea."[4–6] Testimonials claim causal benefits for everything from AIDS improvement, balding relief, cancer cures, and diabetic and arthritic symptom relief to prolonged longevity, weight loss, and cognition-enhancements.[2,7] In conjunction with kombucha's growing popularity, life-threatening human health problems are beginning to be reported. Hepatotoxicity,[8] necrotizing pancreatitis,[9] and several human deaths have been linked to drinking kombucha beverage.[4,10]

A MEDLINE search of the National Library of Medicine located neither human nor animal studies published in the peer-reviewed literature that would support or refute any of the benefits claimed. The sole retrievable document, from the 1960s,[11] also identified the lack of any substantial scientific information about kombucha, and concluded that no preclinical human or animal studies were available. This pilot study with mice contributes to filling that knowledge gap.

The purpose of the present investigation was primarily to explore certain claimed benefits of cognition enhancement, appetitive behaviors, and longevity, and secondarily to optimize use of animal subjects[12,13] by gathering and reporting gross anatomic and physiologic data to help clarify both beneficial and adverse effects of kombucha.

MATERIALS AND METHODS

Animal Subjects

C57-BL/6 mice were received from Charles River Laboratories (Wilmington, MA, USA) as 10- to 12-g weanlings (initial total = 66 mice; males = 32, but 2 males died during acclimation phase, leaving 30, females = 34). Each mouse was individually weighed

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and visually inspected for sex and health, and was randomly assigned to one of eight group housing bins with the restrictions that each bin could contain only one sex, and each bin (solid-bottom, wire-topped, 40.6 cm length × 22.9 cm height × 20.3 cm deep) could maximally house 9 mice.[13]

Throughout the 3-y experiment, mice had ad libitum access to food and beverage; lighting was automatically controlled at light: dark = 12:12 h, environmental temperature was controlled at 21 ± 2°C, there were 12 air changes per hour, woodchip bedding with shredded paper was used for nesting material, and objects were provided for environmental enrichment.[13] Mice within a bin were individually identified by ear tagging such that in the event an individual escaped, the recaptured mouse could be returned to the appropriate bin (e.g., all mice in Bin-1 were ear-tagged with #10's, Bin-2 were tagged with #20's, and so forth through Bin-8, which were ear-tagged with #80's).

After a 2-wk acclimation period, bins of mice were randomly assigned to treatment conditions. During the 21-d preexperimental baseline period, each mouse was handled daily until of tractable temperament, thoroughly accustomed to human voices and routine handling, and reliably trained to voluntarily enter a clear plastic capture/inspection cup when presented. Because this was a survival experiment, all subjects were allowed to experience natural mortality.

Design

The experimental approach used a two-group (treatment, control) longitudinal design with one within-group factor (sex) and dependent measures that included both univariate single observations as well as multivariate repeated measures.[14] Four bins of mice (two of each sex) were randomly assigned to each treatment condition (kombucha treatment or untreated control). The treatment group (n = 32 total mice) consisted of 17 females (Bin-1 = 9 mice, Bin-3 = 8 mice) and 15 males (Bin-6 = 8 mice, Bin-8 = 7 mice). The untreated control group (n = 32 total mice) consisted of 17 females (Bin-4 = 8 mice, Bin-7 = 9 mice) and 15 males (Bin-2 = 7 mice, Bin-5 = 8 mice). In setting $\alpha = 0.05$, this sample size and design would have good analytical power[15] and a high level of discrimination for the interval/ratio level responses.

With the exception of postmortem dissection to recover organs, all the response variables were deliberately selected for their non-invasiveness and minimal disruption of natural behaviors.[13] Given the absence of available animal data, relatively gross dependent measures were used in this initial inquiry.

