Unstable heart rate and temperature regulation predict mortality in AKR/J mice

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Tankersley, Clarke G., Rafael Irizarry, Susan E. Flanders, Richard Rabold, and Robert Frank. Unstable heart rate and temperature regulation predict mortality in AKR/J mice. Am J Physiol Regul Integr Comp Physiol 284: R742–R750, 2003. First published October 10, 2002; 10.1152/ajpregu.00416.2002.—Elderly populations face greater risks of mortality when exposed to changes in environmental stress. The purpose of the following study was to develop an age-dependent susceptibility model that achieved the following three goals: 1) to operationally define homeostasis by assessing the stability and periodicity in physical activity, heart rate (HR), and deep body temperature (Tdb), 2) to specify alterations in activity, HR, and Tdb regulation that signal imminent death, and 3) to test the hypothesis that the decay in homeostasis associated with imminent death incorporates the coincident disintegration of multiple physiological systems. To achieve these goals, the circadian regulation of activity, HR, and Tdb was assessed using radiotelemeters implanted in AKR/J (n = 17) inbred mice at ~190 days of age. During a 12:12-h light-dark cycle, weekly measurements were obtained at 30-min intervals for 48-h periods until each animal’s natural death. The average (±SE) life span of surgically treated animals did not differ from untreated controls (319 ± 12 vs. 319 ± 14 days). Cardiac and thermal stability were characterized by a circadian periodicity, which oscillated around stable daily averages of 640 ± 14 beats/min in HR and 36.6 ± 0.1°C in Tdb. Stable HR and Tdb responses were compared with extreme conditions 3 days before death, during which a disintegration of circadian periodicity was coincident with a fall in the daily average HR and Tdb of ~29 and ~13% lower (i.e., 456 ± 22 beats/min and 31.7 ± 0.6°C), respectively. The results further suggested that multiple predictors of cardiac and thermal instability in AK mice, including significant bradycardia, hypothermia, and a loss of circadian periodicity, forecast life span 5–6 wk before expiration.

survivorship curves; circadian regulation of body temperature; circadian regulation of heart rate; homeostasis; homeostatic instability

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Horwitz (25) measured body weight and the circadian rhythm of $T_{db}$ in rats facing imminent death. These investigators reported a rapid loss in body weight and a disintegration of the circadian rhythmicity in $T_{db}$ regulation to define a period of senescence several days before death. These results are consistent with other studies (20, 32) and suggest that pathophysiological changes (i.e., biomarkers of aging related to circadian function) predict proximal life span better than chronological age.

The statistical model used in the current study characterizes an individual’s circadian regulation of behavioral and physiological responses. Our group described the statistical basis for this model in detail elsewhere (13). In the current article, we hypothesize that mortality risk progresses with age and is proportional to the accumulated number of physiological aberrations. The results suggest that bradycardia, hypothermia, and loss of circadian rhythmicity signal imminent death in AK mice; that is, mortality risk progressively increases as reflected by an accumulation of specific pathophysiological aberrations.

METHODS

Animals. Male, retired breeders of the AKR/J inbred mouse strain were purchased from the Jackson Laboratory (Bar Harbor, ME) at $\sim$180 days of age and were individually housed in the animal facilities at Johns Hopkins University. Water and chow (Agway Pro-Lab RMH 1000) were provided ad libitum. The environments before and during the experiments as well as animal handling were highly standardized (e.g., transparent plastic cages were used containing pine chip bedding without filter tops). All animal protocols were reviewed and approved by the Animal Care and Use Committee of the Johns Hopkins Bloomberg School of Public Health.

Surgical procedures. Activity counts, HR (i.e., confirmed by ECG recordings), and $T_{db}$ were measured simultaneously using a transmitter implant and a radiotelemetry system (Data Sciences, International, St. Paul, MN). The weight of the transmitter (model TA10ETA-F20) was $\sim$3.5 g, and its dimensions were 2-cm long, 1-cm wide, and 0.7-cm deep. At $\sim$190 days of age, the implant surgery was initiated by obtaining the animal’s presurgical weight and anesthetizing each animal with a mixture of acepromazine (0.5 ml at 10 mg/ml) and ketamine (5 ml at 100 mg/ml) at a dose of $\sim$2 $\mu$g. The hair covering the abdomen and chest wall was clipped and further removed using a depilatory. Surgery was performed by placing the animal on a heating pad, applying betadine to the exposed region of skin, and establishing a sterile field surrounding the animal. A midline incision was made to open the abdomen, and the transmitter was inserted and sutured to the abdominal muscle. The negative ECG lead was guided through the muscle and directed subcutaneously to the right shoulder. The positive ECG lead, also guided through the muscle, was directed laterally (left side) and positioned $\sim$1 cm below the rib cage. Both leads were sutured to secure a lead placement resembling lead II in traditional human ECGs. Surgery was completed within 30 min and recovery from anesthesia generally occurred within 60–90 min. After surgery, each animal was placed in a holding cage set on a heating pad for the first 24 h after surgery. Each animal was allowed to recover for at least 2 wk before the start of data collection. Individual cages were refreshed every Friday at 1400, and weekly body weights were recorded. Additional details concerning the telemetry system and output variables have been described elsewhere (16).

