Age-Related Association of Tail Tendon Break Time With Tissue Pentosidin in DBA/2 vs C57BL/6 Mice: The Effect of Dietary Restriction

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In recent years, there has been a growing interest in the development of a panel of biomarkers useful in the evaluation of interventions on aging processes. An ideal marker should change with age, be related to species longevity, and respond to the effects of dietary restriction, which is the only intervention currently known to increase species longevity. In the present study, we compared parameters of collagen aging (i.e., tail tendon break time [TBT] and the glycoxidation product pentosidine) in tendon, ear, and skin of two species of rodents with different life spans: the shorter-lived DBA/2 versus the longer-lived C57BL/6 mouse strains. Both TBT and tissue pentosidine significantly increased with age in both strains of mice. The rate of increase for TBT and pentosidine occurred faster for the DBA/2 compared with the C57BL/6 strain. Dietary restriction significantly inhibited the age-related increase of TBT and pentosidine formation rate in DBA/2 mice. In C57BL/6 mice, the age-related increase of TBT was significantly inhibited by dietary restriction. However, except for tendon at 24 months, pentosidine level was not affected by dietary restriction. These studies show that the rate of collagen aging, as reflected by TBT and glycoxidation, increases proportionally with age, and that these rate increases are related to longevity in two strains of mice. Pentosidine can be monitored with age just as well in a piece of easily accessible ear tissue as in skin or tendon. Thus, pentosidine is expected to be a useful and easily measurable noninvasive marker in future intervention studies on aging.

The ultimate goal of gerontological research is to improve the quality of life in old age. Because of this goal, aging and its underlying causes and interventions are usually studied in hope of alleviating or preventing debilitating processes of old age. In these studies, it is advantageous to use an animal model with a considerably shorter life span than that of a human. Thus, laboratory rodent strains with life spans of 3–5 years are frequently chosen because they develop age-related stigmata and complications similar to those of humans. Recently, interest has focused on the development of a panel of biomarkers useful in the evaluation of possible interventions on aging processes. Because it has been hypothesized that manifestations of aging are most pronounced in tissues with slow turnover, such as connective tissues of skin and tendon (Kohn, 1982), two parameters studied in such tissues as putative biomarkers of aging are tail tendon break time (TBT) (Higgins et al., 1991; Heller and McClearn, 1992) and the protein crosslink pentosidine (Reiser, 1994; Cefalu et al., 1995).

Originally Verzar (1963) demonstrated that the resistance to thermal denaturation of rat tail tendons, measured as TBT, increased with advancing age. Further work by Everitt and colleagues (Everitt et al., 1981, 1983) showed that the anti-aging intervention dietary restriction beginning at an early age in rats retarded the aging rate of tail tendon collagen fibers, as measured by TBT, and inhibited the development of certain disease processes of old age such as renal disease, cardiac enlargement, and tumors. Harrison et al. (1978) and Harrison and Archer (1983) found strong correlations between chronological age and TBT in many inbred and hybrid strains of mice. However, in these same studies, no relationship was found between TBT and longevity.

Since solubility of tail tendon collagen decreases with age, it was suggested early on by Verzar (1963) that molecular crosslinking of the collagen may explain the age-related increase of TBT and the noted effects on collagen solubility. Ever since, the exact chemical structures for these putative markers of senescence have been sought (Calkins, 1981).

One such hypothesis for molecular crosslinking of collagen with age is based on amino-carbonyl reactions of sugars with long-lived proteins, such as collagen, to form fluorescent, yellow-colored protein chemical adducts and cross-links, referred to as nonenzymatic browning or the Maillard reaction (Monnier et al., 1991). Because many of these reactions involve oxidation, the resultant end products have collectively been termed “glycoxidation” (Baynes, 1991). One such product originally isolated from old human collagen is pentosidine, an imidazopyridinium crosslink involving a pentose sugar crosslinked with arginine and lysine residues (Sell and Monnier, 1989).