Open-Field Behaviors

Among the numerous benefits attributed to drinking kombucha is an improvement in mental capacity.[2,7] Kombucha beverage therapy was included in Hans Irion's 1944 Training Course for Pharmaceutical Technical Colleges, where kombucha was recommended for those engaged in strenuous intellectual activity.[7] For the purposes of this study with mice, such behavior was conceptualized as an overt symbol of brain activity, operationalized within the information-processing model[16] to be species-appropriate behaviors of initial environmental curiosity and responsiveness, both of which are considered prerequisites in motivation for learning.[17]

Several open-field behaviors with high face validity were selected from the natural behavioral repertoire of the mouse: time to begin exploring novel environment (Initial Movement, in s), frequency of vertical explorations (count of Rearing), frequency of returning to release/capture location (count of Returning), frequency of manipulating novel objects (count of Novel Object Manipulation), and time to enter or reenter capture cup when presented (Leaving Latency, in s). Although both Initial Movement time and Leaving Latency could also be used as general assessments of an animal's physiologic stress, frequency of grooming (count of Grooming) was also included as a specific measure of such stress.[18]

Each mouse was individually released into a clear glass open-field round maze, 40-cm diameter × 30 cm high, with 2.5-cm blue grids marked on white paper beneath the floor. A defined 7.5-cm × 10-cm mouse release area was marked, and a novel object was placed opposite the mouse, 30.5 cm away. Two observers timed and recorded the mouse behaviors over a 2-min trial. Separate timers were used for 2-min trial timing, Initial Movement time, and Leaving Latency time. The blue grid lines on the white paper placed beneath the glass maze were not visible on trial videorecording, and grid line crossings could not be reliably counted by naked eye observation, so were not included in the behavioral data.

Appetitive Behaviors

Daily estimates of food and beverage consumption were gathered, along with weekly weight measurements, because some estimate of energy acquisition and use is needed in studies that use exploratory behaviors as response variables.[19] Mice were individually weighed each week with an Ohaus LS200 electronic scale (Ohaus Inc., Switzerland) accurate to 0.1 g at 10−40°C. After weighing, each mouse was visually inspected for general condition, then returned to bin mates. Daily estimates of appetitive behaviors were made during each of three intervals: 1) first 3-wk pretreatment as baseline; 2) 3 wk with treatment group receiving kombucha, during which neither group had exercise wheels; and 3) 3 wk after kombucha began, when both groups had access to one running wheel per bin for ad libitum use. Wheel-running activity was not quantified.

Daily food consumption, g/mouse, was estimated by subtracting food remaining from a known quantity provided and dividing by the number of mice in each bin. Visible spillage was collected and included in the food remaining weight; feeding efficiency (i.e., differences in spillage) was not quantified in this study. Animals had ad libitum access to Premium Harvest Deluxe Small Animal Mix (Central Garden and Pet, Algon, WA, USA), which provided minimum crude protein 12%, minimum crude fat 12%, maximum crude fiber 12%; vitamin supplements included in the mix were: ascorbic acid, Vitamin A premix, Vitamin B_2, B_{12}, calcium carbonate, calcium lignin sulfonate, calcium pantothenate, choline chloride, cobalt carbonate, copper sulfate, copper oxide, Vitamin D_3, Vitamin E premix, ferous sulfate, manganous oxide, magnesium oxide, potassium sorbate, sodium selenite, and zinc oxide. Proper tooth wear was ensured by providing Vitakraft Nibble Rings (Vitakraft, D-28295 Bremen, Germany), which provide minimum crude protein 12.2%, minimum fat (oil) 1.8%, minimum calcium 0.9%, minimum phosphore 0.6%, maximum crude fiber 7.7%, maximum moisture 9.9%, and maximum ash 7.75%; rings contained vitamin supplements Vitamin A 2780 IU per pound and Vitamin D# 340 IU per pound. Daily litter inspection revealed no evidence of diarrhea and minimal food spillage.

Daily beverage consumption, mL/mouse, was estimated by subtracting beverage remaining from a known quantity provided and dividing by number of mice in the bin. Untreated control groups received ad libitum water (reverse-osmosis filtration of ordinary community water supply). Treatment group received an ad libitum obligate beverage of 15% solution of kombucha, prepared according to procedures outlined by Frank,[2,7] using items readily available to the average local consumer: 2 L water (domestic supply), 5 g tea (Lipton Loose Tea, blend of orange pekoe and pekoe-cut black tea), 140 g granulated white cane sugar. The 15% (v/v) solution of kombucha/filtered water is a volume-equivalent dose to what has been "recommended" for daily human consumption.[1,2,7] All mice in the treatment group were observed to readily drink the 15% kombucha. Fresh kombucha was prepared and solution provided to the mice was replaced every 7 d, so that at all times the treatment mice were drinking from the same batch.

