Original Article

doi: 10.16926/par.2023.11.27

Physiological response and biomarkers in kickboxing - systematic review

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Authors' Contribution: A – Study Design, B – Data Collection, C – Statistical Analysis, D – Manuscript Preparation, E – Funds Collection

Abstract: Kickboxing is a relatively young sport with growing popularity across the World. There is a rapidly growing need for clear and valid detailed specific information about the effects of kickboxing as an activity. Therefore, this research aimed to collect all legitimate evidence about the acute and chronic physiological response, as well as monitoring and utilization of specific biomarkers in kickboxing. For this systematic review, the authors conducted research in the three relevant scientific bases and one search engine for this specific topic, PubMed, Web of Science, Scopus, and Google Scholar. After title and abstract screening, and review of full-text manuscripts regarding inclusion criteria, 16 studies were included in the review. Studies were sorted according to the type of research methods used. Results revealed a positive chronic impact of kickboxing on the overall athletes' health, speaking of hormonal, blood, and immune parameters. Although, the acute reaction of athletes' organisms includes the increase in levels of cortisol, growth hormone, testosterone, glucose, indicators of lipid peroxidation activity, H+, BEecf, lactic acid, and pyruvic acid, while the levels of myostatin and irisin tend to decrease. Such results confirm kickboxing as a high-demand anaerobic glycolytic activity. Secondly, the currently available analyses of the greatest value for monitoring in competitive kickboxing are hormonal, especially for testosterone & cortisol, immunoassays & saliva assays for immunity tracking, and genetic analyses as auxiliary tools. Further investigations should include females, kickboxing discipline differentiation, weight and age stratification, and differentiation.

Keywords: combat sport, World Association of Kickboxing Organisations (WAKO), biochemistry, sports medicine, monitoring

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Recevied: 22.06.2023; Accepted: 31.07.2023; Published online: 6.09.2023

Citation: Babić M, Pobrić I, Čular D. Physiological response and biomarkers in kickboxing - systematic review. Phys Act Rev 2023; 11(2): 120-137. doi: 10.16926/par.2023.11.27

INTRODUCTION

Kickboxing is one of the youngest combat-striking sports. However, the beginning of kickboxing as an art can be traced to the ancient Japanese and Thai martial arts, especially precedes of Muay Thai and Karate. There is no official consensus about the history of kickboxing as a sport at all. Accessible data tells us about Mike Anderson, a karateka, as the founder of the first kickboxing rules in 1972 (e.g. full contact karate) and the first World Championship in Full Contact in 1974, after which kickboxing gradually started to separate from Karate. Punching techniques, as well as several other techniques, were derived from Boxing and Muay Thai too. Finally, Mike Anderson along with George Brueckner established the World All-Style Karate Organization (later & today- World Association of Kickboxing Organisation) (WAKO) in 1976 [1], and until now it is the main governing amateur kickboxing federation. According to WAKO, there are six competitive contact/combat disciplines, half of them are performed in the ring under full contact rules, while the second half does occur on tatami (8x8m squared foam field) under specific light/semi-contact rules [2]. The mentioned federation was taken into consideration because it is the only kickboxing federation recognized by International Olympic Committee [3]. The rapid growth of kickboxing through the last decades is recorded, especially in Europe, Asia, and America. Moreover, such growth should be accompanied by scientific findings, because there are still many unobserved fields in kickboxing, especially in terms of its impact on health, elite performance, injury rehabilitation, etc.

Kickboxers are, therefore, required to have an optimal motor and technical skills level and be able to perform specific tactical actions during the fight [4]. In addition, elite kickboxers have been found to develop relatively high anaerobic and aerobic capacities [5], which is a guiding light to the further revelation of the complete physiological profile of kickboxers. There is an evident lack of comprehensive literature to conclude desired overall fitness profile of kickboxers. The last published review about the anthropometric and psycho-physiological profile of kickboxing [6] reported that low-fat percentage and emphasized mesomorphic component mark, successful kickboxing athletes, with self-confidence, self-efficacy, motivation and mental toughness as desirable psychological traits, while precise physiological values remained unrevealed.

There is a certain amount of existing literature regarding addressed issues. To begin, hematological parameters are examined due to their very important role in combat athlete status, because combat sports are intermittent, consisting of alternating periods of high- and low-intensity efforts, and pauses during matches [7], which is surely a great challenge for the human body. Histochemical and biochemical methods are covering a wide field of possibly tracked endocrine, genetic, immune, and other parameters of muscle and organism status. A single measurement of a biomarker does not allow precise determination of an individual's health status [8]. Hormones however play a significant role in the overall performance and fitness of athletes. For example, due to the relation with the „fight or flight" mechanism, cortisol as a stress hormone can be used to measure the stress levels of kickboxing competitors. Still, the majority of papers that feature biochemical analyses focus on blood lactates (La). Such results are to be expected as clinical physicians and sports scientists have used lactate threshold (LT) tests for over fifty years because their application is considered extremely useful for recommendations on individual exercise intensity in cardiac patients and trained athletes [9-11].

Therefore, this particular review aims to collect all relevant evidence collected through hematologic, histochemical, and biochemical analyses about: a) the acute and chronic physiological impact of competitive kickboxing on kickboxers' fitness status and health, and b) the current practice of biomarker utilization as evaluation tools for monitoring in competitive kickboxing.

MATERIAL AND METHODS

The whole study is conducted following recent guidelines for performing systematic reviews in sports science [12]. Application of mentioned guidelines includes standardization of identification and screening procedures through Boolean operators and download process, as well as utilization of PICO, PRISMA, and STROBE tools to ensure the quality and transparency of conducted systematic process.

Subjects

The sample for this review is composed of n=16 scientific studies published in English. The overall sample of participants within examined studies is composed of n=242 kickboxers and n=136 participants in control groups. All examined kickboxers are male, and two papers featured underage (junior/youth) participants (12.50%), while others compete in the senior age category (87.50%). All analyzed kickboxers were competitors at the time of measurement, while 75.00% were elite athletes and 25.00% of them were amateur competitors. Although, there is insufficient information about the classification of participants within kickboxing disciplines, with K-1 discipline as the most studied (25.00%), followed by Full Contact (18.75%), Light Contact (12.50%), and Point Fighting (6.25%) while 37.50% of studies did not specify rules of competition form.

Eligibility criteria

Researchers selected three scientific databases for research, PubMed, Web of Science, Scopus, and one search engine: Google Scholar. Databases were selected by their compatibility with the selected topic.

