Medicina Sportiva
Med Sport 17 (4): 211-216, 2013
DOI: 10.5604/17342260.1081300
Copyright © 2013 Medicina Sportiva

D-ASPARTIC ACID: BIOLOGICAL ROLE AND POTENTIAL APPLICATIONS AS DIETARY SUPPLEMENT IN SPORT

Maria Ładyga, Zbigniew Obmiński

Instytut Sportu, Warszawa, Poland

Abstract

Aspartic acid naturally occurs as an L- or D-form. Like many other chemical, aspartic acid demonstrates a varied biological activity dependent on which enantiomer it is in a pair. L-Aspartic acid (L-Asp) belongs to endogenous human amino acids involved in peptides and proteins formation which also partakes in purine and pyrimidine synthesis. D-Aspartic acid (D-Asp) occurs in organisms in a free form and does not create proteins. D-Asp plays an important role in the endocrine and the nervous system (functioning as a neurotransmitter and a neuromodulator). Involvement of D-Asp in the endocrine activity has also been widely analysed. Research into D-Asp role in the synthesis and release of sex hormones in humans have revealed that seminal plasma and spermatozoa contain D-Asp and that there is a direct correlation between D-Asp concentration and the quantity and motility of spermatozoa in semen. It was therefore hypothesized that D-Asp may play an exceptional role in spermatogenesis. These results have prompted the sports market to launch dietary supplements containing D-Asp that is recommended as a compound that enhances testosterone synthesis. The aim of this overview was to present current knowledge about the role of D-Asp in the nervous and endocrine system of animals, especially of humans, and find the answer to the question, could the D-Asp intake be beneficial for professional athletes.

Key words: *D-Aspartic acid – testosterone – steroidogenesis - nutritional supplement*

Introduction

Out of 140 nonproteinogenic amino acids (or non-coded amino acids, because they are not encoded in the genetic code), D-Aspartic acid, also called testosterone „booster", has recently been one of the most popular compounds offered as dietary supplements, which accelerate and enhance testosterone synthesis and as a result increase its natural level. D-Aspartic acid is a D-form enantiomer of aspartic acid which naturally occurs also as an L-form aspartic acid. Like many other chemical, aspartic acid demonstrates a varied biological activity dependent on which enantiomer it is in a pair [1]. L-Aspartic acid (L-Asp) belongs to endogenous human amino acids involved in peptides and proteins formation which also partakes in purine and pyrimidine synthesis. It has been hypothesized that L-Aspartic acid, especially the potassium magnesium aspartate salt, enables us to save the supply of muscle glycogen and/or promote faster glycogen resynthesis during physical exercise. Another hypothesis suggests that L-Asp may improve the capacity for short intensive exercise by generating energy as a substrate for energy production in Krebs cycle and prevent skeletal muscles fatigue, which is, however, yet to be confirmed [2]. In particular the hypothesis about the effectiveness of D-Asp as a compound that increases the synthesis of testosterone in humans requires confirmation. Therefore, the aim of this overview was to collect and organize current scientific knowledge regarding a biological action of D-Asp, the role of D-Aspartic acid in the nervous and endocrine system, in particular in humans and find the answer to the question, could the D-Asp intake be beneficial for professional athletes as a legal form of performance enhancement and androgenic status maintenance.

D-Aspartic acid in animal tissues

D-Aspartic acid was first discovered in 1977 by D'Aniello and Giuditta [3] in the brain of the *Octopus vulgaris* and in the following year it was also found in the peripheral nervous system [4]. Next, the amino acid was identified in neuroendocrine tissues of various groups of animals: opistobranchia sea [5,6], tunicates [7,8], amphibians [9,10], reptiles [11,12] and birds [13,14]. In mammals, D-Aspartic acid was found in mouse's brain [15] and in rats: in testis [16], pineal gland [17], in pituitary and adrenal glands [18,19], in prefrontal cortex and hippocampus [20,21] and a variety of neurons in central nervous system and elongated spermatids [22], in blood plasma of testicular vein, testicular interstitial cells, seminiferous tubules and sperm cells [23] but also in a retina [24], hypothalamus and testis [25] and in the cells of hypothalamus-neurohypophysel system [26].

