March 22nd, 2022

Dear Academic Editor of the *World Journal of Gastroenterology* journal,

We are grateful for the very constructive remarks on our manuscript and here we offer point-by-point response to the reviewer's comments. We believe that the changes we have made based on reviewers' comments improved the quality of this mini review.

All the modifications have been written in red in the revised manuscript.

No	Editor / Reviewer Comment	Authors response to Comment	Changes made to the article All changes are written in red.	Page/line number
	Reviewer 1			
1	In the Abstract: "Supraphysiologic and long-term use of AASs affects all organs leading to cardiovascular, neurological, endocrine, gastrointestinal, renal, and other disorders. It must added that testosteron induces hematological disorders. Induction of erythropoetin. Is there an iron overload as a consequence of increased EPO?	Thank you for pointing out this omission. We have corrected this in both Abstract and Introduction. Due to this paper being focused on androgenic anabolic steroids induced liver disorders we feel that this might be beyond scope of this paper so have not reviewed its significant hematologic effects such as induction of erythropoetin. Hematologic effects of AAS are significant and we appriciate this remark, however due to limited number of relevant studies regarding iron related liver disorders in androgenic anabolic steroid use we were not able to discuss this issue in our paper.	The following change has been made in the manuscript based on this comment: Supraphysiologic and long-term use of AASs affects all organs leading to cardiovascular, neurological, endocrine, gastrointestinal, renal, and hematologic disorders. Supraphysiologic doses and prolonged unsupervised use of AASs has major impact on users affecting all organs and causing cardiovascular, neuroendocrine, neuropsychiatric, renal, gastrointestinal, musculoskeletal, dermatologic, immune disorders and hematologic disorders. Testosterone has a major impact on homeostasis of electrolytes, macromolecules and micromolecules, including alterations in systemic iron balance and erythropoiesis. It has been established that AAS-induced erythropoiesis is mediated by erythropoietin[7]. Most recent studies also imply suppression of hepcidin (a negative regulator of the iron transporter ferroportin) as a mechanism of androgen induced enhanced iron absorbtion and	Page 2 Line 40 Page 3 Line 86-92

			incorporation into red blood cells[8]. New references have been added: Shahidi NT. Androgens and erythropoiesis. *N Engl J Med* 1973; **289**(2): 72-80 [PMID: 4575719 DOI: 10.1056/NEJM197307122890205] Beggs LA, Yarrow JF, Conover CF, Meuleman JR, Beck DT, Morrow M, Zou B, Shuster JJ, Borst SE. Testosterone alters iron metabolism and stimulates red blood cell production independently of dihydrotestosterone. *Am J Physiol Endocrinol Metab* 2014; **307**(5): E456-461 [PMID: 25074984 PMCID: PMC4154071 DOI: 10.1152/ajpendo.00184.2014]	Reference 7 Reference 8
2	In the Introduction: Unmodified testosterone is a rapidly metabolized substance with therapeutic index of 1 resulting in proportional anabolic and androgenic effects. What does that mean?	Thank you for this remark. This was wrongly explained and we have now corrected it and reformulated this sentence.	This is how this part of the text reads now. Unmodified testosterone is rapidly metabolized when administered orally or parenterally and exerts a similar proportion of anabolic and androgenic effects. Chemical modifications of testosterone allow prolonged effective blood concentrations and changes to its pharmacodynamic properties, therefore enhancing desired anabolic or androgenic effects. In its synthetic derivatives used for aestethic and performance-enhancing purposes, testosterone is altered mainly through alkylation and esterification to enhance bioavailability by reducing hepatic metabolism, prolong duration of action and maximize anabolic properties, though all of the synthetic compounds still exert significant androgenic effects.	Page 3 Line 70-79
3	Hepatotoxicity: As far as I remember Thiobarbituric acid material are sialylated glycoproteins. Is that correct?	Thank you for this comment.	The thiobarbituric acid reactive substances (TBARS) assay is commonly used to determine lipid oxidation and antioxidant activity. Ghani MA, Barril C, Bedgood DR, Prenzler PD. Measurement of antioxidant activity with the thiobarbituric acid reactive substances assay. *Food Chem* 2017; **230**:	