Data acquisition and analysis. Each animal was housed individually in a facility maintained at 23–24°C with a light-dark cycle set at 12-h intervals (i.e., lights off occurring at 1800). To conserve the battery life of each transmitter, a majority of the sampling was collected at 30-min intervals during weekend periods beginning at 1700 on Friday and continuing to 0800 on Tuesday. Data acquisition was confined to weekend periods to ensure that measurements were obtained with the animals relatively undisturbed.

The data analysis of activity counts, HR, and $T_{db}$ was restricted to a 48-h period beginning 0800 each Saturday and continuing to 0800 on Monday to assess the circadian pattern of each parameter. The measurement of activity was a cumulative determination obtained from translocation motion counts sampled during the last 10 min of each 30-min interval. $T_{db}$ represented a cumulative average of intra-abdominal temperature during the same 10-min interval as activity. HR measurements were averaged during the same 10-min interval and were then used to determine the HR value by computer software using a peak-detect algorithm to determine the average R-R interval, and then verified by comparing each HR against the corresponding ECG sample. As each animal showed preliminary evidence of body weight loss or reductions in daily average HR or $T_{db}$, continuous sampling was initiated to capture a complete profile of the last weeks preceding death.

A preliminary study of this model included the assembly of a survivorship curve and an assessment of the possible effects of surgery on survivorship. The data set consisted of age-matched untreated (i.e., no surgery) controls (UTC, $n = 43$ mice) and surgically treated (ST, $n = 25$ mice) AK mice. For the most part, all animals experienced similar laboratory conditions, including lighting, temperature, and other housing (i.e., environmental) conditions as described above. From these data, survivorship curves were constructed for UTC and ST groups (see Fig. 1). In 35 of 43 animals of the UTC group, body weight was recorded weekly and shortly after death. The body weight data were plotted as a function of age for the UTC and ST groups (see Fig. 2).

Statistical model. A statistical model was developed to evaluate the circadian periodicity of AK mice using activity counts and $T_{db}$ responses and is reported in detail elsewhere (13). Our model draws on procedures similar to other studies (32) but does not rely on cosinor analysis of circadian periodicity. Briefly, the current model permitted a partitioning of the total variation into three main effects, including individual mouse, day, and hour of the day. During the period of tightly regulated homeostasis, the partial variance attributable to hour of the day (i.e., indicative of the circadian variation) was substantially larger than the other two effects and their interactions. In addition, the partial variance attributable to individual mouse variation, although statistically significant, was small and consistent with a robust genetic role in determining the circadian periodicity in activity counts and $T_{db}$ responses. Therefore, the parameter estimates and statistical variances associated with the shape of the circadian pattern in this sample of AK mice characterized the “typical” homeostatic profile for this strain.

If it is reasonable to suggest that the homeostatic profile is determined by a robust genetic component, then individual mice will mimic the strain-specific average. Furthermore, individual departures from the strain-specific average suggest that nongenetic (i.e., primarily environmental) factors incrementally perturb homeostatic regulation. Initially,
these perturbations are modest and, as hypothesized (9), only a small number of physiological systems are likely to be affected. As other systems compensate for the progressive loss in homeostasis, an increased number of physiological systems are stressed to the point of inadequate compensation. In our statistical model, individual departures of a given response were mathematically defined as the number of standard deviations (i.e., distances) from the strain-specific average. For the circadian periodicity data, individual departures from the strain-specific average were quantified by the integral of the squared difference between two consecutive curves (13). By these methods, six indicators comprising distances corresponding to mean levels and circadian periodicity were established for the three telemetric responses (i.e., activity, $T_{db}$, and HR).