The presence of D-Aspartic acid was also determined in humans in their tooth enamel [27], in the brain of healthy people as well as of those suffering from Alzheimer's disease [28- 31], in fetal blood obtained from umbilical cord as well as in adults' blood

[32], in lenses of human cataracts [33], in prefrontal cortex [34], in erythrocytes membrane of healthy people and those suffering from a chronic kidney disease [35]. Seminal plasma and spermatozoa [36] as well as in pre-ovulatory follicular fluid [37] were also found to contain D-Aspartic acid.

Biosynthesis of D-Aspartic acid in mammals

Initially, D-Asp was claimed to originate exclusively from ingestion and intestinal bacteria. Long et al. were the first to have reported the presence of D-Asp in the cells of pheochromocytoma and rat pituitary tumor cells [38,39]. The conversion of [^{14}C] L-Asp to [^{14}C] D-Asp was then acknowledged by Wolosker et al. [25] in the culture of nervous cells derived from rat embryos, implying two possibilities of D-Asp synthesis: via aspartate racemase or aspartate transaminase. Subsequent years yielded no detailed research on D-Asp synthesis, which, according to Furuchi and Hommy [40], should be blamed on extremely low aspartate racemase activity or insufficient knowledge of optimal culturing lab conditions in which molecular mechanisms of D-Asp are investigated. It was only in 2009 that Topo et al. [41] discovered that aspartate racemase, occurring in many tissues, is involved in the conversion of L-Asp to D-Asp in rats and that pituitary gland was the tissue of its highest enzymatic activity of all the tissues analysed (pituitary gland, testes, brain, liver, kidneys and plasma).

The role of D-Aspartic acid in the nervous system

Previous studies have provided evidence that D-Aspartic acid plays an important role in the endocrine and the nervous systems functioning. D-Asp induces synthesis of the proteins which participate in the development of the nervous system, specifically in the prenatal period or directly after birth and it acts as a neurotransmitter or neuromodulator at synapses. D-Asp participation in the development of the central nervous system is demonstrated by its high transient concentrations in the brain and the retina of 13- to 14-day-old chicken embryos which subsequently decrease to very low level and remain the same for the rest of the chicken's life [13]. Such a high transient accumulation of D-Asp in rat embryos takes place solely in their brain, whereas in the retina it appears but several days after birth only to decline to low levels, similarly to the case of the chickens. Therefore, the highest D-Asp concentrations in the retina of both chickens (which possess a full vision since birth) and rats (which open their eyes on the 12th -13th day of life) occur 4-7 days prior to the animals enveloping their sight [13,32]. Very low concentrations of D-Asp in adult rat brain and high concentrations in rat embryo and newborn brains have also been noted by Wolosker et al. [25].

Numerous findings have supported the hypothesis that D-Asp plays a role as a neurotransmitter and neuromodulator at synapses. In the goldfish retina, D-Asp has been demonstrated to be capable of potentiating the effect of L-glutamate in the light response by about 15-fold [42] and, acting as a selective NMDA receptor antagonist (NMDA receptor in synapses regulates calcium flux in cells), it elicits the release of γ Aminobutyric acid (GABA) which functions as the main inhibitory neurotransmitter in the CNS [43]. In vitro studies have demonstrated that D-Asp is released from the rat cerebellum by depolarizing stimuli induced by potassium ions, just as it occurs for other neurotransmitters. The neurotransmitive role of D-Asp has also been proven by its presence in synaptic vesicle in *Aplysia limacina* [4]. According to Ota et al.[45], D-Asp fulfils to a large extent, if not fully, the function of the classic neurotransmitter i.e. D-Asp particles are synthesized, decomposed and released from presynaptic neurons and then induce reactions in postsynaptic ones.

Involvement of D-Aspartic acid in the hormone regulation

D-Asp involvement in the endocrine activity has also been widely analysed. D-Asp has been reported to negatively regulate melatonin synthesis in the rat pineal gland [46] but to stimulate the pituitary gland to release α-melanocyte-stimulating hormone (α-MSH), which activates skin cells to melanin (brown natural pigment) synthesis [47].

The role which D-Asp plays in the synthesis and release of sex hormones participating in reproduction processes appears to have attracted the richest array of studies. The majority of research confirming D-Asp involvement in the hormone synthesis and release was conducted on rats. It was demonstrated that D-Asp injected in an adult rat led to an increase in luteinizing hormone (LH), testosterone, progesterone and prolactin [16,48]. A significant increase in testosterone and LH synthesis has also been reported in in vitro experiments involving incubation of Leydig cells and pituitary glands with 0,1nM D-Aspartic acid [41]. The incubation of isolated rat hypothalamus with D-Asp, in turn, has indicated that it can elicit the release of GnRH-LHRH (gonadotropin-releasing hormone) that is, the hormone responsible for releasing LH and FSH from the anterior lobe of pituitary gland [47,48].