The purpose of the present study is to compare TBT to pentosidine levels in tendon, ear auricle, and skin in the DBA/2 and the C57BL/6 strains of mice in relationship to age and dietary restriction. These inbred strains of mice have been established as important animal models of aging by the National Institute on Aging (NIA; Hazzard and Soban, 1989). They also have frequently been used in gerontological research because of their independent genetic origins, as reflected by their differences in genealogy.
(Finch, 1990), and because of their differences in longevity, i.e., the shorter-lived DBA/2 strain compared to the longer-lived C57BL/6 strain (Goodrick, 1975; Higgins et al., 1991; Heller and McClearn, 1992). If pentosidine is a valid biomarker of aging, it would be expected that it, as well as TBT, would increase at a faster rate with age in the shorter-lived DBA strain compared with the longer-lived C57 strain and that dietary restriction would retard this rate.

METHODS

Animals. — The characteristics of mice used in experiments, including strain, gender, dietary groups, ages, and numbers, are summarized in Table 1. Mice of the C57BL/6JNia strain (Figure 1) were purchased through the NIA from its contract colonies at Charles River Laboratories (Stone Ridge, NY), whereas mice of C57BL/6NNia and DBA/2NNia strains (Figures 2 and 3) were obtained free-of-charge under the Biomarkers of Aging Project through the NIA from the National Center for Toxicological Research (NCTR, Jefferson, AR). The fewer number of mice represented for the C57BL/6NNia ad libitum group at 24 months in Figure 2 (Table 1) is due to animal death before shipment. The omission of earlier time points for DBA/2NNia mice in Figure 3 is due to the unavailability of these mice at the time of this study due to colony phaseout of dietary restricted mice for this particular strain by the NCTR.

Dietary restriction of mice was initiated by reducing feed to 80% of intakes for ad libitum-fed mice starting at 14 weeks of age, to 70% at 15 weeks, and finally to 60% at 16 weeks.

Tissue procurement and processing. — Mice were anesthetized by an intraperitoneal injection of a 0.3 cc mixture of ketamine/acepromazine (3 mg/0.3 mg). After killing by cervical dislocation, tails, ear auricle, and skin were collected from each mouse and stored at -80 °C. Tails were deskinned and the ventral tendon fibers were removed by dissection for measurement of TBT. Other fibers used for HPLC (high pressure liquid chromatography) analyses were removed, washed with phosphate buffered saline (PBS), extracted with 2:1 chloroform/methanol, and freeze-dried. A piece of dorsal skin was frozen by immersion in a mixture of dry ice/methanol followed by removal of the hairy coat and epidermis by scraping with a surgical scalpel blade. The skin was diced and homogenized in PBS with a polytron (Brinkmann,

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Westbury, NY). After collecting the pellet by centrifugation at 14,000 × g (Du Pont, Wilmington, DE), the insoluble skin was sequentially extracted at 24-hour intervals with 2:1 chloroform/methanol, 1 M NaCl, 0.5 M acetic acid, and digested for 24 hours at 4 °C of 0.1 mg/ml pepsin in 0.5 M acetic acid. A piece of ear auricle was processed similarly to that described for skin. In addition, a piece of ear auricle was diced, followed by extraction with PBS and 2:1 chloroform/methanol. Following extraction, samples were washed three times with water and freeze-dried.