In the first step, the authors selected specific preconditions for the extraction of scientific papers following the PICO framework [13]. Inclusion criteria were defined according to Richardson et al. [14]: 1) Population: kickboxing competitors; 2) Intervention: hematological/biochemical/histochemical analysis before and/or after competitive kickboxing sparring or contest; 3) Comparison: a) longitudinal monitoring and comparisons of kickboxing competitors for estimations of chronic impact, and b) transversal comparisons between individuals in kickboxing and between samples (control and/or combat sport groups) for determination of acute changes caused by kickboxing bout; 4) Outcome: effect of kickboxing on athletes' fitness status overall/segmental, the impact of specific conditions within athlete's body on the sport performance. On the other side, exclusion criteria were the following: studies about pathological issues/injuries, studies in other languages besides English, monitoring of other fitness/anthropometric and/or psychological parameters, studies about kickboxing without biomarker analysis, studies which include combat athlete sample without kickboxing athletes.

In the second step, researchers selected a specific combination of terms and used them in searching through selected databases. Selected specific terms were searched in combinations with Boolean operators [15]: kickboxing AND physiology, kickboxing AND biomarkers, kickboxing AND blood, kickboxing AND lactate, kickboxing AND histochemical, kickboxing AND biochemical, kickboxing AND hormones, kickboxing AND saliva, kickboxing AND influence, kickboxing AND health.

Extraction protocol

Authors assessed studies within extraction protocol from 1/12/2022 to 10/12/2022. The authors initially retrieved n= 289 scientific studies. After removing the duplicates, further screening included a detailed evaluation of retrieved studies within folders. Following selected filtrations, authors assessed eligibility evaluation and finally extracted n=16 studies for inclusion in this systematic review. The procedure of study selection is shown in Figure 1, by Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [16].

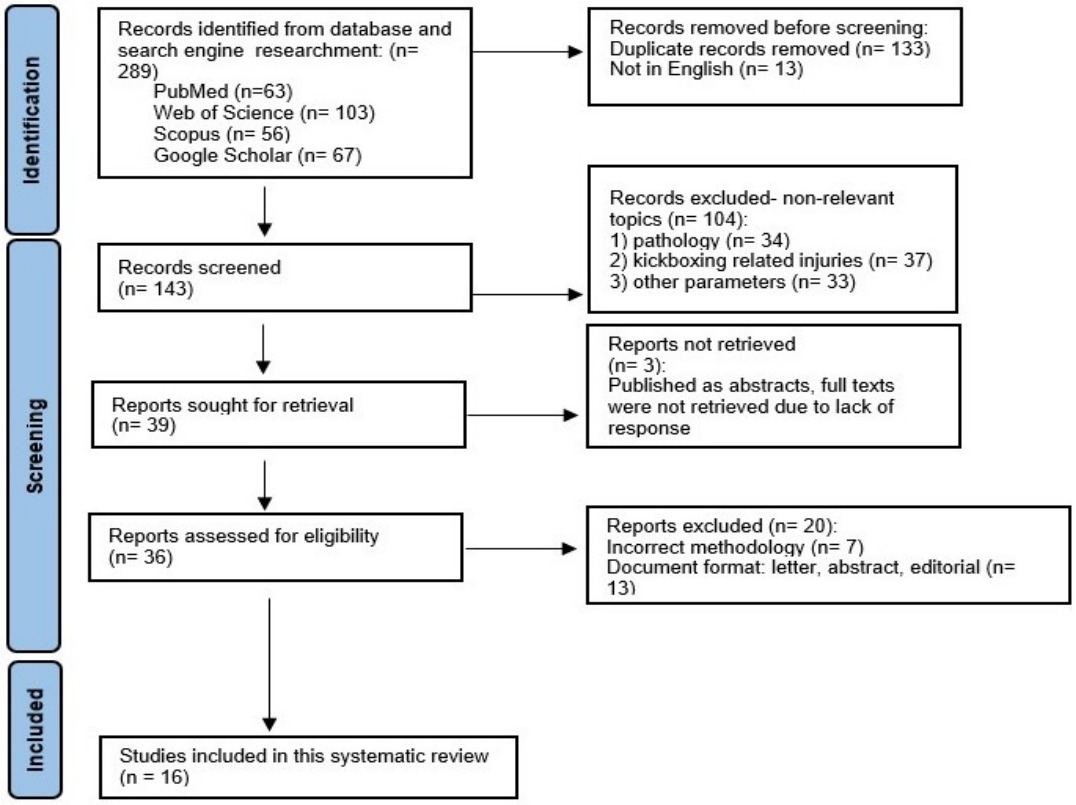

Figure 1. Flow diagram for screening and selection of studies according to Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA).

Quality assessment

Onwards, a quality assessment evaluation was performed. Authors utilized STROBE-„Strengthening the Reporting of Observational Studies in Epidemiology“ checklist [17] to determine the study quality of selected papers.

Statistical Methods

Evaluation of impact within extracted studies was assessed through effect size (ES) calculation for each significant result report, within every selected study. Values were shown and calculated through Pearson's correlation coefficient (r), Cohen's d, and Hedge's d. Cohen's d was used for the impact of the difference between the groups of equal sample size (majority), and Hedge's d was used for different sample sizes. Further analyses were conducted through descriptive statistics in the PC program *Statistica 14*.

RESULTS

The results of the quality assessment process are presented in Table 1. The quality of selected studies was evaluated according to the STROBE score ranking, and results revealed the following stratification: n=4 studies were estimated as “very high-quality“ (18-22 points), n=11 as “high-quality“ (14-17 points), and one (n=1) as “medium quality" (10-13 points). Further, no article was classified as "very low quality" (0–5 points) or "low quality" (6–9 points). Greatest scores according to sections were recorded in 1, 2, 3, and 4 (Title and abstract, Background, Objectives, Study design), within 100% of papers considered as complete. The lowest scores were obtained within sections 13 (16%) and 14 (0%), which represent a brief sample description. Unfortunately, the majority of studies did not exactly elaborate specific characteristics of the participants. The overall average score of all analyzed studies is 16.06 points, which is characterized as “high-quality“. The results of the initial ES evaluation are presented in Table 2.

Table 1. STROBE checklist evaluation results of selected papers (n=16).

