

AAS AND ORGANS DAMAGE: A FOCUS ON NANDROLONE EFFECTS

GIUSEPPE DAVIDE ALBANO¹, FRANCESCO SESSA¹, ANTONIETTA MESSINA², VINCENZO MONDA³, GIUSEPPE BERTOZZI¹, FRANCESCA MAGLIETTA¹, PASQUALE GIUGLIANO³, GIUSEPPE VACCHIANO⁴, MARSALA GABRIELLA⁵, MONICA SALERNO¹

¹Section of Forensic Pathology, Clinical and Experimental Medicine Department, University of Foggia, Ospedale Colonnello D'Avanzo, Viale Degli Aviatori 1, Foggia, Italy - ²Department of Experimental Medicine, Università degli Studi della Campania "Luigi Vanvitelli", Naples, Italy - ³U.O.C., Legal Medicine, Sant'Anna and San Sebastiano Hospital, Palasciano Street, Caserta (Italy) - ⁴U.O.C., Department of Law, Economics and Mathematical Methods, Sannio University, Benevento (Italy) - ⁵Struttura Complessa di Farmacia, Azienda Ospedaliero-Universitaria, Ospedali Riuniti di Foggia, Italy

ABSTRACT

AAS are being used widespread in order to improve athlete's performance, due to its capacity to improve muscle mass and strength, furthermore, recent studies showed a diffuse abuse by adolescents for libido reasons and appearance. Nandrolone is one of the most abused AAS in the world, it has a similar structure and chemical characteristic compared to testosterone. It interacts with different receptors in our body, such as estrogen, progesterone and testosterone, that's why Nandrolone side effects affect the whole human body. Different proteins and genes are involved in testosterone biosynthesis of testosterone, with different regulatory pathways. In this review we focused on nandrolone related male reproductive system damage, in order to show the heavy consequences of AAS abuse. Nandrolone regulates MMP-2 and it could lead to spermatogenesis impairment. Oxidative stress and increase of Ros production could play a leading role in Nandrolone related infertility. Different oncogenic pathways are activated by Nandrolone and more studies need to be done to clear up correlations between this molecule and cancer. Leydig cell testosterone production is influenced by Nandrolone, moreover it was demonstrated to be a dose-dependent mechanism, despite multiple proteins and genes involved in this process were discovered, other regulatory proteins, genes and pathways, need to be studied in deep.

Keywords: anabolic androgenic steroids, nandrolone decanoate, athletes, testis, organ damage.

DOI: 10.19193/0393-6384_2017_6_147

Received November 30, 2016; Accepted May 20, 2017

Introduction

Anabolic androgenic steroids (AAS), represent a large group of synthetic derivatives, produced to prolong the biological activity of the molecule in vivo, as well as to develop molecules that are more anabolic and less androgenic⁽¹⁾. AAS are used for therapeutic reasons in lots of diseases, such as growth delay, osteoporosis, hypogonadism and anemia in elderly people⁽²⁾. However, AAS are commonly used in order to improve athlete's performance, due to its capacity to improve muscle mass and strength; furthermore, recent studies showed a

diffuse abuse by adolescents for appearance and libido matters, and ranked AAS within a selection of most frequently abused 19 drugs between adolescents, including cocaine, heroine, cannabis and ecstasy^(3,4). AAS abuse side effects act on almost all body tissues, indeed most of them have androgen receptors⁽⁵⁾. Side effects include: aggressive behavior, violence, suicide attempts, liver failure, acne, decrease of HDL, hepatic adenoma, hypogonadism^(6,7).

Testosterone, nandrolone, methandienone, and methenolol, are the most commonly abused androgens and among them nandrolone differs from

stanozolol because of its capacity to be converted in estrogens by aromatase, meanwhile stanozolol is a non-aromatizable androgen⁽⁸⁾ (table 1).

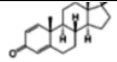
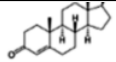
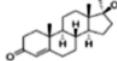
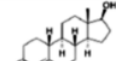
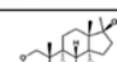
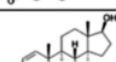
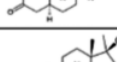
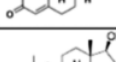
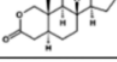
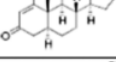
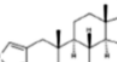
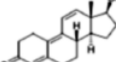
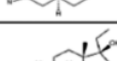
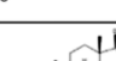
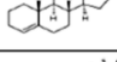
			
Methylsterone		Nandrolone	
Oxandrolone		Boldenone	
Oxymetholone		Methenolone	
Stanozolol		Trenbolone	
Ethylestrenol		Stenbolol	
Fluoxymesterone		Drostanolone	
Dexamethasone			

Table 1: Most frequently abused AAS chemical structure.

Nandrolone is one of the most frequently abused AAS in the world⁽⁹⁾, furthermore it is responsible of apoptosis induction of spermatogenic cells in rats⁽¹⁰⁾, and influences leydig cells production of testosterone⁽¹¹⁾.

Recent studies involving bodybuilders and AAS abusers proved that Nandrolone decanoate (ND) leads to several changes in sperm such as decrease sperm count and percentage and azoospermia. In this review we focused on Nandrolone molecule, its effects on Leydig cells function and its influence on reproductive system, in order to show the consequences of its abuse.

Nandrolone effects on organs

As we mentioned before, nandrolone is one of the AAS most frequently abused in the world⁽⁹⁾. Its structure is pretty similar to testosterone and the basic chemical structure is a perhydrocyclopentanophenanthrene ring system which comprises of three fused six-membered rings (A, B and C) and one five-membered ring (D) (fig. 1)⁽¹²⁾.