The kombucha beverage is a weak tea solution fermented by a zooglea (so-called "living skin"). This zooglea, or lamella, is a symbiotic colony of various bacteria (predominantly *staphylocci* and *pseudomonae*) and various species of yeasts (predominantly *acetobacters, brettenomyces, candidae,* and *saccharomyces*).[1,7,20,21] The culture, widely available at health food stores, is initially thin, translucent, and gelatinous but becomes thicker and more opaque as it ages, developing a disk-shaped appearance resembling a large rubbery pancake, hence the moniker, "mushroom." Each colony produces a new lamella every 7 d, so starter cultures are also readily attainable from other brewers.

Full-strength aliquots of prepared kombucha were sampled from 23 consecutive batches prepared over 1 y and analyzed for specific gravity and salinity with a LaMotte refractometer (BioMarine Aquafauna, Hawthorne, CA, USA), and for glucose, protein, and hemoglobin with Bililabstix Reagent Strips (Miles Inc., Diagnostics Division, Elkhart, IN, USA). This demonstrated specific gravity 1.036–1.073 (mean = 1.056, SD = 0.01); salinity 49–94 parts per thousand (mean = 75.17 ppt, SD = 8.47); pH 2–2.5 (mean = 2.28, SD = 0.253); glucose 500–2000 mcg/dL (mean = 1239, SD = 971). Bililabstix reagents were non-reactive for protein, but positive hemoglobin was believed to be a false-positive reaction from microbial peroxidases, as discussed further on, with standing stability.

A 1-L sample of the 15% (v/v) kombucha/filtered water solution was independently analyzed by Northern Testing Laboratories, Inc. (NTL, Fairbanks, AK, USA), using US Environmental Protection Agency methods for domestic water supply. The 15% (v/v) solution was found to contain copper (0.183 mg/L), iron (0.039 mg/L), manganese (0.212 mg/L), nitrate-N (0.27 mg/L), nitrite-N (0.03 mg/L), calcium (12.4 mg/L), sodium (5.11 mg/L), both alkalinity (<1 mg/L) and hardness (47.8 mg/L) as $CaCO_3$, and Langelier index of 230 units.

The 15% solution had good stability during the 7 d each bottle was standing at room temperature and available for the mice to drink. However, the solution repeatedly tested positive for hemoglobin using Bililabtix. Although the reagents are equally sensitive to myglobin, there was no clearly identified source of either hemoglobin or myglobin. The product insert informed that false-positive results can occur with oxidizing contaminants or in the presence of microbial peroxidases. Given the microbial symbiosis of the kombucha zooglea, the latter explanation seems most parsimonious.

Longevity

Kombucha is claimed[1,2,7] to "prolong life," and is known in France as *Champignon de longue vie,* or "mushroom of long life." In this study, mice were assessed for health and general condition in conjunction with weekly weighing; euthanasia guidelines were not included because longevity was an important response variable, and all mice were allowed to experience natural mortality. Longevity was measured as number of days a mouse lived after arrival as a weanling.

Organs as Indicators of Health

After death, gross dissection was performed to recover individual organs (brain, heart, kidneys, liver, spleen), which were weighed and preserved in 10% formalin solution. Brains were recovered to augment behavioral measures of environmental responsiveness and the claimed benefit on mental capacity.[1,2,7] Hearts were recovered, as kombucha is recommended for those engaged in sport and strenuous exercise,[2,7] with claims that it improves physical capacity[7] and has been claimed[2,7] to reduce blood pressure. Kidneys were recovered based on the claim[2,7] that kombucha removes harmful substances by conjugation with glucuronic acid, which is eliminated with the urine through the kidney, and that it stimulates diuresis,[1] and is efficacious in cases of hardening of the kidneys.[7] Livers were recovered because kombucha is claimed to be a potent detoxifying agent that heals a damaged liver[1,2,7] and because of reports of hepatotoxicity in humans.[8] Spleens were recovered, as kombucha is alleged to stimulate the immune system.[1,2,7]

