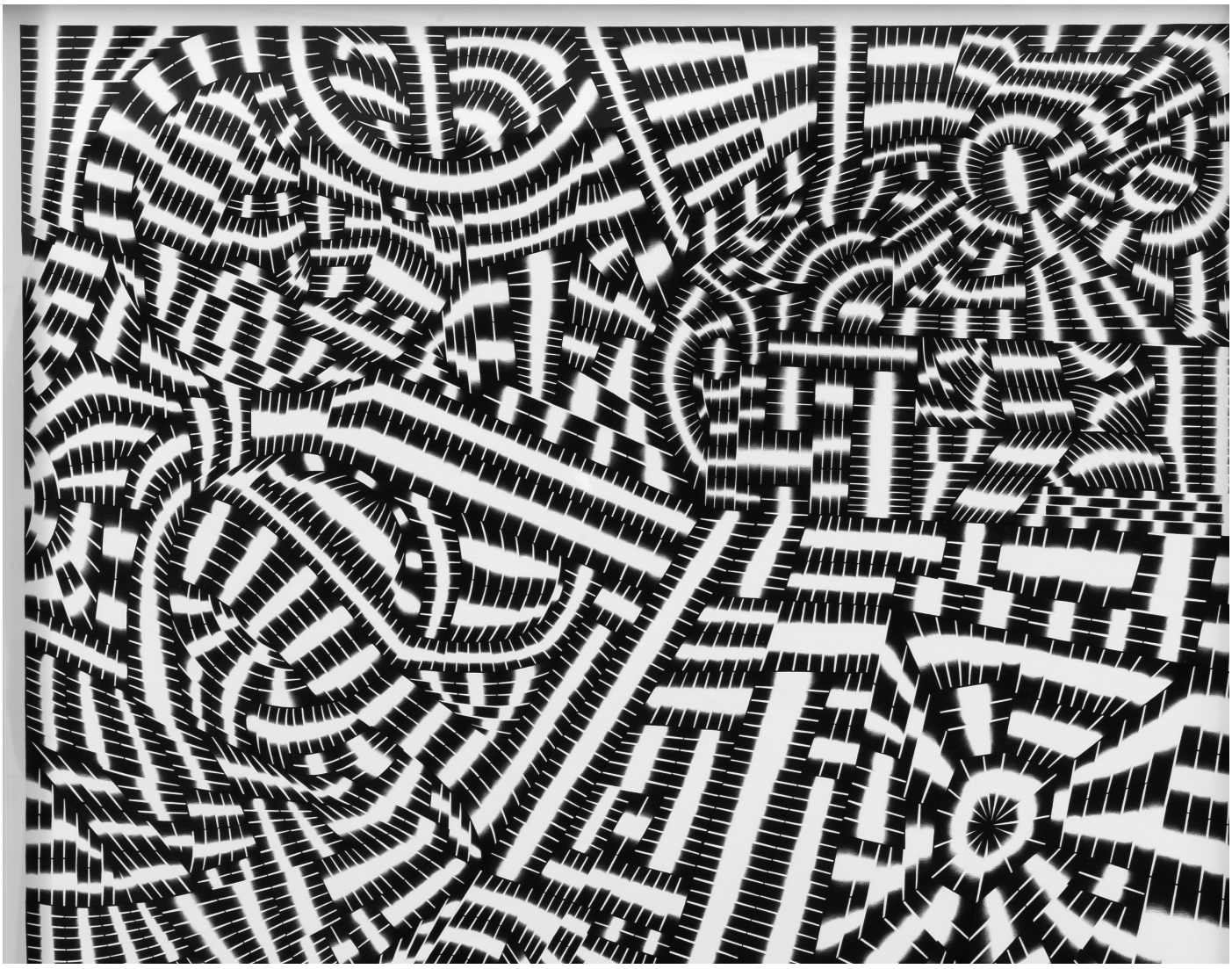
Volume 7, Number 1, April 2023.
ISSN 2459-9484
seemedj.mefos.unios.hr

# SEEMEDJ SOUTHEASTERN EUROPEAN MEDICAL JOURNAL



**Lana Ključarić, Five minutes before sleep (2), 2015**
drawing, ballpoint pen on paper, technical pen, rulers
MLU-3862

Josip Juraj Strossmayer University of Osijek
Faculty of Medicine

*This seemingly simple technique, a ballpoint pen on paper, is an experimental procedure like a stream of consciousness. How many expanses are there that can be conquered? How far does her expressive power go? At the intersection of the artist's inner life and the work of art itself, the drawing technique and its aesthetic effect is the focus of our interest. Through close and detailed observation of each individual work, or through its details and the strokes of a ballpoint pen, we testify to the duality of a person and her work. The drawings are deliberately liberated from linguistic expression, political commentary or psychic tension, but controlling the hand in these efforts towards perfecting and abstract-aesthetic effect is only seemingly opposed to the communicative function of the work. The human need for storytelling, and when, as with Lana Ključarić, is deliberately silenced, it comes to the surface. Through stories, we define ourselves as individuals and as cultures – our personal narratives determine our place in the world and in life, we constantly repeat them until they become a lens through which we observe the reality that surrounds us. We pass them on through generations like a genetic code and adapt them to each individually. We create for ourselves the horizons of our own journeys and the goals behind them. - V. Radoš (2017)*

**Southeastern European Medical Journal (SEEMEDJ)**
**Published by**
University Josip Juraj Strossmayer Osijek
Faculty of Medicine Osijek
**for publisher:** Ivica Mihaljević, MD, PhD, - Dean of Faculty of Medicine Osijek, Croatia



**Published online:**
**http://seemedj.mefos.unios.hr**
**ISSN 2459-9484**

Since 2021 the journal is published with the financial support of the
Ministry of Science, Education and Sports of the Republic of Croatia

## Contents

# Instead of an Editorial: Some Statistics on First Five-Year Period of Publication of Southeastern European Medical Journal (SEEMEDJ), From 2017-2022.

Ines Drenjančević[1], Marija Raguž[1]

1 Faculty of Medicine Osijek, University Josip Juraj Strossmayer Osijek, J. Huttlera 4, 31000 Osijek, ines.drenjancevic@mefos.hr; maraguz@mefos.hr

Southeastern European Medical Journal (SEEMEDJ), has been published by Faculty of Medicine Osijek, University Josip Juraj Strossmayer Osijek, since 2017. At the moment we have published 6 full volumens; this issue is the first of two, of the seventh volume. As a brand new scientific journal we have undergone all phases of development, necessity for recongnition in the scientific community as a desirable destination for quality manuscripts.