Hormone synthesis begins in citosol and it is completed in mitochondria. Star (steroidogenic acute regulatory protein) protein, transfers cholesterol to the inner membrane, and during its delivery

cholesterol is converted in progesterone by a cytochrome P450, family II, subfamily A, polypeptide I (CYP II A1)⁽¹³⁾. Progesterone is then transported to endoplasmic reticulum and converted to progesterone by 3 β -hydroxysteroid dehydrogenases (HSD3 β). The maturation of progesterone to androstenedione is catalyzed by the 17 α -hydroxylase/C17-20lyase (CYP17A1 also known as P45017A1) and finally to testosterone by 17 β -hydroxysteroid dehydrogenase (17 β HSD), steroid dehydrogenase specific for androgens production⁽¹⁴⁾. Androgen pathway involves steroid binding the androgen receptors (ARs), an intracellular ligand-activated transcription factor, and member of the nuclear receptor family, acting on the genome. After complex AR-steroids formation, it migrates into the nucleus and it binds to palindromic DNA sequences, and specifically, to hormone response elements (HRE) initiating gene transcription. Genomic action of ARs is modulated by a large variety of coregulators, which are proteins that target gene expression by enhancing (coactivator) or restraining (corepressor) transcription.

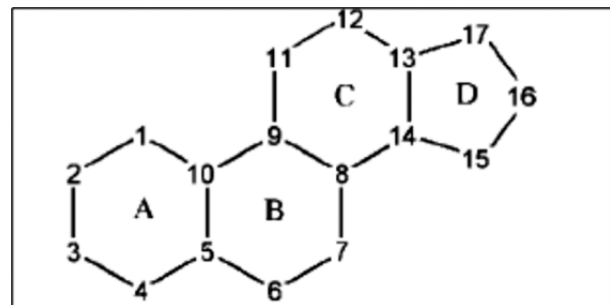


Fig. 1: Base structure of AASs.

However, AASs may also have a direct rewarding or hedonic properties, mediated not so much by their genomic effects, but by non genomic effects⁽³⁹⁾, characterized by:

- 1) rapid onset (seconds to minutes) that is faster than genomic mechanism,
- 2) insensitivity to inhibition of RNA and protein synthesis,
- 3) effects produced by steroids unable to access the nucleus either covalently linked to membrane impermeable macromolecules or in cell lacking a nucleus),
- 4) not usually blocked by classical antagonists due to different steroidal specificity from classical cognate nuclear receptors. These mechanisms are responsible of smooth muscle relaxation, neuromuscular and junctional signal transmission and neuronal plasticity^(37,38).

Endogenous nandrolone is produced in several biochemical reactions, that's why, its main metabolite, norandrosterone, can be detected in human urine⁽¹⁴⁾.

ND interacts with various receptors, such as estrogen, progesterone and testosterone⁽¹⁶⁾ and despite ND has a low affinity to Androgens receptors (AR), it was proved that by injecting a higher dose of ND in rats this lower affinity can be overcome⁽¹⁷⁾. Other effects of ND injection in rats, such as testosterone level in serum, sperm DNA fragmentation level, sperm concentration, were proved to be receptor and dose-independent. Then, this observation could give some evidences about two different models of action, receptor-dependent and receptor independent. Nevertheless, the two mechanisms in testis could be strictly linked. Because of ND influence on AR activity, several effects in different tissues and organs were established. Two weeks of daily ND administration causes alterations in mRNA expression of dopamine receptors, furthermore ND could influence expression of serotonin receptors but these data are not significant yet. These data could suggest a influence between ND and some functions such as aggressive behavior, memory and hippocampal plasticity. Due to his anabolic activity, ND was proved to increase the volume but not the length of of the proximal and distal convoluted tubules of the mouse kidney, furthermore, some studies said that ND doses administered over a long period is responsible for selective nephrotoxicity⁽¹⁹⁾. Prolonged administration of nandrolone in mice causes a dose-dependent oxidative stress and damage: in mice kidneys treated with ND was shown an increase of marker of lipid peroxidation (MDA) and a decrease of antioxidant enzymes activity, such as GR and GPx⁽¹⁸⁾.

Other recent studies findings supported the idea that oxidative stress plays a leading role in Nandrolone mediated neurotoxicity⁽²⁰⁾. Indeed ROS represent a serious hazard for cells because of their oxidizing ability to damage proteins, lipids and DNA⁽²¹⁻²³⁾. Recent studies proved that androgens are neuroprotective in case of minimal oxidative stress levels, otherwise, by the time stress levels are elevated, androgens increase oxidative stress damage. Moreover, with a similar mechanism, ND injection in mice impairs Cardiac function and his ability to react to oxidative stress.

Moreover, other studies showed that ND could have serious side effects on other organ systems, such as in liver, which could lead to liver failure, a

decrease in high-density lipoprotein (HDL) levels and hepatic adenomas⁽⁷⁾.

Testosterone biosynthesis in Leydig cells

The endocrine system consists of dynamic biological processes involved in the regulation of a complex array of physiologic activities. The synthesis of sex steroids in male is elaboratively regulated by various local factors including testicular paracrines/ autocrines in addition to the negative feedback through the hypothalamic-pituitary-gonadal (HPG) axis⁽⁴³⁾. It is commonly known that gonadotropic hormones, such as LH and FSH, influence testis activity and growth. Testosterone production and secretion is carried out by groups of cells called Leydig cells, interposed between the seminiferous tubules, and whose activity is regulated by the pituitary hormone LH⁽²⁵⁾. LH is essential for Leydig cell function because it is the main stimulating hormone of androgen production by adult Leydig cells. LH release from the pituitary gland, may in turn result in a decreased testosterone level, and as a result, testicular atrophy occurs⁽³⁶⁾. Leydig cells synthesize testosterone, using steroidogenesis machinery composed by cholesterol transporters⁽³³⁻³⁵⁾, steroidogenic enzymes, and many regulatory molecules. In the testis, it was established that the binding of LH to its G protein-coupled receptor (LHR) on Leydig cells acutely increases the levels of cAMP and cytoplasmic Calcium, which are both required for steroidogenesis⁽⁵¹⁾.

