The THAI Journal of SURGERY 2007; 28:20-26.
Official Publication of the Royal College of Surgeons of Thailand

The Dependence of the Impulse Mode of the Increment of Some Hormones and Mitotic Activity from Testosterone Production in Older Men

Alexander V. Pechersky, MD, PhD[a]
Vladimir F. Semiglazov, MD, PhD[b]
Oleg B. Loran, MD, PhD[c]
Anatoly I. Karpischenko[d]
Viktor I. Pechersky, MD[e]
Vadim I. Mazurov[f]

[a]*Department of Surgery, Medical Academy of Post-Graduate Studies, St. Petersburg, Russia*
[b]*Surgical Department, N.N. Petrov Scientific-Research Institute of Oncology, St. Petersburg, Russia*
[c]*Department of Urology, Russian Medical Academy of Post-Graduate Studies, Moscow, Russia*
[d]*Department of Clinical Biochemistry and Laboratory Diagnostics, Russian Military-Medical Academy, St. Petersburg, Russia*
[e]*Problem Scientific-Research Laboratory, State Academy of Physical Education of P.F. Lestgaft, St. Petersburg, Russia*
[f]*Department of Therapy, Medical Academy of Post-Graduate Studies, St. Petersburg, Russia*

Abstract

Objective: **After 40 years of age, men have a reduction in the amount of testosterone circulating in their bloodstream. The mechanisms of hypothalamic-pituitary regulation are broken down, including the mechanisms for the pulse mode of incretion. The aim of this research work was to study cause-and-effect links between the development of partial androgen deficiency of aging men (PADAM) and changes in the impulse mode of hormonal regulation in men of older age groups.**

Patients and Methods: **In this study, 9 patients with partial androgen deficiency were analyzed. The age of these patients ranged from 42 to 74 years. The patients were given 40 mg of andriol (testosterone undecanoate) once each morning. All patients were put into one group, for which the research results were compared before and one month after the beginning of androgen-replacement therapy. In order to make the diagnoses of the breakdown in the rhythm of incretion, levels of LH, FSH, and STH, as well as of testosterone, cortisol, and insulin were measured by taking five samples from the serum. The samples were taken once every 20 minutes (in a period of time that covered the whole average period of their impulse incretion.**

Results: **One month after the beginning of androgen-replacement therapy the patients showed an increase in the amplitude of fluctuations of LH (on average by 52.2%), FSH (on average by 126.7%), STH (on average by 81.0%), testosterone (on average by 69.0%), cortisol (on average by 45.5%) and insulin (on average by 76.2%) as compared with the original values before the study began (relative to average values).**

Conclusions: **The reduction in testosterone production as men get older leads to a change from a pulse mode to a tonic mode of incretion of a number of hormones, as well as to a breakdown in the regulation of the cell cycle and an increase in mitotic activity. The inverse development of these changes can be seen among men of older age groups with PADAM who have been given androgen-replacement therapy.**

Key words: **testosterone, PADAM, LH (luteinizing hormone), FSH (follicle stimulating hormone), STH (somatrope hormone), insulin**

Correspondence address : *Dr. A.V. Pechersky, Ph. D., Senior Lecturer, Department of Surgery, St. Petersburg Medical Academy of Post-Graduate Studies. 41 Kirochnaya St., St. Petersburg, 193015, Russia. E-mail: a_pechersky@msn.com*

Introduction

A decrease in the number of cells-producers of testosterone (Leydig cells) among men after 40 years of age leads to a decrease in testosterone production.[1-3] Leydig cells are not able to ensure adequate production of testosterone in response to the increment of luteinizing hormone (LH). The mechanisms for regulation of the system of the gonads-hypophysis-hypothalamus are thereby disrupted, including the mechanism for the impulse mode of incretion.[4-6] Older men show a decrease in the emission of LH, and an increased frequency in the peaks of emission of LH. They also demonstrate a breakdown in impulse increment of testosterone.[3,7-9] The biological value of the rhythm of testosterone increment isn't entirely set.[3]

The aim of this research work was to study cause-and-effect links between the development of partial androgen deficiency of aging men (PADAM) and changes in the impulse mode of hormonal regulation in men of older age groups.

