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CLINICAL STUDY

Effect of aging on serum gonadotropin levels in healthy subjects and patients with nonfunctioning pituitary adenomas

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Abstract

Objective: Nonfunctioning pituitary adenomas (NFPA), which represent about one-quarter of human pituitary tumors, occur in middle or old age. Determination of gonadotropin levels, which are not expected to be high during the early postmenopause in normal women and which are low in women with NFPA, is important to distinguishing hypogonadal status due to the normal decline of gonadal function from that due to hypothlalamic-pituitary dysfunction. The aim of the study was to verify whether this difference still persists in old subjects, despite the physiological decline of gonadotropins in the last decades of life.

Design and methods: The study included 154 healthy subjects (aged 50–104 years) and 47 patients with NFPA (aged 50–80 years). Blood samples were collected after an overnight fast and hormone levels were measured by two immunofluorimetric assays.

Results: In healthy women the highest serum levels of gonadotropins were present in the 50–60 year age group, with a slight but progressive age-associated decrease in serum FSH and LH being observed thereafter. In healthy men serum gonadotropin levels were stable up to 70 years, increased up to 75–85 years and thereafter gradually decreasing. Female patients with NFPA showed levels of gonadotropins which were far lower than controls. Only three patients had levels of both FSH and LH above the 2.5 centile for normal subjects. A high sensitivity and specificity of gonadotropin measurements (about 90%) for the diagnosis of NFPA was observed in female patients aged 50–80 years. In male subjects, a large overlap of gonadotropin values in NFPA and controls, namely over the 50–70 years age range, was observed.

Conclusions: Our study demonstrates that despite the gradual decline of gonadotropin levels in healthy postmenopsausal women, the reduction of both FSH and LH persists in old patients with NFPA, suggesting that measurement of gonadotropin levels could prove useful in the evaluation of pituitary lesions even in old women. More subtle differences seem to occur in male subjects.

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Introduction

It is well established that aging is associated with dramatic changes in gonadotropin secretion in healthy subjects. In women, after the initial elevation of serum gonadotropins that characterizes the menopause, a progressive decline in both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels occurs with age in the later post-menopausal years (1–5). The age-associated changes in gonadotropin levels in men are more subtle and complex than those observed in aging women, probably reflecting the multifactorial origin of andropause (4, 6–11). Although the average length of human life is currently 75–78 years, the upper limit of which may be increased to 85 years in the next 2 years, the normal range of gonadotropin levels in different age groups, particularly in the last decades of life, has so far been seldom used to assess alterations of gonadotropin secretion. Evaluation of serum gonadotropin levels is important in testing for the presence of a pituitary lesion, particularly in conditions characterized by the absence of signs and symptoms of hormone hypersecretion, such as nonfunctioning pituitary adenomas (NFPAs). Indeed, diagnosis of NFPA generally occurs when the tumor has reached the stage of macroadenoma and signs and symptoms of expanding mass and hypopituitarism, particularly hypogonadism, are present (12, 13). In fact, although several lines of evidence indicate that NFPAs are derived from the proliferation of cells which synthesize glycoprotein hormones and/or their α and β subunits, only very rarely does this synthetic capacity result in an *in vivo* hypersecretion,

while in the great majority of patients signs and symptoms of hypogonadism prevail (14, 15). Since the majority of patients present with NFPA in middle or old age, hypogonadal status is generally confused with the normal decline of gonadal function, particularly in women. While during the perimenopause and early postmenopause gonadotropin levels are expected to be high in normal women and low in women with NFPA, it is not clear whether this difference still persists in older subjects, due to the physiological decline of gonadotropins in the last decades of life.

