Acta Endocrinologica 1982, 100: 455–461

# The aging Leydig cell: VI.
## Response of testosterone precursors to gonadotrophin in men

E. P. Murono[1,2], H. R. Nankin[1], T. Lin[1] and J. Osterman[1]

*Medical Service[1], Wm. Jennings Bryan Dorn Veterans' Hospital, Department of Medicine[1] and Department of Physiology[2], University of South Carolina School of Medicine, Columbia, South Carolina 29201, USA*

Abstract. The effects of a single im injection of human chorionic gonadotrophin (hCG) on circulating testosterone precursor levels at 0, 1–6, 24, 48 and 72 h were examined in normal young adults (mean age 34 years) and normal aged men (mean age 74 years). Basal 08.30–09.00 h concentrations of androstenedione and dehydroepiandrosterone were lower in aged men while progesterone levels were not significantly different from young men. A significant biphasic increase of circulating progesterone was observed in young men, characterized by an early peak at 2 h (33% above basal) and a secondary peak at 24 h (49% above basal). In old men there were no increases in circulating progesterone levels following hCG treatment during the early (1–6 h) or late (24–72 h) periods. There were not discernable increases in circulating dehydroepiandrosterone levels following hCG administration in both groups of men. Androstenedione levels in young men did not change during the first 6 h following hCG but increased significantly at 48 and 72 h, while in old men there was a small peak at 4 h (which was not statistically significant) and a secondary significant rise at 48 and 72 h. However, early and late stimulated absolute levels for androstenedione were lower in the aged population. Thus, there are differences in precursor concentrations in the basal state and in response to hCG in aged men.

The administration of human chorionic gonadotrophin (hCG) has been utilized to assess Leydig cell competence (Kirschner et al. 1965; Anderson et al. 1972; Perheentupa et al. 1972). The normal response is characterized by an initial acute release of testosterone with a peak between 2 and 4 h after im hCG administration, which is followed by a more sustained release of testosterone with a peak between 48 and 96 h after treatment (Saez & Forest 1979; Martikainen et al. 1980; Nankin et al. 1980; Padron et al. 1980; Wang et al. 1980). This biphasic response to hCG is observed whether a single or repeated injections of hCG are administered (Saez & Forest 1979). The mechanism for this biphasic response has not been elucidated; however, two principal views currently have been proposed: a) according to one view, the initial acute response represents the secretion of a releaseable pool of testosterone due to hCG stimulation, while the delayed but more sustained response represents increased production after enhanced synthesis of steroidogenic enzymes (Padron et al. 1980); b) according to another view, the explanation for early response is similar, while the delayed secondary increase in testosterone is thought to represent a combination of receptor desensitization and/or selective inhibition of steroidogenic enzymes and restimulation of the testis by persistently evaluated plasma hCG (Cigorraga et al. 1978; Tell et al. 1978; Saez & Forest 1979). More recently, increased Leydig cell responsiveness in the morning, when most studies are started, has been reported (Nankin et al. 1980). These investigators did not

Requests for reprints: H. R. Nankin, M.D., Department of Medicine, Bldg. T28, Wm. Jennings Bryan Dorn Veterans' Hospital, Columbia, S.C. 29201.

see a typical biphasic response in studies started in the evening, but rather found an initial rise and a subsequent further rise.

There is limited information on the pattern of Leydig cell response to hCG in aged men. Rubens et al. (1974) reported decreased responsiveness to hCG in aged men. Harman & Tsitouras (1980) suggested a diminished Leydig cell reserve with age. We previously showed that the aged Leydig cell demonstrates blunted early and late responses to im hCG as assessed by circulating concentrations of testosterone, 17α-OH-progesterone and oestradiol-17β (Nankin et al. 1981).

In the present study we have expanded the examination of Leydig cell function in aged men by measuring circulating levels of testosterone precursors. We herein report serum concentrations of progesterone, dehydroepiandrosterone and androstenedione in the basal state and between 1 and 72 h following a single im injection of hCG.

## Materials and Methods

### *Materials*

The solvents used in this study were of analytical grade and purchased from Fisher Scientific Corp. [1,2,6,7-$^3$H]progesterone (S.A. 114 Ci/mmol), [1,2-$^3$H]dehydroepiandrosterone (S.A. 58.6 Ci/mmol), and [1,2,6,7-$^3$H]-androstenedione (S.A. 86.8 Ci/mmol) were purchased from New England Nuclear, and were purified prior to use by Lipidex 5000 or celite column chromatography. All non-radioactive steroids were from Steraloids and were re-crystallized prior to use.