One of the aims of SEEMEDJ is to provide support for young researchers who are at the beggining of their scientific and professional carriers, such as PhD students. Part of fullfiling that aim is that SEEMEDJ uses the Diamond Open Access model. This means that there are no author processing fees and no fees to access the published papers. There are no article submission charges. The free access to full published manuscripts is available on our web page as well as in HRCAK.

In last five-year period, Southeastern European Medical Journal (SEEMEDJ) has been covered by following databases and archives: **Indexing & Abstracting Services**: DOAJ, Google Scholar (individual articles), OAJI, Publons, Crossref, Dimensions. **Journal Directories**: SHERPA/RoMEO and **Digital Preservation**: Hrčak

Following statistical data have been obtained from the SEEMEDJ archive. From 2017-2022, SEEMEDJ has published 105 scientific articles; 366 authors contributed to manuscripts, of which 6 % (22 authors) published 3 or more articles. Majority of our authors come from Croatia (Figure 1. Geographic origin of authors who have published in SEEMEDJ). However, we have authors from 9 countries and a number of different institutions.

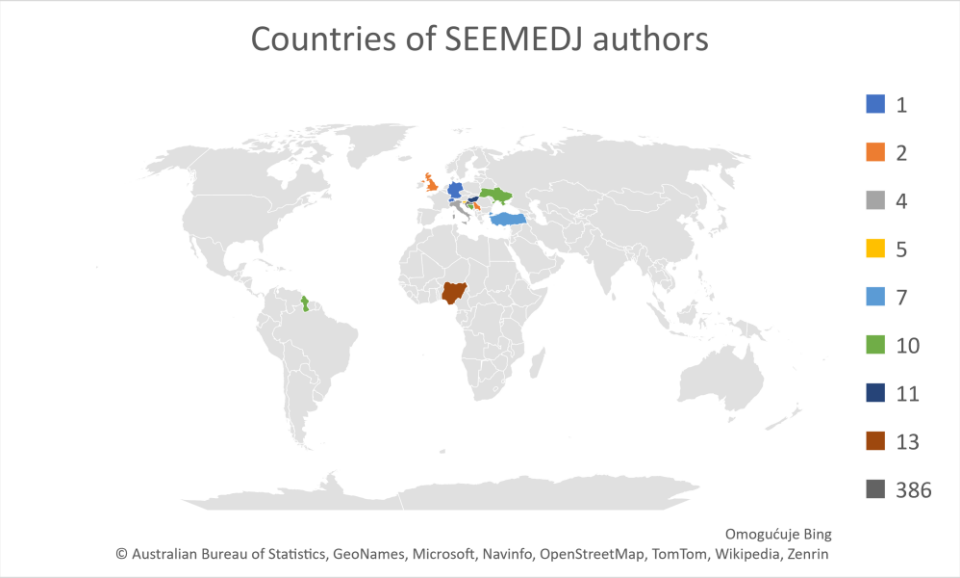
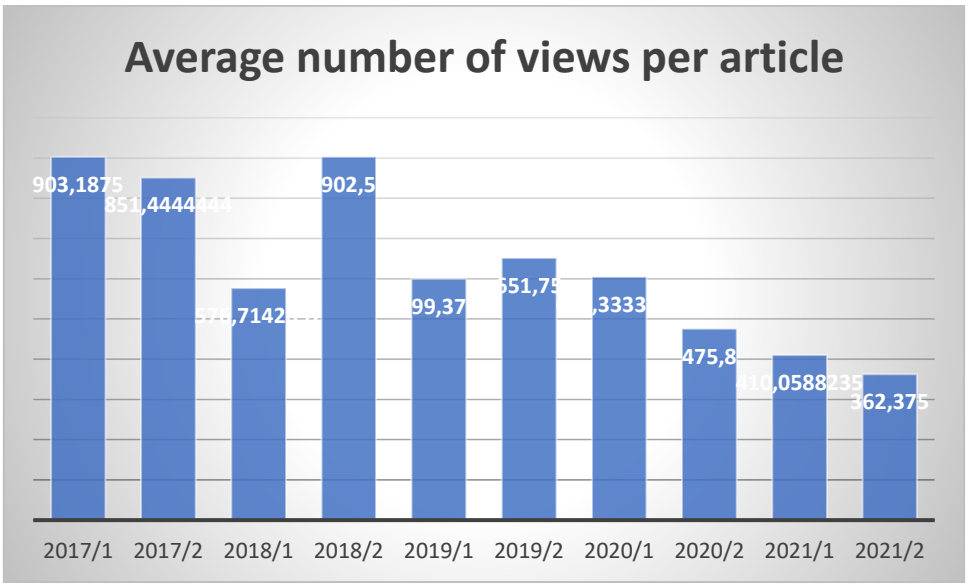



Figure 1.

The average number of views per article  is shown in Figure 2. The range is from 362 to 903 views per issue). At the moment SEEMEDJ has only 6 WoS citation, 25 Scopus and 160 Google Scholar (no duplicates).

Thus, our goals for future period are to increase visibility of the journal, to attract new authors from other countries and to increase quality of published work and subsequently increased citation and achieve indexation in other emintent indexation databases.