Materials and methods

Subjects

The study included 154 healthy subjects (93 females and 61 males) aged between 50 and 104 years. The admittance criteria for the study excluded subjects with any current medical illness, including obesity, or serious illness, such as cancer, myocardial infarction and pulmonary disease, within the previous year, and those using estrogen, antipsychotropic or other medications known to affect pituitary hormone secretion. Overall, the subjects were considered to be in better-than-average health. All women reported the absence of menses for more than 1 year, with histories of previous regular menstrual cycle.

The study also included 47 patients with NFPA (22 females and 25 males), aged between 50 and 80 years. without previous treatments for pituitary lesion. All women reported the absence of menses for more than 1 year and were not receiving estrogen replacement therapy. Criteria for the diagnosis of NFPA were (a) the presence of a sellar mass with or without extrasellar extension detected by magnetic resonance imaging or high resolution CT scans; (b) absence of signs and symptoms of pituitary hyperfunction; (c) normal or moderately high prolactin (PRL) levels (<4000 mU/l) attributable to hypothalamic-pituitary disconnection in patients with very large tumors and (d) histological confirmation of pituitary tumor in surgically excised tissue by light microscopy and, in individual tumors, by immunofluorescence analysis. Four patients had a microadenoma and 43 a macroadenoma. Clinical and biochemical indices of hypothyroidism were present at admittance in 16 patients (34%), 10 of whom also had associated hypoadrenalism (21%). All patients had normal levels of the α-subunit (0.8–4.2 U/l in women and 0.2–0 in men). Body mass index (BMI) was normal (range 18–24). The nature of the study was explained to the subjects before they gave their voluntary informed consent. The study protocol was approved by the local ethics committee.

Hormone assays

Blood samples were collected after an overnight fast. After collection, samples were centrifuged, aliquoted and stored at −20 °C until assay. Serum LH and FSH were measured by two site immunofluorimetric assays in which two monoclonal antibodies are directed against separate antigenic determinants on the hormone molecule (Delfia EG&G Wallac, Milan, Italy) with detection limits of 0.05 and 0.07 U/l, respectively. Serum 17β-estradiol levels and testosterone were measured by immunofluorimetric assay (Delfia EG&G Wallac) with detection limits of 0.05 and 0.4 nmol/l, respectively. The within- and between-assay coefficients of variation of the above-mentioned methods were <8%. Serum α-subunit levels were measured by immunoradiometric assay (Biocode, Liege, Belgium) with detection limit of 0.02 U/l.

Statistical analysis

We used Student's two-sided t test to compare gonadotropin mean levels of NFPA and normal subjects. The Wilcoxon–Mann-Witney rank sum test was also used and produced similar results. Only results obtained from t tests are shown. Bonferroni's multiple comparison test was performed to compare age groups within NFPA or normal subjects. Polynomial regression models were used to describe correlation between hormonal parameters and age. Pearson's product-moment correlation coefficients (r) are shown. Normal values of FSH and LH were defined as those falling within the 2.5 and 97.5 centile range for normal subjects. For this purpose, due to the age-related changes of gonadotropin levels, centiles were calculated using least absolute value models (16). All data are shown as means and standard deviations (S.D.) when appropriate. STATA (Release 5.0) software was used.

Results

Hormone levels in healthy subjects

Serum FSH and LH levels obtained in 154 healthy subjects aged 50–104 years are shown in Figs 1 and 2. In females the highest serum levels of gonadotropins were found in the younger age groups, while during aging a slight but progressive age-associated decrease in serum FSH ($r = -0.45$; $P < 0.001$) and LH ($r = -0.41$; $P < 0.001$) levels was observed (Fig. 1). In particular, a significant decline in FSH was observed in the eighth/ninth decade (52.4 ± 26.1 U/l (80–94 years) vs 70.5 ± 18.8 U/l (50–64 years); $P < 0.05$). Subsequently, a sharp reduction in both FSH and LH levels was observed over the ninth decade, FSH decreasing from 52.4 ± 26.5 U/l (80–94 years) to 28.2 ± 20.9 U/l (≥ 95 years) ($P < 0.01$) and LH from 23.8 ± 14.2 U/l to 12.6 ± 13 U/l ($P < 0.05$; Fig. 1), respectively.