CGMP signaling also stimulates testosterone production, meanwhile, phosphodiesterases have a regulatory function in Leydig cells. The transient synthesis of steroids in response to LH occurs through modulation of a key factor, steroidogenic acute regulatory protein (StAR), whose expression highly correlates with testosterone level. StAR is the rate limiting factor in hormone-dependent steroidogenesis because it is essential for the transport of cholesterol, the precursor of all steroids hormones, from the outer to the inner mitochondrial membrane. The cAMP pathway induces StAR expression and steroidogenesis through activation of several transcription factors. Besides Star activation also requires de novo synthesis of the nuclear receptor 4A1 (NR4A1) also known as NUR77⁽²⁵⁾.

Moreover, induction of NUR77 and STAR involves the Calcium pathway and an increase in intracellular Calcium is nonetheless required. Indeed, cytosolic calcium concentration increases in parallel with testosterone production probably

coming from both extracellular environment and from intracellular calcium store (i.e. endoplasmic reticulum). IGF 1 plays a relevant role in control of Leydig cells number and development. Its production take place also in the testis, in Sertoli, Leydig and peritubular cells derived from immature testis and cultured in vitro⁽³⁹⁾. In several studies conducted in IGF1 knockout mice, failure in maturation of Leydig cells and a reduction of production of testosterone was testified, due to a deregulated expression of testosterone and metabolizing enzymes⁽⁴⁰⁾.

Furthermore, androgens have an autocrine regulation of steroidogenesis in Leydig Cells: a recent study showed AR physically interacts with Nur77, and inhibits the transactivation of Nur77 on steroidogenic enzyme gene promoters, eventually resulting in decreased steroidogenesis in Leydig cells. These findings strongly support the local action of androgen/ AR on testicular steroidogenesis, and may provide an insight into its regulatory mechanism with regard to the steroidogenesis in Leydig cells.

Nandrolone effects on testis and Leydig cells function

Androgen action in the testis, as in other tissues, is mediated through androgen receptor (AR) transcriptional activation. Inside Sertoli cells, testosterone is selectively bound to the androgen receptor and activation of the receptor will result in initiation and maintenance of the spermatogenic process and inhibition of germ cell apoptosis⁽⁴⁸⁾. In testes, ARs are expressed in the somatic Leydig, peritubular myoid and Sertoli cells as well as in rete testis, the epithelial cells of the epididymis, and prostate⁽⁴⁹⁾. Nandrolone interferes with testis growth, development and function. Its administration in rats causes morphological changes, such as reduction of number and size of Leydig cells, cytoplasmic vacuolization, as well as lipid droplet deposition. Moreover, it induces testicular damage by triggering oxidative stress, inflammatory cytokines, matrix metalloproteinases, cell adhesion molecules, apoptotic markers, and DNA damage.

Several studies described quantitative changes in nandrolone decanoate treated rats such as reduction of testis volume and seminiferous tubule length. Moreover, ND treatment in rats decreased number of spermatocytes and spermatids in sperm, and induced sperm DNA fragmentation⁽⁴⁷⁾.

Other studies demonstrated sperm alterations in other parameters, such as sperm count, mobility and morphology. Typical Histological findings were found in Nandrolone treated rats, such as diameter length decrease in seminiferous tubules, as well as basement membrane flattening, degeneration of interstitial cells.

Recent studies showed that despite high doses of Nandrolone may negatively influence Leydig cells, sperm cell and testosterone concentration, in both mature and immature rats, otherwise number of Sertoli cells, testis size and seminiferous diameter changes were observed just in long immature rats. It's commonly known that Nandrolone decrease testosterone levels. This could be an expected finding since AAS activate negative feedback loop of HPA/HPG axis and decrease LH and FSH levels⁽⁴¹⁻⁴²⁾. Otherwise a recent study using in vitro cell coltures, showed no testosterone levels decrease with low doses of Nandrolone, disappearing with higher doses: it's a dose-increase dependent trend. These changes run together with modifications in Star and CYP17A1 genes and proteins expression.

A recent study showed a significant decrease in Star and CYP17A1 genes expression in mice treated with Nandrolone compared with control trained mice, a significative increase of Star and CYP17A1 genes expression in trained mice with no treatment compared with sedentary control, a significative increase of HSD3B1 in nandrolone treated mice, sedentary and trained, compared with not treated control⁽⁴⁴⁾; these data are in agreement with previous studies that showed a increased CYP17A1 gene expression in trained mice treated with linoleic conjugated acid. Moreover, in nandrolone treated mice, both trained and sedentary, a traslocation of MUC1 protein from cytoplasm to nucleus was observed⁽⁴⁵⁾. Traslocation of MUC 1 to nucleus suggests a possible oncogenic role of this protein. Nandrolone decanoate seems to regulate MMP secretion, disrupting Sertoli-germ cell adhesion with consequences on spermatocytes maturation and impairment of blood-testis barrier^(44, 46).

It was proved that aromatizable and non aromatizable androgens like Nandrolone and Stanolozol, could promote leydig cell tumor development trough the induction of aromatase, estrogen and IGF-1 expression⁽⁵⁰⁾. As we mentioned before, administration of Nandrolone leads to increase of lipid peroxidation, heat shock proteins, induces DNA fragmentation and apoptosis.