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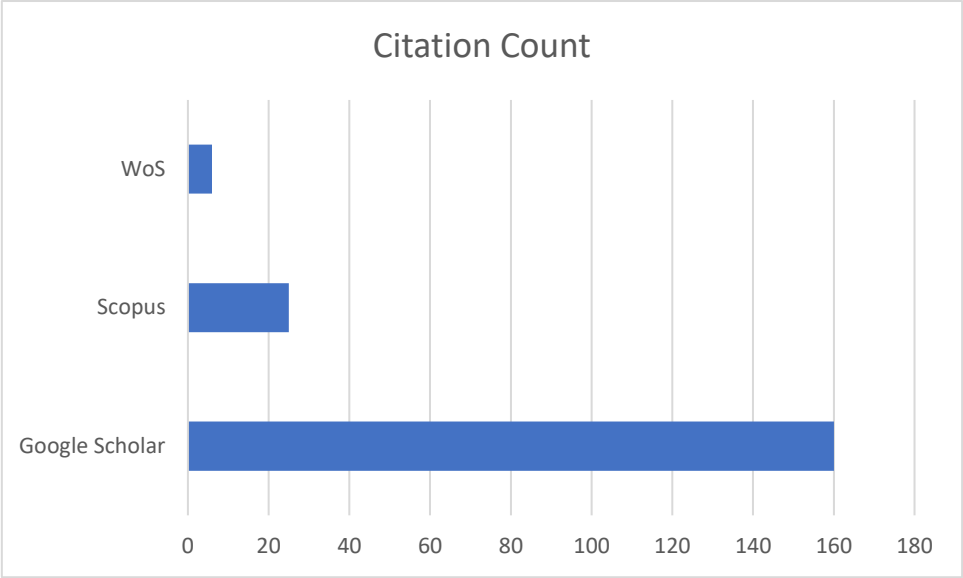
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Figure 3.

Invited review

# Anti-Inflammatory and Antioxidant Response in COVID-19 Infection: Nrf2/HO-1 Pathway

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## Abstract

SARS-CoV-2 virus infection starts with the internalization of the viral particle into the host cells, mainly the upper respiratory system epithelial cells which have the highest expression of the ACE2 receptor which is essential for the internalization process. The pathophysiology of severe forms of COVID-19 disease results not only from direct, cytopathic viral effect but also from immune response dysregulation of the host resulting in hyperinflammatory state and oxidative stress. The nuclear factor erythroid 2-related factor 2 (Nrf2) ability to protect cells and induce a rapid anti-inflammatory and antioxidant response primarily depends on its constitutive cellular expression, which can be affected by numerous endogenous and exogenous factors. The binding of Nrf2 to cellular receptors leads to the transcription of a large number of genes encoding various antioxidant enzymes and other cytoprotective molecules, including heme oxygenase-1(HO-1). Activation of HO-1 results in antioxidant, anti-inflammatory and anti-apoptotic effects. Based on previous studies, the Nrf2/HO-1 pathway provides protection against oxidative stress and inflammatory and immune response which is significant in COVID-19 infection, which is characterized by a strong hyperinflammatory response. This narrative review aims to describe the role of the hyperinflammatory response in the development of COVID-19 infection, with a focus on the NrF2/HO-1 pathway.

(Maričić L*, Mihić D, Šego N. Anti-Inflammatory and Antioxidant Response in COVID-19 Infection: Nrf2/HO-1 Pathway. SEEMEDJ 2023; 7(1); 1-12)

Received: Mar 7, 2023; revised version accepted: Apr 6, 2023; published: Apr 30, 2023

KEYWORDS: COVID-19, SARS-CoV-2, Heme oxygenase-1, Nrf2 factor

## Introduction

Coronaviruses belong to the RNA viruses group with the largest genome among the rest of the RNA viruses (27–37 kb). Virus particles have a characteristic membrane with spikey structured proteins on the outer surface, which is where the name coronavirus (lat. corona, crown) comes from. Three groups of coronaviruses differ genotypically, of which viruses from group III are found only in birds, while viruses from groups I and II are also found in mammals. Coronaviruses mainly cause respiratory system diseases in humans, and at least four variants continuously circulate within the population, especially among young children. Two variants were identified in the mid-1960s (hCoV-OC43 and hCoV-229E), and the other two in the early 2000s (hCoV-NL-63 and hCoV-HKU1). The first coronavirus linked with a more serious illness was identified at the end of 2002. Due to its tendency to cause severe acute respiratory syndrome (SARS), it was named SARS-CoV. It's believed to be of wild animals origin, most likely bats, and was transmitted to humans via infected bats that were prepared and sold as food in China [1]. The SARS-CoV epidemic has affected 29 countries, 8.096 cases were registered, and 774 deaths were confirmed, according to which the estimated mortality was 9.6%. Ten years later, in April 2012., another coronavirus associated with severe forms of respiratory infections emerged, and due to its Middle Eastern origin, this strain has been named MERS-CoV (Middle East Respiratory Syndrome). Concurrently with the sporadic isolation of MERS-CoV, in December 2019., a new coronavirus named SARS-CoV-2 was isolated in the city of Wuhan, China. and rapidly spread, not only in China but also in other continents, therefore becoming the first coronavirus that caused a global pandemic[2,3].

### Disease Background

Despite numerous research, the SARS-CoV-2 origin remains unclear. The most common theory of this virus origin is associated with bats, which are natural reservoirs of the virus, especially considering the high homology of the genome of viruses isolated from bats with the human SARS-CoV-2 virus, which amounts to 96.2%. Initially, infections of humans by the SARS-CoV-2 virus appeared as a classic zoonosis since most of the first confirmed coronavirus cases had direct contact with animals at the market in Wuhan. The first patients were believed to be infected by consuming infected animals that were prepared as food or sold alive, with bats being the most frequently involved [4]. The rapid spread and appearance of the disease among healthcare workers and people who did not have direct contact with infected animals indicated human-to-human transmission. Since it's mainly a respiratory disease and viruses are isolated from the epithelial cells of the respiratory tract, it can be assumed that the main transmission route of the virus between people is droplet transmission, i. e. through aerosols created when speaking, sneezing or coughing [6,5]. Although SARS-CoV-2 has been detected in urine, stool, blood/serum, saliva, and tears, there is no strong evidence that it can be transmitted in such a manner.