Patients and Methods

A total of nine patients with partial androgen deficiency of aging men were observed in this study. Their ages ranged from 42 to 74. In order to be included in the study, patients had to be male, older than 40 years old, and had a decreased level of common testosterone (<10.4 nmol/L) and/or free testosterone (<110.0 pmol/L) in the blood serum6 (Table 1). Patients with infectious diseases of the lower urinal tracts, varicocele, or prostate cancer were excluded from the study. In order to check for these diseases patients were given rectal analysis and transrectal ultrasound scanning of the prostate gland, while prostate-specific antigen (PSA) which was supposed to be no higher than 4 ng/ml was measured in their blood serum. Patients with improper liver function (in other words, whose values of activity of alanine aminotranspherase was greater than 26 ME/L, or aspartate aminotranspherase higher than 25 ME/L and common bilirubin higher than 21 mkmol/L) were also excluded from the study, as were those with a level of creatinine in the blood serum higher than 0.11 mmol/L, those who had had medical treatment over the last three months using antiandrogens or finasterid, and those with trauma of the central nervous system, epilepsy, or other diseases or brain punctures in their medical history.

Patients were given 40 mg of andriol (undecanoate of testosterone) once a day in the morning. All patients made up one group. The research results of this group were compared before and one month after the beginning of androgen-replacement therapy.

Hormonal research and reading PSA levels

The levels of common and free testosterone in the blood serum as well as the level of prostate-specific antigen (PSA) were measured using an immunofermenting method. In order to take these measurements, blood was taken from the veins on an empty stomach at a fixed time (08.00-10.00).[10,11]

The increment of gonadotropin-releasing hormone by the hypothalamus, of other hormones by the adenohypophysis (besides prolactin), and of testosterone by the testicles is done impulsively, and takes place on average once every 90 minutes. Increment of cortisol and insulin is also done impulsively.[6] In order to make diagnosis of the breakdown in the rhythm of increment of LH, follicle stimulating hormone (FSH), and STH as well as of common testosterone, cortisol and insulin, the blood serum was measured in five samples taken at 20-minute intervals; in other words, over the period of time covering the entire average impulsive incretion cycle.[6]

The levels of LH, FSH, and STH as well as of

Table 1 The levels of common and free testosterone and PSA, before and one month after the beginning of androgen-replacement therapy (N = 9)

	Common testosterone (nmol/L)	Free testosterone (pmol/L)	PSA (ng/ml)
$\mu \pm \sigma$ before	17.3 ± 8.2	37.6 ± 20.7	1.9 ± 2. 5
$\mu \pm \sigma$ after 1 month	20.9 ± 10.7	53.9 ± 58.2	1.3 ± 1.0

PSA: prostate-specific antigen

common testosterone, cortisol, insulin and PSA were measured using test kits made by DPS (USA), while free testosterone was measured using test kits made by Diagnostic Systems Laboratories Inc. (USA).

The sensitivity of the methods used for reading levels and the coefficients of variation were, for LH - 0.5 ME/L and 8%; for FSH - 0.5 ME/L and 8%, for STH - 1.3 pmol/L and 8%, for common testosterone - 0.2 nmol/L and 8%, for free testosterone - 0.1 pmol/L and 5.4%, for cortisol - 15 nmol/L and 8%, for insulin - 7.2 pmol/L and 10%, and for PSA - 0.01 ng/ml and 8%.

Statistical analysis

The results of the study were analyzed using a method of dispersion analysis of repeated measurements. The significance of the differences in the changes between the results before and one month after the beginning of androgen-replacement therapy was measured using paired Student criteria. All data in this text and in all tables are given as average values and standard deviations ($M \pm \sigma$). The average values of the changes in parameters ($\bar{d}$) are also given, as are their standard errors ($s_{\bar{d}}$) and the values of the Student criteria (t).[12]

Results

One month after the beginning of androgen-replacement therapy, patients showed an increase in the amplitude of fluctuations of the levels of LH, FSH, STH, common testosterone, cortisol, and insulin as compared to average values (Tables 2-4).