D-Asp has also been reported to regulate testosterone and 17β-estradiol production in male and female amphibian gonads respectively [9,10,49], to stimulate the activity of ovaries and testes in reptiles [50,51] and to affect steoridogenesis activity in the mallard's testes [14].

Further studies have been carried out with an aim to explain the cellular and molecular mechanism which enables D-Asp to influence the synthesis and

release of sex hormones. The observation of rats have revealed that the application of D-Asp increases the level of StAR mRNA regulating sex hormones synthesis in Leydig cells, which stimulates testosterone synthesis [40, 52]. D-Asp function has also been observed in frogs, where it appears to modulate the level of sex hormones in testes by regulation of P450 aromatase activity (P450 aro), the key enzyme responsible for the conversion of testosterone into 17β-estradiol, as well as modulating 5α-reductase type 2 (5α-Red2) which is the catalyst of the conversion of testosterone into 5α-dihydrotestosterone [53].

The role of D-Aspartic acid in the human reproductive system

Research into D-Asp role in the synthesis and release of sex hormones in humans [36] have revealed that seminal plasma and spermatozoa contain D-Asp and that there is a direct correlation between D-Asp concentration and the quantity and motility of spermatozoa in semen, i.e. sperm quality. It was therefore hypothesized that D-Asp may play an exceptional role in spermatogenesis, particularly because it was identified in the spermatozoa nucleus, which, according to the researchers, may indicate D-Asp stimulatory activity in transcription of genes associated with sperm cells maturity. D-Asp concentrations in seminal plasma in men with oligoasthenoteratozoospermia and azoospermia have been demonstrated to be significantly lower (3.07 and 6.7-fold, respectively) in comparison with men with normal semen. However, whether the lower level of D-Asp in these men's semen was the result or the cause of the decreased semen quality has not been determined.

The occurrence of significant amount of free D-Asp in pre-ovulatory follicular fluid was identified in women aged 22-44 who were involved in in vitro fertilization programme (IVF). Significantly high D-Asp concentrations have been identified in younger females (aged 22-34) than in the older ones (aged 35-40) ($P<0.01$). A significant direct correlation has also been found between D-Asp concentrations and the volume of good quality oocytes MII (oocytes in Metaphase II) and the fertility rate (FR). In the older females (aged 35-40) reduced D-Asp concentrations in follicular fluid have been accompanied by a lower oocyte quality and smaller chance of fertilization. According to the researchers, the findings implicate that D-Asp may play a role in female reproduction and that follicular D-Asp concentrations may be used as alternative or additional markers of oocyte quality in IVF patients.

The acute and chronic effects of D- and L-amino acids intake in humans

The presence of endogenous and biologically active simple chemical compounds like amino acids (both as D-form and L-form enantiomers) has prompted pharmaceutical companies to launch dietary supplements containing combinations of such components. The substances containing three branched-chain L-amino acids: leucine, isoleucine, and valine are among the most widely known and recommended ones for athletes and physically active people. Research carried out on athletes has proven that application of amino acids induces the kinds of hormonal reactions which stimulate anabolic and anti-catabolic processes i.e. increased rate of protein synthesis. Athletes engaged in strength training with amino acid supplementation demonstrated higher testosterone levels during intensive training compared to the group with no supplementation [54,55]. Athletes involved in endurance training with „branched-chain amino acid" (BCAA) supplementation were associated with higher post exercise GH level but lower blood lactate concentrations.