### *Radioimmunoassay of serum steroids*

The progesterone antibody was a gift of Dr. A. H. Surve. It cross-reacts with 17α-OH-progesterone, 17α-OH-pregnenolone, pregnenolone, androstenedione, testosterone and oestradiol-17β 2, 5, 1, <0.1, <1 and 5%, respectively. Ether extracted serum samples were chromatographed on celite columns before radioimmunoassay (see below). The intra- and inter-assay coefficients of variation were 4 and 13%, respectively.

The dehydroepiandrosterone (DHEA) antibody was a gift from Dr. A. Kambegawa. It cross-reacts with DHEA sulphate, androstenedione, oestradiol-17β, testosterone, progesterone, cortisol and pregnenolone 5.8, 8.3, 0.01, 0.47, 0.77, 0.04 and 0.01%, respectively. Ether extracted serum samples were chromatographed on celite columns before radioimmunoassay (see below). The intra- and inter-assay coefficients of variation were 15 and 20%, respectively.

The androstendione antibody was a gift of Dr. J. S. D. Winter. It cross-reacts with DHEA, testosterone, progesterone, 17α-OH-progesterone, oestradiol-17β and cortisol 3, 2.8, 1, 0.5, 0.6 and 0.06%, respectively. The radioimmunoassay is performed on ether extracted serum samples after chromatography on celite columns (see below). The intra- and inter-assay coefficients of variations are 14 and 20%, respectively.

### *Celite chromatography of serum samples*

One ml serum samples were extracted with 5 vol diethyl ether and the ether extracted residues were chromatographed on mini-celite columns using the procedure described by Abraham et al. (1972) with modifications. The stationary phase used was ethylene glycol. Samples were eluted first with 2 ml iso-octane, then 2.5 ml iso-octane alone and finally with 2.5 ml of 15% ethyl acetate in iso-octane, which resulted in the isolation of progesterone, androstenedione and DHEA, respectively. Recoveries for each steroid averaged 65–75%. Water blanks chromatographed in parallel with serum samples for each elution fraction demonstrated no detectable effect on total binding in the respective radioimmunoassay.

### *Patients*

The nine elderly men were healthy Caucasian volunteers, ranging in age from 67 to 86 years (mean: 74). All were ambulatory, living at home, taking no medications and they had no known diseases. No abnormalities were noted on screening physical examinations although most of the men exhibited mild prostatic enlargement. The subjects came to the laboratory at 08.00–08.30 h after having breakfast and 3 basal blood samples were taken at 15 min intervals between 08.30 and 09.00 h through an indwelling butterfly needle. These three specimens were averaged to give the basal value for each individual. At 09.00 h hCG (APL-hCG, Ayerst) was given im at a dosage of 40 IU/kg. Blood was collected hourly until 15.00 h when the indwelling needles were removed, and then by venipuncture at 24, 48 and 72 h. Subjects signed a written consent form and the protocol was approved by a Human Studies Committee.

The young control group included 8 healthy physicians and laboratory technicians, ranging in age from 25 to 42 years (mean: 34). They were on no medications and clinically normal. The sampling techniques have been described previously (Nankin et al. 1980) and were similar to those described for the old normals.

Each specimen was analyzed in duplicate for each steroid and the mean values were utilized. All specimens from a single individual were assayed at one time for each steroid. Results are presented as the mean ± standard error (SE). Statistical analyses were performed using Student's *t*-test between groups and the paired *t*-test for calculation within groups.

## Results

*Progesterone response to hCG*

The effect of hCG on circulating progesterone levels is presented in Fig. 1. The mean basal progesterone for young men was 23.4 ng/100 ml ± 4.9. Circulating progesterone levels increased to 31.2 ng/100 ml ± 5.4 at 2 h following hCG treatment, a 33% increase over basal, which was significant ($P < 0.05$). By 3 h following hCG treatment progesterone levels dropped to 25.9 ng/100 ml ± 3.9 and changed very little between 3 and 6 h. A secondary rise in progesterone to 34.8 ng/100 ml ± 6.3 was observed 24 h after hCG treatment and this increase was significant ($P < 0.05$) when compared to basal progesterone. Progesterone levels then gradually decreased at 48 and 72 h.