A recent study shows a significant activity of caspase 3 activity and decrease of germ cell layers in trained rats treated with nandrolone. Although recent studies have shown that exercise training enhanced antioxidant enzyme activities and reduced lipid peroxidation in vital tissues of animals, the testes contain a low amount of antioxidant enzymes compared to other tissues such as the liver and kidney. The testicular mitochondrial membrane is rich in polyunsaturated lipids and the testes may actually be more vulnerable to peroxidative injury. Oxidative stress has been shown to be a major cause of male infertility; a large proportion of infertile men have elevated levels of seminal ROS. Several forms of sperm DNA damage are caused by ROS, e.g. chromatin cross-linking, chromosome deletion and DNA strand breaks (apoptosis)⁽⁶²⁻⁶¹⁾.

Conclusion

Recent data could suggest an influence between ND and some functions such as aggressive behavior, memory and hippocampal plasticity. Moreover, prolonged administration of nandrolone in mice causes a dose-dependent oxidative stress and damage, that could lead to nephrotoxicity and impair cardiac response to oxidative stress. Besides, Nandrolone leads to relevant damages in male reproductive system. Both macroscopic and microscopic findings were found, such as reduction of volume of the testis, reduction of weight, as well as flattening of basement membrane of tubule and degeneration of interstitial cells. It decreases Leydig cell function and testosterone production, activating negative feedback loop, decreasing LH and FSH levels, and causes changes in genes and proteins, such as Star and CYP17A1, production in Leydig cell with a dose-dependent mechanism.

Trough MMP it could damage Blood-Testis barrier and impair spermatogenesis. It was proved that Nandrolone can activate some biochemical signalling pathways, such as, IGF1, aromatase, MUC1, and be involved in cancerogenesis. Nandrolone causes an increase of ROS and oxidative stress, in most of tissues and organs with increase of lipid peroxidation and apoptosis and this mechanism, could be a major cause of male infertility caused by Nandrolone. Nandrolone influence on testosterone biosynthesis should be investigated in order to clarify the mechanisms and find the regulatory genes and proteins involved in. Is not clear how exercise could affect

nandrolone mediated oxidative stress, that's why more studies need to be done in this field. Scientific studies should study in deep the correlations between Nandrolone, tumor development and implicated biochemical mechanisms. People, adolescents, all AAS abusers should be strictly informed about AAS side effects and be aware of all the consequences due to this substance affecting the whole body, that could lead to infertility and severe alterations in testis, Leydig cell function, as well as almost all body tissues and organs⁽⁶²⁻⁷⁴⁾.

References

- 1) Evans NA. Current Concepts in Anabolic-Androgenic Steroids. *American Journal of Sports Medicine* 2004; 32 (2), pp. 534-542. DOI: 10.1177/0363546503262202
- 2) Dohle GR, Smit M, Weber RFA. Androgens and male fertility. *World Journal of Urology* 2003; 21 (5), pp. 341-345. DOI: 10.1007/s00345-003-0365-9.
- 3) Van Amsterdam J, Opperhuizen A, Hartgens F. Adverse health effects of anabolic-androgenic steroids. *Regulatory Toxicology and Pharmacology* 2010; 57 (1), pp. 117-123. DOI: 10.1016/j.yrtph.2010.02.001.
- 4) Pomara C, Cassano T, D'Errico S, Bello S, Romano AD, Riezzo I, Serviddio G. Data available on the extent of cocaine use and dependence: Biochemistry, pharmacologic effects and global burden of disease of cocaine abusers. *Current Medicinal Chemistry* 2012; 19 (33), pp. 5647-5657. DOI: 10.2174/092986712803988811.
- 5) Neri M, Fineschi V, Di Paolo M, Pomara C, Riezzo I, Turillazzi E, Cerretani D. Cardiac oxidative stress and inflammatory cytokines response after myocardial infarction. *Current Vascular Pharmacology* 2015; 13 (1), pp. 26-36.
- 6) Clark AS, Harrold EV, Fast AS. Anabolic-androgenic steroid effects on the sexual behavior of intact male rats. *Hormones and Behavior* 1997; 31 (1), pp. 35-46. DOI: 10.1006/hbeh.1997.1355.
- 7) Frati P, Busardò FP, Cipolloni L, De Dominicis E, Fineschi V. Anabolic androgenic steroid (AAS) related deaths: Autoptic, histopathological and toxicological findings. *Current Neuropharmacology* 2015; 13 (1), pp. 146-159.
- 8) Fineschi V, Michalodimitrakis M, D'Errico S, Neri M, Pomara C, Riezzo I, Turillazzi E. Insight into stress-induced cardiomyopathy and sudden cardiac death due to stress. A forensic cardio-pathologist point of view. *Forensic Science International* 2010; 194 (1-3), pp. 1-8. DOI: 10.1016/j.forsciint.2009.10.025.
- 9) Viggiano A, Chieffi S, Tafuri D, Messina G, Monda M, De Luca B. Laterality of a second player position affects lateral deviation of basketball shooting. *Journal of Sports Sciences* 2014; 32 (1), pp. 46-52. DOI: 10.1080/02640414.2013.805236.
- 10) Shokri S, Aitken RJ, Abdolvahabi M, Abolhasani F, Ghasemi FM, Kashani I, Ejtemaeimehr S, Ahmadian S, Minaei B, Naraghi MA, Barbarestani M. Exercise and supraphysiological dose of nandrolone deconate