In healthy men the relationship between serum gonadotropin levels and age was not linear (Fig. 2). Serum gonadotropins remained stable up to 70 years and thereafter showed an age-associated increase up to

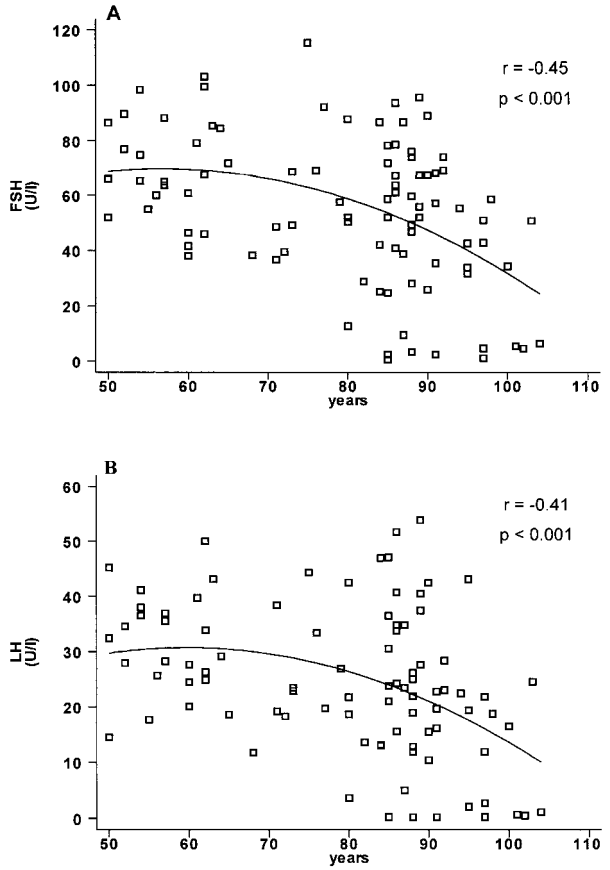

Figure 1 Serum levels of FSH (A) and LH (B) in 93 healthy women aged between 50 and 104 years. The highest serum levels of gonadotropins were present in the younger age groups while during aging a slight but progressive age-associated decrease in serum FSH and LH was observed.

