PURINE METABOLISM IN THE LIGHT OF AEROBIC AND ANAEROBIC CAPACITY OF FEMALE BOXERS – THE PILOT STUDY

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Abstract The aim of the work was to assess the intensity of purine nucleotide degradation during maximum physical exercise. 5 elite female boxers were the subject of the study. Each of them underwent two exercise stress tests in order to evaluate the level of $\dot{V}O_{2peak}$ and the level of anaerobic capacity during a Wingate test. The study involved collecting capillary and venous blood samples at rest and after the exercise test to determine the Acid-Base Balance (ABB), concentration of lactic acid (LA) and purine metabolism nucleotides. The average value of $\dot{V}O_{2peak}$ was 40.92 (SD = 4.087) ml/kg/min, the average anaerobic capacity Ppeak was 7.57 (SD = 0.380) Watt/kg. The workload resulted in significant changes in the level of ABB and LA after both of the exercise stress tests ($p < 0.001$). Concentrations of hypoxanthine (Hx), xanthine (X) and uric acid (UA) in the blood increased significantly after the Wingate test ($p < 0.05$). The level of plasma purine nucleotides at rest and after standard exercise may be a useful tool for monitoring the adaptation of energetic processes in different training phases and support the overload diagnosis.

Key words purine nucleotides, uric acid, Acid-Base Balance (ABB), Wingate anaerobic test, maximal oxygen uptake, anaerobic threshold (AT)

Introduction

Although boxing as a combat sport had its origin in ancient Greece, it was only included in the program of the Olympic Games at the beginning of the 20th century. Until quite recently the sport had been restricted to men. The first boxing World Championship for women was organized in 2001, with inclusion in the Olympic Games in 2012. Despite the many favorable conditions promoting the development of women's boxing, the sport has not been present much in studies devoted to physiology or biochemistry of sports-related effort. The nature of the training process, as well as the high intensity of a boxing match itself (11 total minutes, as 3 rounds of 3 minutes each and one-minute rest between rounds), require boxers to display a high level of anaerobic capacity and a medium level

of aerobic capacity. A training session would be arranged in such a way as to make it possible to develop the aerobic capacity during the preparatory period, as it affects the speed of the athlete in the precomputation period. Time analyses of combat sports show that muscle energetics involve aerobic-anaerobic metabolism depending largely on the intensity of the effort. A high post-effort level of lactic acid in the blood indicates maximal and supramaximal loads (Hübner-Woźniak, Kosmol, Glaz, Kusior, 2006; Guidetti, Musulin, Baldari, 2002). Only an athlete with a high level of capacity is able to attain and maintain such a high level of load without experiencing intensification of fatigue symptoms, resulting in the loss of motor coordination.

The disturbance of the energy homoeostasis of the cell and aggressive hydrolysis of ATP particles during exercise contributes to the production of adenosine monophosphate (AMP), which may in turn, depending on the type of tissue, be subject to different decomposition mechanisms. In skeletal muscles, AMP deamination to inosine monophosphate (IMP) and ammonia involves AMP deaminase enzyme (AMP-d, EC 3.5.4.6). The activity of this enzyme is inhibited by indirect products of the glycolysis process: e.g., 2,3-BPG, a decrease in ATP concentration and reciprocally by those produced as a result of the described IMP reaction. IMP, in turn, is transformed into inosine. Inosine is degraded by purine nucleoside phosphorylase (PNP, EC 2.4.2.1) to hypoxanthine (Hx), which can efflux the muscle and be lost from the adenine nucleotide pool. It is also the only metabolite that is able to be returned to the pool of purine nucleotides of the cell, with the involvement of hypoxanthine-guanine phosphoribosyltransferase (HGPRT, EC 2.4.2.8). A post-exercise re-synthesis of the purine ring is very energy-intensive for the organism, which is why the efficiency of the discussed reutilization system is of great importance for the body. The indicator of the intensity of the purine nucleotide degradation is the hypoxanthine/xanthine (Hx/X) concentration ratio. A high value indicates an effective nucleotide reutilisation mechanism and an increase in the overall pool of available purine bases within the cell. Enzymatic transformation of Hx into X, and eventually into uric acid (UA) with the involvement of the xanthine dehydrogenase enzyme (XDH, EC 1.1.1.204), causes a loss of purine nucleotides from the functioning cell (Hellsten-Westing, Sollevi, Sjödin, 1991; Fox, Palella, Kelley, 1987; Murray, 1971). The final metabolite of nucleotide metabolism, as can be seen in Figure 1, is uric acid (UA).