- increase apoptosis in spermatogenic cells. *Basic and Clinical Pharmacology and Toxicology* 2010; 106 (4), pp. 324-330. DOI: 10.1111/j.1742-7843.2009.00495.x.
- 11) Pomara C, Barone R, Marino Gammazza A, Sangiorgi C, Barone F, Pitruzzella A, Locorotondo N, Di Gaudio F, Salerno M, Maglietta F, Sarni AL, Di Felice V, Cappello F, Turillazzi E. Effects of Nandrolone Stimulation on Testosterone Biosynthesis in Leydig Cells. *Journal of Cellular Physiology* 2016; 231 (6), pp. 1385-1391. DOI: 10.1002/jcp.25272.
 - 12) Turillazzi E, Perilli G, Di Paolo M, Neri M, Riezzo I, Fineschi V. Side effects of AAS abuse: An overview. *Mini-Reviews in Medicinal Chemistry* 2011; 11 (5), pp. 374-389. DOI: 10.2174/13895571179544592.
 - 13) Stocco DM, Wang X, Jo Y, Manna PR. Multiple signaling pathways regulating steroidogenesis and steroidogenic acute regulatory protein expression: More complicated than we thought. *Molecular Endocrinology* 2015; 19 (11), pp. 2647-2659. DOI: 10.1210/me.2004-0532.
 - 14) Kostic TS, Stojkov NJ, Bjelic MM, Mihajlovic AI, Janjic MM, Andric SA. Pharmacological doses of testosterone upregulated androgen receptor and 3-beta-hydroxysteroid dehydrogenase/delta-5-delta-4 isomerase and impaired leydig cells steroidogenesis in adult rats. *Toxicological Sciences* 2011; 121 (2), pp. 397-407. DOI: 10.1093/toxsci/kfr063.
 - 15) Saartok T, Dahlberg E, Gustafsson J-Å. Relative binding affinity of anabolic-androgenic steroids: Comparison of the binding to the androgen receptors in skeletal muscle and in prostate, as well as to sex hormone-binding globulin. *Endocrinology* 1984; 114 (6), pp. 2100-2106. DOI: 10.1210/endo-114-6-2100.
 - 17) Fineschi V, Neri M, Di Donato S, Pomara C, Riezzo I, Turillazzi E. An immunohistochemical study in a fatality due to ovarian hyperstimulation syndrome. *International Journal of Legal Medicine* 2006; 120 (5), pp. 293-299. DOI: 10.1007/s00414-006-0104-z.
 - 16) Pomara C, D'Errico S, Riezzo I, De Cillis GP, Fineschi V. Sudden cardiac death in a child affected by Prader-Willi syndrome. *International Journal of Legal Medicine* 2005; 119 (3), pp. 153-157. DOI: 10.1007/s00414-004-0513-9.
 - 18) Riezzo I, Di Paolo M, Neri M, Bello S, Cantatore S, D'Errico S, Dinucci D, Parente R, Pomara C, Rabozzi R, Turillazzi E, Fineschi V. Anabolic steroid - and exercise - induced cardio-depressant cytokines and myocardial β 1 receptor expression in CD1 mice. *Current Pharmaceutical Biotechnology* 2011; 12 (2), pp. 275-284.
 - 19) D'Errico S, Di Battista B, Di Paolo M, Fiore C, Pomara C. Renal heat shock proteins over-expression due to anabolic androgenic steroids abuse. *Mini-Reviews in Medicinal Chemistry* 2011; 11 (5), pp. 446-450. DOI: 10.2174/138955711795445934.
 - 20) Turillazzi E, Neri M, Cerretani D, Cantatore S, Frati P, Moltoni L, Busardò FP, Pomara C, Riezzo I, Fineschi V. Lipid peroxidation and apoptotic response in rat brain areas induced by long-term administration of nandrolone: The mutual crosstalk between ROS and NF- κ B. *Journal of Cellular and Molecular Medicine* 2016; 20 (4), pp. 601-612. DOI: 10.1111/jcmm.12748.