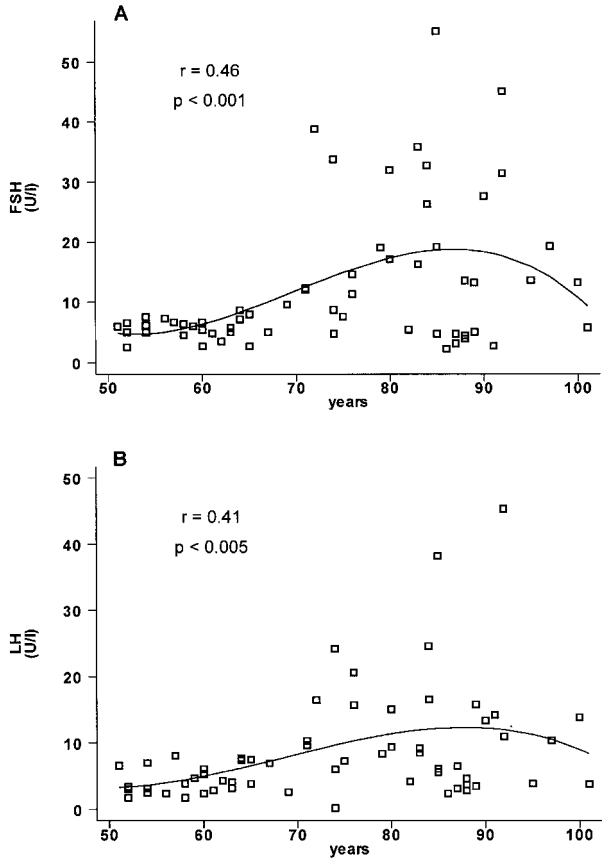

Figure 2 Serum levels of FSH (A) and LH (B) in 61 healthy men aged between 50 and 102 years. The relationship between serum gonadotropin levels and age was not linear. An age-associated increase was observed up to 80–94 years, when the highest serum levels of FSH and LH (18.2 ± 15.4 and 12.0 ± 11.3 U/l, respectively) were reached, followed by a gradual, not statistically significant, age-associated decrease.

around 80–94 years when the highest serum levels of FSH (18.2 ± 15.4 U/l (80–94 years) vs 5.7 ± 1.5 U/l (50–64 years); $P < 0.01$) and LH (12.0 ± 11.3 U/l (80–94 years) vs 4.3 ± 2.0 U/l (50–64 years); $P < 0.05$) were reached, followed by a gradual age-associated decrease, not statistically significant (> 95 years: FSH, 13 ± 5.5 U/l; LH, 8.0 ± 5.0 U/l; P = n.s. vs values for 80–94 years, Fig. 2) When mean gonadotropin levels in healthy female and male subjects were compared, both FSH and LH were higher in females than in males and in both sexes FSH was higher than LH, the LH/FSH ratio always being less than 1 (Figs 1 and 2).

Very low levels of both FSH and LH (<2 U/l), consistent with a functional failure of pituitary gonadotrophs, were found in eight of 55 women aged ≥85 years (Fig. 1). In these subjects, the presence of a pituitary tumor was considered unlikely on the basis of the clinical history and the lack of other pituitary defects. In fact, normal serum levels of free thyroxine (range, 0.4–4.9 mU/l; normal values, 0.26–5), cortisol (range, 150–690 nmol/l; normal values, 140–700 nmol/l) and prolactin (range, 170–470 mU/l; normal values, 100–500 mU/l) were recorded in these subjects. Moreover skull X-rays and visual field assessment were performed in five subjects and were found to be normal.

While in all females serum 17β-estradiol was low (<0.1 nmol/l), comparable with the menopausal decrease in gonadal steroidogenesis, in healthy males testosterone levels showed a progressive age-associated decrease ($r = -0.70$; $P < 0.0001$), with substantially low levels in subjects older than 65 years. In particular, serum testosterone levels lower than those found in adult normal men, i.e. <13 nmol/l, were found in 33% of 65- to 74-year-old men, in 45% of 75- to 84-year-old men and in 79% of men older than 85 years.

Hormone levels in patients with NFPA

The pattern of gonadotropin secretion in patients with NFPA was markedly different from that recorded in healthy normal subjects matched for age. In fact, female patients with NFPA showed levels of FSH and LH which