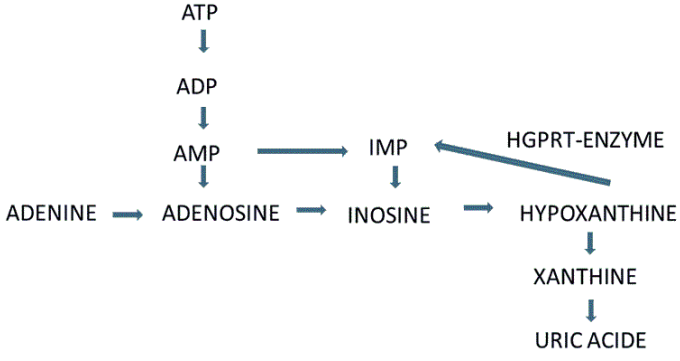

Figure 1. Pathway of purine nucleotide metabolism

Uric acid is a very important antioxidant that is excreted from the body with the urine, among others. The analysis of UA concentration in the blood in sports diagnostics has its limitations. Most likely, they may result from the fact that the lactic acid produced during exercise competes with uric acid over the secretion rate in the proximal tubule of the kidney, decreasing at the same time its excretion with urine (Fox et al., 1987). Therefore, an increase in muscle oxygen potential due to training, limits the rise in lactic acid in the blood during exercise under the same stress, and increases at the same time the excretion of uric acid in the urine. Hypoxanthine has been considered to be an indicator of histotoxic hypoxia over a long period of time. It is also regarded as a marker of adenine nucleotide degradation in the muscle, a marker of energetic stress during exercise, an index of exercise intensity, and may be used in the classification of physical exercise.

It has been indicated in research that hypoxanthine concentration in the blood during exercise depends largely on the exercise duration and intensity. In the study by F. Banaszak and Y. Hellsten-Westing on groups of rowers, swimmers, and long-distance and short-distance runners, they saw the occurrence of an abrupt increase in Hx concentration within the blood during sub-maximum exercises with an intensity above the threshold, maximum and supra-maximum levels (Hellsten-Westing et al.,1991; Banaszak, Rychlewski, 1989). On the other hand, data on the intensity of exercise-induced purine nucleotide degradation in combat sports, particularly in boxing, are very rare in literature.

The aim of the work was to assess the intensity of purine nucleotide degradation during maximum physical exercise as a metabolic response to the changing energetic homoeostasis of the cell.

Methods

Participants

The exercise stress study comprised a group of 5 female boxers − Polish Olympic Representatives of Women's Boxing – and was approved by the Polish Boxing Association. The athletes who qualified for the study form the elite in this sports discipline, having won numerous prizes and awards, including World and European Championship Titles. All examined women were during the follicular menstrual cycle phase. The exercises tests were conducted daily for two consecutive days.

The study was conducted in accordance with the Declaration of Helsinki and the National Statement and Human Research Ethics Guidelines and approved by the IRB (Institute for Research in Biomedicine) at the Poznań University of Medical Sciences (2010-03-04; Ethics Approval Number 232/10). An information sheet was provided to each boxer who was approached to participate in the study, and on agreement to participate, informed written consent was obtained.

Experimental design

Evaluation of aerobic capacity

In order to evaluate the levels of peak oxygen uptake ($\dot{V}O_{2peak}$) and anaerobic threshold (AT), each of the athletes underwent an increasing-intensity physical effort to reach the level of individual maximum capacity or to arrive at the level of refusal. The test was performed using a Kettler cyclo-ergometer. The initial load was 50 Watts (70 RPM) increasing by 15 Watts every 3 minutes at a constant RPM until a maximum load was reached. The exercise stress test was carried out using a Jeager Oxycon Mobile Ergospirometer, and the following circulatory-respiratory

parameters were monitored on a constant basis: heart rate (HR), oxygen uptake per minute ($\dot{V}O_2$), carbon dioxide production (VCO_2), minute ventilation (VE). The threshold load was determined using ventilation method on the basis of the analysis of effort-related changes in respiratory parameters (V-slope method).