### Structure and pathogenesis of SARS-CoV-2 infection

The SARS-CoV-2 genome size is 29.9 kb, and two-thirds of the genome encodes proteins necessary for virus replication. One-third encodes structural proteins: protein E (envelope protein), protein S (spike protein), protein M (membrane protein), and protein N (nucleocapsid protein). Proteins S, M, and E form the virus particle envelope, and protein N, which binds the RNA molecule to itself, is found inside the virus particle [7,8]. The SARS-CoV-2 genome is highly variable, resulting from errors that occur during RNA molecule replication, whereby they accumulate and create new mutations, resulting in the emergence of new viral subvariants or strains. To date, more than 20 strains of this virus have been described. As some mutations cause pathogenicity or virulence modifications (either positive or negative ones), the World Health Organization identifies five variants of concern (VOCs), given their public health impact: alpha (B.1.1.7) which was originally isolated in the UK in late December 2020., beta (B.1.351), which was

first reported in South Africa in December 2020., gamma (P.1), which was first reported in early January 2021. In Brazil, delta (B.1.617.2), first reported in December 2020. In India and omicron (B.1.1.529), first reported in South Africa in November 2021. [9]. Both pathogenicity and virulence of the SARS-CoV-2 result from its ability of cellular internalization, i.e., the ability of the virus to enter cells. The entry of the SARS-CoV-2 virus into the cells is mediated by the S protein of the virus particle and the ACE2 receptor (Angiotensin-Converting Enzyme 2) of the host cells. ACE2 receptor was identified in 2003. and the SARS-CoV-2 and other coronaviruses (hCoV-229E, hCoV-OC43 and hCoV-HKU1) are uptaken via aforementioned receptor. Protein S contains two subunits: the S1 subunit enables viral particle binding to the ACE2 receptor via the receptor-binding domain (RBD), while the S2 subunit enables the fusion of the viral particle and the host cell membranes [10]. In addition to the ACE2 receptor, integrins and CD174-SP are also mentioned as potential receptors for the internalization of SARS-CoV-2 into host cells. There is no strong experimental evidence for these assumptions [11,12]. Since ACE2 has a wide biodistribution in the organism, including the respiratory system, digestive system (small and large intestine), myocardium, kidneys, thyroid gland, liver, brain, and olfactory neuroepithelium, SARS-CoV-2 has a broad tissue and cell tropism, resulting in wide clinical presentation and a clinically diverse manifestation [13]. Despite the broad distribution of these receptors, the respiratory system is predominantly and most severely affected by this viral infection. This can be explained by the fact that the respiratory system epithelial cells have the largest expression of ACE2 receptors, as well as the fact that the main transmission route of the disease is droplet, and the respiratory system mucous membrane is the initial site for the virus entrance into the body. Epithelial cells of nasal mucosa and bronchial epithelial cells are the site of the largest ACE2 receptor expression, while in the alveoli the ACE2 receptor is found only in type II pneumocytes [14]. Upregulated expression of the ACE2 receptor in the respiratory system epithelial cells is affected by proinflammatory cytokines that are produced during infection, especially IL-1$\beta$ and type I and III interferons, which can accelerate its spread and replication [15].

*Clinical characteristics COVID-19 infection*

The respiratory system is the main organ system affected by this disease, but due to broad SARS-CoV-2 virus tropism, other clinical manifestations are possible as the result of other tissues and organs involvement. It's a highly contagious illness that has a wide clinical spectrum, that varies from asymptomatic to mild, severe, and critical (29). Clinically, COVID-19 infection is most often presented as a common cold or a flu-like illness, which in certain individuals can progress to more severe forms, leading to pneumonia and acute respiratory distress syndrome, and also to manifestations that are caused by other tissues and organs involvement [16]. Acute respiratory distress syndrome (ARDS) represents the most severe form of the respiratory system involvement by the SARS-CoV-2 virus and is accompanied by a high rate of severe complications such as sepsis, septic shock, and multiorgan failure, all of which increase the mortality rate. ARDS is characterized by new-onset severe respiratory insufficiency and bilateral pulmonary infiltrates, which occurs as a result of still insufficiently elaborated pathophysiological mechanisms that include the direct viral cytopathic effect, immune response dysregulation (hyperinflammatory state or cytokine storm) as well as increased oxidative stress. The final result is extensive lung parenchyma damage and consequently severely impaired oxygen and carbon dioxide exchange (hypoxemia, hypercapnia) [17]. As previously discussed, SARS-CoV-2 predominantly affects the respiratory system, but other organs and organ systems can also be affected, either as a single organ disease, but most common with the respiratory system involvement. The most common extrapulmonary COVID-19 manifestations involve gastrointestinal and hepatobiliary, hematological, cardiac, renal, and neurological systems [16].

*Immune response in COVID-19 infection*

SARS-CoV-2 virus infection starts by the internalization of the viral particle into the host cells, mainly the upper respiratory system epithelial cells which have the highest expression of the ACE2 receptor which is essential for the internalization process. The viral entry into the cells triggers an immune response whose role is to neutralize the virus and bring the infection under the control. Today, it's considered that the pathophysiology of severe forms of COVID-19 infection results not only from direct, cytopathic viral effect but also from immune response dysregulation of the host resulting in hyperinflammatory state and oxidative stress [18]. By viral particle entrance and the viral RNA release in the host cell cytoplasm begins a cellular response, in addition to the process of virus replication. The cellular response aims to activate the immune response as a protective mechanism of the host against the virus [19]. In this process PRR receptors (Pattern recognition receptors) are essential. These are found in epithelial cells cytoplasm and have role of recognizing PAMP molecules (Pathogen-associated molecular patterns) [20]. These PRR receptors recognize long chains of viral RNA that are formed as intermediates during replication, causing their activation and triggering signaling pathways that include interferon regulatory factor (IRF) and nuclear factor-$\kappa$B (NF-$\kappa$B) [21]. IRF promotes the transcription of interferons I and III, while NF-$\kappa$B promotes the transcription of pro-inflammatory cytokines and chemokines [22]. Interferons, pro-inflammatory cytokines, and chemokines are responsible for chemotaxis and mutual interaction of innate immunity cells: neutrophils, monocytes that differentiate into macrophages, dendritic cells and NK cells. They form the first line of defense of the organism against the SARS-CoV-2 virus. In addition to innate immunity cells, T and B lymphocytes also participate in the immune response to the SARS-CoV-2 virus. After phagocytosis of infected epithelial cells, antigen presenting cells (APC), among which respiratory dendritic cells dominate move to regional lymph nodes and present there the viral antigen, which is expressed on their surface trough molecules of the MHC complex (Major histocompatibility complex), to the naïve circulating T lymphocytes. Viral antigens are recognized by T-cell receptors (TCR) of naïve T lymphocytes, and as a result, their interaction leads to their sensitization, whereby they are activated, proliferate, and migrate to the infection localization. At the infection localization activated cytotoxic T lymphocytes (CD8+) produce antiviral cytokines (INF-$\gamma$, TNF-$\alpha$, IL-2) that inhibit viral replication, chemokines (CXCL-9, 10 and 11) that enhance the chemotaxis of other effector T lymphocytes and cytotoxic molecules (perforin, granzyme B) that kill infected cells, thus trying to eliminate the virus. In addition to classic CD4+ and CD8+ lymphocytes, the so-called unconventional lymphocytes T (uT) that accumulate in the mucous membranes, especially of the respiratory system, are also a part of immune reaction against the SARS-CoV-2 virus. They represent a heterogeneous T lymphocytes group that can recognize non-peptide viral antigens and are not limited to the presentation of antigens via the MHC system like the classic lymphocytes [23]. The humoral immune response is the most effective and long-lasting immune mechanism in terms of host defense against viruses, including the SARS-CoV-2 virus. It's based on the interaction of naïve B lymphocytes with antigen and CD4+ T lymphocytes, the ultimate consequence of which is maturation into plasma cells and the production of specific antibodies [24]. In the host's battle with the SARS-CoV-2 virus antibodies targeted at the RBD domain of the S protein are the most effective because they most successfully block the binding of the virus particle to the ACE2 receptor on the cell surface [25].