Discussion

The endocrine and nervous systems function in a coordinated way, thereby supporting the consistency of the body's inner environment. Although there is an obvious difference in the mechanisms by which these two systems pass along information, each of the two systems features releasing chemical substances as a way of making communication between cells. The endocrine system is a continuation of the central nervous system. Neurosecretorial cells of the hypothalamus combine characteristics of both systems: they get information from higher-lying parts of the central nervous system through synaptic transfers and, at the same time, synthesize hormones which are transported along with the current of axoplasma to the hypophysis. The sensory stimulus is transformed into hormone secretion; such a transformation is called a neuroendocrinal response.[13]

Sending information to the central nervous system is done with the help of a frequency pulse code. This code uses both the frequency of the transfer of nerve impulses and the quantity of nerve impulses in " formed packages".[14] J. Furth was one of the first researchers to use methods of analysis from cybernetics for evaluating the function of the hypophysis in 1967.[15]

Table 2 Average levels of LH, FSH, STH, common testosterone, cortisol, and insulin, before and one month after androgen-replacement therapy (N = 9)

	LH (U/L)	FSH (U/L)	STH (pmol/L)	Common testosterone (nmol/L)	Cortisol (nmol/L)	Insulin (pmol/L)
$\mu \pm \sigma$ before	4.1 ± 2.6	5.5 ± 3.1	96.8 ± 167.2	17.3 ± 8.2	484.1 ± 204.4	68.9 ± 47.3
$\mu \pm \sigma$ after 1 month	2.7 ± 1.6	5.2 ± 3.1	52.8 ± 92.4	20.9 ± 10.7	399.2 ± 154.8	38.7 ± 27.2

Table 3 Differences between the maximum and minimum levels of LH, FSH, STH, common testosterone, cortisol, and insulin, before and one month after androgen-replacement therapy (N = 9)

	LH (U/L)	FSH (U/L)	STH (pmol/L)	Common testosterone (nmol/L)	Cortisol (nmol/L)	Insulin (pmol/L)
$\mu \pm \sigma$ before	1.2 ± 0.6	0.5 ± 0.3	149.6 ± 264.0	3.6 ± 2.1	160.8 ± 79.6	28.0 ± 38.0
$\mu \pm \sigma$ after 1 month	1.2 ± 0.7	0.9 ± 0.5	127.6 ± 228.8	7.8 ± 6.9	206.3 ± 104.6	19.4 ± 12.9

Table 4 Percentage relation of the differences between minimum and maximum levels as compared to average values of the levels of LH, FSH, STH, common testosterone, cortisol, and insulin, before and one month after androgen-replacement therapy (N = 9)

(max-min)/ average value *100%	Values					
	LH (%)	FSH (%)	STH (%)	Common testosterone (%)	Cortisol (%)	Insulin (%)
$\mu \pm \sigma$ before	34.1 ± 16.8	9.0 ± 4.6	101.4 ± 66.8	21.6 ± 9.9	36.5 ± 23.9	33.2 ± 23.1
$\mu \pm \sigma$ after 1 month	51.9 ± 36.1	20.4 ± 17.6	183.5 ± 89.3	36.5 ± 20.7	53.1 ± 29.3	58.5 ± 31.1
Ð 17.8	11.4	82.1	14.8	16.6	25.3	
s_d 7.4	5.071075	25.4	6.6	6.4	8.5	
T 2,405	2,248	3,234	2,260	2,571	2,989	
P p <0.05	p <0.05	p <0.05	p <0.05	p <0.05	p <0.05	

Regulation of the rhythm of increment of hormones of the hypothalamus is done by the suprachiasmatic core of the middle brain.[16] The suprachiasmatic core serves not only as a pacemaker for rhythms, but is also one of the most important centers for integration of the brain. Axons of afferent neurons end in the suprachiasmatic core. These afferent neurons are located in more than 20 sections of the brain.[17]

The system of regulation of rhythms includes three components: neuron-pacemakers, an afferent regulation unit which adapts the work of the pacemaker, and an efferent unit which transfers commands of the pacemaker to the functional target.[18]

The success of the transfer of the biological signal depends not only on the level of the hormone but also on the frequency of the secretion of the hormone. This conclusion is confirmed by the dependency of the correlation of formation of LH and FSH levels on the frequency of secretion of gonadotropin-releasing hormone.[6] The pulse of the rhythm of the formation of hormones from the point of view of cybernetics is related to "discrete messages", which are capable of sending a significantly large volume of information, unlike "non-stop messages" which have a constantly changing size.[14]