**Analytical measurements.** — Tendon breaking time was assayed similar to that described by Harrison and Archer (1978) and previously used and described by us (Kohn et al., 1984; Monnier et al., 1988). Tail tendons were kept wet by immersing them in PBS. One end of the fiber was tied by 4-0 Silk suturing thread (Ethicon, Somerville, NJ) to a weight consisting of a fishing-type 5 oz tin/lead sinker (PSS-5 split shots, Water Gremlin, White Bear Lake, MN). For ease of tying the suture to the weight, each sinker was modified by the addition of a small loop composed of a piece of tin/lead solder (Multicore solders, Westbury, NY). All sinkers with their attached solder-composed loops were adjusted so that all were equally close in weight as measured by an analytical weighing balance (2.78 ± 0.07 g). The other end of the tendon was tied to another piece of...
suture with a loop made at the adjacent end. Each suture loop, together with its attached tendon and weight, was placed on a hook attached, in turn, to an electric switch that initiated a timer by the pull of the weight. The tendon with its attached weight was immersed into a fresh 7 M urea solution at pH 7.5, as described previously (Kohn et al., 1984). The temperature of the urea solution was kept constant at 40 ± 0.5 °C by a surrounding water bath. When the tendon broke, the timer stopped. A total of six tendons were assayed for each mouse.

Methods for preparation of tissue hydrolysates for HPLC were the same as previously described by us (Sell et al., 1996). Briefly, a total of 5 mg of tissue was placed into a 13 × 100 mm screw-capped tube. A total of 2 ml deaerated 6 M HCl was added to the tube, followed by purging the tube with nitrogen and sealing with a Teflon-faced rubber-lined cap. All samples were acid-hydrolyzed for 18 hours at 110 °C. After evaporating the acid, each sample was reconstituted with 1 ml of water containing .01 M heptafluorobutyric acid. After filtering each sample, collagen content was determined for all samples by the hydroxyproline colorimetric assay assuming a collagen content of 14% hydroxyproline by weight (Sell et al., 1996).

Pentosidine was determined by a repetitive injection technique as previously described (Sell et al., 1996). Volumes of ≈ 100 to 200 μl volumes equivalent to 45 μg of hydroxyproline were injected into a C18 reversed-phase analytical column attached to a HPLC apparatus (Waters, Milford, MA) as previously described (Sell et al., 1996). The pentosidine peak was monitored at excitation wavelength 335 nm/emission wavelength 385 nm by an online Jasco 821-FP spectrophotometer (Jasco, Easton, MD). The eluent from 29 to 34 minutes was collected, dried, and reconstituted in 200 μl of .02 M sodium acetate (pH 4.47) and, in turn, 160 μl of this volume was injected into a cation-exchange column as previously described (Sell et al., 1996). Pentosidine eluted approximately 18 minutes. In both reverse phase and ion-exchange separations, the fluorescence detector was interfaced to a computer loaded with Borwin chromatography software for recording and integration of peaks (JMBS Développements, Le Fontanil, France).

Statistics. — Variance homogeneity and normality were tested by the Burt–Foster Q-test and the Shapiro–Wilk W-test, respectively (Shapiro and Wilk, 1965; Anderson and McLean, 1974). The hypothesis of variance homogeneity and normality of data were accepted in Figures 1 and 3 with the logarithmic transformation of data (Anderson and McLean, 1974; Steel and Torrie, 1980) and in Figure 2 without transformation. Data in Figures 1 and 2 were analyzed by regression analysis and a two-way ANOVA (SPSS, Chicago, IL) consisting of each dependent variable (i.e., TBT or tissue pentosidine) versus the independent variables: age (Figures 1 and 2), mouse strains (Figure 1), and dietary restriction (Figure 2). The MSE representing the pooled error variance from this analysis was used, in turn, with the Student-Newman–Keuls multiple comparison test to simultaneously compare all means across independent variables of Figures 1 and 2 (Anderson and McLean, 1974; Steel and Torrie, 1980). For the analysis in Figure 2, adjustment for unequal sample sizes was by the approximation given by Bancroft (1968). Because two means were compared within parameters of Figure 3, a two-tailed Student t-test for unpaired means was used for the comparison (Steel and Torrie, 1980). Power analyses of Figures 2 and 3 were conducted by a test (p = .8) with α = .05 and β = .2 according to Miller and Freund (1977) and Steel and Torrie (1980).