 - 21) Bafunno V, Santacroce R, Chetta M, Peyvandi F, Sessa F, Chinni E, Longo V, Margaglione M. Polymorphic miRNA-mediated gene contribution to inhibitor development in haemophilia A. *Haemophilia* 2012; 18 (6), pp. 1003-1007. DOI: 10.1111/j.1365-2516.2012.02882.x.
 - 22) Monda M, Messina G, Scognamiglio I, Lombardi A, Martin GA, Sperlongano P, Porcelli M, Caraglia M, Stiuso P. Short-term diet and moderate exercise in young overweight men modulate cardiocyte and hepatocarcinoma survival by oxidative stress. *Oxidative Medicine and Cellular Longevity* 2014; art. no. 131024. DOI: 10.1155/2014/131024.
 - 23) Moscatelli F, Messina G, Valenzano A, Petito A, Triggiani AI, Ciliberti MAP, Monda V, Messina A, Tafuri D, Capranica L, Cibelli G, Monda M. Relationship between RPE and blood lactate after fatiguing handgrip exercise in taekwondo and sedentary subjects. *Biology and Medicine* 2015; DOI: 10.4172/0974-8369.1000s3008.
 - 24) Chaves EA, Pereira-Junior PP, Fortunato RS, Masuda MO, de Carvalho ACC, de Carvalho DP, Oliveira MF, Nascimento JHM. Nandrolone decanoate impairs exercise-induced cardioprotection: Role of antioxidant enzymes. *Journal of Steroid Biochemistry and Molecular Biology* 2006; 99 (4-5), pp. 223-230. DOI: 10.1016/j.jsbmb.2006.01.004.
 - 25) Pomara C, Fineschi V, Scalzo G, Guglielmi G. Virtopsy versus digital autopsy: Virtuous autopsy [Virtopsy versus autopsia digitale: autopsia virtuosa] *Radiologia Medica* 2009; 114 (8), pp. 1367-1382. DOI: 10.1007/s11547-009-0435-1.
 - 27) Messina G, Viggiano A, Tafuri D, Palmieri F, De Blasio S, Messina A, De Luca A, Chieffi S, Monda M. Role of orexin in obese patients in the intensive care unit. *Journal of Anesthesia and Clinical Research* 2014; 5 (3), art. n. 1000395. DOI: 10.4172/2155-6148.1000395.
 - 28) Triggiani AI, Valenzano A, Ciliberti MAP, Moscatelli F, Villani S, Monda M, Messina G, Federici A, Babiloni C, Cibelli G. Heart rate variability is reduced in underweight and overweight healthy adult women. *Clinical Physiology and Functional Imaging* 2017; 37 (2), pp. 162-167. DOI: 10.1111/cpf.12281.
 - 29) Valenzano A, Moscatelli F, Triggiani AI, Capranica L, De Ioannon G, Piacentini MF, Mignardi S, Messina G, Villani S, Cibelli G. Heart-rate changes after an ultra-endurance swim from Italy to Albania: A case report. *International Journal of Sports Physiology and Performance* 2016; 11 (3), pp. 407-409. DOI: 10.1123/ijsp.2015-0035.
 - 30) Rinaldi B, Guida F, Furiano A, Donniacuo M, Luongo L, Gritti G, Urbanek K, Messina G, Maione S, Rossi F, De Novellis V. Effect of Prolonged Moderate Exercise on the Changes of Nonneuronal Cells in Early Myocardial Infarction. *Neural Plasticity* 2015; art. n. 265967. DOI: 10.1155/2015/265967.
 - 31) Messina G, Monda V, Moscatelli F, Valenzano AA, Monda G, Esposito T, Blasio SD, Messina A, Tafuri D, Barillari MR, Cibelli G, Chieffi S, Varriale B, Monda M. Role of orexin system in obesity. *Biology and Medicine* 2015; 7 (4), art. n. 1000248. DOI: 10.4172/0974-8369.1000248.
 - 32) Chieffi S, Iachini T, Iavarone A, Messina G, Viggiano A, Monda M. Flanker interference effects in a line bisection task. *Experimental Brain Research* 2014; 232