STROBE checklist		Dopsaj et al., 2013	Rydzik et al., 2022	Azarbayjani et al., 2014	Moreira et al., 2010	Volodchenko et al., 2019	Ouergui et al., 2013	Ouergui et al., 2014	Cimadoro, 2018	Rydzik et al., 2021a	Rydzik et al., 2021b	Salci, 2015	Ouergui et al., 2021	Eken et al., 2021	Kabak et al., 2018	Ouergui et al., 2016	Zubac et al., 2017
Title, abstract	Title, abstract	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Introduction	Background	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Objectives	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Methods	Study design	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Setting					x					x	x					
	Participants	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Variables	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Data source	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Bias	x	x			x		x		x		x		x		x	x
	Study size		x		x		x		x				x				x
	Quantitative variables	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Statistical methods	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Results	Participants	x												x			
	Descriptive results																
	Outcome data	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Main results	x							x	x	x	x	x	x		x	x
	Other analyses	x		x			x		x	x	x	x	x			x	x
Discussion	Key results	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
	Limitations		x	x	x	x				x	x	x	x				x
	Interpretation	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x
	Generalisability	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x
Other information	Funding	x	x	x						x	x						
Total score		18	17	16	15	16	15	14	16	18	18	17	17	15	13	16	18

„blank" = item with absence or lack of information; x = item with complete and explicit information; **In title and abstract**, *Title, Abstract* = informative and balanced summary of what was done and what was found is provided. In the introduction, *Background* = scientific background and rationale for the investigation being reported is explained; *Objectives* = state-specific objectives and/or any pre-specified hypothesis. **In Methods**, *Study design* = key elements of study design are presented early in the paper, *Setting* = setting, locations, and relevant dates for data collection are described. This must include information on the study period (specific dates), sport context (competition level, and competition category), and competition year(s) for all data collected; *Participants* = the eligibility criteria, and the sources and methods of case ascertainment and control selection were giver, as well as the rationale for the choice of cases and controls; *Variables* = all outcomes, exposures, predictors, potential confounders, and effect modifiers were clearly defined, also diagnostic criteria, if applicable; *Data source* = procedure for measurement is described; *Bias* = any efforts to address potential sources of bias were described, *Study size* = a measure of effect size is provided; *Quantitative variables* = Clarification of how quantitative variables were handled in the analyses. If applicable, a description of which groupings were chosen and why should be provided, *Statistical methods* = all statistical methods are correctly described, including those used to control. **In Results**, *Participants* = numbers of individuals at each stage of study were reported—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed, *Descriptive results* = characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders were properly reported, *Outcome data* = numbers in each exposure category were revealed, or summary measures of exposure, *Main results* = statistical estimate and precision (i.e., 95% CI) for each sample or subgroup group examined is provided, *Other analyses* = other conducted analyses were reported—eg analyses of subgroups and interactions, and sensitivity analyses, **In Discussion**, *Key results* = a summary of key results with reference to study objectives is provided; *Limitations* = limitations of the study, taking into account sources of potential bias or imprecision are discussed, *Interpretation* = Paper provides a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence, *Generalisability* = the generalisability (external validity) of the study results is properly disscused. In **Other information**, *Funding* = the funding source of the study is cited or the absence of funding, if applicable.

Table 2. Estimated effect size values of extracted papers separately by the report.

Authors	Relation	Pearson's r	Cohen's d/Hedge's d
Dopsaj et al., 2013	IRF(%) wrestling>kickboxing	-	1.697
	LHD(%) karate>kickboxing	-	1.743
	Hp karate<kickboxing	-	1.170
Rydzik et al., 2022	H+ pre/post	-	0.800
	pCO2 pre/post	-	0.270*
	pO2 pre/post	-	0.580
	HCO3 − pre/post	-	0.880
	BEecf pre/post	-	1.000
	TCO2 x Activeness	0.64	0.880
Azarbayjani et al., 2014	WBC pre/post	-	1.128
	LYM pre/post	-	0.623
	PLT pre/post	-	0.107*
Moreira et al., 2010	Salivary Cortisol pre/post	-	0.900
Volodchenko et al., 2019	MDA pre/post	-	20.960
	DC pre/post	-	30.706
	CAT pre/post	-	10.049
	SH group pre/post	-	8.438
	SOD pre/post	-	6.780
	La pre/post	-	12.893
	MDA/SH pre/post	-	24.182
	MDA/SOD pre/post	-	3.452
	DC/SH pre/post	-	42.888
Ouergui et al., 2013	La 5min after AR/PR	-	3.914
Ouergui et al., 2014	La R1-R3	-	7.934
Cimadoro, 2018	Strikes overall x La	0.76	-
Rydzik et al., 2021a	La pre/post	-	2.230-9.080
	La R interdifference	-	1.250-10.110
Rydzik et al., 2021b	Skin pH x efficiency/effectiveness	0.42-0.43	-
Salci, 2015	La pre/post	-	2.468
Ouergui et al., 2016	Hormones pre/post	-	17.200
	La pre/post	-	19.100
	Glucose pre/post	-	7.800
	T/C ratio /Glucose	-	0.560
Eken et al., 2021	n/a	-	n/a
Kabak et al., 2018	Irisin pre kb/con	-	1.289
	Irisin pre/post kb	-	0.892
	Irisin pre/post con	-	1.503
	Myostatin pre/post kb	-	0.734
Ouergui et al., 2021	La FC/PF pre/post	-	0.479
	La LC/PF pre/post	-	0.771
Zubac et al., 2017	MCV, MCHC, Hb, Hct pre/post	-	0.100*
	MCH, Sodium pre/post	-	0.300
	Calcium pre/post	-	0.600
	Potassium pre/post	-	0.700
	CK	-	0.900
	LDH	-	1.400

pre/post - before and after treatment, IRF(%)- immature reticulocyte fraction, LHD(%)- low hemoglobin density, Hp- Haptoglobin, H+- hydrogen ion concentration, pO2- partial pressure of oxygen, pCO2- partial pressure of carbon dioxide, BEecf- base excess in the extracellular fluid, HCO3- - concentration of bicarbonate ions, TCO2- total molar carbon dioxide concentration, WBC- white blood cell count, LYM- lymphocytes, PLT- platelets, MDA- malondialdehyde, DC- diene conjugates, CAT- Catalase, SH- sulphydryl groups, SOD- catalase and superoxide dismutase activity, La- lactate, MDA/SH, MDA/SOD, DC/SH- coefficients, AR- active rest, PR- passive rest, R- round, R1-3- round 1 to 3, T:C ratio- testosterone/cortisol ratio, FC- Full Contact, LC- Light concact, PF- Point Fighting, kb- kickboxing, MCV- mean corpuscular volume, MCH- mean corpuscular hemoglobin, MCHC- mean corpuscular hemoglobin concentration, Hb- hemoglobin concentration, Hct- hematocrit, LDH- lactate dehydrogenases concentration, CK- creatine kinase concentration, *-effect size below 0.3.