Literature provides reports of D-Asp applied as a dietary supplement to men experiencing reproductive problems due to poor semen quality. Clinical tests conducted by Topo et al. [41] within in vitro fertilization program including men aged 27-37, to whom D-aspartic acid was administered for 12 consecutive days (every morning at breakfast a 10 ml 2,0M solution of sodium D-Asp i.e. 3.12g/10ml) revealed a significant increase in both luteinizing hormone and testosterone after supplementation. In 20 out of 23 subjects (87%) the sodium D-aspartic acid intake caused LH concentration in blood to rise by 33.3% in comparison with baseline values, from 4.2 ± 0.5 to 5.6 ± 0.9 mIU/ml. The effects produced by D-Asp were dependent on the administration time – a significant increase in LH concentrations was recorded after 12 days. Once the supplementation finished, LH concentration was decreasing and although it remained at a higher level than the baseline values, the difference was not statistically significant. Similarly, testosterone concentrations in blood serum increased significantly after a 12-day treatment with D-Asp from 4.5 ± 0.6 to $6.4 \pm 0,8$ ng/ml, i.e. by 42% ($P<0.0082$) and decreased on the third day after supplementation, but in contrast to LH concentration, it was significantly higher ($P<0.01$) then baseline values. This was ascribed to the stimulation of D-Asp accumulated in testes because a parallel in vitro experiment demonstrated the accumulation of significant amounts of D-Asp in rat's testes still for the next 3 days after the supplementation [41].

The findings reported by Topo et al. [41] led to D-Asp being recommended to athletes as a component of supplements, which accelerate and enhance testosterone synthesis and as a result increase its natural level via physical training. This might be of interest to professional athletes as intensive training sometimes disrupts the anabolic-catabolic balance measured by cortisol to testosterone concentration ratio in blood [57]. The

parameter was introduced 25 years ago and it is used to monitor adaptation during physical training but also to assess start stress tolerance. A sharp decrease in testosterone concentration during competition has been proven to reduce physical capacity and mood [58], which may affect the sports competition results.

The available literature is lacking in compelling data which could confirm that D-Asp indeed restrains the decrease in endogenous testosterone induced by physical training load. Similarly, the effect of legal supplements recommended to sustain androgen syntheses in athletes also remains to be proven in clinical research and the application of exogenous testosterone, its analogues and precursors, is forbidden in professional sport. Therefore, D-Asp supplementation may appear to be an attractive and legal way of androgenic status maintenance and pharmacological enhancement of training effects.

Does the D-Asp intake is healthy for humans?

A question arises, whether it is safe for healthy people to use D-Asp supplementation which after 12 days caused testosterone concentration to rise by 42% in the analysed men's blood serum [41]. The reported effect of increased testosterone synthesis as a result of sodium D-aspartic acid supplementation in men with low sperm quality (oligoasthenoteratozoospermia and azoospermia) may be considered as beneficial. We may assume that D-Asp supplementation would improve the health or quality of life of men who experience its deterioration in the second half of life, which is partly genetically determined [59] but may also be linked to age-related androgen deficiency, commonly referred to as andropause [60,61]. Testosterone level, which is decreasing with age [62,63], modifies the effects of genetic factors on hippocampal volume [64], increases the risk of ischemic heart disease [65], intermittent claudication [66] and diabetes [67]. Generally, age-related andropause increases male mortality rate [68].

The process of aging often involves diminishing androgenes production in men, which is what gave rise to the investigation whether pharmacological supplementation of these hormones will mitigate andropause effects and influence the resistance training effects in middle-aged men in "The Andro Project" programme [69]. Testosterone precursors, called pro-hormones on the dietary supplements market, were administered to a study group aged 35-65 within 12 weeks, 200mg/day. The researchers used androstendione and androstendiol, physiologically weak androgens which are converted to active androgen-testosterone in human body. In the study group on androstendione a slight increase (16%) of total testosterone concentration was observed after a month but it then decreased to baseline values owing to endogenous hormone synthesis inhibition. No improvement in adaptation to training load was noted and the observed increase in HDL may indicate an increased risk of ischemic heart disease.

In another study involving men at the age of 30 to 56, 300 mg of androstendione was administered daily for 4 weeks. At the end of the experiment, no alterations to the total testosterone concentrations were reported. However, after a week's supplementation, the researchers noted a significant increase in free testosterone, dihydrotestosterone (DHT) and estradiol, whereas, in the fourth week HDL appeared to have decreased [70]. Another experiment involving young men aged 19-29 who were taking androstendione, revealed similarly adverse changes in lipid profile and no expected influence on their strength development [71]. Therefore, on the basis of the above research it may be concluded that the testosterone precursors, androstendione and androstendiol, can adversely affect men's lipid profile, which, in turn, increases the risk of ischemic heart disease. The available literature provides no answer to the question of how D-Asp supplementation affects the lipid profile.