Evaluation of anaerobic capacity

In order to evaluate the level of anaerobic capacity, a standard 30-second Wingate Maxima capacity test was carried out using a Monark 824 E cyclo-ergometer. The primary test was preceded by a 5 min warmup period at approx. 50 Watts, followed by a 5 min break. External loading was estimated individually at 7.5% of body weight. During the test the athletes were encouraged to maintain maximum effort for 30 secs. Recorded results were analysed using Monark Anaerobic Test Software (ver. 3.0.1, Sweden). The following values were marked during the test: mean power (Pmean), total work (Wtot), peak power (Ppeak), fatigue index (FI % decrease in the power output within 30 s), time to obtain peak power (Tpp), and time of maintaining peak power (Tmax).

Biochemical analysis

Venous and capillary blood samples were collected at rest and 10 minutes after the exercise was finished. Despite the fact that the maximum lactic acid (LA) concentration during the exercise occurred between the 3rd and 8th minute after the exercise was completed, the LA concentration in the 10th minute correlated to Hx increase during the exercise, which peaked 10−15 minutes after exercising.

Capillary blood was marked for Acid-Base Balance (ABB) measurement using a Roche Cobas b121. All values were measured immediately after collection. Lactic acid concentrations were assayed enzymatically spectrophotometrically, measuring the increase in the absorbance of NADH at a wavelength of 365 nm.

Blood plasma was used for the concentration of oxypurines (hypoxanthine, xanthine) and uric acid using high-performance liquid chromatography (HPLC) on a Hewlett-Packard 1050 equipped with a UV detector. A Hypersil ODS 100 × 4.6mm 5µm column and a Hypersil ODS 20 × 4 mm 5µm precolumn by Alltech was used for the marking process. The carrier phase included a buffer of the following composition: 1% methanol + 4% buffer – 100 mmol/L KH_2PO4 with a pH of 5.8. The flow velocity was 1.0 ml/min. The substances were identified by comparing the retention time of the sample under examination and reference compounds of a known composition. Leaching proceeded in the system in the following order: UA, Hx, X. Measurements were performed at a wavelength of 254 nm.

Statistical analysis

The obtained results were used as the average (M) and standard deviation (SD). All of the statistical analyses were performed while using Dell Statistica (data analysis software system), version 13, downloaded from www.software.dell.com The normality of the data distribution was verified by using the Shapiro–Wilk tests. The t-test was employed to evaluate the influence of the exercises on the assessed indices. The relationship between the variables was tested while using Pearson's correlation. A p-value < 0.05 was considered to be significant.

Results

The sample group comprised boxers from different weight categories, from flyweight (up to 54 kg) to heavyweight (up to 81 kg) aged from 23 to 29. A detailed anthropometric description of the boxers is presented in Table 1.

Table 1. Anthropometric characteristics of the boxers

Variable	n = 5	M ±SD	Min	Max
Age (y)		24.8 ±2.19	23	29
Body mass (kg)		68.8 ±9.04	52	78
Body high (cm)		170.6 ±6.94	159	177
BMI (kg/m^2)		23.5 ±1.39	20.5	24.9

Table 2 shows the evaluation of anaerobic capacity enabling performance of physical effort in a short period of time and of maximal or submaximal intensity, involving mostly the activity of fast-twitch muscle fibers. The phosphagen qualities in the sample group members were described using the following indicators (PP, MP, TOPP, TMPP), and the glycolytic qualities using (TW, FI) indicators.

Table 2. Evaluation of the anaerobic capacity of the boxers

Variable	n = 5	M ±SD	Min	Max
P_{mean} (Watt)		432.46 ±45.726	351.89	503.780
P_{peak} (Watt)		517.32 ±49.139	428.44	580.570
P_{peak} (Watt/kg)		7.57 ±0.380	7.01	8.230
T_{pp} (sek.)		6.83 ±1.719	4.44	9.580
T_{max} (sek.)		4.60 ±1.182	2.68	6.300
W_{tot} (J/kg)		192.28 ±8.879	180.99	203.019
FI (%)		14.82 ±2.155	12.47	18.910

P_{mean}: mean power; P_{peak}: peak power; T_{pp}: time to obtain peak power; T_{max}: time of maintaining peak power; W_{tot}: total work; FI: fatigue index.

Table 3 shows the parameters of aerobic capacity, i.e. peak oxygen uptake ($\dot{V}O_{2peak}$) and the percentage value of peak oxygen uptake at the AT (anaerobic threshold, $\%\dot{V}O_{2peak}$). In addition, to show the body's reaction to the exercise stress test, the table contains the values of HR, VE and exercise load.