RESULTS

Analytical Methods and Tools

All descriptive and inferential statistics were prepared using SYSTAT Version 6.0.[22] For the valid application of parametric analyses of variance (e.g., MANOVA) and related procedures, certain basic assumptions about the raw data must be met, particularly that the data are normally distributed with homoscedastic variances and additive factor levels.[23] Most traditional multivariate methods work best with moderately correlated dependent variables,[15] but depend on data vectors being normally distributed.[24] Because applying parametric analyses to data that violate the test's assumptions can lead to spurious conclusions, the γ statistics for normality of data distribution[15,22–24] were checked for each dependent variable. Non-normality, defined as $|\gamma| > 1$, was corrected using commonly employed transforms expected to produce normality.[15,22–24] In this way, when a MANOVA on the transformed data shows significant differences between means, the researcher has greater confidence that real differences actually exist, at which time it is reasonable to conduct single-factor ANOVA as a post hoc procedure.[15,22–24]

Exploratory Open-Field Behavior Findings

The raw behavioral data are summarized in Table I.

To better achieve the normal distribution assumption of multivariate analyses, all count data (Rearing, Returning, Grooming, and Novel Object Manipulation) were square-root transformed, and for all continuous process data (Initial Movement and Leaving Latency), measured in seconds, a natural log transform was used before performing inferential analysis.[15,23,24]

Mulivariate analysis of variance (MANOVA) of the entire open-field behavioral response matrix showed no overall significant effect of kombucha (Wilks' $\lambda = 0.72$, $F(6,20) = 1.24$, $P = 0.32$), a significant overall effect of sex (Wilks' $\lambda = 0.92$, $F(6,155) = 2.13$, $P = 0.05$), and a significant overall interaction of kombucha with sex (Wilks' $\lambda = 0.87$, $F(6,155) = 3.62$, $P < 0.05$). However, in the univariate F-tests ($\alpha = 0.05$, df = 1, 155), which are the customary post hoc procedures for MANOVA,[15,22–24] a significant effect of kombucha was observed for frequency of rearing ($F = 12.54$, $P < 0.001$). There was also a significant sex effect on frequency of rearing ($F = 8.047$, $P = 0.005$) and a significant sex effect on frequency of novel object manipulations ($F = 3.941$, $P = 0.049$)

Descriptive statistics (see Table 1) showed that control females reared more than kombucha females; however, kombucha males reared more than control males. Also, as seen in Table 1, kombucha males manipulated the novel objects more than control males, and more than females in either treatment condition.

Appetitive Behavior Findings

The raw data for estimated food consumption and beverage consumption, as well as measured body weight, are summarized in Table II.

A General Linear Model approach to multivariate analysis of variance[22,23] was performed separately for each appetitive behavior repeatedly measured at three times: 1) pretreatment baseline, 2) kombucha or water only, without additional exercise, and 3) kombucha or water only, with added exercise.

As is common with mammals, the male mice were larger, and

TABLE I.

SUMMARY OF ENVIRONMENTAL AWARENESS AND RESPONSIVENESS MEASURES, BY TREATMENT GROUP AND SEX

Group	Initial movement (sec)	Rearing (count)	Grooming (count)	Manipulated object (count)	Return (count)	Leaving latency (sec)
Kombucha						
Females						
Mean	8.5	12.1	1.2	7.2	5.7	7.5
SD	7.9	4.5	1.4	4.0	2.5	7.2
Males						
Mean	7.1	12.7	0.7	9.4	4.7	10.7
SD	4.4	4.5	1.0	4.7	2.2	18.3
Plain water						
Females						
Mean	8.1	15.0	1.2	7.6	6.5	8.6
SD	5.5	7.7	1.6	4.3	2.8	10.1
Males						
Mean	8.7	8.4	0.7	7.4	4.1	12.8
SD	9.9	5.6	0.7	4.6	2.1	13.1

SD, standard deviation.