Neither has there been research done to explain whether the form of D-Asp application has any impact on biological effect. The dietary supplements available in Poland which are mainly composed of D-aspartic acid (in most cases), sodium-D-aspartate salt or chelated calcium D-aspartic acid are offered in capsules or powder whereas the experiment revealing 42% increase in testosterone concentration in blood serum involved the application of sodium D-aspartic acid. Furthermore, there is an issue of the bioavailability of D-form enantiomers, including D-Asp, in humans, which is being investigated in studies concerning e.g. food processing since these processes may entail a conversion of all the natural L-amino acids (LAAs) into their mirrored isomers, D-amino acids (DAAs). Some DAAs have been demonstrated to be toxic in vivo, e.g. D-serine damages proximal renal tubules in rats [72,73]. The danger, however, does not apply to D-Asp as it has been confirmed to be an endogenous compound in humans. Nevertheless, it is important to bear in mind that the following questions posed at The 1st International Conference on D-Aspartic Acids on The Awaji Island [74] remain largely unresolved:

- What are the interactions of DAAs as compared to LAAs in the binding to the active sites of proteases, such as trypsin, chymotrypsin and pepsin in the digestive tract?
- Do metabolic interactions, antagonisms or synergisms, among free and protein-bound DAAs occur in vivo?
- Do DAAs and D-peptides alter the normal microflora of the intestine?
- How do biological effects of DAAs vary, depending on whether they are consumed in the free state or as part of a food protein?

Summary

The above research findings indicate that D-Asp supplementation could be a legal form of performance enhancement and androgenic status maintenance for professional athletes, or a treatment mitigating the effects of andropause. However, there is currently insufficient empirical evidence to determine how effective D-Asp stimulatory activity is in testosterone synthesis or what health benefits can be expected with long-term use.

Declaration of interest

The authors report no conflicts of interest.