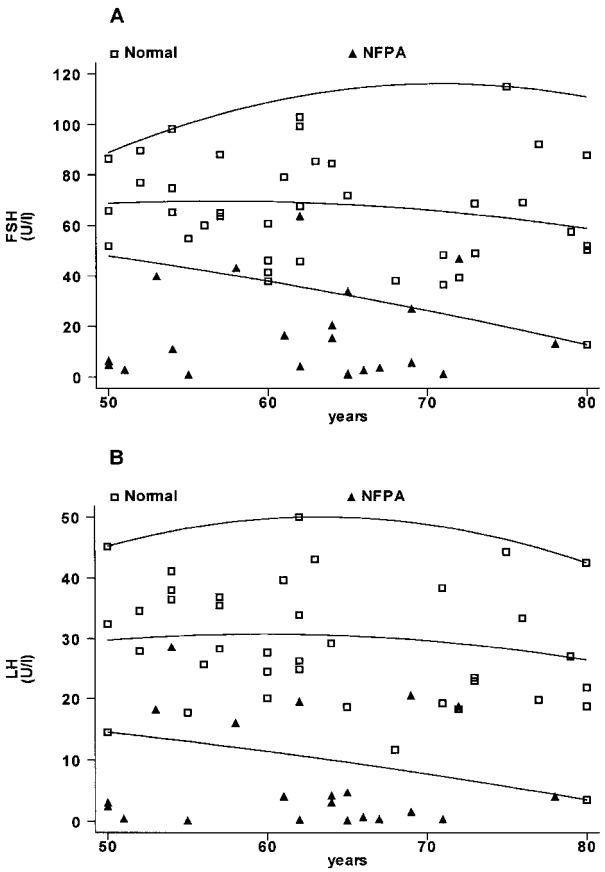

Figure 3 Serum levels of FSH (A) and LH (B) in 38 healthy women and 22 women with NFPA aged between 50 and 80 years. Levels of FSH and LH above the 2.5 centile for normal subjects were recorded in only three patients with NFPA while normal levels of either FSH or LH were observed in 1 and 3 patients with NFPA, respectively.

were far lower than those observed in healthy women aged 50–80 years (Fig. 3 and Table 1). Only three patients had levels of both FSH and LH comparable to those observed in normal postmenopausal controls (above the 2.5 centile for normal subjects), while normal levels of only one gonadotropin were found in four additional patients (FSH in 1 patient and LH in 3 patients; Fig. 3). From the analysis of data obtained in patients with NFPA and normal subjects adjusted for age, the accuracy of FSH and LH measurement for the diagnosis of NFPA appears very high in females in the 50–80 year age range, both FSH and LH showing a high sensitivity and specificity (about 90%). In the few patients with gonadotropin levels consistent with the postmenopausal status, the presence of a gonadotropin secreting adenoma was considered unlikely on the basis of simultaneous elevation of both LH and FSH and/or absence of abnormal gonadotropin response to thyrotropin-releasing hormone (TRH, 200 μg i.v.; data not shown).

In male patients serum FSH levels were similar to those observed in age-matched control subjects, the only exception being a significant FSH reduction in 70- to 80-year-old men with NFPA who did not show the age-associated increase in FSH that occurred in normal controls (Fig. 4A and Table 2). As far as serum LH levels were concerned, though patients with NFPA showed a significant reduction in LH in comparison with normal subjects in all age groups (Table 2), values obtained in individual patients largely overlapped those found in normal subjects (Fig. 4B).

Discussion

This study confirms and extends previous observations on the influence of age on gonadotropin levels in healthy subjects and provides new evidence for the usefulness of FSH and LH evaluation in the diagnosis of NFPA in older patients. Our study confirms the progressive decline of both FSH and LH with age in normal postmenopausal women, that probably involves changes occurring at the level of both the hypothalamic gonadotropin releasing hormone (GnRH) pulse generator and the pituitary gonadotroph (1–5). Despite this age-associated gonadotropin decline, in the majority of

Table 1 Serum FSH and LH levels in healthy women and in women with NFPAs in different age groups.

		Normal		NFPA		
	Age group	*n*	mean ± S.D.	*n*	mean ± S.D.	***P* value**
FSH (U/l)	50–59	13	72.4 ± 14.5	7	15.3 ± 18.1	< 0.0001
	60–69	13	66.2 ± 23.1	12	16.0 ± 18.5	< 0.0001
	70–80	12	60.7 ± 27.8	3	20.1 ± 23.7	< 0.05
	Total	38	66.5 ± 22.2	22	16.4 ± 18.1	< 0.005
LH (U/l)	50–59	13	31.9 ± 8.8	7	9.8 ± 11.1	<0.005
	60–69	13	28.4 ± 10.7	12	4.9 ± 7.2	<0.0005
	70–80	12	26.0 ± 11.8	3	7.6 ± 9.7	<0.05
	Total	38	28.8 ± 10.5	22	6.8 ± 6.7	<0.0001

* Normal vs NFPA. *n*, number of subjects.