*Hyperinflammatory immune response and cytokine storm in COVID-19 infection*

Immune system dysregulation in response to the presence of the SARS-CoV-2 virus, and not only the cytopathic effect of the virus, is responsible for development of more severe clinical forms of the COVID-19 infection [18]. Hyperinflammatory response is a condition

resulting from uncontrolled activation of the immune system, which is primarily manifested by increased production and release of pro-inflammatory cytokines, which is known in clinical practice as a cytokine storm or cytokine release syndrome. The current focus in the development of hyperinflammatory syndrome pertains to dysregulation of the host's innate immunity response cells in the presence of the SARS-CoV-2 virus [26]. The basis of this theory is pyroptosis, the programmed cell death of infected cells, which is activated during viral RNA replication. This is why pyroptosis is also known as an inflammatory form of programmed cell death. During pyroptosis, various molecules that can function as stimulators of innate immunity cells are released [27,28]. Among these is IL-1 $\beta$, whose high levels are detected in the serum and bronchoalveolar lavage of patients suffering from severe forms of COVID-19 disease [29]. By disruption of infected cells during pyroptosis parts of viral RNA that act as PAMP molecules (Pathogen-associated molecular patterns) and other viral proteins are released. These are recognized by previously described PRR receptors of the surrounding cells and trough the IL-1R signaling pathway stimulate transcription and production of proinflammatory cytokines such as IL-6, IL-8, IL-10, TNF-$\alpha$, INF-$\gamma$, MCP, MIP and others. They lead to chemotaxis of macrophages and other effector cells of innate immunity as well as T lymphocytes which further enhance the immune response, cause local damage of cells and tissues and additionally produce cytokines resulting in constant amplification and stimulation of the immune response. Finally, produced cytokines also enter the circulation and can cause damage in distant tissues and organs, which explains the occurrence of multiorgan failure in critical patients with COVID-19 [30,31]. The hyperinflammatory state or cytokine storm is considered one of the main factors in the development of the most severe forms of COVID-19 infection, which is acute respiratory distress syndrome (ARDS) and associated multiorgan failure [32]. Multiorgan failure is promoted by activation of endothelial cells mediated by TNF-$\alpha$ and IL-1$\beta$, which brings to increased expression of selectin P, fibrinogen and von Willebrand factor, as well as induced tissue factor release, which initiates the coagulation cascade and the formation of clots, especially at microcirculatory level [33].

*Oxidative stress in COVID-19 infection*

The production of reactive oxygen species and oxidative stress is one type of the host response to the presence of viruses in its cells and is part of the overall defense against infection, due to active participation of reactive oxygen species in elimination of pathogens. Also, several reactive oxygen species are involved in intercellular and intracellular signaling pathways of the immune system cells [34]. There are several assumed mechanisms of increased reactive oxygen species concentration in COVID-19 infection. The general mechanism is based on the renin-angiotensin-aldosterone system, that is, the interaction of angiotensin II and NADPH oxidase. Angiotensin II is known as a stimulator of NADPH oxidase, which generates reactive oxygen species and oxidative stress. Under physiological conditions, ACE2 catalyzes the conversion of angiotensin II to angiotensin, which doesn't affect NADPH oxidase, but moreover, has an antioxidative effect. During the SARS-CoV-2 virus infection, the availability of "free" ACE2 decreases due to its binding to the virus and/or entry into cells. The result is an increased concentration of angiotensin II and NADPH oxidase stimulation, which causes an increased production of reactive oxygen species and oxidative stress [35,36]. Therefore, the inflammatory reaction intensity and the degree of innate immune response dysregulation (excessive activation of monocyte-macrophage system cells) are proportional to oxidative stress level, and reactive oxygen species together with proinflammatory cytokines lead to the cell-tissue damage present in patients suffering from severe forms of COVID-19.

*Nrf2/HO-1 signaling pathway in COVID-19 infection*

The relation between the hyperinflammatory state and oxidative stress represents a great challenge in patients suffering from COVID-19

infection, especially in terms of discovering molecular pathways that could potentially be therapeutic targets and whose modulation could reduce the inflammatory response and oxidative stress, essential elements in the development of severe forms of COVID-19 disease (Figure 1).