			195-207 [PMID: 28407901 DOI: 10.1016/j.foodchem.2017.02.127]	
4	Anabolic steroids induce collagen deposition. Is Kupffer cell acitivation the sole cause? The authors mention themselves that stellate cell activation plays a major role. I suggest to include stellate cell acitivation in Fig. 1	We appreciate this comment.	The figure has been modified as suggested.	
5	The pathophysiology of peliosis remains rather ambiguous. Possible role of vascular growth factors?	Thank you for this insightfull remark. There is indeed lack of data regarding pathophysiological mechanisms of peliosis hepatis, however, we added some available information regarding this issue.	The following text has been added to this paragraph. So far, disruption of hepatic extracellular matrix and direct endothelial cell injury have been suggested as generating mechanisms for liver peliosis. It has also been proposed that steroid-induced hepatocyte hyperplasia results in mechanical obstruction of hepatic vascular system resulting in dilatation and loss of endothelial barrier function [9, 28, 46]. Molecular mechanisms in pathophysiology of peliosis remain poorly understood, however an important role of vascular endothelial growth factor (VEGF) has been suggested in recent studies. VEGF induces angiogenesis, capillary permeability, and proliferation of endothelial cells in liver and other tissues. Tzirogiannis et al. found major protective effect of vascular endothelial growth factor (VEGF) which almost totally reversed the extent of peliosis in a model of cadmium-induced toxic liver injury and peliosis, implying that it preserves endothelial cell function[47]. However, a study of peliosis hepatis in a patient with follicular lymphoma found elevated VEGF and suggested lesions could be caused by elevation of VEGF and its angiogenic effects[48]. An animal model study of peliosis hepatis associated with melanoma supports a concept that a tumor-derived factor such as VEGF could induce development of peliosis due to its endothelial cell proliferation effect[49].	Page 8 Line 232-247

			These conclusions indicate a need for further research pathophysiology in peliosis hepatis as well as AASs and their impact on growth factors. References have been added: Tzirogiannis KN, Papadimas GK, Kondyli VG, Kourentzi KT, Demonakou MD, Kyriakou LG, Mykoniatis MG, Hereti RI, Panoutsopoulos GI. Peliosis hepatis: microscopic and macroscopic type, time pattern, and correlation with liver cell apoptosis in a model of toxic liver injury. *Dig Dis Sci* 2006; **51**(11): 1998-2006 [PMID: 17053957 DOI: 10.1007/s10620-006-9242-x] de la Mano EP, Martín-Sánchez G, López RL, Galán MAF, Ríos ST, Jiménez MJM, de Morales JMGR, Santos MAC, Núñez GM. Peliosis hepatis associated with follicular lymphoma with a rise in vascular endothelial growth factor and anaemia of inflammation. *Ecancermedicalscience* 2018; **12**: 882 [PMID: 30679949 PMCID: PMC6345076 DOI: 10.3332/ecancer.2018.882] Edwards R, Colombo T, Greaves P. "Have you seen this?" peliosis hepatis. *Toxicol Pathol* 2002; **30**(4): 521-523 [PMID: 12187943 DOI:10.1080/01926230290105686]	Reference 49 Reference 50 Reference 51
6	Cholestasis: Does a polymorphism of MRPs play a role?	Thank you for pointing out this omission. We have now added some new information regarding MRPs and drug induced liver injury.	We have added the following information into the section *Cholestasis*: Additionally, the role of multidrug-related proteins (MRP2, MRP3 and MRP4) is important in bile acid transfer as a complimentary mechanism. It was shown that testosterone metabolites are supstrates of MRP2 protein, which has reduced activity in Dubin-Johnson syndrome (DJS). The effect of AAS in DJS has not been investigated to our	Page 8 Line 217-221