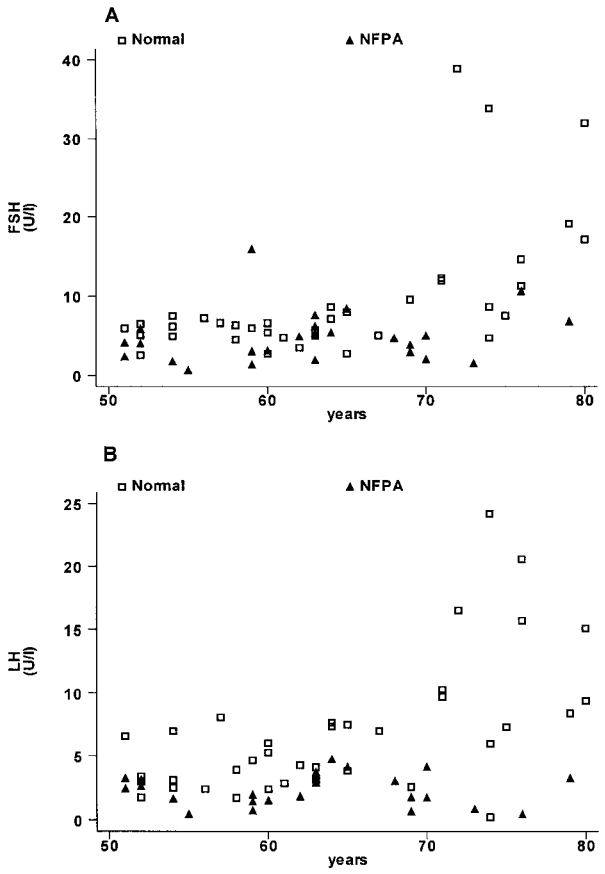

Figure 4 Serum levels of FSH (A) and LH (B) in 37 healthy men and 25 men with NFPA aged between 50 and 80 years. A large overlapping of gonadotropin values was observed between controls and patients with NFPA, namely in the 50–70 year age range. Older men with NFPA did not show the age-associated increase in FSH that occurred in normal controls.

women serum levels of both LH and FSH were definitively higher than those recorded in premenopausal subjects even in the ninth decade of life. Conversely, in about 20% of women aged ≥85 years both FSH and LH levels were definitely low (< 2 U/l), suggesting that an almost complete failure of gonadotropin secretion, in the presence of a preserved function of the remaining pituitary cells, may occur in a subset of old normal subjects.

The age-associated changes in gonadotropin levels in men were more subtle. In fact, the levels of both FSH and LH remained stable up to 70 years of age and thereafter an age-associated increase up to the eighth decade, followed by a gradual decrease was shown. According to other reports (6, 11, 17), in our series of healthy men a progressive decrease of gonadal steroid levels, leading to reduced total testosterone levels in subjects older than 65 years, was observed. Since free testosterone was not evaluated in this study, an underestimation of the hypogonadal condition in elderly men, due to the age dependent increase in sex hormone-binding globulin (SHBG) (18), cannot be excluded. In addition to the progressive decrease in testicular Leydig cell number and function leading to testosterone secretion decline, alterations at the hypothalamic-pituitary level seem to be responsible for the subsequent reduction in the levels of gonadotropins (4, 7–10).

Women with NFPA aged 50–80 years showed levels of both FSH and LH which were far lower than those observed in healthy women. Only three of 22 patients had levels of FSH and LH above the 2.5 centile for normal subjects, while normal levels of only one gonadotropin were found in four additional patients. In patients with gonadotropin levels consistent with postmenopausal status, the presence of a gonadotropinoma was considered unlikely on the basis of the simultaneous elevation of both LH and FSH, which is unusual in this type of tumor, and/or absence of abnormal gonadotropin response to TRH, which characterizes gonadotropinomas (19, 20). However, since gonadotropinomas and NFPA share a number of biochemical and morphological characteristics (14, 15, 19–22), this possibility cannot be excluded. From the analysis of data obtained in normal subjects and in patients with NFPA in the 50–80 year age range,

Table 2 Serum FSH and LH levels in healthy men and men with NFPAs in different age groups.