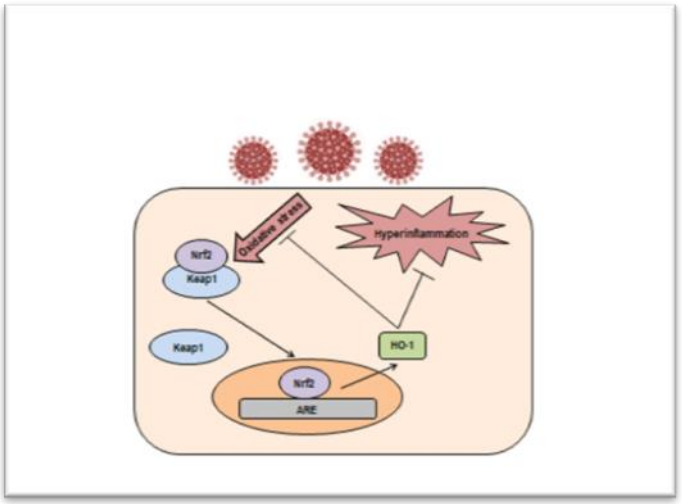


Figure 1. Nrf2/HO-1 signaling pathway in COVID-19 infection

The transcriptional factor Nrf2 (Nuclear factor erythroid 2-related factor 2, Nrf2) is one of the most frequently researched molecules in terms of the cellular anti-inflammatory and antioxidant response in viral infections. The ability of Nrf2 to protect cells and induce a rapid anti-inflammatory and antioxidant response primarily depends on its constitutive cellular expression, which can be affected by numerous endogenous and exogenous factors [37]. The basic binding site of Nrf2 is the promoter region of DNA, ARE region (antioxidant response element), responsible for the transcription of genes whose products are part of the antioxidant response [38]. The binding of Nrf2 to the ARE region leads to the transcription of a large number of genes encoding various antioxidant enzymes and other cytoprotective molecules. These are enzymes that participate in the metabolism of iron and heme (heme oxygenase-1), enzymes that participate in the direct neutralization of reactive oxygen species (NADPH quinone reductase, superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase), as well as enzymes that participate in the synthesis and regeneration of glutathione and NADPH (glutamate cysteine ligase, glutathione reductase, glucose-6-phosphate dehydrogenase) [39]. In addition to the previously discussed effect of Nrf2 by binding to the ARE region, there are also findings of its direct anti-inflammatory effect, independent of gene expression. Kobayashi et al. described in their research that Nrf2 can reduce the expression of pro-inflammatory cytokines (IL-1, IL-6) in activated macrophages by directly inhibiting their transcription in the cell nucleus [40]. Heme oxygenase-1 (HO-1) is commonly researched enzyme that is produced as a result of the interaction of Nrf2 and the ARE region. It is involved in the catabolism of pro-oxidant heme, which results in numerous effects, including antioxidant, anti-inflammatory and anti-apoptotic effects. HO-1 catalyzes the reaction that breaks down heme and produces iron ($Fe^{2+}$), which is stored in the cell in the form of ferritin, carbon monoxide (CO) and biliverdin. Biliverdin is quickly converted by biliverdin reductase into bilirubin, which has higher electrophilicity than biliverdin and a stronger antioxidant effect. In addition to heme degradation products, HO-1 also has direct beneficial effects that may have certain implications for the development of the COVID-19 infection. The antiviral effect of HO-1 is based on several mechanisms. The basic mechanism involves an increase in the interferon I level, which has been proven to be suppressed in cells infected with the SARS-CoV-2 virus. Interferon I, in addition to interfering with viral RNA replication, also enhances the cytolytic effect of innate immunity cells against cells infected with the SARS-CoV-2 virus [41]. Studies on animal models also indicate a direct anti-inflammatory effect of HO-1 mediated by a reduction in the production of pro-inflammatory cytokines [42]. Considering the antioxidant, anti-inflammatory and antiviral effects of Nrf2, its reduced concentration can result in increased oxidative stress, enhanced immune response, as well as faster and uncontrolled virus replication [40,43].

### Clinical implications of NrF2/HO-1 pathway in COVID-19 infection, possible therapeutic approach

Research shows that dysregulation of the immune system and increased expression of oxidative stress are essential in the development of the most severe forms of the COVID-19 infection [26,44], therefore reduced Nrf2 values result in a worse clinical course and severe clinical forms of the disease. The key question research has addressed is the mechanisms of Nrf2 activation. A possible explanation is that the Nrf2 expression is mainly affected by endogenous or exogenous stimulus, and not by its spontaneous transcription because its expression is inhibited in basal conditions, which is why its activation is also one of the cellular defense mechanisms [45]. Viremia acts as a direct activator of Nrf2, and it could be expected that as a result, the serum concentrations of Nrf2 are higher in viral infections [46]. The activation of Nrf2 results in antiviral activity, some authors assume that its lower values in viral infections, especially in severe ones, may also be the result of a direct viral effect on its reduced expression in order to insure a smoother replication [38]. The presence of complications such as bacteriemia and sepsis can inhibit Nrf2 activation [47-49]. This is in line with research conducted on patients with COVID-19 infection [50-52] in which patients with the most severe form of the disease have lower Nrf2 values. When analyzing other exogenous activators of Nrf2, 1,25-dihydroxy vitamin D should be pointed out in COVID-19 infection. In line with numerous studies lower values of 1,25-dihydroxy vitamin D are associated with a worse outcome, a more severe clinical picture, considering that a lower value of the vitamin is correlated with reduced Nrf2 activation. As proven in numerous studies, in patients with COVID-19 infection, Nrf2 is affected by the oxygen therapy, which leads to increased oxidative stress and inflammatory response and has an activating effect on Nrf2 [53-56]. Oxygen administration is required in severe forms of the disease, which results in additional oxidative stress that increases Nrf2 expression. However, patients with the most severe forms of the disease have lower Nrf2 values. One of the explanations is that the application of mechanical ventilation i.e. longer exposure to FiO2 results in decreasing in antioxidant and anti-inflammatory effects, and the period and expression of Nrf2 [52,57]. Although for now there is not much clinical research, these have indicated the correlation of serum HO-1 level with worse clinical outcomes and clinical findings [58]. A large amount of evidence, primarily based on animal models, support the idea that Nrf2 and HO-1 can provide protection against oxidative stress as well as inflammatory and immune responses [59]. Many studies indicate the importance of Nrf2 and its equivalents in embryonic development, stress signaling and aging [60]. Moreover, Nrf2-dependent genes, such as HO-1 provide a cytoprotective effect and play a crucial role in the development of oxidative and aging-related disorders. A more detailed analysis of microRNAs involvement in Nrf2/HO-1 regulation could provide new ideas for the treatment of many diseases (malignant, autoimmune). The involvement of Nrf2 in reductive stress in an animal model of protein aggregation cardiomyopathy indicates the contribution of this transcriptional regulator of antioxidant genes in other pathological situations related to unbalanced redox homeostasis. Due to animal models, our knowledge of the Nrf2/HO-1 pathway in controlling physiology and disease progression advanced significantly, but additional clinical research concerning individual pathological conditions is needed. In this review, we systematically presented the immune response as part of the COVID-19 infection. We focused on the development of anti-inflammatory and antioxidant responses, with an emphasis on the Nrf2/HO-1 pathway. It should be emphasized that clinical studies have been described, the connection with the severity of the disease, using mechanical ventilation. The above is intended to encourage future research to consider a therapeutic approach through Nrf2 activation.