The pattern of progesterone response to hCG was significantly different in old men. Basal levels were 16 ng/100 ml ± 2.8, although lower than basal progesterone in young men, this difference was not statistically significant. There was no discernable elevation of circulating progesterone levels in old men at any time following hCG stimulation. In fact, at 24, 48 and 72 h following hCG treatment progesterone levels were 20–30% lower (although not significant) than the basal level. The dissociated response of circulating progesterone between the two age groups is reflected by the significant differences in corresponding steroid levels between 2 and 48 h following hCG treatment.

*Dehydroepiandrosterone response to hCG*

The effects of hCG on circulating DHEA levels in young and old men are presented in Fig. 2. In young men the mean basal DHEA level was 334 ng/100 ml ± 72. Following hCG stimulation there were no statistically significant early or late elevations of circulating DHEA levels. We have not evaluated the normal changes for diurnal fall in DHEA between 09.00 and 15.00 h to determine whether hCG alters the early pattern.

In old men the mean basal DHEA level was 125 ng/100 ml ± 28 which was statistically lower than the control concentration in young men ($P < 0.02$). Although hCG treatment did not significantly increase circulating DHEA levels, there was a small early (peak at 4 h) and late (broad peak between 24–72 h) response. At 24, 48 and 72 h following hCG the circulating DHEA levels averaged 32, 32

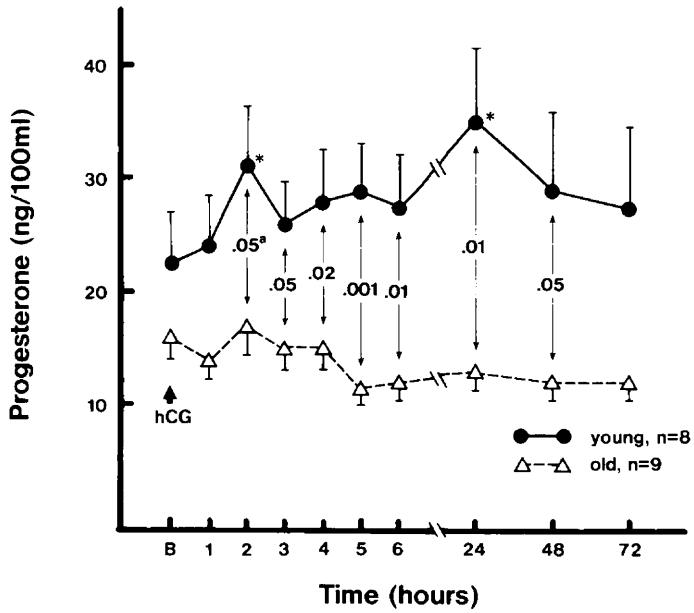


*Fig. 1.*
Effect of hCG on circulating progesterone levels in young and old men. * = $P < 0.02$ when compared with young basal; a = $P$ value when corresponding absolute values in each age group are compared.

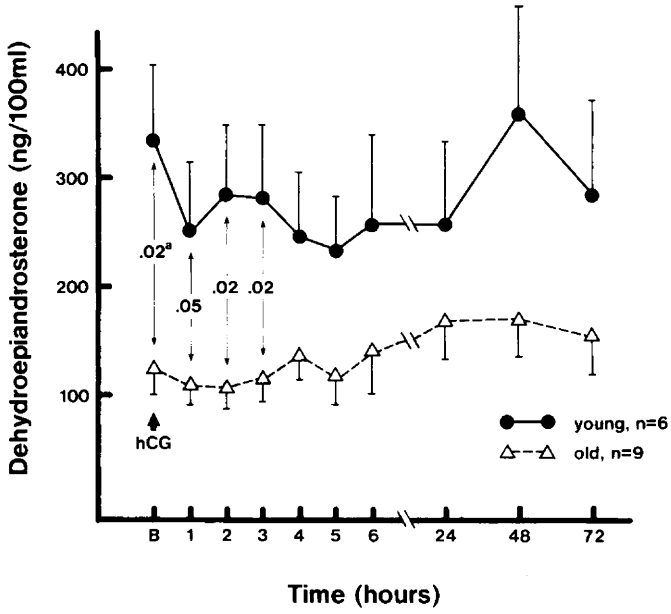


*Fig. 2.*
Effect of hCG on circulating dehydroepiandrosterone levels in young and old men. a = $P$ value when corresponding absolute values in each age group are compared.