		Normal		NFPA		
	Age group	*n*	mean ± S.D.	*n*	mean ± S.D.	***P* value**
FSH (U/l)	50–59	12	5.8 ± 1.4	9	4.3 ± 4.6	n.s.
	60–69	13	5.8 ± 2.1	11	4.9 ± 1.9	n.s.
	70–80	12	17.7 ± 11.2§	5	5.2 ± 3.7	< 0.005
	Total	37	9.7 ± 8.5	25	4.8 ± 3.3	< 0.005
LH (U/l)	50–59	12	4.0 ± 2.1	9	1.9 ± 1.0	< 0.01
	60–69	13	4.9 ± 2.0	11	2.7 ± 1.2	<0.005
	70–80	12	12.0 ± 6.7§	5	2.0 ± 1.6	< 0.005
	Total	37	6.9 ± 5.4	25	2.3 ± 1.3	< 0.0001

* Normal vs NFPA, § $P < 0.001$ vs 50–59 years and 60–69 years. *n*, number of subjects.

accuracy of FSH and LH measurement for the diagnosis of NFPA was very high, both FSH and LH showing a high sensitivity and specificity (about 90%). Therefore, despite the decline in FSH and LH observed in healthy postmenopausal women during aging, the reduction of gonadotropin levels remained statistically significant even in old patients with NFPA.

In male patients with NFPA, serum FSH levels were similar to those observed in healthy subjects, the only exception being a significant reduction in FSH levels in 70- to 80-year-old patients who did not show the age-associated increase in FSH that occurred in normal controls. This may be interpreted as an absent response of gonadotrophs to the sex steroid-mediated negative feedback. Similarly, despite the large overlap with normal subjects, patients with NFPA showed a significant reduction in LH in all age groups.

In conclusion, the present data indicate that despite the gradual decline of gonadotropin levels in healthy postmenopausal women, the reduction in both FSH and LH persists even in old patients with NFPA, suggesting that gonadotropin measurement could prove useful in the evaluation of pituitary lesions even in old women.

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References

1 Kwekkeboom DJ, de Jong FH, Van Hemert AM, Vandenbroucke JP, Valkenburg HA & Lambert SWJ. Serum gonadotropins and α-subunit decline in aging normal postmenopausal women. *Journal of Clinical Endocrinology and Metabolism* 1990 **70** 944–950.

2 Rossmanith WG, Scherbaum WA & Lauritzen C. Gonadotropin secretion during aging in postmenopausal women. *Neuroendocrinology* 1991 **54** 211–218.

3 Rossmanith WG. Gonadotropin secretion during aging in women. *Experimental Gerontology* 1995 **30** 369–381.

4 Lamberts SWJ, van den Beld AW & van der Lely AJ. The endocrinology of aging. *Science* 1997 **278** 419–424.

5 Santoro N, Banwell T, Tortoriello D, Lieman H, Adel T & Skurnick J. Effects of aging and gonadal failure on the hypothalamic-pituitary axis in women. *American Journal of Obstetrics and Gynecology* 1998 **178** 732–739.

6 Gray A, Feldman HA, McKinlay JB & Longcope C. Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachussets Male Aging Study. *Journal of Clinical Endocrinology and Metabolism* 1991 **73** 1016–1025.

7 Kaufman JM, Giri M, Deslypere JP, Thomas G & Vermeulen A. Influence of age on the responsiveness of the gonadotrophs to luteinizing hormone-releasing hormone in males. *Journal of Clinical Endocrinology and Metabolism* 1991 **72** 221–224.