To quantify the time-dependent sequence of physiological events that might forecast homeostatic decay and imminent death, inflection points were computed using a downward trend in the distances for the six indicators (in addition to body weight). Because the distances were normalized for differences in absolute units, mean levels, and variances, a paired comparison could be performed to statistically determine differences in the time course among the indicators. To compute the inflection points, smoothing splines (33) were used to adjust the mean level and circadian periodicity to generate a smoother version of these distance data (1) as we described elsewhere (13). Briefly, activity, $T_{db}$, and HR were examined in each individual to identify departures from both the strain-specific mean level and circadian periodicity. The inflection point for each departure was defined by calculating the second derivative of each fitted spline and computing a maximum. Because the calculated splines were directed downward in nonincreasing functions, the inflection points coincided with extrapolated ages at which the second derivative was maximized.

The statistical modeling required inspection of >25,000 ECG recordings to verify or recalculate HR responses and acquire complete data sets for eight mice. Deficiencies in implant function, electrode placement, computer acquisition, and recording accuracy were factors that precluded the inclusion of a larger sample size. Statistical comparisons using paired $t$-tests were performed on the age at inflection or age at death. Statistical significance was established at an $\alpha$ level of 0.05.

The median survivorships for the ST and UTC groups were 314 and 306 days with ranges of 219–470 and 197–548 days, respectively (Fig. 1). Near the two extremes of each survivorship curve, the ST and UTC groups diverged, suggesting that the ST group experienced fewer deaths during the early age period, but the UTC group had more long-lived members. However, the average (±SE) life span was not significantly different between the ST and UTC groups (i.e., 319 ± 12 vs. 319 ± 14 days of age; $P > 0.05$).

**RESULTS**

**Survivorship curves.** The median survivorships for the ST and UTC groups were 314 and 306 days with ranges of 219–470 and 197–548 days, respectively (Fig. 1). Near the two extremes of each survivorship curve, the ST and UTC groups diverged, suggesting that the ST group experienced fewer deaths during the early age period, but the UTC group had more long-lived members. However, the average (±SE) life span was not significantly different between the ST and UTC groups (i.e., 319 ± 12 vs. 319 ± 14 days of age; $P > 0.05$).

**Body weight.** Body weight is plotted as a function of age for the ST and UTC groups in Fig. 2. At -180 days, a time point before surgery, the two groups were significantly ($P < 0.05$) different in average body weight by -2 g. After the surgical treatment in the ST group, a significant ($P < 0.01$) loss in body weight occurred relative to the UTC group. The difference in body weight was maintained between the ST and UTC groups until ~280 days on average, at which time a precipitous fall in body weight associated with imminent death was observed in both ST and UTC groups. Although group differences in body weight were prominent, there were no detectable differences between the ST and UTC groups with respect to age at the body weight inflection or age at death.

**Circadian pattern and mean level of activity, $T_{db}$, and HR.** Deficiencies in telemeter function, electrode placement, computer acquisition, and recording accuracy were factors that excluded eight mice. In addition, the determination and verification of HR responses...
were, in many ways, limiting factors. As a result, comprehensive data sets, including activity, T<sub>db</sub>, and HR responses, were obtained in 17 AK mice.

In Fig. 3, a summary of the circadian pattern of activity, T<sub>db</sub>, and HR shows the time-dependent averages (±SE) for a 48-h period for two stages of life: 2–3 wk after surgery (i.e., stage of robust homeostasis) and 3 days before death (i.e., stage of severely compromised homeostasis). The circadian pattern in activity 2–3 wk after surgery was characterized by two peaks occurring...
during the dark phase; the prominent peak appeared several hours before the dark-to-light transition (Fig. 3A). The activity approached zero during the light phase. In contrast, the average activity was significantly ($P < 0.01$) diminished 3 days before death and there was no observable change in activity in response to the light-dark cycle. In addition, the group variance in activity 2–3 wk after surgery was greater relative to the activity counts 3 days before death. These results suggested that inactivity was associated with imminent death; a response that was homogenous among individuals. The average circadian pattern of $T_{db}$ is shown in Fig. 3B. The circadian pattern of $T_{db}$ 2–3 wk after surgery indicated that temperature regulation was tightly controlled between a minimum of 35.7 ± 0.1°C and a maximum of 37.5 ± 0.1°C. The decline in thermal stability 3 days before death was characterized by a significant ($P < 0.01$) hypothermia, ranging between 30.5 ± 0.9 and 32.4 ± 0.6°C. A loss in circadian periodicity of $T_{db}$ responses was evident as indicated by an absence of synchronized adjustments to the light-dark phase. Also, a small group variance in $T_{db}$ at 2–3 wk after surgery indicated a homogenous response among individual AK mice. In contrast, there was a relatively large group variance 3 days before death, which was indicative of heterogeneous $T_{db}$ responses among individual mice.