## Conclusion

One of the important features of COVID-19 infection is hyperinflammatory response and due to it, the role of the Nrf2/HO-1 pathway is extremely important. Previous research proposed many mechanisms that positively or negatively affect the activation of Nrf2 and HO-1, as well as their correlation with inflammatory response markers. All further studies concern the factors that affect its activation, including the exogenous factors, as well as the consequences of the adverse effects of the patient's comorbidities on the activation of the Nrf2/HO-1 pathway.

**Acknowledgement.** None.

## Disclosure

**Funding.** No specific funding was received for this study.
**Competing interests.** None to declare.

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**Author contribution.** Acquisition of data: LM, DM, NŠ
Administrative, technical or logistic support: LM, DM, NŠ
Analysis and interpretation of data: LM, DM, NŠ
Conception and design: LM, DM, NŠ
Critical revision of the article for important intellectual content: LM, DM, NŠ
Drafting of the article: LM, DM, NŠ
Final approval of the article: LM, DM, NŠ
Guarantor of the study: LM, DM

Review article

# The Physiology of Thermoregulation in Exercise: A Brief Review

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## Abstract

During physical exercise, the production of heat in the working skeletal muscles increases, imposing heat stress on the body. Thermoregulatory mechanisms induce adjustments of cutaneous vascular conductance and thus skin blood flow (SkBF), sweating rate, and increased cardiac output to achieve thermal homeostasis. The response depends on the intensity, type, duration of exercise, and environmental temperature: during extreme exercise in a hot environment SkBF can attain up to 7 L/min compared to 300 mL/min at rest whereas the sweating rate can reach as high as 4 L/h. Due to opposing non-thermal reflexes, the thermoregulatory response of SkBF during exercise differs from that at rest: the threshold to induce vasodilation in the skin is shifted to higher body core temperature and the sensitivity of the "SkBF to-core temperature" slope is altered. Regular training induces better adaptations to physical stress which enable sportsmen to eliminate additional heat more optimally. The review emphasizes physiological mechanisms involved in thermoregulation during exercise and exposes some thoughts regarding the estimation t of the core temperature in humans, as well as some new approaches for an up-to-date assessment of parameters important for appropriate heat dissipation thereby maintaining core temperature.

(Lenasi H*, Šijanec J. The Physiology of Thermoregulation in Exercise: A Brief Review. SEEMEDJ 2023; 7(1); 13-27)

Received: Mar 20, 2023; revised version accepted: Apr 6, 2023; published: Apr 30, 2023

KEYWORDS: exercise, thermoregulation, skin microcirculation, vasodilation, sweating

## Introduction

Humans as homeothermic organisms need to maintain their body temperature irrespective of relatively high fluctuations in their external or internal environment. During resting conditions, it is mainly external temperature that is fluctuating, whereas, during exercise, the most profound temperature perturbation occurs in the internal environment (1, 2). Though, when exercising in extreme environmental temperature conditions, this additionally impacts the human thermoregulatory system (3, 4).

Thermoregulation encompasses reflex mechanisms enabling that heat elimination from the body equals heat production thus maintaining a constant body core temperature. Core temperature is regulated by a classical negative feedback loop including sensors (central and peripheral thermoreceptors), thermoregulatory center and effectors (2, 5). The information on temperature perturbation is relayed by thermoreceptors to the thermoregulatory center located in the central nervous system which compares the sensed value with the more or less flexible reference temperature value ("set-point") and accordingly switches on and off effector mechanisms to eliminate the perturbation and return the core temperature to the "set-point" value (2, 5). The main effectors include skin arterioles and sweat glands, brown adipose tissue and skeletal muscles. In addition, behavioral changes contribute to thermoregulation (2, 5).

Heat is eliminated from the body by four distinct physical principles, namely radiation, conduction, convection and evaporation (passive and active). The physiological thermoregulatory mechanisms in response to core temperature perturbation adjust the fractions of the four physical principles according to the body's needs: during rest, the majority of heat is eliminated by radiation (60%) and only 20% by passive perspiration, while during exercise the majority of heat elimination is achieved by active evaporation and convection (2, 6).

## Physiological principles of thermoregulation during exercise

While skeletal muscles consume approximately 1.5 mL oxygen/min/kg body weight in resting conditions, their oxygen consumption might reach up to 150 mL/min/kg during intensive exercise (4, 5, 7), implying additional heat burden that during exercise may exceed 1,000 Watt (W) as compared to only 70 W at rest. A thermal load of this magnitude would raise the core temperature by 10C every 10 minutes if there were no thermoregulatory mechanisms (4). Accordingly, thermoregulatory reflexes are activated to eliminate this additional heat from the body to the environment, respectively preserving thermal homeostasis.

### *Thermoregulatory center*

Situated in the preoptic area of the anterior hypothalamus, the thermoregulatory center is believed to comprise a "cold-sensitive" and "hot-sensitive" area activated by a corresponding temperature perturbation (8–11). Yet, the exact anatomic location, the neurotransmitters involved, and the effector pathways are not precisely known in humans as most of the information has been obtained from animal model experiments (8). The center is regarded as an integrator, integrating thermal information from the periphery and core (2): in response to the mismatch between the perturbation and the reference signal, an autonomic involuntary thermoregulation response is mediated by descending projections from the preoptic area, leading to activation of different thermo-effectors, mainly through the sympathetic pathways (9, 12, 13). An important contribution of oxytocin, modulating autonomic and behavioral mechanisms underlying thermoregulation at both central and peripheral levels has been implicated (14).

### *Thermoreceptors*

The thermoregulatory center receives and integrates information on temperature perturbation from the central and peripheral thermoreceptors, respectively (2). Central thermoreceptors are located mainly in the

central nervous system (brain, the spinal cord), although other parts, such as blood vessels and skeletal muscles have been implicated (2). However, the most important ones are part of the thermoregulatory center in the preoptic area and anterior hypothalamus (2, 8, 9). The peripheral thermoreceptors are scattered in the skin throughout the body. Similarly, as for the central ones, there are two types of anatomically distinct peripheral thermoreceptors: warmth receptors and cold receptors, whose distribution and density differ regarding skin site (2, 5, 9).

