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# AGING ALTERS ZONATION IN THE ADRENAL CORTEX OF MEN

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## ABSTRACT

Whereas aging has been shown to be associated with striking reductions in circulating levels of adrenal androgens in humans, the alteration in adrenal function that occurs in aging has not been identified. We sought to determine if there are changes in the zonation of the adrenal in aging men by performing histomorphologic analyses of adrenal specimens that had been obtained at autopsy following sudden death due to trauma. We evaluated adrenals from 21 young men (20-29 yrs) and 12 older men (54-90 yrs); inclusion criteria required the presence of medullary tissue in the specimen and fixation within the first 24 hrs postmortem. Sections stained with H/E were examined microscopically and areas of the cortex that included adjacent medullary tissue were chosen for quantitative evaluation by use of a computerized image analysis system. The average width (arbitrary units, pixels) of the zona reticularis and that of the combined zonae fasciculata/glomerulosa were determined from sections stained for reticulum fibers. The zona reticularis represented 37.1 +/- 1.9% of the total cortical width in the young men, which was significantly greater than that of the older men ( 27.1 +/- 3.3%, P =0.0082). The zona fasciculata/glomerulosa to zona reticularis ratio in the young men (1.84 +/- 0.15) was significantly less that that of the older men ( 3.29 +/- 0.47, P = 0.0011). There was no significant difference in the total width of the cortex in young compared to older men. These data suggest that aging results in alterations within the cortex of the adrenals in men such that there is a reduction in the size of the zona reticularis and a relative increase in the outer cortical zones. A reduced mass of the zona reticularis could be responsible for the diminished production of dehydroepiandrosterone and dehydroepiandrosterone sulfate that occurs during aging.

Adrenal androgens are C19 steroids such as dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DS) that arise primarily from the adrenal cortex. Adrenal androgen production in humans varies both as a function of developmental state and according to physiologic and pathophysiologic circumstance (1-5). During young adulthood, adrenal synthesis of DHEA and DS is usually high, and is believed to occur primarily in the inner cortical zone, the zona reticularis. We and others find that in contrast to the zona fasciculata, wherein cortisol is produced and which has abundant 3β–hydroxysteroid dehydrogenase (3β-HSD), the zona reticularis of the human adrenal contains high concentrations of dehydroepiandrosterone sulfotransferase and little, if any 3β-HSD (6-8). Thus, the functional differences in the steroidogenic pathway in the zona reticularis compared to that in the zona fasciculata involves the preferential synthesis of delta 4,3 ketosteroids in the outer cortical zones but production of delta 5 steroids such as DHEA and DS in the zona reticularis.

In many studies it has been noted that prolonged stress due to illness or trauma has divergent effects on adrenal androgen secretion compared to that of corticosteroids, with secretion of DHEA and DS being frequently reduced in such circumstances (1, 3). The mechanisms responsible for stress associated reductions in adrenal androgen production are unknown. With aging, it has been noted that there are progressive reductions in circulating concentrations of DHEA and DS (1-5). Some investigators have proposed that aging is associated with a reduction in the conversion of C21 steroids to C19 steroids, due to reduced efficiency of the 17-20 lyase activity of the enzyme 17-hydroxylase (5). It is conceivable that functional alterations in the adrenal steroidogenic pathway may accompany structural changes in the adrenal cortex during aging, as has been described for other major alterations in adrenal function (9). To address this issue, we conducted the current study of adrenal zonation in young compared to older men.

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## MATERIALS AND METHODS

Adrenal glands were obtained at the time of autopsy from men who expired within 3 hrs after trauma. For inclusion in the study, we required that the adrenal be fixed within 24 hours after death and that the sample be a full thickness specimen containing medullary tissue. Paraffin embedded specimens from 21 young men, 20-29 yrs old, and from 12 older men, greater than 50 years of age (range = 54-90 yrs), were chosen for study from a larger group of archived samples that were screened for inclusion. Seventeen of the men were white and sixteen were black; the racial distribution was similar among the young and older group. These studies were approved by the Institutional Review Board of the University of Alabama at Birmingham.