As shown in Fig. 3C, the circadian pattern in HR responses was tightly regulated between a minimum of 572 ± 9 and a maximum of 702 ± 13 beats/min in homeostasis. Before death, HR was significantly ($P < 0.01$) depressed, ranging between 411 ± 30 and 509 ± 39 beats/min. The two time periods also differed with respect to the variance in HR among individuals within the group. There was a homogenous HR response among individuals as suggested by a relatively small group variance 2–3 wk after surgery. With imminent death, a heterogeneous HR response developed among individual mice characterized by a relatively large group variance.

Indexes of homeostatic instability. In Fig. 4A, representative traces of HR and $T_{db}$ circadian patterns, compressed in time, are shown from a single animal. Figure 4, B and C, shows the number of standard deviations, a measure of distances, from the group mean level and circadian pattern, respectively, for the same animal. At ~210 days of age (i.e., 2–3 wk after surgery), the animal demonstrated stable circadian patterns and mean levels of HR and $T_{db}$. With increasing age, there were incrementally greater deviations in the mean level and circadian pattern of HR and $T_{db}$ from the earlier strain-specific profile. In this particular animal, abrupt downward inflections occurred between 295 and 320 days of age.

The average age at inflection and the interval in days between the inflection and death for each of seven variables are depicted in Fig. 5, A and B. In Fig. 5A, there were no statistical differences among the seven variables in the average age at inflection. In Fig. 5B, the interval between ages at inflection and death revealed several statistical differences. The average inflection points in the mean level in HR and the circadian pattern in $T_{db}$ were the most proximal indicators of imminent death. On average, these variables inflected between 20 and 23 days before death and were significantly ($P < 0.05$) later in occurrence than the other indicators. At ~40 days before death, the mean level in activity was the first indicator on average to demonstrate an inflection; however, this signal was also the most variable. Inflections indicative of reduced body weight, depression in daily mean $T_{db}$ response, and disintegration in circadian periodicity of activity and HR occurred on average between 34 and 39 days before death and were not significantly ($P > 0.05$) different from each other. Whereas the reported statistical results were based on complete data sets in 8 of 17 mice, an extensive examination of the entire data set ($n = 17$ mice) using parametric and nonparametric (Wilcoxon signed rank test) analyses produced similar results.

**DISCUSSION**

The current study describes an experimental mouse model that demonstrates predictable signs of homeostatic decay as defined by specific regulatory changes in activity, HR, and $T_{db}$. The observations suggested that conspicuous declines in the daily average $T_{db}$ and body weight were accompanied by the disintegration of circadian periodicity in activity and HR, events that occurred 5–6 wk before death (Fig. 5B). A fall in the daily mean HR and a loss of circadian pattern in $T_{db}$ were the final events, which occurred on average 3–4 wk before death. Although a decline in mean activity level may have served as the earliest signal of ensuing homeostatic decay, the large variability associated with this indicator may have reduced its reliability. Collectively, these observations suggest that there is a predictable sequence of pathophysiological events associated with imminent death; i.e., the disintegration of different physiological systems occurs at different periods of homeostatic decay. The sequence of pathophysiological events appears to be independent of life span (Fig. 5A). Furthermore, the data provide a “yardstick” to evaluate the degree of vulnerability of each animal based on a relatively short list of pathophysiological changes. The results of the current study are consistent with others (24, 32) in suggesting that biomarkers of aging are more suitable predictors than chronological time in assessing age-dependent susceptibility.

Response heterogeneity was a conspicuous feature in this model. The heterogeneity in life span resulted in a strain-specific survivorship curve ranging from ~200 to 550 days of age (Fig. 1). Hypothetically, if genetic determinants were a predominant factor that governed life span in this model, mice of the AK inbred strain would have a very narrow range of life span. The range in survivorship suggests that nongenetic determinants (including both intra- and extrauterine environmental factors) played a substantial role in governing life
span. One obvious environmental factor involved the effects of surgery and the transmitter implant. As shown in Fig. 1, the shape of the survivorship curve for the ST group differed slightly from the UTC group. Two possible explanations may be associated with the difference in shape. First, the effect of surgery at ~190 days of age may have eliminated weak individuals at the outset, which shifted the initial portion of the survivorship curve to the right by ~10 days relative to the UTC group. Second, the effect of the implant may have reduced the life span of longer-lived animals (owing to the cumulative effects of additional stress), moving the latter portion of the survivorship curve leftward by ~70 days relative to the UTC group. Nonetheless, a substantial proportion of the ST curve remained within the 95% confidence interval of the UTC group.