References

1. Saladino R, Botta G, Crucianelli M. Advances in the synthesis of bioactive unnatural amino acids and peptides. *Mini Rev Med Chem* 2012; 12: 277-300.
2. http://www.drugbank.ca/drugs/DB00128
3. D'Aniello A, Giuditta A. Identification of D-aspartic acid in the brain of *Octopus vulgaris*. *J Neurochem* 1977; 29: 1053–57.
4. D'Aniello A, Giuditta A. Presence of D-aspartate in squid axoplasm and in other regions of the cephalopod nervous system. *J Neurochem* 1978; 31: 1107–8.
5. Fieber LA, Carlson SL, Capo TR, Schmale MC. Changes in D-aspartate ion currents in the *Aplysia* nervous system with aging. *Brain Res* 2010; 1343: 28-36.
6. Scanlan C, Shi T, Hatcher NG, at.al. Synthesis, accumulation, and release of D-aspartate in the *Aplysia californica* CNS. *J Neurochem* 2010; 115: 1234-44.
7. D'Aniello A, Vetere A, Padula L. Occurrence of free D-amino acids in the gametes, embryos, larvae and adults of the sea-squirt *Ciona intestinalis*. *Comp Biochem Physiol* 1992; 102B: 795–7.
8. D'Aniello A, Spinelli P, De Simon A, at al. Occurrence and neuroendocrine role of D-Asp and NMDA in *Ciona intestinalis*. *FEBS Lett* 2003; 552: 193–8.
9. Di Fiore MM, Assisi L, Botte V, D'Aniello A. D-Aspartic acid is implicated in the control of testosterone production by the vertebrate gonad. Studies on the female green frog *Rana esculenta*. *J Endocrinol* 1998; 157: 199–207.
10. Raucci F, Assisi L, D'Aniello S, et al. Testicular endocrine activity is upregulated by D-aspartic acid in the green frog, *Rana esculenta*. *J Endocrinol* 2004; 182: 365–76.
11. Assis L, Botte V, D'Aniello A, Di Fiore MM. Enhancement of aromatase activity by D-aspartic acid in the ovary of lizard *Podarcis sicula*. *Reproduction* 2001; 121: 803–8.
12. Raucci F, Santillo F, D'Aniello A, et al. D-aspartate modulates transcriptional activity in harderian gland of frog, *Rana esculenta*: morphological and molecular evidence. *J Cell Physiol* 2005; 204: 445–54.
13. Neidle A, Dunlop DS. Developmental changes of free D--aspartic acid in the chicken embryo and in the neonatal rat. *Life Sci* 1990; 46: 1517–22.
14. Di Fiore MM, Lamanna C, Assisi L, Botte V. Opposing effects of D-aspartic acid and nitric oxide on tuning of testosterone production in mallard testis during the reproductive cycle *Reprod Biol Endocrinol* 2008; 6: 28–36.
15. Morikawa A, Hamas H, Inoue T, et al. Determination of free D-aspartic acid, D-serine and D-alanine in the brain of mutant mice lacking D-amino acid oxidase activity. *J Chromatogr B Biomed Sci Appl* 2001; 5: 119-25.
16. D'Aniello A, DiCosmo A, DiCristo C, et al. Involvement of D-aspartic acid in the synthesis of testosterone in rat testes. *Life Sci* 1996; 59: 97–104.
17. Lee JA, Homma H, Sakai K, et al. Immunohistochemical localization of D-aspartate in the rat pineal gland. *Biochem Biophys Res Comm* 1997; 231: 505–8.
18. Lee JA, Long Z, Nimura N, et al. Localization, transport, and uptake of D-aspartate in the rat adrenal and pituitary glands. *Arch Biochem Biophys* 1997; 385: 242–49.
19. Sakai K, Homma H, Lee J, et al. D-Aspartic acid localization during postnatal development of rat adrenal gland. *Biochem Biophys Res Commun* 1997; 235: 433–36.
20. Schell MJ, Cooper OB, Snyder SH. D-Aspartate localizations imply neuronal and neuroendocrine roles. *Proc Natn Acad Sci* 1997; 94: 2013–18.