hence weighed more at pretreatment than female mice (Wilks' $\lambda = 0.278$, $P < 0.001$). The larger males also consumed more food than the females (Wilks' $\lambda = 0.659$, $P < 0.001$), and the larger males consumed more liquid (Wilks' $\lambda = 0.925$, $P = 0.007$), compared to females. This existing baseline sex-difference in food consumption, liquid consumption, and weight existed before treatment with kombucha and persisted throughout the experiment. As seen in Table II at Time-1, before beginning kombucha treatment, the treatment mice weighed slightly more than the control mice (mean control female = 13.1 g, mean treated female = 13.3 g; mean control male 15.1 g, mean treatment male = 15.5 g); the anticipated between-gender weight difference was already manifest at weanling age. This same difference is also seen in Table II at Time-3, after treatment with kombucha had commenced and voluntary exercise was added. Control females gained 16.5 g compared to treatment females, who gained only 13.6 g, a within-gender weight change difference of 2.9 g. This same effect was also seen in the male mice (see Table II, Time-3). Control males gained 19.5 g compared to 15.4 g weight gain in treatment males, a within-gender weight change difference of 4.1 g. Both male and female mice treated with kombucha did not gain as much weight as untreated controls.

TABLE II.

SUMMARY OF DAILY APPETITIVE BEHAVIORS, AND WEEKLY WEIGHT OF MOUSE, BY TREATMENT CONDITION AND SEX, WITH AND WITHOUT EXERCISE

	Food consumption (g/mouse)			Beverage consumption (ml/mouse)			Weight (g)		
	Time-1	Time-2	Time-3	Time-1	Time-2	Time-3	Time-1	Time-2	Time-3
Kombucha									
Females									
Mean	3.7	7.2	5.1	3.3	3.7	3.1	13.3	20.3	26.9
SD	1.4	1.3	1.1	1.2	0.6	1.1	2.3	1.1	3.7
Males									
Mean	4.4	7.1	4.6	3.1	3.7	3.6	15.5	24.5	30.9
SD	1.8	1.9	0.7	0.7	0.8	1.1	3.3	1.7	4.4
Controls									
Females									
Mean	4.5	7.5	6.0	3.1	3.6	3.7	13.1	20.4	29.6
SD	1.9	2.0	0.9	1.1	0.6	1.5	2.3	1.2	4.6
Males									
Mean	5.2	6.8	4.2	3.5	4.3	4.4	15.1	24.9	34.6
SD	2.1	2.4	0.6	0.7	1.2	0.8	3.3	1.7	4.8

Time-1 is pretreatment baseline, Time-2 is kombucha only no additional voluntary exercise, Time-3 kombucha continues and exercise wheels are added.

SD, standard deviation.

TABLE III.

DESCRIPTIVE SUMMARY OF MOUSE WEIGHT, ORGAN SIZE, AND LONGEVITY BY TREATMENT GROUP AND SEX

	2-wk weight (g)	Post-mortem weight (g)	Longevity (d)	Brain (g)	Liver (g)	Heart (g)	Kidneys (g)	Spleen (g)	Spleen (cm)
Kombucha									
Females									
Mean	14.7	22.5	783	1.2	2.2	0.2	0.2	0.4	2.3
SD	0.8	5.8	114	0.2	1.8	0.1	0.2	0.4	0.6
Males									
Mean	18.5	22.9	745	1.1	2.2	0.2	0.2	0.3	2.0
SD	0.9	5.7	55	0.2	2.3	0.1	0.1	0.1	0.2
Controls									
Females									
Mean	15.5	28.6	769	1.2	2.5	0.2	0.2	0.3	2.1
SD	1.0	6.4	87	0.3	1.3	0.2	0.1	0.6	0.6
Males									
Mean	18.9	32.6	719	1.3	2.9	0.3	0.3	0.4	2.0
SD	1.5	8.8	139	0.2	2.7	0.2	0.1	0.6	0.6

SD, standard deviation.