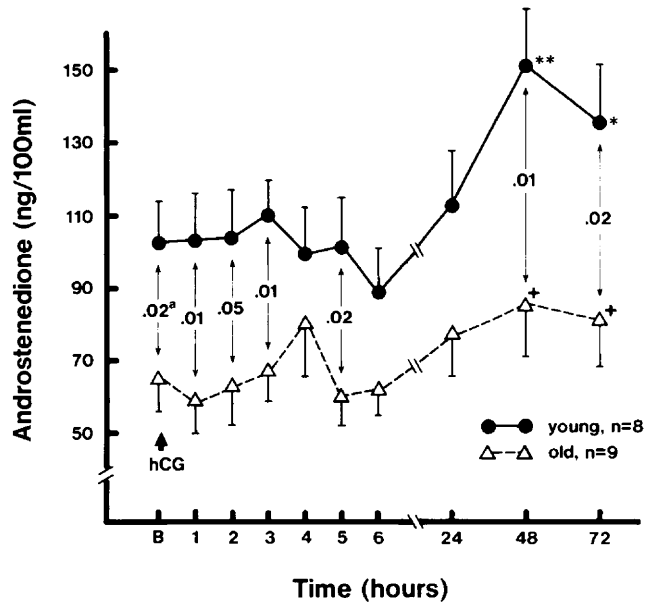


*Fig. 3.*
Effect of hCG on circulating androstenedione levels in young and old men. *, ** = $P < 0.05$ and 0.01, respectively when compared with young basal; + = $P < 0.05$, when compared with old basal; a = $P$ value when corresponding absolute values in each age group are compared.

and 22% higher than basal. Following hCG stimulation, only 1–3 h DHEA levels of old men were significantly lower than the comparable levels of young men.

*Androstenedione response to hCG*

The effects of hCG on circulating androstenedione levels in young and old men are presented in Fig. 3. In young men the mean basal circulating androstenedione level was 103 ng/100 ml ± 11. Following hCG treatment there were no significant early responses, although it is possible that hCG altered the normal diurnal fall in circulating androstenedione levels between 09.00 and 15.00 h. However, significant elevations above basal were observed at 48 and 72 h following hCG ($P < 0.01$ and 0.05, respectively) which averaged 47 and 31% above basal, respectively.

In old men mean basal circulating androstenedione level was 65 ng/100 ml ± 9, which was significantly lower ($P < 0.02$) that the circulating mean basal for the younger group. There was an early rise in androstenedione (peak at 4 h) which was 23% above basal, but this increase was not statistically significant. The increases at 48 and 72 h following hCG treatment, however, were statistically significant ($P < 0.05$ for both time periods) and represented 31 and 25% increases over basal, respectively.

## Discussion

The present studies of circulating precursor steroids demonstrate differences in basal concentrations and responses to a single im hCG injection in young and old men.

In a previous publication we reported blunted early (1–6 h) and late responses (24–72 h) of circulating testosterone and 17α-OH-progesterone to a single im hCG injection in old men, while only the late response of oestradiol-17β in old men was reduced when compared to young men (Nankin et al. 1981). In the present study we were unable to detect a progesterone rise 1–6 or 24–72 h following hCG in old men. The present results in young normal adult males agree with the results of Wang et al. (1980) who reported a biphasic response of serum progesterone following infusion with human luteinizing hormone (hLH), partially agree with the findings of Martikainen et al. (1980) who reported an increase in circulating progesterone levels 8 h following a single injection of hCG in young adult men, but differ with the results of Vermeulen & Verdonck (1976) who reported no effect on circulating progesterone levels following hCG administration for 3 consecutive days in adult men. The per cent increases in circulating progesterone levels in young men following hCG treatment in the present study were small, i.e. 33% at the 2 h peak and 49% at 24 h. This may be due partially to the primarily adrenal origin of plasma progesterone in man (Vermeulen & Verdonck 1976). We have no explanation for the actual fall in circulating progesterone 24, 48 and 72 h following hCG treatment in old men, although Martikainen et al. (1980) reported a similar pattern in circulating progesterone levels in a group of young adult men 72–168 h following a single injection of hCG.

We were unable to detect significant elevations in circulating DHEA at any time following hCG treatment in young men, although it is possible that hCG treatment altered the normal diurnal fall in DHEA between 09.00 and 15.00 h. In previous studies Nieschlag et al. (1973) reported a significant increase in circulating DHEA levels following treatment for 4 consecutive days with hCG, while Vermeulen & Verdonck (1976) and Pirke et al. (1977) reported little or no effect of hCG injection for 3 consecutive days on circulating DHEA in adult males, and Wang et al. (1980) found that infusion with hLH had no effect on circulating DHEA levels in young normal adult males. Because DHEA is primarily secreted by the adrenal cortex (Vermeulen & Verdonck 1976; Pirke et al. 1977), the amounts secreted by the testis probably are sufficiently diluted to obscure detecting significant elevations in this steroid. With aging, DHEA levels decrease approximately 60–70% (Vermeulen & Verdonck 1976; Pirke et al. 1977; Murono et al. 1982). This contrasts with testosterone and 17α-OH-progesterone which are secreted primarily from the testis and which decrease only 20–30% with aging (Vermeulen & Verdonck 1976; Murono et al. 1982). The principal source of DHEA which is reduced with age must be that which is contributed by the adrenal. Thus in aged men, the contribution by the testis to the total circulating DHEA should increase, and we thought that hCG induced elevations in circulating DHEA levels would be detected. Although a biphasic response of DHEA to hCG was observed in old men, the early

and late responses were not significantly higher than basal. Pirke et al. (1977) reported very little effect of hCG treatment for 3 consecutive days on circulating DHEA levels in a group of old normal males. The generally blunted steroidogenic responses of old Leydig cells (Nankin et al. 1981) apparently negated significant elevations in circulating DHEA levels in aged men.

In a previous study involving 10 aged men, the basal morning androstenedione concentration was 17% lower than the basal level measured in younger men, but this difference was not significant ($P < 0.14$). In the present population of 9 old men (composed of the same population making up the earlier study, but excluding one man who did not participate in this hCG injection study) the basal androstenedione concentration averaged 35% lower than the mean androstenedione level of younger males, and this difference was significant ($P < 0.01$). We feel that the present results reflect the generally reduced steroidogenic competence of the aged testis (Rubens et al. 1974; Pirke et al. 1977; Nankin et al. 1981). We were unable to detect significant elevations in circulating androstenedione levels during the first 6 h following hCG in young men, although hCG may have altered the normal pattern in diurnal fall of this steroid between 09.00 and 15.00 h. A previous study by Martikainen et al. (1980) reported no elevations in circulating androstenedione in young adult men during the first 4 h following a single hCG injection. However, they reported significant elevations in circulating androstenedione (approximately 75% above basal) between 48 and 96 h following hCG. In the present study we observed significant elevations between 48 and 72 h, with a 47% increase over basal at 48 h. Vermeulen & Verdonck (1976) also observed a significant elevation (52% above basal) in circulating androstendione levels in adult men following 3 consecutive daily injections of hCG and Wang et al. (1980) reported increases in androstenedione concentrations 16–36 h following infusion with hLH in healthy young adult men. Although a small early response (peak at 4 h following hCG) was observed in the present old men, only the secondary peak at 48 and 72 h was statistically significant. The per cent increases above basal at 48 and 72 h were 31 and 25%, respectively, which were less than those observed in young men; and the absolute levels attained during this time were also significantly less than the levels attained in young men.

In summary, the present results demonstrated that basal precursor steroid levels and the responses of young and aging men to im hCG differ. For progesterone, basal concentrations in both young and old men were comparable. In response to hCG, young men demonstrated both early and late responses to hCG with peaks at 2 and 24 h, respectively, while old men demonstrated no response. Basal DHEA concentrations in old men were significantly lower. There was no demonstrable response of DHEA to hCG in the young men, while there was a modest biphasic increase in old men, which was not statistically significant. The basal androstendione concentration in old men was significantly lower than in young men. In response to hCG circulating androstenedione levels in both age groups increased only after 48 and 72 h, but the absolute increases were significantly lower in the aged population. These results demonstrate reduced capacity of old men to secrete testosterone precursors in response to hCG. Furthermore, because there was no significant build up of testosterone precursors in this study and a previous study (Nankin et al. 1981), there does not appear to be a specific enzymic defect at the level of the smooth endoplasmic reticulum in the aged testis.

## Acknowledgments

These studies were supported by the Veterans' Administration and the National Institute of Aging/National Institutes of Health Grant No. 1 R01 Ag 01217-03 REB. Appreciation is expressed to J. Kilgore, J. Lane and K. Gettys for technical assistance, to D. Barwick for the typing of the manuscript and to R. Hearn for the artwork.

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Received on July 28th, 1981.