Table 3. Evaluation of aerobic capacity based on the analysis of $\dot{V}O_2$, VE, HR, and load with peak- and AT-level effort

Variable	n = 5	Peak exercise			Anaerobic threshold (AT)		
		M ±SD	Min	Max	M ±SD	Min	Max
$\dot{V}O_2$peak (ml/kg/min)		40.92 ±4.087	32.6	46.2	26.86 ±1.801	24.50	30.00
VE (l/min)		111.80 ±10.089	96.0	128.0	44.00 ±9.690	30.00	60.00
HR (beat/min)		187.00 ±6.300	176.0	197.0	146.40 ±9.040	131.00	159.00
Load (Watt)		257.00 ±20.120	230.0	275.0	158.00 ±27.928	125.00	200.00
% $\dot{V}O_2$peak (%)					66.30 ±6.57	54.11	75.15

$V\dot{O}_2$: oxygen uptake per minute; VE: minute ventilation; HR: heart rate.

Table 4 presents the biochemical reaction to the loads applied during both exercise stress tests. The change in the level of concentration of Acid-Base Balance and lactic acid in the blood proved to be statistically significant in

both tests ($p < 0.01$). The second level of degradation of the AMP, measured by the post-effort concentration levels of Hx, X, and UA, showed statistically significant changes in biochemical parameters after the anaerobic capacity evaluation test ($p < 0.05$) and for the Hx ($p < 0.01$).

Table 4. Concentration levels of oxypurines, uric acid, lactic acid and Acid-Base Balance in both active and resting state in the blood of the female boxers undergoing aerobic and anaerobic capacity tests

Variable	n = 5	WAnT		Incremental Stress Test	
		rest	post-exercises	rest	post-exercises
		M ±SD	M ±SD	M ±SD	M ±SD
Hx (µmol/L) **/NS		7.22 ±2.799	13.32 ±1.87	7.18 ±1.734	15.70 ±6.354
X (µmol/L) */NS		0.89 ±0.452	1.44 ±0.673	1.69 ±0.469	2.16 ±0.721
Hx/X NS/NS		10.33 ±4.22	11.32 ±4.57	4.23 ±2.31	7.18 ±2.77
Hx+X (µmol/L) */*		8.12 ±3.04	14.77 ±2.36	8.87 ±1.48	17.89 ±6.71
UA (µmol/L) */NS	1	136.97 ±43.527	312.01 ±70.821	160.30 ±73.270	206.16 ±59.618
LA (mmol/L) **/**		1.5 ±0.561	11.91 ±1.32	1.49 ±0.175	10.88 ±1.299
BE (mmol/L) **/**		−0.58 ±1.539	−11.269 ±1.122	0.34 ±0.839	−12.24 ±1.560
HCO_3^- (mmol/L) **/**		23.82 ±1.218	15.58 ±0.794	24.24 ±0.722	14.92 ±1.116
pH **/**		7.41 ±0.016	7.25 ±0.022	7.42 ±0.010	7.26 ±0.022

LA: lactic acid; BE: acid-base balance; HCO_3^-: sodium bicarbonate; Hx: hypoxanthine; X: xanthine; UA: uric acid.
NS: not statistically significant, *$p < 0.05$, **$p < 0.01$.

Discussion

Women's boxing, as a relatively new sport discipline, is lacking an in-depth description of the physiological and biochemical responses in the body to training-related effort. A detailed analysis of the results of studies carried out among different population groups and among boxers of different ranks will make it possible to shape their physiological profiles in the future.

The authors of this paper obtained an oxygen capacity indicator ($\dot{V}O_{2peak}$) of 40.92 ±4.087 ml/kg/min with a HR of 187 ±6.3 beats/min, VE 111.80 ±10.089 l/min and AT 66.30 ±6.57 % $\dot{V}O_{2peak}$, which proves that the level of oxygen capacity is good. No correlation between the body mass nor weight category of the boxers and the $\dot{V}O_{2peak}$ value was observed in this research. The highest and the lowest value of peak oxygen uptake was observed for boxers in the super-middleweight category. It indicates that there are other factors determining the capacity of oxygen consumption by the body, apart from body mass. Chatterjee's study from 2006 showed that the average value of $\dot{V}O_{2max}$ for 45 women from the Indian boxing team fluctuated at approx. 48.6 ±6.8 ml/kg/min. (Hellsten-Westing Norman, Balsom, Sjodin, 1993; Imamura et al., 1999). This difference in the oxygen uptake is a result of the different testing procedure, where treadmill tests were used, and our tests were carried out using a cyclo-ergometer. From the physiological point of view, the activity carried out with the use of a cycle ergometer