Results

In Figure 1, significant main effects due to age (p < .0001) and strain (p < .0001) were noted for TBT, showing a progressive and significant (p < .0001) increase with age for both the C57BL/6 and DBA/2 strains. However, the pattern and the rate of the increase in TBT differed between the two strains as noted by a significant (p < .0001) Age × Strain interaction. For DBA mice, a linear increase was noted with age in which the mean TBT was progressively and significantly larger for each increment of age. In comparison, for C57BL/6 mice, a curvilinear increase was observed which again got progressively and significantly larger at each age increment (Figure 1). At 5 and 17 months, TBT increased more rapidly in DBA/2 compared with C57BL/6 mice. However, after age 17 months, TBT increased at a much more rapid rate in C57BL/6 mice, reaching break times approximating those observed for old DBA/2 mice at 25 months (Figure 1). Thus, significant differences in TBT between strains were noted at 5 (p < .01) and 17 (p < .01) months, but not at 25 months (p > .05).

Similar to TBT, significant main effects due to age (p < .0001) and strain (p < .01) were also noted for pentosidine levels in tendon, ear auricle, and skin with a significant Age × Strain interaction noted for all three tissues (p = .0001, .004, .0001, respectively). In general, the rates of increases in pentosidine level with age in these tissues closely paralleled those observed for TBT, with some noticeable exceptions. First, levels overlapped extensively at 5 months between strains. Thus, mean levels at 5 months were not significantly (p > .05) different between the two strains (Figure 1). Second, unlike TBT, levels measured in all three tissues were not significantly (p > .05) different at 5 versus 17 months in the C57BL/6 strain. Third, the rate of the increase occurring between 17 and 25 months in the C57BL/6 strain varied somewhat with tissue. In tendon and ear auricle, there was no significant (p > .05) difference in levels between DBA/2 and C57BL/6 mice at 25 months. In contrast, for skin, levels at 25 months remained significantly (p < .001) greater for DBA/2 compared with C57BL/6.

In Figure 2, TBT was followed in relationship to age and dietary restriction in C57BL/6 mice. The results showed significant main effects for both age (p < .0001) and dietary restriction (p < .001). As shown by regression analysis, TBT significantly increased with age in both ad libitum-fed (p < .0001) and dietary restricted (p < .0001) mice. Also, as expected, the increase in the ad libitum group was inhibited by dietary restriction as observed by the significant main effect for this variable in the ANOVA. However, in the comparison of individual group means (Figure 2), TBT was significantly greater at 18 months (p < .01) and 24 months (p < .001) for ad libitum versus dietary restricted mice (Fig-
Likewise, pentosidine significantly increased with age in both ad libitum and dietary restricted mice as observed in tendon (p < .0001), ear auricle (p < .0001), and skin (p < .0004). However, the main effect due to dietary restriction was nonsignificant for the three tissues (p = .169, .629, and .069, respectively) as well as the Age × Diet interaction (p = .185, .504, and .437). In the comparison of group means in Figure 2, except for tendon at 24 months (p < .05), there was no difference (p > .05) in levels between dietary groups at 12, 17, and 24 months.

Differences between ad libitum-fed versus dietary restriction were reevaluated in DBA/2 mice. Unlike the previous C57BL/6 mice, consistent differences existed in TBT and pentosidine for all tissues at 26 months (Figure 3). Mean TBT was significantly (p < .05) less for dietary-restricted compared with ad libitum-fed mice (Figure 3). Likewise, pentosidine levels in tendon and ear auricle were significantly (p < .05) less in dietary restricted mice. Although skin pentosidine showed similar results, the difference in this case was not significant (p > .05).

Since several major comparisons for pentosidine of Figures 2 and 3 were found nonsignificant, a power analysis was made to determine the minimum numbers needed to detect with P = .8 significant differences between means of one SD when using α = .05. For Figure 2, differences between ad libitum versus dietary restricted mice in ear and skin levels at 24 months were determined at n = 7 and 14, respectively. In Figure 3, this comparison for skin levels became significant at n = 8.