- (4), pp. 1327-1334. DOI: 10.1007/s00221-014-3851-y
- 33) Stocco DM, Wang X, Jo Y, Manna PR. Multiple signaling pathways regulating steroidogenesis and steroidogenic acute regulatory protein expression: More complicated than we thought. *Molecular Endocrinology* 2005; 19 (11), pp. 2647-2659. DOI: 10.1210/me.2004-0532.
- 34) Rone MB, Midzak AS, Issop L, Rammouz G, Jagannathan S, Fan J, Ye X, Blonder J, Veenstra T, Papadopoulos V. Identification of a dynamic mitochondrial protein complex driving cholesterol import, trafficking, and metabolism to steroid hormones. *Molecular Endocrinology* 2012; 26 (11), pp. 1868-1882. DOI: 10.1210/me.2012-1159.
- 35) Messina G, Zannella C, Monda V, Dato A, Liccardo D, De Blasio S, Valenzano A, Moscatelli F, Messina A, Cibelli G, Monda M. The beneficial effects of coffee in human nutrition. *Biology and Medicine* 2015; 7 (4), art. no. 1000240, .DOI: 10.4172/0974-8369.1000240.
- 36) Messina A, De Fusco C, Monda V, Esposito M, Moscatelli F, Valenzano A, Carotenuto M, Viggiano E, Chieffi S, De Luca V, Cibelli G, Monda M, Messina G. Role of the orexin system on the hypothalamus-pituitary-thyroid axis. *Frontiers in Neural Circuits* 2016; 10 (AUG), art. n. 66. DOI: 10.3389/fncir.2016.00066.
- 37) Liu PY, Death AK, Handelsman DJ. Androgens and cardiovascular disease. *Endocrine Reviews* 2003; 24 (3), pp. 313-340.
- 38) Heinlein CA, Chang C. The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. *Molecular Endocrinology* 2002; 16 (10), pp. 2181-2187. DOI: 10.1210/me.2002-0070.
- 39) Messina G, Palmieri F, Monda V, Messina A, Dalia C, Viggiano A, Tafuri D, Messina A, Moscatelli F, Valenzano A, Cibelli G, Chieffi S, Monda M. Exercise causes muscle GLUT4 translocation in an insulin-independent manner. *Biology and Medicine* 2015; 7 (Specialissue3), art. n. 006. DOI: 10.4172/0974-8369.1000S3007.
- 41) Grevik N, Strahm E, Garle M, Lundmark J, Sthle L, Ekström L, Rane A. Long term perturbation of endocrine parameters and cholesterol metabolism after discontinued abuse of anabolic androgenic steroids. *Journal of Steroid Biochemistry and Molecular Biology* 2011; 127 (3-5), pp. 295-300. DOI: 10.1016/j.jsbmb.2011.08.005.
- 42) Alen M, Rahkila P, Reinilä M, Vihko R. Androgenic-anabolic steroid effects on serum thyroid, pituitary and steroid hormones in athletes. *The American Journal of Sports Medicine* 1987; 15 (4), pp. 357-361. DOI: 10.1177/036354658701500411.
- 43) Messina G, Di Bernardo G, Viggiano A, De Luca V, Monda V, Messina A, Chieffi S, Galderisi U, Monda M. Exercise increases the level of plasma orexin A in humans. *Journal of Basic and Clinical Physiology and Pharmacology* 2016; 27 (6), pp. 611-616. DOI: 10.1515/jbcpp-2015-0133.
- 44) Barone R, Pitruzzella A, Marino Gammazza A, Rappa F, Salerno M, Barone F, Sangiorgi C, D'Amico D, Locorotondo N, Di Gaudio F, Cipolloni L, Di Felice V, Schiavone S, Rapisarda V, Sani G, Tambo A, Cappello F, Turillazzi E, Pomara C. Nandrolone decanoate interferes with testosterone biosynthesis altering blood-testis barrier components. *Journal of Cellular and Molecular Medicine* 2017; Article in Press. DOI: 10.1111/jcmm.13092.
- 45) Barone R, Macaluso F, Catanese P, Gammazza AM, Rizzuto L, Marozzi P, Lo Giudice G, Stampone T, Cappello F, Morici G, Zummo G, Farina F, Di Felice V. Endurance exercise and conjugated linoleic acid (CLA) supplementation up-regulate CYP17A1 and stimulate testosterone biosynthesis. *PLoS ONE* 2013; 8 (11), art. n. e79686. DOI: 10.1371/journal.pone.0079686.
- 46) Bafunno V, Bury L, Tiscia GL, Fierro T, Favuzzi G, Caliandro R, Sessa F, Grandone E, Margaglione M, Gresele P. A novel congenital dysprothrombinemia leading to defective prothrombin maturation. *Thrombosis Research* 2014; 134 (5), pp. 1135-1141. DOI: 10.1016/j.thromres.2014.08.028.
- 47) Tahtamouni LH, Mustafa NH, Hassan IM, Ahmad IM, Yasin SR & Abdalla MY. Nandrolone decanoate administration to male rats induces oxidative stress, seminiferous tubules abnormalities, and sperm DNA fragmentation. *Jordan Journal of Biological Sciences* 2010; 3, 165-174.
- 48) Dohle GR, Smit M, Weber RFA. Androgens and male fertility. *World Journal of Urology* 2003; 21 (5), pp. 341-345. DOI: 10.1007/s00345-003-0365-9.
- 49) Vornberger W, Prins G, Musto NA, Suarez-Quian CA. Androgen receptor distribution in rat testis: New implications for androgen regulation of spermatogenesis. *Endocrinology* 1994; 134 (5), pp. 2307-2316. DOI: 10.1210/endo.134.5.8156934.
- 50) Chimento A, Sirianni R, Zolea F, De Luca A, Lanzino M, Catalano S, Andò S, Pezzi V. Nandrolone and stanozolol induce leydig cell tumor proliferation through an estrogen-dependent mechanism involving IGF-I system. *Journal of Cellular Physiology* 2012; 227 (5), pp. 2079-2088. DOI: 10.1002/jcp.22936.
- 51) Messina G, Valenzano A, Moscatelli F, Salerno M, Lonigro A, Esposito T, Monda V, Corso G, Messina A, Viggiano A, Triggiani AI, Chieffi S, Guglielmi G, Monda M, Cibelli G. Role of autonomic nervous system and orexinergic system on adipose tissue. *Frontiers in Physiology* 2017; 8, art. n. 137. DOI: 10.3389/fphys.2017.00137.
- 52) Ledda C, Cocuzza S, Salerno M, Senia P, Matera S, Rapisarda V, Loreto C. Occupational exposure to Mount Etna's basaltic dust: Assessment of mutagenic and cytotoxic effects. *Molecular Medicine Reports* 2017; 15 (5), pp. 3350-3354. DOI: 10.3892/mmr.2017.6380.
- 53) Perillo L, Esposito M, Caprioglio A, Attanasio S, Santini AC, Carotenuto M. Orthodontic treatment need for adolescents in the Campania region: the malocclusion impact on self-concept. *Patient Prefer Adherence*. 2014 Mar 19;8:353-9. doi: 10.2147/PPA.S58971.
- 54) Carotenuto M, Gimigliano F, Fiordelisi G, Ruberto M, Esposito M. Positional abnormalities during sleep in children affected by obstructive sleep apnea syndrome: the putative role of kinetic muscular chains. *Med Hypotheses*. 2013 Aug; 81(2): 306-8. doi: 10.1016/j.mehy.2013.04.023.
- 55) Esposito M, Gallai B, Roccella M, Marotta R, Lavano F, Lavano SM, Mazzotta G, Bove D, Sorrentino M, Precenzano F, Carotenuto M. Anxiety and depression levels in prepubertal obese children: a case-control study. *Neuropsychiatr Dis Treat*. 2014 Oct 3;10:1897-