The effect of the surgical implant was further evaluated by comparing the body weight between ST and UTC groups (Fig. 2). After a substantial weight loss after surgery, the average body weight of the ST group remained lower than the UTC group until death. The lower body weight of the ST group may have provided a survival advantage because food restriction and body

Fig. 4. Representative traces for HR and \( T_{db} \) of an individual AK mouse are depicted. Longitudinal data of HR and \( T_{db} \) responses were analyzed to generate a departure measurement based on the number of standard deviations (i.e., distances) from the mean level (B) and the standardized distances in circadian pattern (C). Bold lines in B and C represent smoothing splines derived from departure data shown by the thin lines. Number of standardized distances abruptly decreases after an inflection that is specific for each variable. Arrows for the mean levels and circadian periodicities show computed inflection points as defined by the age at which the second derivative is maximized in HR and \( T_{db} \) (see METHODS).
The daily mean levels and circadian patterns in HR and $T_{db}$ changed significantly between young adulthood (i.e., 2–3 wk after surgery) and 3 days before death (Fig. 3). Between-animal variability in the HR and $T_{db}$ measurements was also greater 3 days before death. Individual differences in activity were not the proximal cause of the increased between-animal variability, because every animal became inactive with imminent death. Likewise, the degree of bradycardia and hypothermia was not observed in young adulthood when animals were inactive during the light phase. Although changes in HR and $T_{db}$ were coincident with activity in young adulthood, it appears reasonable to infer that during the period of homeostatic decline the extreme bradycardia and hypothermia were not dependent on simply a reduction in activity. Furthermore, a significant decline in mean daily $T_{db}$ response occurred, on average, ~14 days before a significant reduction in the mean daily HR (Fig. 5B), suggesting that individual mice tolerated a longer period of hypothermia relative to the period of bradycardia.

Several previous reports demonstrate age-dependent physiological changes in rodent models using longitudinal designs. Wax and Goodrick (47) demonstrated age-dependent losses in wheel running activity associated with imminent death in mice. Although the mechanisms that lead to losses of circadian pattern are uncertain, the disintegration of $T_{db}$ periodicity in the present study generally occurred more proximal to death relative to the decay in the other two circadian regulated variables, activity and HR (Fig. 5B). McDonald and associates (23) suggested that the fall in the daily average $T_{db}$ seen in older rats was related to the rapid loss in body weight and a mechanism of decreased neuropeptide Y sensitivity (4). Whether or not a similar mechanism has a role in the current study is unclear. An additional question that remains unresolved concerns the dichotomy between HR and $T_{db}$ regulation. That is, the loss of circadian periodicity in HR preceded the persistent bradycardia, whereas the disintegration of the circadian periodicity in $T_{db}$ occurred subsequent to the hypothermic response. As the integration of these essential physiological systems unravels during homeostatic decay, maintenance of circadian periodicity in $T_{db}$ and mean HR may provide certain benefits to the survival of the organism.

In summary, the data suggested that at least two levels of homeostatic incompetence existed in AK mice facing imminent death. The first level occurred 5–6 wk before death and was characterized by an abrupt decline in body weight and daily mean $T_{db}$. Within the same period, there was disintegration in circadian regulation of activity and HR. A second level occurred 3–4 wk before death and was distinguished by an abrupt decline in daily mean HR and the attrition of circadian periodicity in $T_{db}$ responses.

**Perspectives**

Temperature regulation is controlled by the preoptic-anterior hypothalamus and is dependent on setpoint temperature (10). Because the current results demonstrated that the circadian pattern in $T_{db}$ continued to fluctuate around a depressed daily average, a shift downward in setpoint temperature may have occurred ~5 wk before death. Many blood-borne substances can alter hypothalamic setpoint temperature, including immunoadjuvants and inflammatory cytokines (5, 6, 14, 15, 34, 46). In the present study, many of the AK mice exhibited an enlarged thymus gland on autopsy, an observation consistent with the development of lymphatic carcinomas known to occur in this inbred strain (18, 19, 41). One possible mechanism to explain the decline in $T_{db}$, HR, and body weight involves cachectic processes accompanying the lymphatic carcinogenesis (12, 26, 43).

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The original intention in developing the current model was to address public health issues surrounding the associations between increased daily mortality rates and changes in environmental levels of ambient air pollution and temperature. For example, the population with the greatest mortality risk in association with daily fluctuations in air pollution consists of individuals who are elderly or patients with chronic cardiopulmonary disease (3, 8, 9, 28, 30, 42). A similar association is valid regarding abrupt ambient temperature changes (2, 17). We have postulated that one factor common to these populations, which defines an elevated vulnerability to modest levels of environmental stress, is severe homeostatic instability (5). While the current model mimics specific aspects of physiological aging introduced in other studies (23, 25), the results suggest that 5–6 wk before represents a period of homeostatic instability in AK mice.

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REFERENCES