Table 3. Table of included studies (n=16).

Authors	n	Age	Kickboxing level	Kickboxing discipline	Performed analysis	Given parameters
Dopsaj et al., 2013	14 (33 control)	Under 18	Professional	n/a	Hematological, oxidative stress, and immune status analysis	Hb, MCV, absolute reticulocyte count, IRF(%), LHD(%), serum iron, TIBC, sTfR, serum ferritin, IL-6, IgG, IgA, IgM, IgE, Hp, hs-CRP
Rydzik et al., 2022	14	19-35	Champion level	K-1	Acid-base balance (ABB) analysis	H+ , pCO2 , pO2 , HCO3 −, BEecf, TCO2
Azarbayjani et al., 2014	13	18-24	National/state champions	n/a	Blood analysis	Leukocyte [WBC, LYM] erythrocyte [RBC, HGB, HCT], PLT
Moreira et al., 2010	20	23±4	Amateur	n/a	Saliva analysis	Salivary cortisol, Salivary IgA abs, Salivary IgA rate
Volodchenko et al., 2019	18	17.29± 0.31	High level	n/a	Saliva analysis	Products of lipid peroxidation, MDA, DC, indicators of the antioxidant system, SOD, SH-group concentration
Ouergui et al., 2013	18	18.5±1.85	Well trained	FC	blood lactate analysis	La
Ouergui et al., 2014	18	18.5±1.85	8.2±0.9 years of experience	FC	blood lactate analysis	La
Cimadoro, 2018	8	24.3±1.8	Semi-professional	K-1	blood lactate analysis	La
Rydzik et al., 2021	24	19-28	Category A (elite)	K-1	Skin pH analysis	pH level
Rydzik et al., 2022	15	23.9±4.6	Elite	K-1	Blood lactate analysis	La
Salci, 2015	10	19.3±1.6	Competitors amateurs	LC	Blood lactate analysis	La
Ouergui et al., 2021	18	20–23	Competitors amateurs	FC, LC, PF	Blood lactate analysis	La
Eken et al., 2021	12 (101 control)		Professional	n/a	DNA isolation analysis	ADRA2A rs1800544, CC, CG, GG genotype, C and G alleles
Kabak et al., 2018	10 (10 control)	20.20±1.62	Amateur	n/a	Blood analysis	Irisin, Plasma myostatin
Ouergui et al., 2016	20	21.3±2.7	regional and national level	n/a	Hormonal, lactate, and glucose analysis	cortisol, testosterone, GH, T:C ratio, glucose, La
Zubac et al., 2017	10	22.1±4.1	National Champions	n/a	Urine, histological and biochemical analysis	Erythrocytes; MCV; MCH; MCHC; Hb; Hct%; %PV; LDH; CK; sodium, potassium, calcium, Usg

TIBC- iron binding capacity total, IL-6- interleukin 6, sTfR- soluble transferrin receptor, IgG, IgA, IgM, IgE- immunoglobulins, hs-CRP- high-sensitivity C-reactive protein, RBC- red blood cell count, HGB- hemoglobin, ADRA2A- gene region, rs1800544- polymorphism, GH- growth hormone, %PV- plasma volume change, Usg- urine specific gravity values.

ES analysis revealed a strong impact/ES of obtained results, with low ES found only within three (sub)reports. Additionally, this study represents Level I of evidence according to the medical guidelines [18] due to its systematic nature.

The majority of analyzed studies were conducted on relatively young subjects of post-pubertal age, with an overall age range varying from 16 to 35 years. Further,

competitive athletes were analyzed in 15 of 16 papers. There is an evident lack of analyses conducted within tatami combat disciplines, with light contact competitors analyzed twice, and point fighting once, while there is zero evidence about kick light competitors at all. Tatami combat disciplines in kickboxing make up a whole unexplored field in sports science, and therefore they need to be examined well in future experimental work. Unfortunately, the majority of studies focused on lactate analysis. Consequently, studies with analysis of numerous biomarkers, with one or several methods are of great importance for precise evaluation of competitor's status. Certain biomarkers can be measured with both histochemical and biochemical analysis, so authors of this review for further systematization primarily considered methods that were used for examination in extracted papers.

Table 4. Papers where histochemical analyses were performed (n=5).

Authors	Histochemical parameters	Values	
		Pre	Post
Moreira et al., 2010**	Salivary cortisol (ng · ml-1)	38.5±19.0	57.5±21.0*
	Salivary IgAabs (µg · ml-1)	54.0±25.0	58.5±55.0
	Salivary IgArate (µg · min.-1)	20.8±14.0	19.3±20.0
Ouergui et al., 2016	GH (µg/mL)	0.6±0.6	11.4±4.1*
	Testosterone (µg/mL)	3.2±1.3	4.5±1.6*
	Cortisol (µg/mL)	97.7±37.1	143.7±29.4*
Kabak et al., 2018	Irisin (µg·ml-1)	2.26±0.60	1.43±0.50*
	Myostatin (pg·ml-1)	2652.3±960.9	2914.5±1011.6*
Dopsaj et al., 2013	Ferritin (µg·L-1)	88.6±35.5 (63.1–114.1)	
	IL-6 (pg·ml-1)	1.73±0.84 (1.19–2.27)	
	sTfR (mg·L-1)	1.29±0.38 (1.12–1.49)	
Eken et al., 2021	ADRA2A Genotype GG	25%	
	ADRA2A Genotype CG	33.3%	
	ADRA2A Genotype CC	41.7%	
	Allele C	58.3%	
	Allele G	41.7%	

** - saliva analysis, I- before treatment, II- after treatment, *- significantly different values, ''- different values (p=0.08)

Table 5. Papers where hematological analyses were performed (n=3).