Food Consumption

Once treatment with kombucha began, there was a significant main effect of kombucha on food consumption alone (Wilks' $\lambda = 0.890$, $P < 0.001$) and a significant interactive effect of kombucha with sex on food consumption (Wilks' $\lambda = 0.814$, $P < 0.001$). The MANOVA F-tests ($\alpha = 0.05$, df = 1,156) for food consumption were not significant in the treatment period without exercise ($F = 1.484$), but became significant for sex ($F = 58.56$, $P < 0.001$) and sex-treatment interaction ($F = 26.61$, $P < 0.001$) when metabolic demands of exercise were added. Food consumption was a gross estimate, but, as seen in Table II, food consumption patterns were mixed. Treated females ate less than control females at both Time-1 (mean difference = 0.3 g/mouse/d) and this within-gender difference increased with added exercise (mean difference = 0.9 g/mouse/d). The pattern for males differed. Kombucha-treated males ate more than controls at Time-2 (mean difference = 0.3 g/mouse/d), but the pattern reversed with addition of exercise at Time-3 (mean difference = 0.8 g/mouse/d). Treated females consumed more than controls, but treated males consumed less than controls.

Liquid Consumption

Once treatment with kombucha began, there was also a significant main effect of kombucha on liquid consumption (Wilks' $\lambda = 0.926$, $P = 0.008$). MANOVA F-tests ($\alpha = 0.05$, df = 1, 156) revealed a sex interaction effect ($F = 5.377$, $P = 0.022$). Both effects persisted when metabolic demands were increased by exercise; there was a main effect of kombucha ($F = 10.95$, $P < 0.001$). Liquid consumption estimates are also gross measures, but, as seen in Table II, both treatment and control females increased liquid consumption at Time-2. However, when volitional exercise was added to kombucha treatment, at Time-3, control females continued to increase liquid consumption whereas liquid consumption by females treated with kombucha declined. These are small, <0.1-mL changes in mean liquid consumption. The same pattern is evident within male mice. Control males drank more than treated males at all three time periods, and with the addition of voluntary exercise, control males increased liquid consumption while kombucha-treated males declined somewhat. Again, these are gross estimates and show small, <1.0-mL, changes.

Weight Change

All weanling mice were expected to, and did, gain weight. However, once treatment with kombucha began, a significant difference in weight was observed between treatment groups (Wilks' $\lambda = 0.870$, $P < 0.001$), while the expected between-sex differences in weight continued. Once the metabolic demands of exercise were added, however, the within-sex difference in weight increased. At pretreatment baseline (Time-1) female groups' weight differed by 0.17 g, and the male groups' weight differed by 0.47 g. During the kombucha-without-exercise condition (Time-2), female groups' weight differed by 0.13 g and the male groups' weight differed by 0.52 g. This within-sex between-group difference in weight gain was magnified with the addition of exercise. When metabolic load of exercise was added (Time-3) the female groups' weight difference increased to 2.6 g and male groups' weight difference increased to 3.72 g. Concomitantly, kombucha-drinking mice of both sexes ate more and drank more, although litter inspection revealed no evidence of diarrhea.

Organ Findings

The last mouse died 1018 d after the experiment began, and all organs recovered were preserved in 10% formalin solution for later analysis. The raw data on longevity, postmortem weight, and organ weight are summarized in Table III.

As previously discussed, parametric analyses require normally distributed data[15,22–24] and, with the exception of postmortem weight, univariate responses for organ measures were not normally distributed at the cell level, thus distributions were normalized, individually, using customary transforms.[15,23,24] The following were noted: initial weight at week 2 (Z-transform), brain weight (Z-transform), liver weight (log10 transform), heart weight (Z-transform), left kidney weight (Z-transform), right kidney weight (Z-transform), spleen weight (Z-transform), spleen length (log10 transform), and longevity (square root transform).

General linear model analysis of variance[15,22] was performed on the transformed multivariate data matrix for organs and longevity. A significant effect of treatment (kombucha versus control) was observed in the overall response matrix: Wilks' $\lambda = 0.128$, $F(10,35) = 23.902$, $P < 0.001$. Univariate F-tests are the customary post hoc procedure in MANOVA[15,22–24] and these ($\alpha = 0.05$,