8 Johnson L, Zane R, Petty CS & Neaves WB. Attenuation of luteinizing hormone secretory burst amplitude as a proximate basis for the hypoandrogenism of healthy ageing in men. *Journal of Clinical Endocrinology and Metabolism* 1992 **75** 707–713.

9 Veldhius JD, Urban RJ & Dufau ML. Differential responses of biologically active luteinizing hormone secretion in older versus young men to interruption of androgen feedback. *Journal of Clinical Endocrinology and Metabolism* 1994 **79** 1736–1770.

10 Vermeulen A. Role of the hypothalamo-pituitary function in the hypoandrogenism of healthy aging. *Journal of Clinical Endocrinology and Metabolism* 1992 **75** 704–706.

11 Morley JE, Kaiser FE, Perry HM, Ping P, Morley PMK, Stauber PM *et al.* Longitudinal changes in testosterone, luteinizing hormone and follicle-stimulating hormone in healthy older men. *Metabolism* 1997 **46** 410–413.

12 Snyder PJ. Gonadotroph cell adenomas of the pituitary. *Endocrine Review* 1985 **6** 552–563.

13 Faglia G, Spada A, Beck-Peccoz P, Persani L, Ambrosi B & Colombo P. Clinically nonfunctioning pituitary adenomas. Advances and perspectives in pituitary adenomas. In *New Trends in Basic and Clinical Research*. Excerpta Medica International Congress Series 961, pp 373–382. Amsterdam: Elsevier, 1991

14 Asa SL, Gerrie BM, Singer W, Horvath E, Kovacs K & Smyth HS. Gonadotropin secretion *in vitro* by human pituitary null cell adenomas and oncocytomas. *Journal of Clinical Endocrinology and Metabolism* 1987 **80** 1011–1019.

15 Jameson JL, Klibanski A, Black PM, Zervas NT, Lindell CM, Husu DW *et al.* Glycoprotein hormone genes are expressed in clinically nonfunctioning pituitary adenomas, *Journal of Clinical Endocrinology and Metabolism* 1987 **80** 1472–1478.

16 Bloomfield P & Steiger W. Least absolute deviation curve-fitting. *SIAM Journal of Scientific and Statistical Computing* 1980 **1** 290–301.

17 Nahoul K & Roger M. Age-related decline of plasma bioavailable testosterone in adult men. *Journal of Steroid Biochemistry* 1990 **35** 293–299.

18 Maruyama Y, Aoki N, Suzuki Y, Sinohara H & Yamamoto T. Variation with age in the levels of sex-hormone binding plasma proteins as determined by radioimmunoassay. *Acta Endocrinologica* 1984 **106** 428–432.

19 Snyder PJ, Muzyka R, Johnson J & Utiger RD. Thyrotropin-releasing hormone provokes abnormal follicle-stimulating hormone (FSH) and luteinizing hormone responses in men who have pituitary adenomas and FSH hypersecretion. *Journal of Clinical Endocrinology and Metabolism* 1980 **51** 744–748.

20 Daneshdoost L, Genarelli TA, Bashey HM, Savino PJ, Sergott RC, Bosley TM & Snyder PJ. Recognition of gonadotroph adenomas in women. *New England Journal of Medicine* 1991 **324** 589–594.

21 Saccomanno K, Gil-del-Alamo P, Bassetti M, Reza-Elahi F & Spada A. *In vitro* detection of glycoprotein production and secretion by human nonfunctioning pituitary adenomas. *Journal of Endocrinological Investigation* 1993 **16** 109–114.

22 Gil-Del-Alamo P, Pettersson KSI, Saccomanno K, Spada A, Faglia G & Beck-Peccoz P. Abnormal response of luteinizing hormone beta subunit to thyrotrophin-releasing hormone in patients with nonfunctioning pituitary adenoma. *Clinical Endocrinology* 1994 **41** 661–666.

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