**DISCUSSION**

The present study compared the age-related relationship between TBT and pentosidine in DBA/2 and C57BL/6 mice and the effects of dietary restriction on these parameters. Although experiments were also made to determine the relationship between pentosidine and protein glycation, these results are part of a detailed study described elsewhere (Sell, 1997) and hence have not been addressed here. In short, these results show that glycation of collagen measured as furosine progressively increased with age and significantly responded to the effects of dietary restriction. Conversely, glycohemoglobin levels measured by the boronate affinity column method significantly declined with age for mice of Figures 1 and 2. This age-related decrease has also been observed by other investigators using the same method (Katz et al., 1990; Novelli et al., 1995) and is suggested to be due to differences in red blood cell turnover with age in rodents (Katz et al., 1990). Our results show no specific strain effect for glycohemoglobin levels for mice of Figure 1. However, in Figure 2, levels did respond to dietary restriction at ages 12 and 18 months, but not at 24 months. This difference in response of glycohemoglobin levels to dietary restriction with age in rodents has also been previously reported (Katz et al., 1990).

Mice used in these experiments were chosen based upon immediate availability from the suppliers. Because two strains, genders, and sources of animals were used in experiments (Table 1), an evaluation was made in the consistency of values for parameters of Figures 1–3. Separate plots were thus made for each parameter versus age consisting of individual data points for C57BL/6 mice of Figure 1 versus Figure 2, and DBA/2 mice of Figure 1 versus Figure 3 (plots not shown). However, these evaluations failed to reveal any differences in TBT and pentosidine measured in different tissues except for the oldest aged mice examined where values for females tended to be higher in comparison to males. The latter observations were apparent for TBT, tendon pentosidine, and ear auricle pentosidine for C57BL/6 mice (Figures 1 and 2), and ear auricle and skin pentosidine for DBA/2 mice (Figures 1 and 3). However, given that different sources of mice were used, that high variability existed for these measurements within old animals, and that gender was not directly compared in this study, no conclusion as to the gender effect could be made.

Previous work by Fu et al. (1992, 1994) showed a parallel increase in pentosidine formation and the crosslinking of tendon collagen, as assessed by gel electrophoresis, when rat tail tendons were incubated with glucose. Likewise, Richard et al. (1991) showed a parallel increase in pentosidine and TBT when tail tendons were incubated with ribose. However, because the lag phase and the percentage increase of pentosidine and TBT were not the same over the incubation period, there appears to be some dissociation between the two events. These observations suggest that other crosslinks or factors may be responsible for the large observed increase of TBT during this type of incubation. Undoubtedly, pentosidine would contribute to the observed increase in TBT if the estimates of Vater et al. (1979) are correct.

TBT as measured by the time it takes to denature collagen in a concentrated urea solution has previously been used to measure the degree of collagen crosslinking (Heller et al., 1987) and as an indicator of the collagen aging rate (Harrison et al., 1978). Many previous studies have shown TBT to be significantly and strongly correlated with chronological age (Verzár, 1963; Everitt, 1971; Harrison et al., 1978; Harrison and Archer, 1983). Less clear, however, is its relationship to biological age.

Using many different mouse genotypes of various inbred and hybrid strains varying in life spans, Harrison and colleagues (Harrison et al., 1978; Harrison and Archer, 1983) have shown a strong relationship between TBT and chronological age, but not mean life span. However, in the comparison of two wild-type mouse species, Harrison and Archer (1983) found that the collagen aging rate increased twice as rapidly in the shorter-lived *Mus musculus* in comparison to the longer-lived *Peromysus leucopus*, suggesting that TBT is a measure of biological age. However, in the evolutionary tree, these two rodent species are fairly distant, and thus the validity of comparing these two species in gerontological research is in question (Spratt and Austad, 1996).

In a separate study by Bochantin and Mays (1981), TBT increased logarithmically with age and increased at a faster rate in the shorter-lived Sprague-Dawley in comparison to the Fischer 344 strain of rats. TBT strongly correlated with maximum life span, but not with mean life span.
Likewise, previous work by Higgins et al. (1991) showed that the rate of increase in TBT occurred faster for the shorter-lived DBA/2 compared with the longer-lived C57BL/6 strain of mice between the ages of 2 and 10 months. This age range was chosen (a) because collagen aging rate is linear during this time period, and (b) to minimize the variability and nonlinearity problems known to occur with TBT in old-aged rodents. Thus, TBT relationship to longevity was not studied. In a subsequent study by Heller and McClearn (1992), TBT was measured longitudinally by surgically removing tendons from tails of DBA/2 and C57BL/6 mice, and their hybrid crosses, at four different ages (2, 5, 10 and 15 months). As previously noted, collagen aging rate increased more rapidly in the DBA/2 strain in comparison to C57BL/6 strain.

The present study compared TBT with pentosidine determined in tendon, ear, and skin of DBA/2 and C57BL/6 mice. As previously noted by Higgins et al. (1991) and Heller and McClearn (1992), collagen aging rate as assessed by TBT increased more rapidly at early and middle ages (<15 months) in the DBA/2 in comparison to the C57BL/6 strain. However, at late ages (>17 months), the rate accelerated in the C57BL/6 strain so that it caught up to that noted for the DBA/2 strain (Figure 1). Likewise, the age-related pattern of the pentosidine increase approximated that noted for TBT except that tissue differences were observed. In skin, pentosidine remained significantly less at 25 months in the C57BL/6 versus DBA/2. In contrast, in tendon and ear, there were no statistical differences in pentosidine levels between these two strains at 25 months (Figure 1).

The reason for the accelerated rates of TBT and pentosidine formation in the DBA strain is not immediately apparent. However, these mice are not noted for their particular heartiness and suffer from high morbidity. This observation is reflected in their survival curve (Sprott and Austad, 1996), which shows a marked decrease in mean versus maximum life span. By comparison, the survival curve of the C57BL/6 strain is found to be rectangular (Goodrick, 1975; Turturro and Hart, 1992; Sprott and Austad, 1996). In a recent study comparing the survival curve of C57BL/6 to that of DBA/2 mice (Sprott and Austad, 1996), a small difference was noted to exist in maximum life spans between these two strains (150 vs 130 weeks), while a larger difference existed in mean life spans (120 vs 88 weeks). As observed from these curves, mortality starts as early as 15 weeks of age in DBA mice. The facts that TBT and pentosidine increase so rapidly in DBA mice and that oxidative processes are required for their formation suggest that oxidative stress, possibly of genetic origin, may shorten mean life span of this strain.

One of the pathologic processes present in the DBA strain, and possibly associated with their early demise (Sheldon et al., 1995), is their susceptibility to ocular diseases such as glaucoma and cataracts (Cohen-Salmon et al., 1989; Sheldon et al., 1995). In one study, cataract was one of the most frequently diagnosed ocular lesions in this strain (Sheldon et al., 1995). Interestingly, pentosidine has been associated with human lens senescence and cataractogenesis probably induced through an oxidative stress type of mechanism involving ascorbate (Nagaraj et al., 1991).

Another factor that highly influences TBT and pentosidine level is dietary restriction. This anti-aging intervention is known to increase mean and maximum life span and at the same time delay many age-associated disease processes such as tumorigenesis (Weindruch and Walford, 1982), leukemia (Shimokawa et al., 1993), and renal disease (Everitt et al., 1983). Previous studies have shown that the physiological aging rate of collagen as determined by TBT is also delayed (Everitt et al., 1981, 1983). As shown in Figure 2, a small but significant inhibition of the age-related increase of TBT was found to occur at 18 and 24 months in dietary restricted C57BL/6 mice in comparison to the ad libitum controls. Likewise, 'TBT was significantly decreased by dietary restriction in DBA/2 mice at 26 months in comparison to the ad libitum-fed mice (Figure 3).

Since dietary restriction has been shown to delay the aging process, this intervention would be expected to also retard the age-related increase of pentosidine as well. Except for tendon at 24 months, the results showed no significant effect of dietary restriction on pentosidine formation in C57BL/6 NNia mice (Figure 2). However, dietary restriction significantly delayed pentosidine formation in tendon and ear of DBA/2NNia mice (Figure 3). Given the variability and small number of mice used, a further analysis of the statistical power of the study suggested that some of the comparisons that were found nonsignificant may become significant if a greater number of mice were represented in treatments. This was evidenced for levels of ear and skin at 24 months in Figures 2 and skin in Figure 3.

Similarly, Reiser (1994) also reported no specific age effect of dietary restriction in reducing pentosidine in aortic collagen of C57BL/6 NNia mice, the same strain as used here (Figure 2). In the Reiser study, pentosidine was not detected in tail tendon and skin, suggesting that the assay used may not have been sensitive enough to detect the low levels present in these tissues. Conversely, Cefalu et al. (1995), working with Brown-Norway rats, showed that the age-related increase of pentosidine in skin was significantly inhibited by dietary restriction at 17 and 29 months of age.

In conclusion, evidence presented shows TBT and tissue pentosidine increasing at a faster rate in the shorter-lived DBA/2 strain in comparison to the longer-lived C57BL/6 strain of mice at early age (5 months), middle age (17 months) and, to a much lesser extent, late age (25 months). These findings would be expected if pentosidine is a valid biomarker of aging. However, as noted, the degree of rectangularity of the survival curve is not the same for the two strains. Thus, it is uncertain whether the accelerated rates of TBT and pentosidine formation in the DBA strain represent a true aging effect or are simply due to an inherited pathological weakness resulting in spontaneous mortality beginning at a young age. Ideally, it would be advantageous to conduct a longitudinal study in which sequential values are obtained from a group of DBA mice destined to live a long time compared with the shorter-lived DBA mice. Such a comparison would give much more insight into whether this marker system is truly connected with longevity.

In these studies, both TBT and pentosidine were found to increase with age in more or less parallel fashion. However,
because the response to dietary restriction was not the same between these two parameters in C57BL/6 mice, and because the age-related response in pentosidine level among tissues did not exactly match that observed for TBT, these results further support the notion of some dissociation between TBT and pentosidine. Undoubtedly, other glycoxidative products yet to be discovered probably play an important role in explaining the age-related increase in TBT. These findings, as well as the noted tissue differences and variabilities, suggest that: (1) pentosidine by itself may not be robust in the prediction of longevity, but instead may have to be used in concert with other markers of aging; (2) different species and strains used in future studies may have to be independently assessed for their age-related response to dietary restriction; and (3) future experimental designs involving pentosidine measurements must be rigorously controlled.

In the present study, the age-related increase of pentosidine could be followed just as easily in a piece of ear tissue as that found in skin or tendon. Ear clipping and notching are common methods to individually identify rodents in animal husbandry. Thus, it is expected that pentosidine would be a useful and easily measured noninvasive biomarker in future intervention studies on aging.

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GERON CORPORATION–SAMUEL GOLDSTEIN
DISTINGUISHED PUBLICATION AWARD

The Geron Corporation–Samuel Goldstein Distinguished Publication Award lecture will be presented at 1:30 p.m. Monday, November 17, 1997 at the Annual Scientific Meeting of The Gerontological Society of America in Cincinnati, Ohio. The lecture will be presented by William T. Cefalu, a co-author of the article selected by the Editorial Board of the Journal of Gerontology: Biological Sciences as the best published in the six issues of that journal starting May 1996 and ending March 1997.

The article chosen was:


The award will again be given at the 1998 Annual Meeting for the best article published in the journal starting May 1997 and ending March 1998.