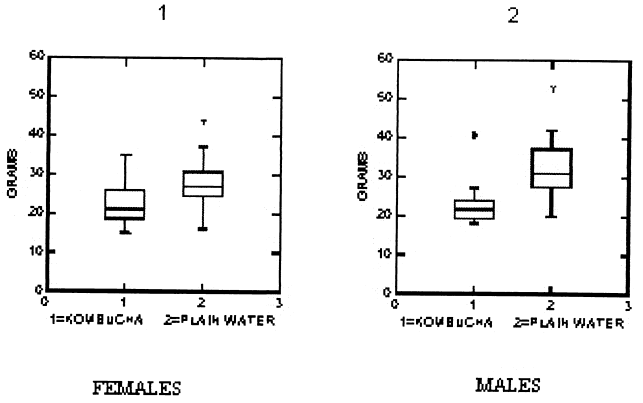

FIG. 1. Comparison of raw post-mortem mouse weights by treatment group and sex.

df 1, 44) showed no significant main effect of treatment for heart, kidneys, or spleen weight. However, significant effects of treatment were observed for postmortem body weight ($F = 40.459$, $P < 0.001$), as seen in Figure 1.

Significant effects of treatment were also observed for brain weight ($F = 5.524$, $P = 0.023$), liver weight ($F = 5.929$, $P = 0.019$), and spleen length ($F = 28.995$).

A significant main effect of sex was observed for only postmortem body weight ($F = 20.603$, $P < 0.001$) and spleen length ($F = 17.707$, $P < 0.001$). No effect of sex was detected in any of the other organs recovered.

Three interactive effects of treatment with sex were noted: longevity ($F = 123.698$, $P < 0.001$), postmortem body weight ($F = 3.484$, $P = 0.069$) with males weighing more than females, and spleen length ($F = 16.219$, $P < 0.001$). The spleens of kombucha-drinking mice were longer than untreated controls (males' mean = 1 mm longer, females' mean = 1.5 mm longer).

Longevity

A significant main effect of treatment was observed ($F = 211.863$, $P < 0.001$), as well as a significant effect of sex on longevity ($F = 209.607$, $P < 0.001$), and a significant treatment-sex interactive effect ($F = 123.698$, $P < 0.001$). As seen in Figure 2, kombucha-drinking mice lived longer than untreated controls (kombucha males mean = 26 d longer than control males, kombucha females mean = 14 d longer than control females).

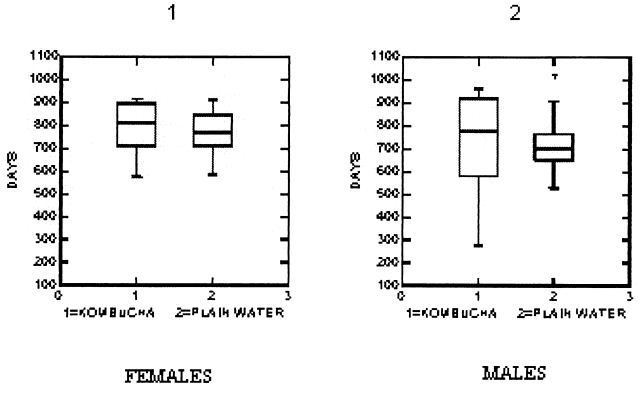

FIG. 2. Comparison of raw days of longevity by treatment group and sex.

DISCUSSION

Kombucha is not a magic potion; it is simply not fully understood. Despite the numerous benefits claimed for kombucha, few have published empiric support with preclinical animal or human studies. This pilot study using mice offers some qualified support for the claimed benefits of weight reduction, longer life, and increased environmental awareness or responsiveness, as seen in open-field behaviors. However, the effects of inhibited weight gain, longer life, and increased open-field behaviors did not come without physiologic cost, in terms of splenomegaly and hepatomegaly, both of which are congruent with reported adverse health effects in humans.[4,8,9] These findings suggest that kombucha may have an effect on digestion, perhaps affecting nutrient absorption in some way. Further research will be needed to clarify the causal mechanisms and processes by which kombucha produces such benefits or side-effects, as they remain currently unknown.

Weight Reduction

Although it cannot be concluded from this study that kombucha contributes to weight loss, the data show that chronic consumption of kombucha did inhibit weight gain in mice, from weanlings through senescence. Kombucha-drinking mice of both sexes did not gain as much weight as did untreated controls of the same sex, even though the 15% kombucha solution contained additional glucose (250–500 mcg/dl). One possibility is that free xanthines from the tea may be increasing metabolism, such as seen in the kombucha-without-exercise condition, and that additional exercise merely amplifies this basic process. Although preventing weight gain isn't the same as showing weight loss, the findings tend to support the claims[2,7] that drinking kombucha may contribute to weight loss. Again, however, the mechanisms and processes remain unknown. A main effect of weight loss would need to be established more directly, such as with an obese animal model treated with kombucha.