Hematological parameters	Dopsaj et al., 2013	Azarbayjani et al., 2014		Zubac et al., 2017	
		Pre	Post	Pre	Post
Hb ($g \cdot L^{-1}$, $g \cdot dL^{-1}$)	151.4±5.2 (144.5–158.3)	14.91	14.01*	14.5 ±0.5	14.6 ±0.5
HCT (%)	-	46.36	43.10*	43.1 ±1.2	43.2 ±1.8
Reticulocytes ($x10^{12} \cdot L^{-1}$)	0.036±0.011 (0.021–0.051)	-	-	-	-
IRF (%)	0.26±0.04* (0.24–0.28)	-	-	-	-
LHD (%)	1.77±0.76* (1.01–2.53)	-	-	-	-
WBC (10^3/mL)	-	7.80	10.30*	-	-
RBC (10^3/mL)	-	5.71	5.57*	-	-
MCV (fL)	88.8±2.1 (86.9–90.7)	-	-	89.1 ±3.8	88.9 ±3.7
Erythrocytes (pL)	-	-	-	4.87 ±0.3	4.86 ±0.2
LYM (10^3/mL)	-	2.40	4.11*	-	-
PLT (10^3/mL)	-	196.50	260.20*	-	-
MCH (pg)	-	-	-	29.8 ±1.04	30.1 ±1.3
MCHC (g/dL)	-	-	-	32.9 ±6.5	32.5 ±5.0

The authors of the first study in Table 4 [19] tested the hypothesis that salivary cortisol will increase, while immunoglobulin A (Iga) will decrease after a kickboxing match. Selected concentrations were measured with immunosorbent assays on a sample of 20 kickboxers. Results revealed that sports performance during kickboxing matches did not influence any investigated immunity markers (salivary IgArate, IgAabs, and flow rate) while the concentration of salivary cortisol significantly increased. The second study [20] revealed a detailed profile of kickboxers, wrestlers, and karatekas, as well as differences between them. Ferritin, interleukin 6, and soluble transferrin receptor were analyzed with immunoassay, and results between groups did not significantly differ. Still, kickboxers had the highest score on ferritin and IL-6, with the lowest score on sTfR. Obergui et al. [20] observed hormonal changes during a kickboxing match. Concentrations of cortisol, testosterone, and growth hormone had significantly increased. GH increased equally post-combat for winners and losers. It is suggested that GH increases with the intensity of exercise [21]. Speaking of testosterone, kickboxing combat was able to simulate the increased testosterone synthesis induced by completing attacking movements as shown in judo before [21,22]. Cortisol increase was expected due to high-demand kickboxing conditions. Researchers from the fourth study [23] compared irisin and myostatin concentrations between the kickboxing athletes and a morphologically similar group of sedentary people before and after HIIT. Irisin concentration decreased in both groups during HIIT exercise, while 3 and 6 hours after activity irisin remained significantly higher among kickboxing athletes. Meanwhile, myostatin concentrations increased during activity, but 3 hours after the event myostatin among kickboxers significantly decreased, unlike the sedentary group where myostatin level remained increased. The last study from Table 4 [24] includes a genetic analysis of kickboxing athletes where authors tried to determine the difference in allelic distribution of rs1800544 polymorphism between professional kickboxers and the sedentary control group. ADRA2A rs1800544 polymorphism with C and G alleles and CC, CG, and GG genotypes were not statistically different between groups. Looking within the kickboxing group, C allele and CC genotype are the most frequently found parameters, and respectively, this study represents one part of the future genetic profiling of kickboxing competitors.

The primary purpose of the cardiovascular system during physical exertion is to deliver oxygen to exercising muscles to support the metabolic demands of respiring mitochondria [25]. Speaking of elite sports performance, cardiovascular parameters play an important role in the control of physiological training/competition loads. Dopsaj et al. [20] conducted a hematological analysis on combat sports athletes and compared results between groups (sports). The percentage of immature reticulocyte fraction among kickboxers was significantly lower in regards to wrestlers, while low Hb density was significantly lower among kickboxers compared to the results of karatekas. It should be emphasized that the hematological values of all observed athletes were within healthy reference values. Such results lead to the conclusion that kickboxing has a positive chronic impact on cardiovascular health. On the other hand, the second paper from Table 5 [26] analyzed the difference in the impact between aerobic and anaerobic activity on acute hematological response. The authors concluded that a one-session RAST (running an anaerobic speed test) can cause more changes in the hematological profiles of kickboxers; so, it may be a reason that kickboxing relies on anaerobic power [26]. The authors of the last study [27] monitored possible changes in selected parameters during the tapering period (two weeks), affected by voluntary body-mass loss and exercise-induced muscle damage (EMID). As can be seen in Table 5, no significant changes were observed. These results can be interpreted in several ways. Acute responses to rapid weight loss include hypohydration and reduced plasma volume [28], which increases blood viscosity. Such effects impair cardiovascular efficiency [29] and increase the risk of acute cardiovascular problems [30]. For instance, a study conducted on boxing competitors found that rapid weight reduction mainly achieved by dehydration might cause a decrease in erythropoietin with the consequent reduction in erythropoiesis and neo cytolysis,

resulting in decreased tHb_{mass} (total hemoglobin mass) [28]. Later work by Reljić et al. reported a significant decrease in tHb_{mass}, erythropoietin, reticulocytes, haptoglobin, triiodothyronine (FT_3), and free androgen index [31]. After all, the reason for the lack of changes reported in the last study (Table 4) could be the opposite impact of training load and rapid weight loss practice on monitored parameters which annulated possible significant shifts. However, hematological parameters are of great value for athletes' acute cardiac capacity evaluation, as well as for chronic adaptations on training, or the training program itself.