involves a much lower muscle mass, hence the lower level of oxygen uptake. A similar level of maximum oxygen uptake was described in a study by H. Imamura et al. (1999). The average level of $\dot{V}O_{2max}$ was 42.7 ±5.1 ml/kg/min. Furthermore, P. Chatterjee's studies from 2006 indicate that one 2-minute long round increased HR up to 197 ±7 beat/min. This points to a maximal, and sometimes even supramaximal, work intensity (Chatterjee, Banerjee, Majumdar, 2006). The energetics of muscle activity at such a level of intensity is based mostly on phosphagen and glycolytic-lactic metabolism. The 30-second Wingate test allowed us to assess a given boxer's predisposition to such intense effort. The maximum power level in the sample amounted to 517.32 ±49.139 Watts and 7.57 ±0.380 Watt/kg body mass, with the average power level of 432.46 ±45.726 Watts (6.41 ±0.296 Watt/kg). Maximum power was not correlated to the body mass. The highest and the lowest value of the studied indicator occurred among the women boxers in the super-middleweight category. Due to the limited number of studies and works on the capacity of female boxers, it was necessary to compare the obtained results with the findings of C. Doria et al. (2009). A comparative analysis involved juxtaposition of karate and boxing as the training patterns for both disciplines are similar. The average maximum power, describing the potential of ATP resynthesis, in the group of karate kata and kumite women was 6.5 ±0.3 Watt/kg on average and was comparable with the values attained by the women boxers. Moreover, metabolic reaction and effort-related concentration of lactic acid resulting from the applied exercise loads were quite similar (LA for karate was 12.4 ±2.2, for boxing, 11.91 ±1.32 mmol/L). Extensive studies by E. Hübner-Woźniak, A. Kosmol, A. Głaz, A. Kusior (2006), focusing on the body's reaction to exercise loads in combat sport athletes, enabled a substantive analysis of relationships existing between many biochemical and physiological indicators. These were carried out, however, in a group of men. The maximum exercise load applied in both tests triggered the development of tissue hypoxia, leading to an imbalance in the ATP/ADP relation and to a disturbance of the pace of ATP resynthesis. As a consequence, an increase in the amount of ATP decomposition metabolites in blood was noticed, such as: Hx, X, and UA. Moreover, statistically significant effort-related changes in biochemical parameters were observed during the Wingate test ($p < 0.05$). Moreover, statistically significant effort-related changes in biochemical parameters were observed during the Wingate test for LA, BE, HCO_3^-, pH, Hx ($p < 0.01$) and X, UA ($p < 0.05$). The analysis of correlation between the effort-related increase in the amount of Hx, and UA, and physical capacity indicators, show a high negative correlation between the maximum and average power and the effort-related increase of UA in the blood ($r = –0.9247$, $p < 0.05$), and time to obtain peak power and increase of Hx ($r = –0.8913$, $p < 0.05$). The post-exercise difference in the intensity of purine nucleotide degradation (Hx/X) is lower among the competitors subjected to the Wingate test, which may prove a higher loss of nucleotides from their entire pool available within the cell. On the other hand, a post-exercise higher total of nucleotides measured with the Hx+X sum occurred after the Incremental Stress Test, which may prove a faster and less energy-intensive re-synthesis of nucleotides with an involvement of the HGPRT enzyme. This confirms the theory that high training stimuli trigger an increased activity of the HGPRT enzyme and a return of Hx to the nucleotide pool. It is not leached from the muscles due to its transformation into UA. Similar findings were obtained by J. Zieliński and K. Kusy (2012) who carried out a study in a group of sprinters and triathletes – athletes of different physiological profiles − and demonstrated that athletes exposed to higher training loads display a higher increase in the concentration of Hx in the blood after exercise tests. At the same time, an analysis of purine nucleotide turnover and the measurement of HGPRT activity shows the development of more energy-efficient patterns of purine chain synthesis among sprinters, compared to athletes exposed to smaller training loads (Zieliński, Kusy, 2012). High training loads applied to running practice patterns triggered a development of beneficial adaptive changes and an