			knowledge, whereas it was found that pregnancy and oral contraceptives increase bilirubin levels in women with DJS [42].	
	Reviewer 2			
1	Most of currently available data on AAS adverse effects in this article are based on case reports, in vitro studies, and animal model studies, while there is a lack of randomized controlled trials and systematic studies, which needs to be mentioned in the discussion.	Thank you for pointing out this omission, we have added this into the main text.	The following text has been added. Prospective studies with supraphysiologic doses are difficult to gain IRB approval due to ethical and legal considerations [18], therefore randomized controlled trials are lacking and in this mini review we discuss data from case reports, *in vitro* studies and studies on animal models.	Page 4 Line 120-121
	Reviewer 3			
1	The authors mentioned "latest case reports regarding adverse effects of AASs in dietary supplements" in Core tip, but there are few case reports in the main text.	We appreciate this remark and we have added some more case reports into the main text.	The following has been added. Patil V. et al reported three cases of AAS-induced hepatotoxicity which all manifested differently. First case is a 31-year-old male who developed cholestatic liver injury after two months of oxymetholone use, second case is a 24-year-old male who developed well differentiated HCC and multiple hepatic adenomas after three years of intramuscular testosterone decanoate and daily oral stanozolol use and third case is a 36-year-old male with steatohepatitis after two months of intramuscular nandrolone decanoate use [36] And: El Sherrif *et al* report two cases of cholestatic DILI caused by the short-term use of AAS-containing dietary supplement Mass-Drol. The authors found that inhibition of expression of genes *ATPB81* and *ABCB11* by AAS may play a crucial role as the underlying	Page 7 Line 186-191 Page 7 Line 210 - 217

			mechanism of cholestatic injury. This leads to impaired bile salt transport and also reduced excretion of different hepatic ectoenzymes. Indeed, both patients presented with cholestasis with marked hyperbilirubinemia, but lack of GGT rise. The authors postulated that individuals who are carriers of c.2093G>A mutation in *ABCB11* may be specifically susceptible to cholestatic hepatic injury caused by AAS due this mechanism[11]. References have been added: Patil V, Jothimani D, Harika K, Hakeem AR, Sachan D, Vij M, Rela M. Versatility of Anabolic Androgenic Steroid-Induced Hepatotoxicity. *J Clin Exp Hepatol* 2022; **12**(1): 216-221 [PMID: 35068803 PMCID: PMC8766528 DOI: 10.1016/j.jceh.2021.03.003] Erlinger S, Arias IM, Dhumeaux D. Inherited disorders of bilirubin transport and conjugation: new insights into molecular mechanisms and consequences. *Gastroenterology* 2014; **146**(7): 1625-1638 [PMID: 24704527 DOI: 10.1053/j.gastro.2014.03.047]	Reference 38 Reference 44
2	The discussion on the disease mechanism is not deep enough.	We appreciate this comment and we have added some more information on disease mechanisms.	The following has been added in the section about *Cholestasis*: El Sherrif *et al* report two cases of cholestatic DILI caused by the short-term use of AAS-containing dietary supplement Mass-Drol. The authors found that inhibition of expression of genes *ATPB81* and *ABCB11* by AAS may play a crucial role as the underlying mechanism of cholestatic injury. This leads to impaired bile salt transport and also reduced excretion of different hepatic ectoenzymes. Indeed, both patients presented with cholestasis with marked hyperbilirubinemia, but lack of GGT rise. The authors postulated that individuals who are carriers of c.2093G>A mutation in *ABCB11* may be specifically susceptible to cholestatic hepatic injury	Page 7-8 Line 210-221

			caused by AAS due this mechanism [11]. Additionally, the role of multidrug-related proteins (MRP2, MRP3 and MRP4) is important in bile acid transfer as a complimentary mechanism. It was shown that testosterone metabolites are supstrates of MRP2 protein, which has reduced activity in Dubin-Johnson syndrome. The effect of AAS in DJS has not been investigated to our knowledge, whereas it was found that pregnancy and oral contraceptives increase bilirubin levels in women with DJS [42]. And in the section about *Peliosis hepatis*: So far, disruption of hepatic extracellular matrix and direct endothelial cell injury have been suggested as generating mechanisms for liver peliosis. It has also been proposed that steroid-induced hepatocyte hyperplasia results in mechanical obstruction of hepatic vascular system resulting in dilatation and loss of endothelial barrier function [9, 28, 46]. Molecular mechanisms in pathophysiology of peliosis remain poorly understood, however an important role of vascular endothelial growth factor (VEGF) has been suggested in recent studies. VEGF induces angiogenesis, capillary permeability, and proliferation of endothelial cells in liver and other tissues. Tzirogiannis et al. found major protective effect of vascular endothelial growth factor (VEGF) which almost totally reversed the extent of peliosis in a model of cadmium-induced toxic liver injury and peliosis, implying that it preserves endothelial cell function[47]. However, a study of peliosis hepatis in a patient with follicular lymphoma found elevated VEGF and suggested lesions could be caused by elevation of VEGF and its angiogenic effects[48]. An animal model study of peliosis hepatis associated with melanoma supports a concept that a tumor-derived factor such as VEGF could induce development of peliosis due to its	Page 8-9 Line 232-247