Evaluation of changes in biochemical parameters seems to be an important part to consider regarding the prediction of success in sports [32]. As young athletes grow toward adulthood, their hormonal shifts tend to be intensified, as well as variations of other parameters. Therefore, to exactly estimate someone's biological age, such analyzes are still irreplaceable. A better understanding of these parameters can provide new insights into the quantification of training and competition loads, assisting the development of sport-specific conditioning programs [21]. Looking at Table 6 many biomarkers were observed, yet almost all papers feature different ones. Dopsaj et al. [20] used biochemical methods to access certain blood (iron-related), oxidative stress, and immune parameters in combat athletes. Iron-related parameters were reported to be within their reference ranges as expected. Kickboxers achieved significantly greater values of haptoglobin in regards to karatekas, and higher values of haptoglobin and hs-CRP than subsamples of wrestlers and karatekas. Although values of IgE, IgG, IgA, and IgM were lower among kickboxers, still values were within their reference ranges. Authors calculated centroids for Oxidative stress parameters (karate professionals: -0.867, wrestlers: 1.075, and kickboxers: -0.011), which means that wrestling has slightly better physical training contents for enhancement of the antioxidant defense system. The second study along with other measurements conducted a biochemical analysis of glucose in the blood. Values of glucose significantly increased during the match, regardless of the final match result (win/lose). The steady increase of glucose during the kickboxing combat may be attributed to an enhanced cortisol activation of gluconeogenesis through enhanced substrate delivery to the liver [21,33]. Nevertheless, cortisol is not the only hormone responsible for enhanced glucose release and epinephrine was shown to be the most important counterregulatory hormone that is capable of increasing glycogenolysis and gluconeogenesis [21,34]. A third study from Table 6 combined urine, biochemical, and hematological analysis with tensiomyography on the sample of kickboxers during their tapering, and weight loss period. EIMD markers significantly decreased throughout this study by –74.4% and –29.4% for CK and LDH, respectively. Authors suggest that initial CK concentration has been enhanced by previous mesocycles. Greater increases in serum CK activity after EIMD was associated with a shorter Tc (contraction time), a measure of estimated MHC- I proportion [27]. The quality of kickboxing preparations for competition surely depends on such tapering timings where competitors' body has enough time to recover from EMID. Respectively, competitors with a greater amount of MHC- II fibers should have a longer tapering period. Volodchenko et al. [32] conducted a biochemical analysis of salivary parameters after standard kickboxing training. Observed parameters were found to be significantly different before and after training, excluding pyruvic acid concentration. The results demonstrate the engagement of the antioxidant protection system and the increased use of thiol-containing compounds to neutralize free radicals [32]. The lack of pyruvic acid change could be explained with relatively light stimuli (one training). The last observed study in Table 6 [35] studied a possible impact of a kickboxing match (3 rounds) on acid-base balance (ABB) and total molar CO_2 concentration (TCO_2). Following the kickboxing match, all observed parameters were found to be significantly different. Such results indicate the great role of anaerobic metabolism during the kickboxing match. Glycolytic metabolism exercise significantly decreases the levels of bicarbonate as a main factor in neutralizing hydrogen ions in the blood. This process occurs according to the

Table 6. Researches where biochemical analyses were performed (excluding La-only analyses) (n=5).

Authors	Biochemical parameters	Values	
Dopsaj et al., 2013	-Serum iron (μmol·L-1)	15.1± 2.6 (12.3-17.9)	
	-TIBC (μmol·L-1)	62.5± 5.9 (57.4-67.6)	
	-Haptoglobin (g·L-1)	1.09± 0.48* (0.64-1.54)	
	-hs-CRP (mg·L-1)	0.80± 0.97 (0.41-1.19)	
	-IgE (g·L-1)	15.3± 12.4 (8.3–22.3)	
	-IgG (g·L-1)	11.51± 2.09 (10.4–12.6)	
	-IgA (g·L-1)	1.89± 0.50 (1.59–2.19)	
	-IgM (g·L-1)	1.07± 0.39 (0.79–1.35)	
	-Malondialdehyde (MDA) (μmol·L-1)	0.912± 0.176 (0.782-1.042)	
	-Advanced oxidation protein products (AOPP) (μmol·L-1)	20.1± 3.7 (14.9-25.3)	
	-Pro-oxidant antioxidant balance (PAB) (HK units)	358± 87 (282-434)	
	-Reactive oxygen metabolites (ROMs) (Carr U)	260± 47 (214-306)	
	-SH-groups (mmol/L, μmol/L)	0.564± 0.072 (0.504-0. 624)	
	-Superoxide dismutase (SOD) (U·L-1)	75± 10 (66-84)	
		Pre	Post
Ouergui et al., 2016	-Glucose (mmol/L)	5.0±0.4	8.1±1.2*
Zubac et al., 2017	-LDH (U/ L-1)	262± 7	184± 19.4*
	-CK (U/ L-1)	600± 7	154± 71.8*
	-Sodium (mmol/L)	140± 1.7	141± 1.5
	-Potassium (mmol/L)	4.3± 0.4	4.5± 0.5
	-Calcium (mmol/L)	2.3± 0.1	2.4± 0.1
Volodchenko et al., 2019**	-Diene conjugates (DC) (μmol/L)	24.46± 0.31	37.79± 0.53*
	-Catalase (CAT) (μkat/L)	41.71± 0.35	47.85± 0.79*
	-Pyruvic acid (PA) (μmol/L)	22.21± 0.37	23.31± 0.56
	-Lactic acid- saliva (LA) (mM/L)	0.48± 0.08	1.95± 0.14*
	-Malondialdehyde (MDA) (μmol·L-1)	4.57± 0.25	9.81±0.25*
	-SH-groups (mmol/L, μmol/L)	2.08± 0.16	0.85± 0.13*
	-Superoxide dismutase (SOD) (U·L-1)	2.07± 0.17	3.48± 0.24*
Rydzik et al., 2022	-H+ (nmol/L)	37.9± 3.3	54.0± 9.8*
	-pCO2 (mmHg)	37.2± 3.3	31.8± 2.6*
	-pO2 (mmHg)	77.2± 6.0	85.6± 8.5*
	-HCO3 − (mmol/L)	24.6± 1.3	14.9± 1.6*
	-BEecf (mmol/L)	0.5± 1.2	11.9± 2.7*
	-TCO2 (mmol/L)	24.1± 1.3	15.8± 1.4*

*- saliva analysis

following equation: H+ + HCO3 − → H2O + CO2 ↑ [35]. Speaking of TCO_2, a positive correlation was found regarding the activity of the attack, which was expected.

Blood lactate concentration measurement is a wide-adopted method for evaluations of exercise intensity and athletes' adaptation to training. During intense exercise in humans, the lactate concentration can increase by up to 40 mmol/L in working muscles and 25 mmol/L in plasma [36-39]. Still, there is controversy about the causally consequential relationship between lactates and physical work. Many observations suggest that lactate production is likely to have value rather than be hazardous but the beneficial effects are seldom preached [40]. Reported effects of lactate production and consequently acidosis include the greater release of O2 from hemoglobin for working muscle fibers (the Bohr Effect), stimulation of ventilation, enhancement of muscle blood flow, and afferent feedback to the CNS to increase cardiovascular drive [40-44]. Speaking

of specific values within kickboxing, Point Fighting seems to be the least demanding discipline in terms of anaerobic requirements. Every lactate value serves to certain movement tactics and metabolism-related challenges outside and within the organism, and measurements of them provide insight into the current state of the human organism. Besides the discipline rules and discipline-specific tactics, maximal and mean values, thresholds, growth intensity and other properties of the lactate system also depend on the individual's anthropometric features, fitness level, overall health, task specificity, opponent's fitness level, etc. As can be seen in Table 7, lactate concentration significantly and rapidly increased after all observed protocols. Ouergui et al. [45,46] and Cimadoro [47] measured kickboxers' lactate values complemented with performance tests following the kickboxing match. In addition, Ouergui et al. [21,45,46,48], Salci [49], and Rydzik et al. [50] used heart rate (HR) monitoring as a complementary method, while a majority of observed studies [21,45-49] implemented rating of perceived exertion (RPE).

Results/curves of HR and RPE were reported to be distinctly close correlated with the lactate curve. The second study from Table 6 [45] suggests that blood lactates increased significantly during the combat and reached 14.93 ± 0.71 mmol·L^{-1} for the active rest (AR) group and 14.87 ± 0.69 mmol·L^{-1} for the passive rest (PR) group, meanwhile, AR had significantly lower levels of lactates at the 5th and 10th minutes after the match. Cimadoro [47] reported that 58% of the variance in lactates can be explained by the total number of blows in the first two rounds. Ouergui et al. [21] suggest that the rate of lactate production during kickboxing matches is classified as strongly anaerobic (>6 mmol·L^{-1} · 5 min^{-1}). Second, winners and losers did not differ from each other in hormonal, physiological, and physical parameters, while winners used more attacking techniques, especially jab cross, and the roundhouse kick. Salci [49] revealed a significant reduction of the hamstring/quadriceps strength ratio between the first and third rounds. Only a study that compared kickboxing disciplines mutually, suggests that effort-pause ratios were 1:2, 1:3, and 1:4 for full contact, light, and point fighting combats, while their HR did not significantly differ [49]. Listed ratios suit the lactate results for each discipline as shown in Table 7.

Table 7. Papers with lactate analysis included (n=7).

Author(s)	Blood lactate concentration (mmol·L^{-1})	
Ouergui et al., 2013	1R[1]	8.63±0.87
	2R[1]	11.72±0.85*
	3R[1]	14.93±0.71*
Ouergui et al., 2014	1R[2]	8.82±0.73*
	2R[2]	11.75±0.92*
	3R[2]	14.87±0.69*
Cimadoro, 2018	Before	1.9±1.1
	After	15.3±1.6*
Rydzik et al., 2022	1R	11.3±1.4*
	2R	13.1±1.2*
	3R	14.6±1.9*
Salci, 2015	1R-first/second fight	7.22 ± 1.75/6.80 ± 1.12
	2R-first/second fight	10.92 ± 2.53/10.01 ± 2.97*
	3R-first/second fight	12.14 ± 2.21/11.49 ± 3.18*
Ouergui et al., 2021	Light Contact pre/post	2.2 ± 0.5/15.8 ± 4.0**
	Full Contact pre/post	1.9 ± 0.5/15.2 ± 5.3*
	Point fighting pre/post	1.9 ± 0.5/13.2 ± 2.6*
Ouergui et al., 2016	Before	2.0±0.6
	After	14.0±1.8*

1R- first round, 2R- second round, 3R- third round, [1,2]- same sample, [1]- active rest group, [2]-passive rest group

DISCUSSION

Hormonal shifts

Hormones as signal molecules play an important role in the overall performance and fitness status of athletes. Extracted studies include information about several hormones including cortisol (blood and saliva), testosterone, growth hormone (GH), irisin, and myostatin. The prematch cortisol values seem to be higher than the normal values reported in the literature [19,51,52]. In addition, cortisol levels could be higher before competition due to an increase in stress [19]. Cortisol is a well-known stress hormone and measurements of this hormone can give accurate information about the stress level of competitors. Results show that winners had lower concentrations before and after the match in regards to losers although winners utilized more attack techniques. Such facts could be explained by the different psychological approaches found between winners and losers, competitive experience level, and attack-related stress release. A possible correlation between attack techniques and psychosocial stress release (cortisol decrease) could also be an answer for higher testosterone levels among amateurs because amateurs tend to perform attack actions as a psychosocial stress-relief mechanism, which are correlated with higher testosterone levels [21,50]. Other studies [53] suggest that psychosocial stressors, as well as physical stressors, increase lactate levels. Testosterone is the key hormone for combat sports, due to its impact on muscle properties, power, assertiveness, competitiveness, and aggressiveness, as a mechanism inherited from the very first humans. According to the review [54] about hormones in combat sports, a moderate increase of testosterone was observed after a match among combat competitors with a greater increase among amateurs than professionals, which could be explained by the inversely proportional relationship between experience and vigilance level. The authors of the current review suggest that cortisol and testosterone have great potential for monitoring psycho-physical status among combat athletes. Physical activity stimulates the excretion of GH, which stimulates the synthesis of proteins, cellular glycolysis, bone growth, and fat consumption. The revealed result suggests that the intensity of full-contact combat was sufficient to stimulate the pituitary gland, stimulating GH production, with no differences observed between winners and losers. The following analyzed hormones were irisin and myostatin. Irisin is a myokine released into the bloodstream by cleavage of the fibronectin type III domain-containing protein 5 (FNDC5) triggered by muscle contraction [55]. The selected hormone is associated with the browning response and thermogenesis of white adipose tissue (WAT). In this process, irisin induces the mitochondrial uncoupling protein (UCP)-1 and other brown fat-like genes in WAT, shifting WAT toward a beige/brown phenotype and leading to gene expression, morphological changes and mitochondrial activity typical of the brown adipose tissue (BAT) [56]. From obtained results, it can be concluded that kickboxing chronically positively affected the increase of irisin concentrations, while acute responses were inverted. Speaking of myostatin, inhibition of this hormone leads to muscle hyperplasia and hypertrophy. Myostatin inhibitors can improve athletic performance and therefore there is a concern these inhibitors might be abused in the field of sports [57]. Results of analyzed kickboxers show significant inhibition of myostatin 3 hours after the fight, which represents a valuable insight into muscular responses to kickboxing matches. However, there is an evident lack of hormonal analyzes in kickboxing, with only one conducted study per analyzed hormone (except cortisol). Further experiments should include other hormones potentially relevant for kickboxing performance and activity, e.g. adrenaline, noradrenaline, glucagon, insulin, estrogen, thyroxine, and Insulin-like Growth Factors (IGF), etc.