increase of HGPRT enzyme activity. On the other hand, Y. Helleten-Westing, B. Norman, P.D. Balsom, B. Sjodin (1993) studies show that effort up to the level of refusal causes a loss of Hx and inosine, leading to a decrease in the cellular ATP pool by approx 9%. A total restoration of ATP to its rest level involves slow and energy-demanding *de novo* synthesis of purine rings (Tullson, Terjung, 1990). The created Hx may subsequently be included into the purine nucleotide pool or leached together with inosine and UA through the kidneys or intestines (Harkness, Simmonds, Coade, 1983; Sorensen, Levinson, 1975). The UA formed by means of decomposition is an antioxidant used as a substrate in the non-enzymatic reaction of oxidation to allantoin (Hellsten et al., 2001). The carried out tests demonstrated that a stronger effort-related degradation of purine nucleotides occurred among the athletes during the a 30-second maximal power test. This is also additionally supported by a higher effort-related increase in the UA concentration level in the blood. Comparing previous studies conducted by the authors involving a group of karate athletes who displayed a lower level of purine nucleotide degradation during a Wingate test (measured by the ratio of Hx/X) and a higher effort-related increase of the amount of Hx and X in blood, it may appear that the difference is most probably a result of a higher aerobic capacity of the karate athletes ($\mathring{V}O_{2max}$ 51.3 ml/kg/min) and their better anaerobic capacity (maximum power of 530.81 ±77.697 Watt) than the women boxers (Domaszewska, Laurentowska, Michalak, Kryściak, Rakowski, 2008).

Given the presented findings, an analysis of the activity of HGPRT would be reasonable, although it turned out to be impossible due to methodological constraints. In the light of studies conducted by T. Rychlewski and J. Zieliński focusing on the use of measurement of the Hx concentration level as a marker of cellular energy crisis, the introduction of biochemical diagnostics of such a type as a tool for marking post-exercise changes in skeletal muscles seems well-justified (Zieliński, Kusy, 2012; Rychlewski et al., 1997). A comparison of exercise-induced changes of ABB indicators and LA concentration provided evidence that the type of physical activity performed did not influence exercise-induced changes of these parameters; they were comparable in both of the studied tests. That is why LA concentration during exercise as an indicator of the intensity of ischaemic lesions occurring within the cell is not sensitive enough. Based on literature and our previous research, we claim that the measurement of Hx concentration within the blood after a completed exercise is a more sensitive marker of the described lesions. An analysis of the obtained results indicates a statistically significant increase in Hx after exercise with reference to the lack of a statistically significant change after the Incremental Stress Test. However, it should not downgrade the usefulness of traditional physiological and biochemical markers in sports diagnostics, but rather serve as an indicator that would allow for a more precise optimization of training loads and, in consequence, make it possible to prevent overtraining in combat sports, which is often conducive to injury. D.R. Howell et al. (2017) in their research, provided evidence that there are no serious body injuries during fights affecting physiological or cognitive functions of the body.

Conclusions

The level of aerobic and anaerobic capacity in the analyzed sample group of female boxers is similar to that found in world literature on the subject. The biochemical reaction to the applied test workloads was comparable to the reaction found among athletes practicing other combat sports.

The level of plasma purine nucleotides at rest and after standard exercise may be a useful tool for monitoring the adaptation of energy processes in different training phases and support the overload/overtraining diagnosis. These markers may expand traditional physiological and biochemical methods of diagnostic processes, e.g. lactic acid, HR_{max}, and HR_{AT}. In our opinion, the changes in plasma Hx and X concentration are sensitive metabolic indicators of the exercise's status. These parameters possibly provide indirect information about the potential energetic status of the muscle, especially in highly trained athletes in which no significant adaptation changes are detected when examined by means of commonly acknowledged biochemical parameters.

Acknowledgments

This statement is to certify that all authors have seen and approved the manuscript being submitted. We warrant that the article is the authors' original work. We warrant that the article has not received prior publication and is not under consideration for publication elsewhere. On behalf of all co-authors, the corresponding author shall bear full responsibility for the submission. All authors agree that author list is correct in its content and order and that no modification to the author list can be made without the formal approval of the Editor-in-Chief. The authors declare no conflicts of interest.

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