902. doi: 10.2147/NDT.S69795.
- 56) Esposito M, Parisi L, Gallai B, Marotta R, Di Dona A, Lavano SM, Roccella M, Carotenuto M. Attachment styles in children affected by migraine without aura. *Neuropsychiatr Dis Treat.* 2013; 9: 1513-9. doi: 10.2147/NDT.S52716.
- 57) Esposito M, Gallai B, Parisi L, Castaldo L, Marotta R, Lavano SM, Mazzotta G, Roccella M, Carotenuto M. Self-concept evaluation and migraine without aura in childhood. *Neuropsychiatr Dis Treat.* 2013; 9: 1061-6. doi: 10.2147/NDT.S49364.
- (58) Verrotti A, Carotenuto M, Altieri L, Parisi P, Tozzi E, Belcastro V, Esposito M, Guastaferro N, Ciuti A, Mohn A, Chiarelli F, Agostinelli S. Migraine and obesity: metabolic parameters and response to a weight loss programme. *Pediatr Obes.* 2015 Jun; 10(3): 220-5. doi: 10.1111/ijpo.245.
- 59) Pasquali D, Carotenuto M, Leporati P, Esposito M, Antinolfi L, Esposito D, Accardo G, Carella C, Chiovato L, Rotondi M. Maternal hypothyroidism and subsequent neuropsychological outcome of the progeny: a family portrait. *Endocrine.* 2015 Dec; 50(3): 797-801. doi: 10.1007/s12020-015-0564-3.
- 60) Esposito M, Ruberto M, Gimigliano F, Marotta R, Gallai B, Parisi L, Lavano SM, Roccella M, Carotenuto M. Effectiveness and safety of Nintendo Wii Fit Plus™ training in children with migraine without aura: a preliminary study. *Neuropsychiatr Dis Treat.* 2013; 9: 1803-10. doi: 10.2147/NDT.S53853.
- 61) Franzoni E, Matricardi S, Di Pisa V, Capovilla G, Romeo A, Tozzi E, Pruna D, Salerno GG, Zamponi N, Accorsi P, Giordano L, Coppola G, Cerminara C, Curatolo P, Nicita F, Spalice A, Grosso S, Pavone P, Striano P, Parisi P, Boni A, Gobbi G, Carotenuto M, Esposito M, Cottone C, Verrotti A. Refractory absence seizures: An Italian multicenter retrospective study. *Eur J Paediatr Neurol.* 2015 Nov; 19(6): 660-4. doi: 10.1016/j.ejpn.2015.07.008.
- 62) Morandi A, Bonnefond A, Lobbens S, Carotenuto M, Del Giudice EM, Froguel P, Maffei C. A girl with incomplete Prader-Willi syndrome and negative MS-PCR, found to have mosaic maternal UPD-15 at SNP array. *Am J Med Genet A.* 2015 Nov; 167A(11):2720-6. doi: 10.1002/ajmg.a.37222.
- 63) Matricardi S, Spalice A, Salpietro V, Di Rosa G, Balistreri MC, Grosso S, Parisi P, Elia M, Striano P, Accorsi P, Cusmai R, Specchio N, Coppola G, Savasta S, Carotenuto M, Tozzi E, Ferrara P, Ruggieri M, Verrotti A. Epilepsy in the setting of full trisomy 18: A multicenter study on 18 affected children with and without structural brain abnormalities. *Am J Med Genet C Semin Med Genet.* 2016 Sep; 172(3): 288-95. doi: 10.1002/ajmg.c.31513.
- 64) Esposito M, Gimigliano F, Ruberto M, Marotta R, Gallai B, Parisi L, Lavano SM, Mazzotta G, Roccella M, Carotenuto M. Psychomotor approach in children affected by nonretentive fecal soiling (FNRFS): a new rehabilitative purpose. *Neuropsychiatr Dis Treat.* 2013; 9: 1433-41. doi: 10.2147/NDT.S51257.
- 65) Esposito M, Precenzano F, Sorrentino M, Avolio D, Carotenuto M. A Medical Food Formulation of Griffonia simplicifolia/Magnesium for Childhood Periodic Syndrome Therapy: An Open-Label Study on Motion Sickness. *J Med Food.* 2015 Aug; 18(8): 916-20. doi: 10.1089/jmf.2014.0113.
- 66) Santamaria F, Esposito M, Montella S, Cantone E, Mollica C, De Stefano S, Mirra V, Carotenuto M. Sleep disordered breathing and airway disease in primary ciliary dyskinesia. *Respirology.* 2014 May; 19(4): 570-5. doi: 10.1111/resp.12273.
- 67) Esposito M, Roccella M, Gallai B, Parisi L, Lavano SM, Marotta R, Carotenuto M. Maternal personality profile of children affected by migraine. *Neuropsychiatr Dis Treat.* 2013; 9: 1351-8. doi: 10.2147/NDT.S51554.
- 68) Esposito M, Marotta R, Gallai B, Parisi L, Patriciello G, Lavano SM, Mazzotta G, Roccella M, Carotenuto M. Temperamental characteristics in childhood migraine without aura: a multicenter study. *Neuropsychiatr Dis Treat.* 2013; 9: 1187-92. doi: 10.2147/NDT.S50458.
- 69) Perillo L, Esposito M, Contiello M, Lucchese A, Santini AC, Carotenuto M. Oculal traits in developmental dyslexia: a preliminary study. *Neuropsychiatr Dis Treat.* 2013; 9: 1231-7. doi: 10.2147/NDT.S49985
- 70) Carotenuto M, Esposito M, Cortese S, Laino D, Verrotti A. Children with developmental dyslexia showed greater sleep disturbances than controls, including problems initiating and maintaining sleep. *Acta Paediatr.* 2016 Sep; 105(9): 1079-82. doi: 10.1111/apa.13472.
- 71) Mazzeo F, Motti ML, Messina G, Monda V, Ascione A, Tafuri D, Palmieri F, Messina A, Monda M. Use of nutritional supplements among south Italian students of physical training and sport university. *Curr Top Toxicol.* 2013; 9: 21-26.
- 72) Viggiano E, Monda V, Messina A, Moscatelli F, Valenzano A, Tafuri D, Cibelli G, De Luca B, Messina G, Monda M. Cortical spreading depression produces a neuroprotective effect activating mitochondrial uncoupling protein-5. *Neuropsychiatr Dis Treat.* 2016; 12: 1705-10. doi:10.2147/NDT.S107074.
- 73) Messina G, Valenzano A, Moscatelli F, Triggiani AI, Capranica L, Messina A, Piombino L, Tafuri D, Cibelli G, Monda M. Effects of Emotional Stress on Neuroendocrine and Autonomic Functions in Skydiving. *J Psychiatry* 2015; 18(4). doi:10.4172/2378-5756.1000280.
- 74) Mazzeo F, Monda M, Messina G, Santamaria S, Messina A, Montesano M, Monda V, Tafuri D. Doping in Italy: An analysis of its spread in ten years *Biol Med.* 2016; 8: 1000263 doi: 10.4172/0974-8369.1000263.

Corresponding author

Dr. MONICA SALERNO

Section of Forensic Pathology, Clinical and Experimental
Medicine Department

University of Foggia

Ospedale Colonnello D'Avanzo

Viale Degli Aviatori 1

71100 Foggia

(Italy)