Genetics

Nearly 200 genetic polymorphisms have been found to influence sports performance traits, and over 20 polymorphisms may condition the status of the elite

athlete [58]. Still, an advantageous genotype not always translates into the phenotype of a champion, since a variety of psychological and environmental factors still influence gene expression [59]. Current evidence about the possible ideal genetic profile in kickboxing is almost non-existent. Only study about genetics in kickboxing that was recently conducted by Eken et al. [24] could determine only proportions of selected alleles (C, G) and genotypes (CC, CG, GG) on ADRA2A gene among kickboxers, but values were not significantly different from the control group. Talent/sports performance phenotype identification based on DNA testing is likely to be of limited value at present, and that field testing, which is essentially a higher-order 'bioassay', is likely to remain a key element of talent identification in both the near and foreseeable future [58,60].

Immune parameters

Immune status is a very important aspect of the overall functionality and living of human organisms. Regular physical activity chronically enhances immunity quality, along with other factors, such as optimal diet and habitat. Intense/prolonged acute exercise triggers an immunosuppression period that lasts longer in unfit individuals [61]. Contrary to that, performing kickboxing matches does not influence acute: saliva flow rate, salivary IgA absolute concentration, or salivary IgA secretion rate [19]. The second study [20] reported the immune profile of kickboxers, while their results were within the healthy referent ranges. Authors of the work suggest that athletes undergoing extreme training regimes can suffer from short-term immunosuppression and increased infection risk, which implies the possible applicability of immunoassay in sports, as a monitoring tool for evaluation of acute immune status. Although, only two studies analyzed parameters of immune status, more experimental work is needed for the final confirmation of such results.

Acid-base balance

During intense physical activities, blood pH can be reduced up to 6.8 (7.4 is optimal). At rest, an equilibrium is maintained between the pulmonary gas pressures (pO2 and pCO2) in the human body, and the buffering system in the blood consists of hydrogen ion (H+) acceptors, which include bicarbonate ions (HCO3−), proteins, amino acids, hydrogen phosphate ions (HPO4 2−), and hemoglobin contained in erythrocytes [35]. Muscle fatigue occurs due to H+ accumulation because the mitochondrial function and enzymatic activity are impaired [62] and as a consequence, the production of glycolytic energy is disrupted [62-64], as well as muscle functions too. Robergs et al. [65] pointed out that part of the H^+ comes from ATP hydrolysis ($ATP^{4-} + H_2O \rightarrow ADP^{3-} + HPO^{2-}_4 + H^+$), and that reducing pyruvate to lactate ($pyruvate^- + NADH + H^+ \rightarrow lactate^- + NAD^+$) consumes H^+. Further, the maintenance of a higher concentration of HCO3− results in faster removal of H+ from muscle cells [62,66]. The maintenance of alkalinity in intracellular fluids enables a faster removal of H+ from muscle cells resulting in delayed muscle fatigue which occurs due to increased acidosis [65]. Looking at Table 5, there is no doubt that kickboxing requires great anaerobic capacity close to maximal, with the continuous growth of blood lactates in parallel relation with the match time. The significant growth of H+ and decrease of HCO3- during the match confirm such a statement. From the revealed results it can be concluded that point fighting (PF) has slightly lower requirements of anaerobic capacity, due to larger pauses caused by the central judge, while other disciplines have reported similar lactic values. In general, H+, HCO3-, La, and other measured significantly changed acid-base parameters can be sensitive biomarkers for monitoring overall competitors' fatigue, functional status, and alkalinity capabilities.

CONCLUSION

Winners tend to have lower concentrations of cortisol compared to other competitors. On the other side, testosterone affects muscle properties, power, assertiveness, competitiveness, and aggressiveness, and all competitors tend to have moderately increased levels of testosterone after the match. Speaking of GH, results show that the intensity of full-contact (only observed discipline) combat is sufficient to stimulate GH production. Kickboxing has been shown to chronically increase irisin concentrations, while acute responses were inverted. Results from studies regarding myostatin show significant inhibition of myostatin 3 hours after a fight, providing a better insight into the acute muscular responses to kickboxing. From a hematologic perspective, kickboxing has a positive impact on blood health, as all measured parameters were within healthy reference ranges, with slightly higher values of hemoglobin, haptoglobin, and high sensitivity C-reactive protein. Biochemical/histochemical analyzes reported an acute increase in glucose, indicators of lipid peroxidation activity, H+, BEecf, lactic acid, and pyruvic acid, as well as the chronic increase of ferritin and interleukin 6. Kickboxing seems to be strongly anaerobic glycolytic activity according to obtained values of blood lactates with an average value of 14.15 ± 2.4 after the third round. Finally, according to the available evidence, the parameters of immune status did not change. Reported immune profile suits as the parameter of chronic impact, while the salivary immune parameters represent the acute immune systems response to kickboxing. The majority of reported parameters overall can be successfully used as biomarkers of elite athletes' status. Endocrinal, acid-base, and immune analyses are substantial and irreplaceable tools for the evaluation of an elite kickboxer's acute fitness status. On the other hand, hematologic parameters, and biochemical/histochemical profiling seem to be useful to scientists because they allow insight into chronic body adaptations to kickboxing training and competition periods, or chronic adaptations before entering kickboxing. Finally, genetic testing in kickboxing could be utilized as an auxiliary tool in processes of talent identification, to determine someone's potential for a certain discipline within kickboxing, and such processes should be conducted with awareness regarding ethical considerations. Further work on listed fields should especially include female samples, kickboxing discipline differentiation, gender, weight, and age stratifications.

Funding: This work was supported by the Croatian Science Foundation under Project Grant No. [IP2020-02-3366]. The sponsors did not have any role in the study design, data collection, analysis,decision to publish, or preparation of the manuscript.

Institutional Ethical statement: Ethical approval for this research was obtained in advance through the Croatian Science Foundation project (IP-2020-02- 3366), approved by the Ethics Committee and in accordance with the Ethical codex of the Croatian Science Foundation. Research has been conducted in accordance with the principles expressed in the Declaration of Helsinki.

Data Availability Statement: Data supporting reported results can be found within scientific bases in which authors have conducted the review, through the following links: https://scholar.google.com/; https://pubmed.ncbi.nlm.nih.gov/; https://www.webofscience.com/wos/woscc/basic-search/

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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