Tissue sections were stained with hematoxylin and eosin (H/E) and also by use of Gridley's silver staining method with nuclear fast red as counterstain to reveal reticulum fibers. The H/E sections were used to assess overall characteristics of the specimens and to establish areas on which morphometric image analysis was to be conducted. The cortex was defined as those cells laying between the medulla and the adrenal capsule; areas in which medullary veins and their associated cortical cuff cells were present were not chosen for quantitative analysis due to the complexity of the cortex in such regions. By use of Image Pro-Plus computerized image capture and analysis system (Media Cybernetics, Silver Spring MD), we measured the average width of the cortex from the H/E stained sections at 4x power. Adjacent sections stained for reticulum fibers, which aided in distinguishing the columns of cells comprising the zona fasciculata from the compact, irregularly arranged clusters of cells of the zona reticularis, were viewed at 10x power for measurements of the thickness of the zona reticularis and of the combined zonae fasciculata + glomerulosa. In a few instances when this staining pattern proved ambiguous, the H/E stained sections were used. If evidence of obvious heterogeneity in cortical thickness existed, we performed analyses of up to 3 different areas to obtain a representative average thickness. Although irregularities in the borders of the cortical zones can virtually obviate simple quantitative analyses, the computerized image analysis system that we employed overcomes such difficulties. All measurements were conducted on coded sections in which the age of the donor was not apparent. The data for thickness of the cortical zones were normalized by expressing the thickness of each zone as a % of the whole cortical thickness. Also, we calculated the ratio of the thickness of the combined zonae glomerulosa/fasciculata to that of the zona reticularis. The data for the young group were compared to that of the older group by t test or by Wilcoxon test, as appropriate.

## RESULTS

The average total cortical thickness (arbitrary units, pixels) of the young men (246 +/- 19, Mean +/- SE) was slightly higher than that of the older men (225 +/- 12). The thickness of the zona reticularis in the older men (62 +/- 9.4) was reduced by one-third compared to that of the young men (93 +/- 12); this reduction approached statistical significance (P = 0.079). Moreover, the % of the cortical thickness attributed to cells of the zona reticularis was significantly lower (P = 0.008) in the old men than in the young men (Fig. 1). The median % of cortex identifiable as zona reticularis in young men was 37%, while in the older men, it was only 24%. Conversely, the thickness of the outer 2 cortical zones was slightly greater in the older men (164 +/- 11) than in the young men (153 +/- 11). Finally, the proportion of outer cortical zone thickness (glomerulosa + fasciculata) to that of the zona reticularis was strikingly higher (P = 0.0011) in the older men than in the young men (Fig. 2).

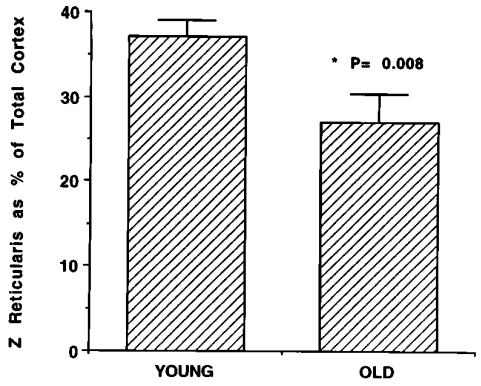


**Figure 1.** Effect of aging on the % of the adrenal cortex occupied by the zona reticularis.

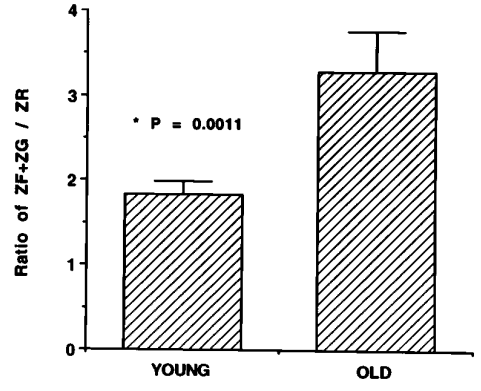


**Figure 2.** Effect of aging on the relative sizes of the zonae fasciculata/glomerulosa compared to the zona reticularis.

## DISCUSSION

The control of adrenal androgen production has received a great deal of attention over the past few years and the impact of aging on adrenal androgen secretion is the subject of considerable investigation presently. There is no doubt that production of steroids such as DHEA and DS declines dramatically during aging (1-5). Stress due to many factors also can give rise to reductions in circulating levels of DHEA and DS (1, 3), which is suggestive perhaps that the mechanisms that cause short term (presumably) reductions in DHEA/DS production in stress may also contribute to the decline in adrenal production of such steroids over the course of decades of life. Our current findings demonstrate that there are morphologic changes in the adrenal cortex

with aging such that the cells generally believed to be responsible for production of DHEA and DS occupy proportionately less of the cortical mass in older men than in young men. Indeed, the ratio of the width of the outer 2 cortical zones (glomerulosa + fasciculata) to that of the zona reticularis in young men is only about half that of the older men. Since the width of the cortex in the older men was slightly less than that of young men, then these data imply a substantial quantitatively important remodeling of the cortical zones in aging.