21. Wang H, Wolosker H, Pevsner J, et al. Regulation of rat magnocellular neurosecretory system by D-aspartate: evidence for biological role(s) of a naturally occurring free D-amino acid in mammals. *J Endocrinol* 2000; 167: 247–52.
22. Sakai K, Homma H, Fukushima JA, et al. Localization of D-aspartic acid in elongate spermatids in rat testis. *Arch Biochem Biophys* 1998; 351: 96–105.
23. D'Aniello AJ, Lee M, Petrucelli L, Di Fiore MM. Regional decreases of free D-aspartate levels in Alzheimer's disease. *Neurosci Lett* 1998; 250: 131–34.
24. Lee JA, Homma H, Tashiro K, et al. D-Aspartate localization in the rat pituitary gland and retina. *Brain Res* 1999; 838: 193–99.
25. Wolosker H, D'Aniello A, Snyder SH. D-Aspartate disposition in neuronal and endocrine tissues: ontogeny, biosynthesis and release. *Neuroscience* 2000; 100: 183–89.
26. Wang H, Wolosker H, Morris JF, et al. Naturally occurrence free D-aspartate is a nuclear component of cells in the mammalian hypothalamus–neurohypophyseal system. *Neuroscience* 2002; 109: 1–4.
27. Helfman PM, Bada JL. Aspartic acid racemization in tooth enamel from living humans. *Proc Natl Acad Sci* 1975; 72 2891–94.
28. Man E, Sandhous ME, Burg J, Fisher GH. Accumulation of D-aspartic acid with age in human brain. *Science* 1983; 222: 1407–8.
29. Fisher GH, D'Aniello A, Vetere A, et al. Free D-aspartate and D-alanine in normal and Alzheimer brain. *Brain Res Bull* 1991; 26: 983–5.
30. Fisher GH, D'Aniello A, Vetere A, et al. Quantification of D-aspartate in normal and Alzheimer brain. *Neurosc Lett* 1992; 143: 215–8.
31. Fisher GH, Lorenzo N, Abe H, et al. Free D- and L-amino acids in ventricular cerebrospinal fluid from Alzheimer and normal subjects. *Amino Acids* 1998; 15: 263–9.
32. Dunlop DS, Neidle A, McHale D, et al. The presence of free D-aspartic acid in rodents and man. *Biochem Biophy Res Commun* 1986; 142: 27–32.
33. D'Aniello A, D'Onofrio G, Pishetola M. Determination of D--aspartate content in lenses of human cataracts using a new method of hydrolysis to minimize racemization of amino acids. *Ital J Biochem* 1987; 36: 322–4.
34. Hashimoto A, Kumashiro S, Nishikawa T, et al. Embryonic development and postnatal changes in free D-aspartate and D-serine in the human prefrontal cortex. *J Neurochem* 1993; 61: 348–51.
35. Perna AF, D'Aniello A, Lowenson D, et al. D-Aspartate content of erythrocytes membrane proteins is decreased in uremia: implication for the repair of damaged proteins. *J Am Soc Nephrol* 1997; 8: 95–104.
36. D'Aniello G., Ronsini S., Guida F, et al. Occurrence of D--aspartic acid in human seminal plasma and spermatozoa: possible role in reproduction. *Fertil Steril* 2005; 84: 1444–49.
37. D'Aniello G, Grieco N, Di Filippo MA, et al. Reproductive implication of D-aspartic acid in human pre-ovulatory follicular fluid. *Human Reprod* 2007; 22: 3178–83.
38. Long Z, Homma H, Lee JA, et al. Biosynthesis of D-aspartate in mammalian cells. *FEBS Lett* 1998; 434: 231-5.
39. Long Z, Lee J, Okamoto Y, et al. D-Aspartate in a prolactin--secreting cloning strain of rat pituitary tumor cells (GH3). *Biochem Biophys Res Commun* 2000; 276: 1143-7.
40. Furuchi T, Homma H. Free D-aspartate in mammals *Biol Pharm Bull* 2005; 28: 1566-70.