Information from the central nervous system, which is transferred in the form of nerve impulses which follow one after another at regular intervals and are united into packets, is transformed into an impulse rhythm of formation of hormones. The ability of neurons of the suprachiasmatic core, unlike neurons of other sections of the brain which have endogenic rhythm, to transform a series of rhythms into single impulses allows this to be achieved.[19]

Increment of the majority of hormones of the adenohypophysis has an impulse character which complies with the short period of their decomposition. Unlike other hormones of the front part of the hypophysis, prolactin is formed in a tonic mode. This type of increment is made up of a "continuous message" and, accordingly, caries less information. The mode of prolactin increment is determined by regulation by dopamine (DA), which also forms in tonic mode.[16] Prolactin is a phylogenetically older hormone, and its increment mode is less complete as compared to that of other hormones of the adenohypophysis which have an impulse increment mode. As a result, the volume of information carried through increment of prolactin is significantly inferior to the analogous value for the majority of other hormones which formed at later stage of evolution.

The development of PADAM is accompanied by a breakdown in the impulse regime of hormone increment by the adenohypophysis. These changes lead to the limitation and distortion of the information being transferred, which regulates a whole series of physiological processes including proliferative activity.[20] Thus LH is the stimulator of synthesis of IGF-1 in Sertoli's cells. IGF-1 strengthens the expression of LH receptors on Leydig cells, and thereby activates steroidogenesis. FSH has a slowing-down influence on the creation of transformation growth factors in Sertoli's cells. Transformation growth factors reject steroidogenesis in Leydig cells.[6] In relation to this, the frequency of the rhythm of formation of gonadotropin-releasing hormone, which determines the correlation between LH and FSH, has a direct influence on the level of cell growth factors and accordingly on cell proliferation.

As testosterone production goes down as men get older, the agreement between the central and peripheral core of the hypothalamus-hypophysis-gonad system is broken down. When changes in the testes take place as men get older (smaller quantity of Leydig cells), those patients who have PADAM demonstrate that the impulse incretion of gonadotropin-releasing hormone and LH is not accompanied by an adequate impulse increment of testosterone. The central nervous system understands this state to be an even deeper manifestation of androgen deficit. There is thus a compensatory increase in the levels of gonadotropin-releasing hormone, LH, and FSH, using a mechanism of inverse feedback.[6] Despite the increase in the level of testosterone, the mode for testosterone increment remains non-physiological, and gradually becomes tonic. The fact that there is a significant decrease in the level of fluctuation of common testosterone in men with PADAM one month after androgen-replacement therapy as compared to before the treatment ($p<0,05$) (Tables 2-4) proves this conclusion.

The reaction of Leydig cells in tonic mode to the impulse formation of gonadotropin-releasing hormone and LH among men with PADAM is accompanied by a gradual transition to a tonic regime of hormone increment by the hypophysis and hypothalamus. The lower levels of the amplitude of impulse increment of LH an FSH among patients observed in this study before treatment as compared to analogous values one month after androgen-replacement treatment (Tables 3, 4) prove this conclusion.

Apparently, a decrease in impulse increment of hypothalamus-hypophysis hormones is additionally determined by the suppression of activity of neuron-pacemakers of the suprachiasmatic core due to the transition to tonic regime of the periphery endocrine organs (testosterone) and, accordingly, changes in the characteristics of the afferent signal which is received as part of the mechanism for negative inverse feedback.

The long and continuous (in the tonic mode) influence of gonadotropin-releasing hormone leads to de-sythesization of this hormone's receptors on gonadotropin cells, and to the suppression of the increment of LH and FSH, despite the remaining deficit of testosterone.[6,21] Thus, among those patients with PADAM who were studied, the original levels of LH and FSH did not exceed the normal referential interval (Table 2).

The use of analogues of gonadotropin-releasing hormone, which have a suppressing influence on gonadotropin cells of the hypophysis as well as on Leydig cells,[6] is based on this effect. Suppression of the impulse incretion of gonadotropin-releasing hormone, in turn, is reflected in the correlation of LH and FSH, and in the formation of cell growth factors. A vicious circle is formed. Melatonin together with a change in the expression of its receptors in the suprachiasmatic core has a significant influence on regulation of neuron-pacemakers.[18]

An increased level of prolactin, which is observed among patients with PADAM,[22] leads to the impulse increment of gonadotropin-releasing hormone and, consequentially, of the impulse rhythm of production of LH, FSH, and testosterone, as well as to the suppression in impulse increment of STH.[6] This conclusion is proven by the significantly lower fluctuation in the levels of the hormones among patients with PADAM before androgen-replacement therapy as compared with values one month after the given therapy (Tables 2-4).