Prior studies have suggested alterations in the enzymology of the adrenal cortex of humans as a consequence of aging. The reductions in adrenal androgen production, in the absence of impaired cortisol formation, have suggested to some investigators the possibility of altered 17-20 lyase activity of the enzyme 17 hydroxlyase in aging (5). We believe that another explanation also is possible, which is, nevertheless, compatible with the above suggestion. We (8) and others (6,7) find that the zona reticularis contains abundant DHEA-sulfotransferase, which favors the delta-5 steroid pathway, but has little, if any, 3β-hydroxysteroid dehydrogenase (3β-HSD). The zona reticularis also contains 17-hydroxylase, which with the above enzyme pattern, gives it the ability to produce prodigious quantities of DHEA and DS but little in the way of delta 4,3 ketosteroids from cholesterol or pregnenolone as substrate. If, as our data suggest, there is a reduced mass of the zona reticularis in aging but no reduction in the mass of the zona fasciculata, then one might anticipate that the results of dynamic adrenal testing would reveal reduced DHEA output but not of cortisol in response to ACTH or CRH challenges.

The factors responsible for functional and morphologic zonation of the adrenal cortex are not known. Prolonged stress or ACTH administration has been shown to cause lipid depletion in the adrenal cortex (9). In adrenals obtained from individuals who died suddenly as the result of trauma or from those obtained at surgery in association with nephrectomy, the zona reticularis has little if any 3β-HSD (7, 8) or its messenger RNA (10). On the other hand, it has been reported that this enzyme is present throughout the cortex of adrenals obtained at autopsy from individuals who probably suffered considerable and prolonged antemortem stress due to severe illness (7). Others have suggested that there is no change (11) or even increases (12) in the size of the zona reticularis with aging. Those studies did not employ the sort of morphometric evaluations that are possible with image analysis systems available today and were, consequently, generally qualitative in nature. Another qualitative study did, however, suggest that there was altered zonation with aging, due to increased irregularity of the cortical zones (13), an observation recently made in passing by others as well (10). We concur (data not shown) that the border between the zona reticularis and the zona fasciculata becomes increasingly tortuous with aging. Thus, aging, through unknown means, appears to cause qualitative and quantitative alterations in adrenocortical zonation. The apparent reduction in the size of the zona reticularis may be an important cause for impaired production of adrenal androgens in aging.

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## REFERENCES

1. **Parker LN, Odell W.** 1980 Control of adrenal androgen secretion. Endo Rev 1:392-410.

2. **Orentreich N, Brind JL, Rizer RL, Vogelman JH.** 1984 Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J Clin Endocrinol Metab 59:551-555.

3. **Parker CR Jr.** 1993 Control of adrenal androgen production in normal development and aging. Sem Repro Endocrinol 22:313-317.

4. **Belanger A, Candas B, Dupont A,** et al. 1994 Changes in serum concentrations of conjugated and unconjugated steroids in 40- to 80-year-old men. J Clin Endocrinol Metab 79:1086-1090.

5. **Liu CH, Laughlin GA, Fischer UG, Yen SSC.** 1990 Marked attenuation of ultradian and circadian rhythms of dehydroepiandrosterone in postmenopausal women: Evidence for a reduced 17,20-desmolase enzymatic activity. J Clin Endocrinol Metab 71: 900-906.

6. **Kennerson AR, McDonald DA, Adams JB.** 1983 Dehydroepiandrosterone sulfotransferase localization in human adrenal glands: a light and electron microscopic study. J Clin Endocrinol Metab 56:786-790.

7. **Sasano H, Mason JI, Sasano N, Nagura H.** 1990 Immunolocalization of 3β–hydroxysteroid dehydrogenase in human adrenal cortex and in its disorders. Endocrine Pathol 1:94-101.

8. **Parker CR Jr, Faye-Petersen O, Mason JI, Grizzle WE, Falany CN, Stankovic AK.** 1995 Ontogeny and topology of 3β-hydroxysteroid dehydrogenase (HSD) and dehydroepiandrosterone sulfotransferase (DST) in the fetal and adult human adrenal. Proceedings of the annual meeting of the Society for Gynecologic Investigation (Abstract P257).

9. **Neville AM, O'Hare MJ**. 1982 The human adrenal cortex. Springer-Verlag, Berlin. pp 16-40.

10. **Endoh A, Kristiansen SB, Casson PR, Buster JE, Hornsby PJ**. 1996 The zona reticularis is the site of biosynthesis of dehydroepiandrosterone and dehydroepiandrosterone sulfate in the adult human adrenal cortex resulting from its low expression of 3β–hydroxysteroid dehydrogenase. J Clin Endocrinol Metab 81:3558-3565.

11. **Parker LN, Lifrak ET, Mamadan MB, Lai MK**. 1983 Aging and the human zona reticularis. Arch Andrology 10:17-20.

12. **Cooper ERA**. 1925 The suprarenal gland. In: Histology of the more important human endocrine organs at various ages. Oxford Univ Press, London, p50.

13. **Kreiner VE, Dhom** G. 1979 Altersveranderungen der menschlichen Nebenniere. Zbl Allg Pathol U Pathol Anat 123: 351-360.