Longevity

Regardless of sex, kombucha-drinking mice lived significantly longer than untreated controls, with the greatest variability shown in the kombucha-drinking males. Considering that the average lifespan for the C57/BL-6 mouse is approximately 2 y, the observed effect on longevity represents about a 5% longer life for the males and a 2% longer life for the females. This would tend to support the claims[1,2,7] that drinking the tea-beverage kombucha from the so-called "mushroom of long life" contributes to longer life. Beyond human testimonials, a main effect of increased longevity has not been documented with other species, but could be explored using those who would voluntarily drink the kombucha. It is known that simple caloric restriction and weight reduction contributes to longer life in rats and humans and that may be a major mechanism at work in this study. As with other claimed benefits for kombucha, the exact mechanisms and processes remain unknown.

Open-Field Behaviors

Kombucha-drinking mice showed significantly increased behaviors indicative of increased environmental awareness and responsiveness. The slightly higher glucose available in the kombucha solution might have contributed to explorations that were more energetic. Free xanthines from the tea product might also produce this effect. Replication of these pilot efforts would need to include a simple sugar-water solution and a tea-water solution as additional controls to fully clarify this finding.

Internal Organs

In the organs recovered, significant differences were observed for brain weight (decreased), liver weight (increased), and spleen length (increased). The smaller brain found in kombucha-drinking mice of both sexes may simply be a proportionate and parallel manifestation of the overall smaller body size for kombucha-drinking mice, given the differences in weight also observed. The smaller brains may also be due, in some part, to reduced nutrient availability if kombucha interfered with digestion and nutrient extraction as the weanling mice were maturing. This calls into question whether kombucha should be given to developing children, and no data exist on safety during pregnancy for mother or fetus. The observed increased spleen length in kombucha-drinking mice could reflect a mild splenomegaly from the chronic ingestion of microorganisms in the kombucha solution. The hepatomegaly observed in kombucha-drinking mice of both sexes appears congruent with the reports of human hepatotoxicity.[8] A full and complete explanation for the splenomegaly and hepatomegaly will require more extensive and definitive biochemical analysis of the kombucha solution.

The composition of the zooglea varies geographically due to inclusion of wild yeasts.[7,20,21] The basic biochemistry of the kombucha beverage remains largely unknown, but it has been shown[7,20–24] to vary due to geographical location, tea type,[25] sugar type,[26] incubation time,[26] and temperature.[26] Thus, no two solutions ever produce exactly the same final beverage

Limitations

Any study has limitations, particularly a pilot study employing gross response variables. One limitation here is that mice were group-housed seven to nine mice per bin, and the single running wheel may have affected feeding behavior and dominance hierarchy within the bin. Housing each mouse alone would eliminate that potential effect, as well as render measures of feeding efficiency more precise. Suitable individual housing would also permit collection of excrement for fecal and urinalysis, and permit more refined measures of appetitive behaviors. Additional metabolic data such as serum analyses are also needed, particularly liver function tests. Because the mice lived for several years, other behavioral responses could also be added, and more direct cognitive tasks such as learning or problem-solving could be added. However, locomotor responses are subject to confounding effects of age-related disabilities such as arthritis, senility, and so forth. Logistical improvements in observing locomotor activity would be achieved by using higher-contrast grid paper, of a type easily seen on videotape.

In summary, this study with mice demonstrated that chronic kombucha ingestion contributed to significantly inhibited weight gain, increased environmental awareness and responsiveness, and prolonged life. However, chronically drinking kombucha also contributed to longer spleens and enlarged livers. These findings in mice parallel both the health benefits[2,7] and dangerous side-effects[8,9] being reported in humans.

Nonetheless, people still brew and consume the beverage with worldwide popularity. The fact that reports are now appearing from various countries[8,9] of people suffering severe adverse health effects attributed to kombucha, including death, further points out an important need for additional empiric evidence and greater depth of understanding beyond individual testimonials.

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