Cortisol has an influence on the regulation of rhythms of the suprachiasmatic core, which is proven by the high concentration of receptors of corticosteroids in the suprachiasmatic core.[23] Any deviation in production of cortisol from the norm, whether it be a significant decrease in production (when patients have adrenalectomy),[24,25] or an increase in cortisol levels together with a reduced amplitude of its impulse increment (when patients have PADAM) (Tables 2-4), has a suppressive effect on neurons-pacemakers of the suprachiasmatic core.

Insulin resistance, which goes along with PADAM,[22] leads to the exhaustion of β cells and to a breakdown in the impulse increment of insulin.6 Before androgen-replacement therapy was given to patients with PADAM, these patients had a significantly lower amplitude of fluctuation in their insulin levels ($p<0.05$) (Tables 2-4).

The signal, which regulates mitotic activity, is sent through G-protein, which ensures that the hormonal signal is sent a number of times. G-protein in its activated form stimulates the synthesis of cyclical adenosine monophosphate from adenosine triphosphate through adenylate cyclase, and stimulates the synthesis of cyclical guanosine monophosphate from guanosine triphosphate through guanylate cyclase.

This leads to the launch of the cascade mechanism for activating inner-cell proteins. The phosphorylation-dephosphorylation process is the fundamental mechanism for making the biological effect of "secondary" messengers within cells. Phosphorylation is the most important post-translation modification of protein molecules. Phosphorylation activates or inhibits the fermentive activity of protein molecules. Dephosphorylation leads to inactivation of the ferment and to a return to the original state by stopping the transfer of the mitotic signal. Hormonal regulation of mitotic activity of normocytes is discreet and impulsive. The change from the impulse formation of hormones to a tonic increment regime inhibits the onset of the physiologically necessary dephosphorylation phase. The signal chain, which caries the mitogenic signal, takes on a continually active state (çthe pressed button effecté). The cell is thereby held in a regime of constant mitotic activity. The activity of adenylate cyclase and guanylate cyclase gets an additional stimulus from ions of Ca^{++} and free radicals-products of peroxide oxidation of lipids.[26,27] The levels of the latter go up among patients with age-related deficiency of testosterone production.[22]

The daily dose of the testosterone preparation which enters the blood plasma when making androgen-replacement therapy among patients with PADAM shouldn't exceed the average daily production of testosterone in healthy men: 7 mg/day.[6] Considering that about half of the mass of testosterone undecanoate is made up of undecanoate, while a part of the preparation is lost in the stomach-intestinal tract, the optimum dose per capsule of testosterone undecanoate is 40 mg per day. Transdermal gel of testosterone would best be given in the amount of 2-5 mg of testosterone per day.[28] Giving a larger dose per day leads to suppression of the body's own production of testosterone, with loss of increment rhythm. Thus, restoration of regulation, which should be done by testosterone, does not take place.

Consequently, the physiological rhythm of increment of hormones helps the neuroendocrine system to complete its main function, which is integration of various biological processes into a single organism. These processes take place on the molecular, cell, tissue, organ, and system levels. An age-related decrease in the production of peripheral hormones (including PADAM) leads to a loss of impulse rhythm and the establishment of a tonic increment mode for a whole series of hormones, as well as to an increase in mitotic activity. The reverse development of these changes can be observed in men of older age groups with PADAM who are given androgen-replacement therapy. The restoration of the physiological regime of increment of testosterone, in turn, is the main criteria by which the success of androgen-replacement therapy can be judged.

REFERENCES

1. Suoranta H. Changes in the small blood vessels of the adult human testis in relation to age and to some pathological conditions. Virchows Arch. A Pathol Anat 1971; 352: 165-81.
2. Neaves WB, Jobnson L, Porter JC, et al. Leydig cell numbers, daily sperm production, and serum gonadotropin levels in aging men. J Clin Endocrinol Metab 1984; 59: 756-63.
3. Dedov II, Kalinichenko SYu. Age-related androgen deficit. Moscow: Prakticheskaya Medicina; 2006.
4. Bremner, et al. Loss of circadian rhythmicity in blood testosterone levels with aging in normal men. J Clin Endocrinol Metab 1983; 56: 1278-81.
5. Gray A, Feldman HA, McKinlay JB, Longcope C. Age, disease, and changing sex-hormone levels in middle-aged men: Results of the Massachusetts male aging study. Clin Endocrinol 1991; 73: 1016-25.
6. Lavin N. Endocrinology. Moscow: Praktika; 1999.
7. Veldbuis JD, Urbun RJ, Lizarralde G, et al. Attenuation of luteinizing hormone secretory burst amplitude as a proximate basis for the hypoandrogenism of healthy aging in man. J Clin Endocrinol Metab 1992; 75: 707-13.
8. Kraiser FE, Morley JI. Gonadotropins, testosterone, and the aging male. Neurobiol Aging 1994; 15: 559-63.
9. Veldbuis JD, Zwart A, Mulligan T, et al. Muting of androgen negative feedback unveils impoverished gonadotropin-releasing hormone/luteinizing hormone secretory reactivity in healthy older men. J Clin Endocrinol Metab 2001; 86: 529-35.
10. Morales A, Bain J, Ruijs A, et al. Clinical practice guidelines for screening and monitoring male patients receiving testosterone supplementation therapy. Internat J Impot Res 1996; 8: 95-7.
11. Loran OB, Segal AS, Supryaga OM. Andriol in treatment of secretory sterility and climacteric syndrome among men. Urol Nephrol 1999; 3: 41-4.
12. Glantz SA. Primer of biostatistics. Moscow: Practica; 1999.
13. Green P, Stout W, Tailor D. Biologiya. Moscow: Mir; 1993. p. 291-2.
14. Gubanov N I. Moscow: Biofizika; 1978.
15. Furth J. Pituitary cybernetics and neoplasia. Harvay Lect 1967; 63: 47-71.

16. Kettail VM, Arki RA. Pathophysiology of the endocrine system - SPB-Moscow: Nevsky Dialect; 2001.
17. Ugryumov MV. Mechanisms of neuroendocrinal regulation. Mscow: Nauka; 1999.
18. Klein DC, Moore RY, Reppert SM. Suprachiasmatic nucleus: The mind's clock. N.Y: Oxford Univ press; 1991.
19. Mirmiran M, Kok JH, Boer K, Wolf H. Prenatal development of human circadian rhythms: Role of the fetal biological clock. Neurosci Behav Rev 1992; 16: 371-8.
20. Pechersky AV, Mazurov VI, Semiglazov VF, Karpishenko AI. Method of restoration of the impulse regime of hormonal regulation among men with partial age-related androgen deficit (PADAM). Patent for invention No 2235501. Priority invention April 02, 2002. Registered in the state register of inventions of the Russian Federation, September 10, 2004.
21. Montanari E, et al. Etiopathogenesis of benign prostatic hyperptrophy. Arch Ital Urol Androl 1995; 67: 7-12.
22. Pechersky AV, Semiglazov VF, Loran OB, et al. The effects of changes in testosterone level on the development of cytokines among patients with prostate cancer. Thai J Surg 2006; 27: 56-63.
23. De Kloet ER, Rosenfeld P, Van Eekelen AM, et al. Stress, glucocorticoids and development. Progr Brain Res 1988; 73: 101-20.
24. Arduinin D, Rizzo G, Parlatti E, et al. Modification of ultradian and circadian rhythms of fetal heart rate after fetal-maternal adrenal gland suppression: double blind study. Prenatal Diagnosis 1986; 6: 409-17.
25. Arduinin D, Rizzo G, Parlatti E, et al. Loss of circadian rhythms of fetal behavior in a totally adrenalectomized pregnant woman. Gynecol Obstet Invest 1987; 23: 226-9.
26. Karpishenko AI. Medicinal Laboratory Diagnostics. SPB: Intermedika; 2001.
27. Berezov TT, Korovkin BF. Biological Chemistry. Moscow: Medicina; 2004.
28. Pechersky A. Adequacy of Androgen-Replacement Therapy Among Patients with PADAM. Gender Medicine 2006; 3(A): 62.