			endothelial cell proliferation effect[49]. These conclusions indicate a need for further research pathophysiology in peliosis hepatis as well as AASs and their impact on growth factors. References have been added: Tzirogiannis KN, Papadimas GK, Kondyli VG, Kourentzi KT, Demonakou MD, Kyriakou LG, Mykoniatis MG, Hereti RI, Panoutsopoulos GI. Peliosis hepatis: microscopic and macroscopic type, time pattern, and correlation with liver cell apoptosis in a model of toxic liver injury. *Dig Dis Sci* 2006; **51**(11): 1998-2006 [PMID: 17053957 DOI: 10.1007/s10620-006-9242-x] de la Mano EP, Martín-Sánchez G, López RL, Galán MAF, Ríos ST, Jiménez MJM, de Morales JMGR, Santos MAC, Núñez GM. Peliosis hepatis associated with follicular lymphoma with a rise in vascular endothelial growth factor and anaemia of inflammation. *Ecancermedicalscience* 2018; **12**: 882 [PMID: 30679949 PMCID: PMC6345076 DOI: 10.3332/ecancer.2018.882] Edwards R, Colombo T, Greaves P. "Have you seen this?" peliosis hepatis. *Toxicol Pathol* 2002; **30**(4): 521-523 [PMID: 12187943 DOI:10.1080/01926230290105686]	Reference 49 Reference 50 Reference 51
3	Figure 1 is poor to explain the detailed molecular mechanism of the diseases, and needs to be refined and embellished.	We appreciate this remark.	The figure has been refined as suggested.	
4	Some references, such as the literature cited by the data of epidemiological survey, are too old and may not be enough to represent the current situation.	Thank you for this observation, we have amended epidemiological data.	The following changes have been made: Although the precise number of AAS users is difficult to determine, it is estimated that the prevalence across the world is 1-5% [15]. A study from 2013 found that the prevalence of AAS use among male elite college students in the US during their lifetime is about 20% [16].	Page 4 Line 102 – 108

			In Norway high school population a prevalence of 4% was found [17]. While it is difficult to establish a true prevalence of AAS use due to underreporting of this socially undesirable behavior, all the surveys nevertheless found significantly higher AAS use in male compared to female persons and majority of AAS users are or were professional or near professional athletes [15]. References have been added: Anawalt BD. Diagnosis and Management of Anabolic Androgenic Steroid Use. *J Clin Endocrinol Metab* 2019; **104**(7): 2490-2500 [PMID: 30753550 PMCID: PMC6517163 DOI: 10.1210/jc.2018-01882] Buckman JF, Farris SG, Yusko DA. A national study of substance use behaviors among NCAA male athletes who use banned performance enhancing substances. *Drug Alcohol Depend* 2013; **131**(1-2): 50-55 [PMID: 23688842 PMCID: PMC3763820 DOI: 10.1016/j.drugalcdep.2013.04.023] Jenssen IH, Johannessen KB. Aggression and body image concerns among anabolic androgenic steroid users, contemplators, and controls in Norway. *Body Image* 2015; **12**: 6-13 [PMID: 25261635 DOI: 10.1016/j.bodyim.2014.08.009]	Reference 15 Reference 16 Reference 17
5	NFKB is not cytokine.	Thank you for pointing this out.	The following change has been made: It is known that AAS induce infiltration of liver with inflammatory cells, which together with activated Kupffer cells favor a pro-inflammatory state by releasing NFκB and inflammatory cytokines TGF-β1, TNFα and IL-1b.	Page 5 Line 140

Please address all correspondence regarding this revised manuscript to me at:
martina.smolic@mefos.hr

We look forward to your favorable decision.

Sincerely,

Professor Martina Smolić, M.D., Ph.D.
Vice Dean for Science and Postgraduate Study Programs
Faculty of Dental Medicine and Health
Church Street 21, Osijek, Croatia
Josip Juraj Strossmayer University of Osijek//
Faculty of Medicine
J. Huttlera 4, Osijek, Croatia
Phone: +385-91-224-1484
martina.smolic@mefos.hr//martina.smolic@fdmz.hr