41. Topo E, Soricelli A, D'Aniello A, et al. The role and molecular mechanism of D-aspartic acid in the release and synthesis of LH and testosterone in humans and rats. *Reprod Biol Edcrinol* 2009; 7: 120–30.
42. Ishida AT, Gordon G. D-Aspartate potentiates the effects of L-glutamate on horizontal cells in goldfish retina. *Proc Natl Acad Sci* 1981; 78: 5890–4.
43. Cha JJ, O'Brien DR, Dowling JE. Effects of D-aspartate on excitatory amino acid-induced release of [3H] GABA from goldfish retina. *Brain Res* 1986; 376: 140–8.
44. Hopainen I, Kontro P. D-aspartate release from cerebellum astrocytes: Modulation of the high K-induced release by neurotransmitter amino acids. *Neuroscience* 1990; 36: 115–20.
45. Ota N, Shi T, Sweedler JV. D-Aspartate acts as a signaling molecule in nervous and neuroendocrine systems. *Amino Acids* 2012; 43: 1873-86.
46. Ishio S, Yamada H, Hayashi M, et al. D-Aspartate modulates melatonin synthesis in rat pinealocytes. *Neurosci Lett* 1998; 249: 143–6.
47. Pampillo M, Scimonelli T, Bottin MC, et al. The effect of D-aspartate on luteinizing hormone-releasing hormone, α-melanocyte-stimulating hormone, GABA and dopamine release. *Neuroreport* 2002; 13: 2341–44.
48. D'Aniello A, Di Fiore MM, Fisher H, et al. Occurrence of D-Aspartic acid and N-methyl-D-aspartic acid in rat neuroendocrine tissues and their role in the modulation of luteinizing hormone and growth hormone release. *FASEB J* 2000; 14: 699–714.
49. Raucci F, Di Fiore MM. D-Asp: a new player in reproductive endocrinology of the amphibian Rana esculenta. *J Chromatogr B Analyt Technol Biomed Life Sci* 2011; 879: 3268-76.
50. Raucci F, D'Aniello S, Di Fiore MM. Endocrine role of D--aspartic acid in the testes of lizard Podarcis s. sicula. *J Endocrinol* 2005; 187: 347–59.
51. Raucci F, Di Fiore MM. The reproductive activity in the testis of *Podarcis s. sicula* involves D-aspartic acid: a study on c-kit receptor protein, tyrosine kinase activity and PCNA protein during annual sexual cycle. *Gen Comp Endocrinol* 2009; 161: 373–83.
52. Nagata Y, Homma H, Matsumoto M, Imai K. Stimulation of steroidogenic acute regulatory protein (StAR) gene expression by D-aspartate in rat Leydig cells. *FEBS Lett* 1999; 454: 317–20.
53. Burrone L, Raucci F, Di Fiore MM. Steroidogenic gene expression following D-aspartate treatment in frog testis. *Gen Comp Endocrinol* 2012; 175:109-17.
54. Kraemer WJ, Ratames NA, Volek JS, et al. The effect of amino acid supplementation on hormonal responses to resistance training overreaching. *Metabolism* 2006; 55: 282-91.
55. Sharp CP, Pearson DR. Amino acid supplements and recovery from high-intensity resistance training. *J Strength Con Res* 2010; 24: 1125-30.
56. De Palo EF, Gatti R, Cappellin E, et al. Plasma Lactate, GH and GH-binding protein levels in exercise following BCAA suplementation in athletes. *Amino Acids* 2001; 20: 1-11.
57. Aldercreutz H, Harkonen M, Kuoppasalmi K, et al. Effect of training on plasma anabolic and catabolic steroid hormones and their response during physical exercise. *Int J Sports Med* 1986; 7: 27-8.
58. Obminski Z., Lerczak K., Mroczkowska H., Witek K. Changes in psycho Physiological indices in Male volleyball players Turing 5-day international tournament. *Pol J Sports Med* 2012; 28: 67-73.
59. Frederiksen H., Christiansen K. The influence on genetic factors on functioning and exercise in second half of life. *Scand J Med Sports* 2003; 13: 9-18.
60. Jankowska EA, Szklarska A, Łopuszańska M, Medraś M, Are age and educational level the determinants of hormonal parameters considered as indices of andropause? *Pol Merkuriusz Lek* 2004; 94: 323-7.
61. Lukas WD, Campbell BC, Ellison PT. Testosterone, aging, and body composition in men from Harare, Zimbabwe. *Am J Hum Biol* 2004; 16:704-12.
62. Vermeulen A, Kaufman JM. Ageing of the hypothalamo--pituitary-testicular axis in men. *Horm Res* 1995; 43: 25-8.
63. Mitchell R, Hollis S, Rothwell C, Robertson WR. Age-related changes in the pituitary-testicular axis in normal men: lower serum testosterone results from decreased bioactive LH drive. *Clin Endocrinology* 1995; 42: 501-7.
64. Panizzon MS, Hauger RL, Eaves LJ et al. Genetic influence on hipocampal volume differ as a function of testosterone level in middle- age men. *Neuroimage* 2012; 59: 1123-31.
65. Cao J, Zou BP, Wang H, et al. Sex hormones and androgen receptor: risk factor of coronary heart disease in elderly men. *Chin Med Sci J* 2010; 25: 44-9.
66. Yeap BB, Alfonso H, Chubb SA, at al. Lower plasma testosterone or dihydrotestosterone, but not estradiol, is associated with symptoms of intermittent claudication in older men. *Clin Endocrinol (Oxf)* 2013; 79:725-32.
67. Cao J, Li J, Hao W, Li X, et al. Correlation of sex hormone and androgen receptor with diabetes mellitus in elderly men. *Aging Male* 2011; 14: 162-7.
68. Araujo AB, Dixon JM, Suarez EA, et al. Clinical review: Endogenous testosterone and mortality in men: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2012; 96: 3007-19.
69. Broeder CE, Quindry J, Brittingham K, et al. The Andro-Project: physiological and hormonal influences of androstendione supplementation in men 30 to 65 years old participating in a high-intensity resistance training program. *Arch Intern Med* 2000; 160: 3093-104.
70. Brown GA, Vukowich MD, Martini ER, et al. Endocrine responses to chronic Androstendione intake in 30- to 56-year--old men. *J Clin Endocrinol Metab* 2000; 85: 4074-80.
71. King DS, Sharp RL, Vukovich MD, et al. Effect of oral androstendione on serum testosterone and adaptations to resistance training in young men: a randomized control trial. *JAMA* 1999; 281: 2012-18.
72. Williams RE, Lock EA. D-Serine-induced nephrotoxicity: possible interaction with tyrosine metabolism. *Toxicology* 2004, 201, 231–8.
73. Maekawa M, Okamura T, Kasai N, et al. D-Amino-acid oxidase is involved in D-serine-induced nephrotoxicity. *Chem Res Toxicology* 2005; 18: 1678–82.
74. Friedman M. Origin, Microbiology, Nutrition, and Pharmacology of D-Amino Acids. *Chemistry & Biodiversity* 2010; 71: 1491-530.

Accepted: November 28, 2013
Published: December 20, 2013

Address for correspondence:
Maria Ładyga
Instytut Sportu
ul. Trylogii 2/16
Warszawa, Poland
maria.ladyga@insp.waw.pl

Zbigniew Obmiński: zbigniew.obminski@insp.waw.pl

Authors' contribution
A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection