Lifelong Treatment with Oral DHEA Sulfate Does Not Preserve Immune Function, Prevent Disease, or Improve Survival in Genetically Heterogeneous Mice

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OBJECTIVES: To determine whether lifelong exposure to dehydroepiandrosterone sulfate extends the lifespan or retards immune senescence in mice.

DESIGN: Double-blind, placebo-controlled intervention trial.

SETTING: A specific pathogen-free rodent vivarium.

PARTICIPANTS: 120 mice bred as a cross between CB6F1 females and C3D2F1 males.

INTERVENTION: DHEAS at 100 μg/mL in drinking water from weaning until death.

MEASUREMENT: Age at death, cause of death, antibody production after erythrocyte immunization, and T cell subset profiles in peripheral blood at ages 8 and 18 months.

RESULTS: DHEAS ingestion did not lead to a significant increase in mean or maximal longevity: the 95% confidence interval for DHEAS effect on mean lifespan ranged from +35 days to −80 days. There were no significant effects of DHEAS on incidence of lethal illnesses, except for a trend toward higher levels of mammary adenocarcinoma in DHEAS-treated females and mouse urinary syndrome in DHEAS-treated males. DHEAS treatment did not improve the ability of middle-aged mice to produce antibody to a foreign particulate antigen, and it did not alter the proportions of age-sensitive T cell subsets in middle-aged animals.

CONCLUSION: Although differences among species in pharmacokinetics complicate interpretation of studies in which DHEA or DHEAS is administered to rodents, our data provide no support for the idea that chronic exposure to this steroid retards immune senescence or prevents late life illness.
J Am Geriatr Soc 47:960–966, 1999.

Key words: DHEAS; lifespan; immunity; senescence

The observation that plasma concentrations of the adrenal steroid dehydroepiandrosterone sulfate (DHEAS) fall dramatically throughout adult life[1] have for decades prompted speculation that administration of this human hormone might retard the development of late life illness. Although DHEA is assumed to be the active form of this steroid, more than 99% of the hormone in serum is in the soluble, sulfated form DHEAS, from which DHEA is liberated in those tissues that contain steroid sulfatases. Epidemiologic evidence that postmenopausal women with relatively high DHEAS levels had low risks of breast cancer[2] and that low DHEAS levels predicted high risks of bladder cancer[3] and cardiovascular disease[4] were consistent with the idea that DHEA might protect against a wide range of late life diseases. Some of the metabolic effects of DHEA[5] have also prompted speculation that it may serve to protect against the atherogenic effects of adult-onset diabetes mellitus.

Interest in DHEA or DHEAS as a preventative or therapeutic agent has also been stimulated by reports of its effects on cancer and immunity in aged rodents. Research on possible anti-oncogenic effects has employed either carcinogen-treated mice or mice of strains that show a genetic predisposition to spontaneous neoplasia. Thus, for example, ingestion of very high levels of DHEA (typically 0.6% of food intake, equivalent to about 500 mg/kg body weight per day) have been reported[6] to diminish induction of lung tumors in A/J mice, and colon tumors in BALB/c mice, as well as spontaneous mammary tumors in the C3H-A^{vy}/A strain. DHEA administration has also been reported to reduce the incidence of renal disease and proteinuria in rats and mice, and to inhibit the development of diabetes in leptin-resistant db/db mice.[6] DHEAS ingestion at lower doses (100 μg/mL of drinking water, approximately equivalent to 4 mg/kg of body weight/day) has been reported to have dramatic effects on mouse immune function, fully preventing age-associated changes in cytokine production and age-dependent declines in production of antibodies in primary and secondary immunizations with protein antigens[7,8] and inhibiting development of a range of autoimmune and inflammatory mediators in treated mice.[9]

These studies, along with the widespread use of nonprescription DHEA preparations by individuals interested in the alleged antigeric properties of this hormone, encouraged us to carry out a longitudinal, double-blind, prospective study of the effects of DHEAS ingestion on immunity, disease risk, and survival in mice. For this effort we selected the UM-

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0002-8614/99/$3.50

HET3 stock, bred as the progeny of CB6F1 females and C3D2F1 males; this breeding scheme produces a genetically heterogeneous population of mice in order to minimize the risks of testing for an effect in a single genetic background.[10] We report here that lifelong administration of DHEAS, at levels approximately fourfold greater than those employed in recent human clinical trials, improves neither survival nor immune status in mice.

METHODS

Mice

UM-HET3 mice were produced by the Core Facility for Aged Rodents of the University of Michigan's Older Americans Independence Center. All mice in the test population have (BALB/cByJ x C57BL/6J)F1 mothers and (C3H/HeJ x DBA/2J)F1 fathers and are thus genetically distinct full sibs. The mice were housed in adjacent cages in same-sex groups (initially three males/cage or four females/cage; these densities declined in later life as a result of mortality) and given free access to water and laboratory chow diet. Sentinel mice in the colony are tested quarterly for a range of antiviral titers and parasitic infections; all such tests were negative throughout the experimental interval, and the animals can, therefore, be considered specific pathogen free. To avoid seasonal effects, which have been shown in other laboratories to influence mouse lifespan,[11] the experimental group was composed of four subgroups of approximately equal size born in August 1994, February 1995, May 1995, and August 1995. Male and female mice were assigned separately at weaning to either the control or the DHEAS treatment group by use of a random number table.

DHEAS Treatment

DHEAS was purchased from Aldrich Chemical Company (Milwaukee, WI), diluted into purified water to a concentration of 100 μg/ml, and sterilized by filtration. A fresh stock was made each week, and stored at 4° until use. To minimize degradation of the steroid, water supply bottles were changed each Monday, Wednesday, and Friday, and all water bottles were light-shielded by aluminum foil. Water without DHEAS was used for control animals. This protocol is based closely on that shown previously to preserve immune function in aging mice.[7–9] Water bottles were prepared in a coded protocol by an individual who was not otherwise involved in the experiment so that technicians involved in the assessment of the mice were blinded to treatment condition.

Assessment of T Lymphocyte Subsets

Peripheral blood samples (400 μL) were taken from each mouse by tail venipuncture at 8 months and again at 18 months of age and examined for their proportions of a series of T lymphocyte subsets by two color flow cytometric methods that have been described in detail previously.[12]

Assessment of Immune Competence by Induction of Antierythrocyte Antibodies

At approximately 22 months of age (range: 20.7 to 23.9) each mouse received an intraperitoneal injection of 200 μL containing 5×10^8 washed sheep erythrocytes in sterile phosphate buffered saline. Mice were bled (150–200 μL) by tail venipuncture 14 days later, and clarified sera were tested for antierythrocyte antibodies by hemagglutination using twofold dilutions from a 1:48 starting concentration. For the hemagglutination test, 25 μL of a 2% erythrocyte suspension were mixed with 50 μL of diluted serum, and agglutination was assessed 1 hour later. Titers, expressed as the reciprocal of the most dilute sample to show hemagglutination, were log-transformed before statistical analysis.

Health Assessment and Necropsy

Each mouse was inspected at least twice each weekday and at least once per day on weekends and weighed once per month. Animals that seemed to be ill were placed on a "watch list" for more frequent inspection and weighed weekly. Animals judged to be severely ill, based on a symptom check list (lethargy, rapid weight loss, bleeding or ulcerated tumor, inability to eat or drink), were declared moribund and were humanely killed for pathological assessment. The decision to submit a live but moribund mouse to necropsy was made by individuals who were not aware of the treatment condition for the animals. Mice found dead in their cages were also submitted for necropsy. Details of the necropsy procedure, involving histological assessment of a wide range of tissues, have been given elsewhere.[10]

Statistical Analysis

Intergroup differences in mean life span and in antibody titers were assessed using an analysis of variance method, taking treated males, treated females, untreated males, and untreated females as independent groups. Differences between treatment groups and between sexes in the incidence of fatal lesions and incidental pathological findings were assessed by two-tailed Fisher's exact test, with Bonferroni correction for multiple comparisons in certain instances mentioned in the text. The effects of DHEAS treatment on T cell subset levels were assessed by two-tailed unpaired Student's *t* test, with Bonferroni correction as specified in the text. The results of the hemagglutination assays were evaluated by two-factor analysis of variance (gender × treatment), because preliminary data had suggested an effect of gender on antibody response in some mouse populations.

RESULTS

Life Span

Our primary working hypothesis was that lifelong exposure to DHEAS would extend mouse life span by retarding the development of late life illnesses. We based our sample size on the assumption that mean life span of the control group would be approximately 800 days, with a standard deviation of ~150 days. Under these circumstances a group size of n = 60 per treatment would have 80% power to detect an effect of 77 days, i.e., about 10% of the mean longevity. Early in the study it became clear that a small number (eventually n = 9/60 = 15%) of the male mice were dying of a syndrome, the Mouse Urinary Syndrome (MUS), that occurs only in group-housed male mice and is thought to represent the effects of psychological stress imposed by the development of dominance hierarchies in all-male groups.[13,14] Because these males died at much earlier ages than the other members of the population (see below), we performed the main life span analysis without consideration of the mice that died of this urinary syndrome.

Figure 1 shows the cumulative survival curves for control mice and for DHEAS-treated mice, excluding all MUS

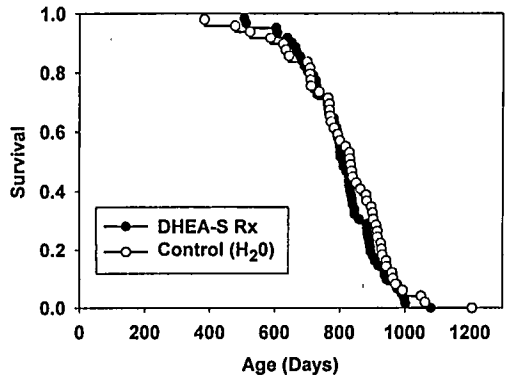

Figure 1. Survival curves for control and DHEAS-treated mice, excluding the nine male mice that died of murine urinary syndrome. Each symbol represents one mouse. See Table 1 for summary statistics.

deaths. Although there is a slight trend toward earlier deaths among the DHEAS-treated mice, the difference is not statistically significant ($P = .27$ by log-rank test). Table 1 shows mean life span statistics separately for female mice and for males with and without the urinary syndrome. DHEAS had no significant effect on longevity in either male or female mice; the slightly shorter life span in the DHEAS-treated male mice seems to reflect the higher incidence of MUS among the DHEAS-treated males. Considering all mice, the 95% confidence interval for the difference in longevity between control and treated mice is −35 to 80 days; i.e., we can be 95% confident that DHEAS does not extend life span in this mouse stock by more than 35 days, i.e., about 4% of mean life span, and it might lead to a shortening of life span by as much as 80 days, i.e., about 10%.

Pathology

Mice found dead or discovered to be severely ill were subjected to a thorough histopathological analysis both to determine the most likely cause of death (or morbidity) and to catalogue nonlethal (i.e., incidental) lesions that might be altered by lifelong exposure to DHEAS. Table 2 presents the probable causes of death for each of the four groups (control vs DHEAS and males vs females). Cause of death could not be determined in 15/120 (13%) of the mice, either because of advanced autolysis or because the mouse suffered from multiple lesions, each of which is likely to have contributed to its illness. Nonneoplastic diseases were the sole cause of death in only 18 of the mice (15%), consistent with our findings in an analysis of another stock of four-way cross mice.[10] Our experimental design does not provide sufficient statistical power to test for any but the strongest possible effects of DHEAS on the cause of death inasmuch as with a total n = 120, even the most common cause of death (lymphoma of various forms) accounts for only 22 deaths. Nonetheless, we note that all six of the cases of fatal mammary adenocarcinoma were found in the DHEAS-treated group ($P = .04$ by two-tailed Fisher's exact test, but $P = .17$ when males are excluded). Diagnoses of MUS, the stress-related urinary syndrome seen only in males, were more common in the DHEAS-treated males (7/29 = 24%) than in the control males (2/31 = 6%), but the difference is not statistically significant ($P = .28$).

We also tabulated distribution among treatment groups of a number of nonlethal lesions that are nonetheless of potential interest: cataract, cerebral mineralization, hepatocellular adenoma, membranous glomerulonephritis, ovarian cyst, pancreatic islet cell hyperplasia, and spongiform encephalopathy. Among these, DHEAS treatment had a significant effect only on membranous glomerulonephritis, which was diagnosed in 37% (19/51) of the controls and 16% (11/69) of the DHEAS-treated mice. This difference is significant by Fisher's exact test ($P = .01$) before adjustment for multiple comparisons, but a Bonferroni correction suggests that the significance ($P = .07$) is only marginal and could represent a chance occurrence in a series of comparisons. There was no effect of sex on any of these incidental lesions except for the unsurprising absence of ovarian cysts in males and the tendency for pancreatic islet cell hyperplasia to be somewhat more common in males (41/60 = 68%) than in females (26/60 = 43%; $P = .01$ by Fisher's exact test.)

Antibody Responses to Immunization

As a result of previous reports indicating that DHEAS administration, at 100 μg/mL of drinking water, could prevent the decline in late life of humoral immune responses,[7] we tested each mouse at approximately 22 months of age for the ability to generate antibodies to a particulate antigen, sheep

Table 1. Mean Life Span for DHEAS Treated and Control Mice: Breakdown by Sex and MUS Diagnosis*

Group	DHEAS RX	Control
All Females	800 ± 19, N = 40	809 ± 30, N = 20
All Males	758 ± 32, N = 29	803 ± 34, N = 31
Males w/o MUS	818 ± 25, N = 22	825 ± 31, N = 29
Males w/MUS	572 ± 72, N = 7	472 ± 128, N = 2
All Mice	782 ± 17, N = 69	805 ± 23, N = 51

* Values are means ± standard errors for the indicated number of animals. MUS refers to the mouse urinary syndrome, thought to be a result of social stress in group-housed males, as described more fully in the text.

Table 2. Probable Causes of Death in DHEAS Treated and Control Mice*

Cause of Death	Female DHEAS	Female Control	Male DHEAS	Male Control
Fibrosarcoma	7	6	1	0
Lymphoma	12	3	2	5
Mammary CA	6	0	0	0
MUS	0	0	7	2
Neoplastic (other)	6	5	7	9
Nonneoplastic†	3	3	4	8
Pulmonary CA	1	0	4	4
Undetermined‡	5	3	4	3
Totals	40	20	29	31

* Abbreviations: CA = adenocarcinoma; MUS = murine urinary syndrome.
† Includes deaths from congestive heart failure (n = 11), enamel organ dysplasia (2), endometritis (2), hepatocellular adenomas, mammary fibroadenoma, and renal amyloidosis.
‡ Includes mice in which more than a single disease was considered to have contributed to death as well as mice in which autolysis was too far advanced to permit a confident diagnosis.

erythrocytes (SRBC). We chose this immunogen because of previous evidence showing that responses to SRBC declined strongly with age in mice.[15] Table 3 shows the results of this experiment. There were no significant effects of DHEAS treatment on titers of antierythrocyte antibodies in either male or female mice.

T Lymphocyte Subset Distributions

As mice age, they show systematic changes in the relative proportions of certain T cell subsets in peripheral blood, including increases in the proportion of CD4 and CD8 cells with the memory phenotype, decreases in the proportion of CD4 cells with the naïve or virgin phenotype, increases in the proportion of CD4 and CD8 T cells expressing high levels of the plasma membrane pump P-glycoprotein,[16] and a decline in CD4 cell numbers. All of these changes have previously been documented in the UM-HET3 stock,[12] and the shift from naïve to memory populations has also been noted in aging humans (reviewed in ref. 17). We, therefore, examined control and DHEAS-treated mice at two ages, 8 and 18 months, to see if the steroid altered the relative proportions of any of these age-sensitive subsets. Figure 2 presents the results of these determinations as means ± standard errors. There was no statistically significant effect of DHEAS treatment on any of the T cell markers in mice tested at 18 months. Two subsets, CD4V (naïve CD4 cells) and CD4P (CD4 cells with P-glycoprotein expression) showed a significant effect of DHEAS treatment at the 8 month test point using criteria that are not adjusted for multiple comparison artifacts. DHEAS seemed to lead to relatively low levels of CD4P cells and relatively high levels of CD4V cells, in each case consistent with the idea that DHEAS treatment might be acting to retard the development of age-associated shifts in subset distribution. After Bonferroni correction, however, only the effect on CD4P levels remains significant, and only marginally so ($P = .048$). The lack of any effect of DHEA on most of the age-sensitive subsets at 8 months, and the lack of any DHEA effect on subsets examined at 18 months, suggest that the effect, if it exists at all, is transient and inconsistent.

DISCUSSION

Rodent models present with both advantages and disadvantages for evaluation of interventions designed to prevent or retard late life illness. The major advantage of rats and mice is their relatively short life span. It is exceptionally difficult to conduct a study in which a putatively protective drug is administered to healthy human subjects over more than a small percentage of the human lifespan. Lifelong prospective studies of drug effects in rodents might be able to provide insights into beneficial or toxic effects of substances under consideration for long-term human intervention trials, but interpretation of the results of these trials must include consideration of differences between the animal and human species.

Previous studies of long-term administration of DHEA or DHEAS to rodents have been few, and have focused on specific strains of experimental animals selected because they exhibit unusually high risks of early life neoplasia or autoimmune disease. Ingestion of DHEA at ~450 mg/kg body weight/day extends the life span of (NZB x NZW)F1 mice, a strain that develops severe lupus-like autoimmunity within the first year of life.[18] High-dose DHEA ingestion also improved survival of tumor-prone C3H-A^{vy}/A mice, although formal survival analysis of the published data shows that the difference between treated and untreated mice was only marginally significant ($P = .09$) when the experiment was terminated at 18 months of age. DHEA ingestion is also said to have extended the lifespan of both C57BL/6 mice and Sprague-Dawley rats, but the paper in question did not present any data to support this assertion.[6]

In contrast, our own data provide no support for the hypothesis that DHEAS ingestion extends life span in mice. Certain features of our experimental design deserve emphasis to help put this negative result into context. Our experimental approach used mice bred using a four-way cross method to avoid the possible objection that any effects noted might be limited to a single mouse genotype. Four-way cross mouse populations have long been recommended to experimental gerontologists as an inexpensive way to generate rodent populations with reproducible genetic heterogeneity[19] but have only recently been exploited for such studies.[10,20] Although the use of this four-way cross stock does not prove that DHEAS treatment could not influence longevity in some other variety of mouse or rat, it does suggest that our findings are not likely to reflect strain-specific idiosyncrasy. Secondly, we used a sufficiently high sample size to provide relatively tight confidence limits on the possible effect of the DHEAS treatment. Pooling both sexes and including all 120 mice, the best estimate of the effect size (DHEAS longevity minus control longevity) is −23 days, with a 95% confidence interval of −80 to 35 days. Thus we can conclude with 95% confidence that the effect of DHEAS on lifespan is less than 35 days, or about 4% of the mean lifespan, and that DHEAS treatment is equally likely to have a life-shortening effect of 80 days, about 10% of the lifespan.

The dose used for our intervention, 100 μg/mL of drinking water, provides an intake equivalent to approximately 4 mg/kg body weight. This dose is thus much less than that ingested by mice given DHEA in their food at levels of 0.4% to 0.6% (equivalent to about 500 mg/kg body weight) but slightly higher than the levels (~1 mg/kg body weight) considered safe for use in human investigation.[21–23] Since long-term exposure to even 1 mg/kg daily can have mild androgenizing effects in humans,[23] it is unlikely that doses much higher than 4 mg/kg would be suitable for long-term administration in the context of a preventative trial. In our study, we used DHEAS, which unlike DHEA is water soluble, in place of the DHEA used in some of the previous mouse and human studies, in part because we wished to follow closely the protocol reported to prevent immune senescence in

Table 3. Antierythrocyte Responses by DHEAS Treated and Control Mice*

Group	DHEAS RX	Control
Females	2.15 ± 0.07, n = 35	2.05 ± 0.08, n = 17
Males	2.19 ± 0.07, n = 25	2.27 ± 0.08, n = 25

* Values given are the logarithm of the antierythrocyte antibody titer in sera drawn 14 days after immunization, ± the standard error of the mean for the indicated numbers of mice. There is no significant effect of DHEAS treatment. Negative sera, i.e., sera that failed to cause hemagglutination at a dilution of 1:48, were assigned a value of 1.68 = log (48). These negative sera represented 20% of the males (regardless of DHEAS exposure), 35% of the control females, and 34% of the DHEAS treated females; there was no effect of DHEAS treatment on the proportion of negative sera.

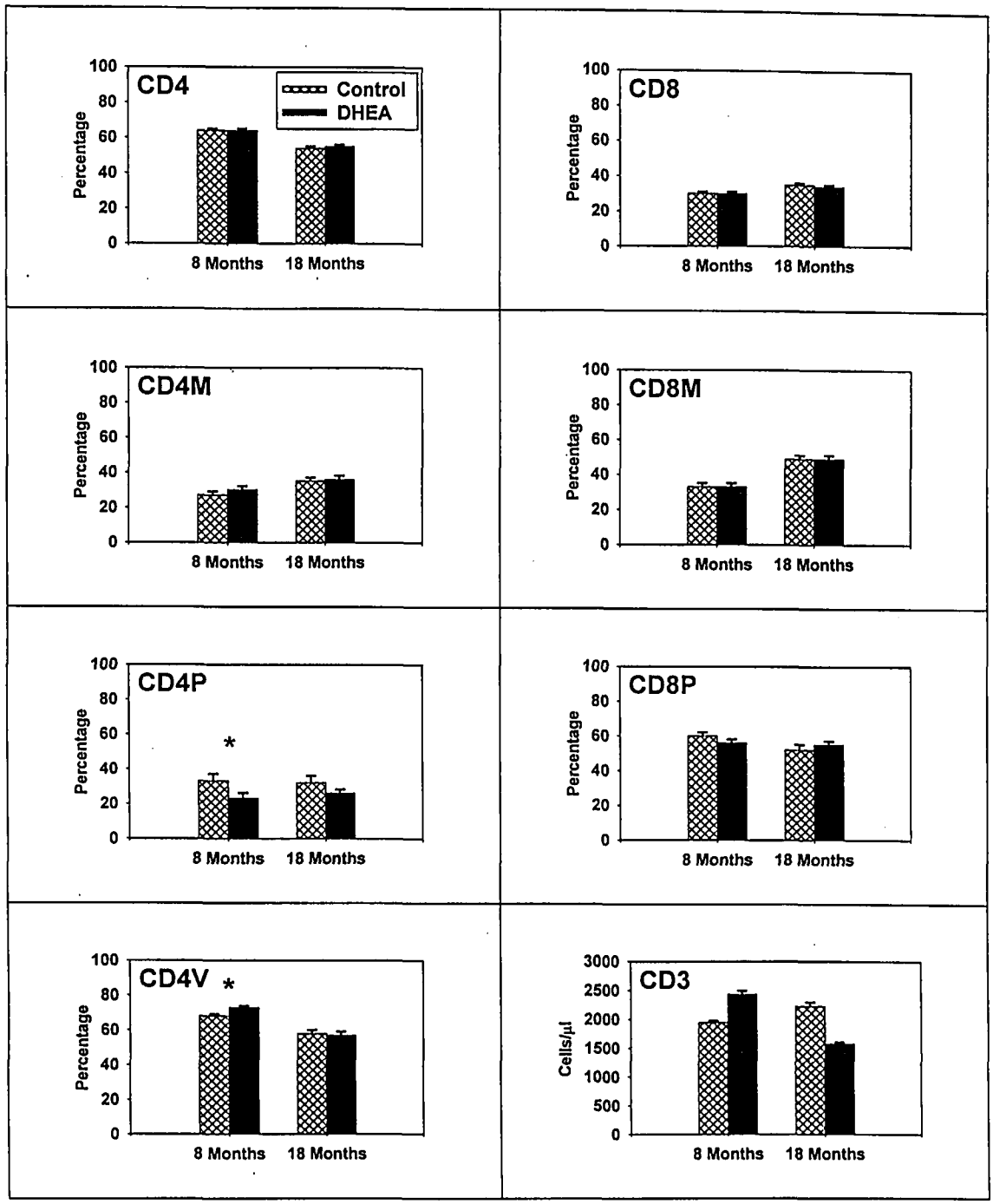

Figure 2. Proportions of T cell subsets in the peripheral blood of control and DHEAS treated mice at 8 and 18 months of age. Each bar shows mean and standard error. Number of mice in each bar ranges from 35 to 49 at 8 months and from 28 to 45 at 18 months. Asterisks indicate a significant effect of the DHEAS treatment at $P < .05$ prior to correction for multiple comparisons. Abbreviations: CD4 M = CD4 memory; CD8 M = CD8 memory; CD4V = CD4 virgin; CD4P and CD8P = CD4 and CD8 cells with high level P-glycoprotein expression.

mice[7,8] and, in part, because the rapid conversion of ingested DHEA to DHEAS[22–24] makes these two forms of the agent biologically equivalent.

We were unable to develop a reliable assay for DHEAS detection in mouse serum using commercially available radioimmunoassay kits because we found that mouse serum contained substances, possibly steroid-binding proteins, that interfered with the assay method. Addition of diluted mouse serum to buffers or human sera containing known amounts of DHEAS led to inconsistent results that were dependent on the source of the mouse serum and the assay pH. Methods that separate DHEA from interfering substances by solvent extraction or chromatography have estimated the level of total DHEA in mouse serum at 2 to 10 nmol/L.[25–27] Male mice that were fed a diet containing DHEA at ~450 mg per day per kg body weight, achieved serum levels of DHEA that were

only 110 nmol/L, i.e., about 20-fold lower than those seen in normal adult humans.[26] Injection of DHEA at 1.2 mg/mouse/day (about 30 mg/kg body weight per day) has been reported to produce serum DHEA levels of only 117 nmol/L in samples taken 16 hours after the last of three daily injections.[25] These data suggest that DHEA is metabolized or excreted more rapidly by mice than by humans and make it relatively hazardous to extrapolate our findings to predict the outcome of analogous human clinical interventions. The dose we selected, 100 μg/mL of water, was chosen because of reports that chronic ingestion at this dose prevented immunosenescent decline, even though the reports of functional enhancement did not include measures of serum DHEA concentration.[7,8] Studies of rats have suggested that DHEA-fed female rats achieve 30-fold higher serum concentrations than males,[28] but we found no differences between male and female mice in the extent of the DHEAS effect on life span or antibody responses.

Daynes and his colleagues have reported that oral DHEAS ingestion, for periods of 9 to 50 weeks at the dose we used for this study, prevents or repairs many of the immunological alterations seen in aging mice. The benefits reported include higher levels of antibody produced after immunization by ovalbumin,[7] lower levels of tissue-specific autoantibody, serum amyloid P protein and IL-6,[9] and restoration of the ability to produce high levels of IL-2 after in vitro activation.[8] Age-related elevations in spontaneous and induced production of IL-10, a potent suppressor of some forms of cell-mediated immune response, are also reported to have been corrected by a 3-week exposure to DHEAS-supplemented drinking water.[29] Acute exposure of aged mice to DHEA (a single injection at the time of immunization) has been reported to improve the humoral response to influenza vaccination and to protect vaccinated mice against influenza infection.[30,31]

In contrast, we find no evidence that chronic exposure to DHEAS improves the humoral immune responses of mice, using as stimulus a particulate antigen, sheep erythrocytes, known from previous work to elicit strong responses in young mice but weaker responses in aged mice.[15] Human clinical trials designed to test the immunostimulatory properties of DHEA or DHEAS have also proven disappointing. Thus administration of oral DHEA (50 mg/day for a 4-day period) to older volunteers did not improve the response to influenza vaccination in a double-blind study; indeed, placebo recipients generated higher titers of antibody to one of the three influenza strains included in the vaccine.[32] Oral DHEA also failed to improve the response of human volunteers to tetanus immunization,[21] and did not produce any significant change in responses to influenza vaccination. Another study used subcutaneous DHEAS injection in the context of influenza vaccination and found no significant effect of the steroid on antibody titers to any of the three component viruses although there was a trend ($P = .06$) toward improved titers to the H3N2/Beijing virus when baseline titers were subtracted.[33] Our data on antierythrocyte antibody responses are, thus, largely consistent with this body of negative results, though at variance with early reports of dramatic improvement in humoral immunity in mice chronically exposed to DHEAS in their drinking water[7] at the same dose used in our study. Age-dependent change in T cell subsets, which are retarded by a lifespan-extending caloric restriction protocol[34] and which do correlate with interindividual differences in lifespan,[35] do not seem to be altered in a consistent way by the DHEAS treatment.

The hypothesis that exogenous DHEA or DHEAS treatment might diminish the risk of late life illnesses was based originally on epidemiological work suggesting lower risks of cardiovascular disease,[4] bladder cancer,[3] and breast cancer[2] in people whose DHEAS levels were relatively high and by studies in rodents at high risk for spontaneous or induced neoplasia.[6] Interpretation of the epidemiological findings has been complicated, however, by long-term follow-up studies that found no significant relationship between serum DHEAS levels and ischemic heart disease or cardiovascular disease in men or women.[36–38] High levels of DHEAS do not seem to protect men or women from cognitive impairment[39] or to confer protection against the development of abnormal glucose tolerance.[40] Among premenopausal women, high DHEAS levels are associated with relatively low breast cancer incidence, but increased DHEAS seems to increase breast cancer risk among postmenopausal women,[41,42] consistent with suggestions that DHEA effects may depend on current and prior history of end-organ exposure to gonadal steroids.

Nor have DHEA intervention trials shown encouraging results. A 6-month trial of oral DHEA at 50 mg/day produced no changes in lipid profiles, insulin sensitivity, or body fat, except for a small decline in high density lipoprotein levels in women, but it did lead to a 2-fold increase in androgen levels in the female volunteers.[23] A subsequent 1-year trial using a 100 mg/day dose also led to increased androgen levels in women but no change in lipid or apolipoprotein measures, insulin sensitivity, nitrogen balance, or bone mineral density. A small (15%) increase in knee muscle strength in the male subjects (compared with about 6% in the placebo group) was not seen in women, and lumbar muscle strength was unaltered in both sexes. A 20-week trial of DHEA at 50 mg/mL was interpreted by the authors as showing positive effects on a variety of immune parameters, including T cell and B cell mitogenic response, mitogen-stimulated production of IL-2 and IL-6, and NK-mediated cytotoxicity.[23,43] At least some of these claims, however, seem likely to reflect a multiple comparisons artifact because the "positive" findings were extracted from a series of repeated measurements, at several times after the initiation of DHEA exposure, in which treated and untreated subjects were only occasionally distinct, whereas the analysis used did not make use of a post-hoc test that adjusts α-levels for multiple comparisons. Another double-blind study of DHEA ingestion (50 mg/day for 3 weeks with placebo cross-over) also reported an increase in NK function but found a decline in helper T cell number and T cell mitogen responses.[24] On balance, then, there is little compelling evidence that extended (3 to 52 week) exposure to DHEA alters objective measures of health status in humans, except possibly for undesirable androgenic effects in women. Our own data are consistent with this pessimistic appraisal in that life-long treatment with DHEAS did not increase life span or diminish disease risks and may have conferred some increased risk of illnesses, such as mammary cancer in females and urinary syndrome in males, that are influenced by sex steroid levels.

ACKNOWLEDGMENTS

The authors thank Gretchen Buehner, Mike Eisenbraun, Cindy Hendrickson, George Kunz, Luann Linsalata and

Joyce Peterson for assistance in this project, and Dr. Raymond Daynes for advice on DHEAS administration.

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