*Central thermoreceptors*

During exercise, the central thermoreceptors receive the information of increased core temperature (in the range of 2°C to 4°C) (2, 5) When a certain temperature threshold, usually denoted as a "set-point" value, has been reached, effector mechanisms are activated for heat elimination which subsequently establishes thermal homeostasis (2, 5, 9). The exact physiological background of the set-point has not been known; the role of various "transient-receptor potential" (TRP) membrane channels, similarly as for the cold sensing, have been implicated in the activation of these "hot-sensitive" neurons (9, 13, 15). It is worth mentioning that the "set-point" has a rather variable value, as for example during fever it is shifted to higher values (2). Similarly, the value of the "set-point" changes during exercise, i. e. the system is operating at a higher temperature steady state during exercise as compared to resting conditions (2, 5, 7). Moreover, additional factors importantly affect the value of the "set-point", such as the circadian rhythm (6, 16), and sex as well as the phase of the menstrual cycle in premenopausal women (17–19), consequently also impacting exercise performance.

Apart from these reflexes of the negative feedback mechanisms, some of the effector mechanisms are activated before an increase in core temperature, at the very beginning of exercise, denoting a "central command" and assumingly reflecting the anticipatory, feed-forward impulses from the prefrontal, supplementary and premotor cortex (5, 11, 20).

*Peripheral thermoreceptors*

In addition, the hypothalamus also receives information on the temperature changes over the skin surface relayed by peripheral thermoreceptors, specialized free nerve endings embedded in the skin dermis all over the skin surface (1, 2, 5). Changes in surface skin temperature are sensed before the core temperature is altered and, in this respect, peripheral thermoreceptors represent a protection mechanism that diminishes too extensive fluctuations of core temperature during resting conditions, (5), and might be regarded as an additional feed-forward thermoregulatory mechanism (2, 5, 9). During exercise, the response differs: peripheral thermoreceptors are thought to modulate the response of the thermoregulatory center during exercise (2, 6, 15, 21). As explained later, at the beginning of exercise, vasoconstriction in the skin is sufficient to cause temporal storage of excessive heat produced by the working muscles inducing an increase in core temperature which is then sensed by the central thermoreceptors, and reflexes for heat dissipation are activated (2, 4, 6). In fact, during exercise when a new steady-state core temperature is established, mean skin temperature decreases during exercise because of the increased evaporative cooling of the skin caused by sweating (2), increasing the temperature gradient between the body surface and the environment, thus making conductive-convective heat elimination more efficient (2, 5). In a warm environment and when skin temperature is high, the mechanisms of heat elimination are activated at lower core temperature, i.e. the threshold for heat elimination is lowered (3, 4, 11, 22–24), while the threshold is increased in a cool environment when skin temperature is low (25, 26). Peripheral thermoreceptors have been shown to have a great potential for adaptability, especially when exercising in a hot environment; nevertheless, the mechanisms of these adaptations have not been fully understood (15, 24, 27).

*Increased core temperature induces fatigue and might lead to hyperthermia*

The increased core temperature has been linked to "central fatigue", which is speculated to represent a protection mechanism from potential brain injury (10, 28, 29). Fatigue and exhaustion are warning signs that exercise should be stopped or adjusted to reduce brain temperature increase. Brain temperature is determined by its metabolic activity and its perfusion, reflecting the temperature of blood (2, 15, 30). Many studies are being conducted on how to reduce brain heating during exercise, accordingly, improving thermoregulatory mechanisms, prolonging exercise duration and improving sports performance. The application of central pre-cooling or whole-body cooling has been advocated, being achieved by drinking cold drinks, applying cooled gel sacks, or whole-body cooling using special cool water-recycling suits (31–33); yet the best regime needs to be defined. As evaporative-heat loss is imminently connected with water loss, the role of an appropriate replacement of fluid and electrolyte could not be overemphasized (1, 15, 34). Hyperthermia exaggerates fluid loss, inducing a self-perpetuating, vicious cycle of both hyperthermia and hypovolemia, potentially leading to heat stroke and hypovolemic shock (1, 2, 5, 35).

*Cutaneous circulation is the main effector of thermoregulation during physical exercise*

In response to increased heat stress during exercise, the thermoregulatory center activates reflexes to transfer additional heat to the body surface from where heat is dissipated into the surroundings, accordingly, establishing a new steady state.

Besides an increase in cardiac output during exercise, skin perfusion is increased due to profound vasodilation of skin arterioles, reducing cutaneous vascular conductance (CVC) and increasing skin blood flow (SkBF). These mechanisms enable an efficient heat transfer to the skin from where it is eliminated and significantly increase the surface area for heat exchange between the body and the environment. The vasodilation of skin micro-vessels can induce an immense increase of SkBF compared to resting conditions, i.e. from 300 mL/min in thermoneutral conditions (while in a cold environment as low as 100 mL/min) up to 7 L/min during exercise in a hot climate (4, 7, 24). The regulation of vascular tone in skin microcirculation is very complex, including neural and local mechanisms (21, 36–39).

A decrease in vascular tone is mainly achieved by the withdrawal of the sympathetic tone regulating CVC as the skin arterioles throughout the body are innervated by sympathetic vasoconstrictor nerve fibers (11, 21, 36, 39–41). In addition, non-glabrous non-acral parts of the skin also receive vasodilatory sympathetic fibers which mediate active vasodilation (21, 36, 39). It is speculated that during exercise, these fibers contribute to active vasodilation in non-acral parts of the skin, such as the trunk, skin of the forehead and face, and non-acral parts of extremities (11, 21, 36). While vasoconstrictor fibers mainly mediate their action via noradrenaline acting on $\alpha$-adrenergic receptors, the exact neurotransmitter of the sympathetic vasodilatory fibers remains questionable: proposed potential (co)transmitters include acetylcholine (ACh), substance P, calcitonin-gene-related polypeptide (CGRP) and nitric oxide (NO) (21, 36, 38). Respectively, the active role of endothelium seems to importantly contribute to active vasodilation in the skin (38, 39, 42). Special anatomical features present in skin microcirculation of glabrous acral parts, such as fingers, toes, palms, feet, ear lobes and nose, are arteriovenous anastomoses, direct connections between arterioles and venules (43, 44) which when open significantly increase the rate of heat elimination (24, 36, 43, 45).

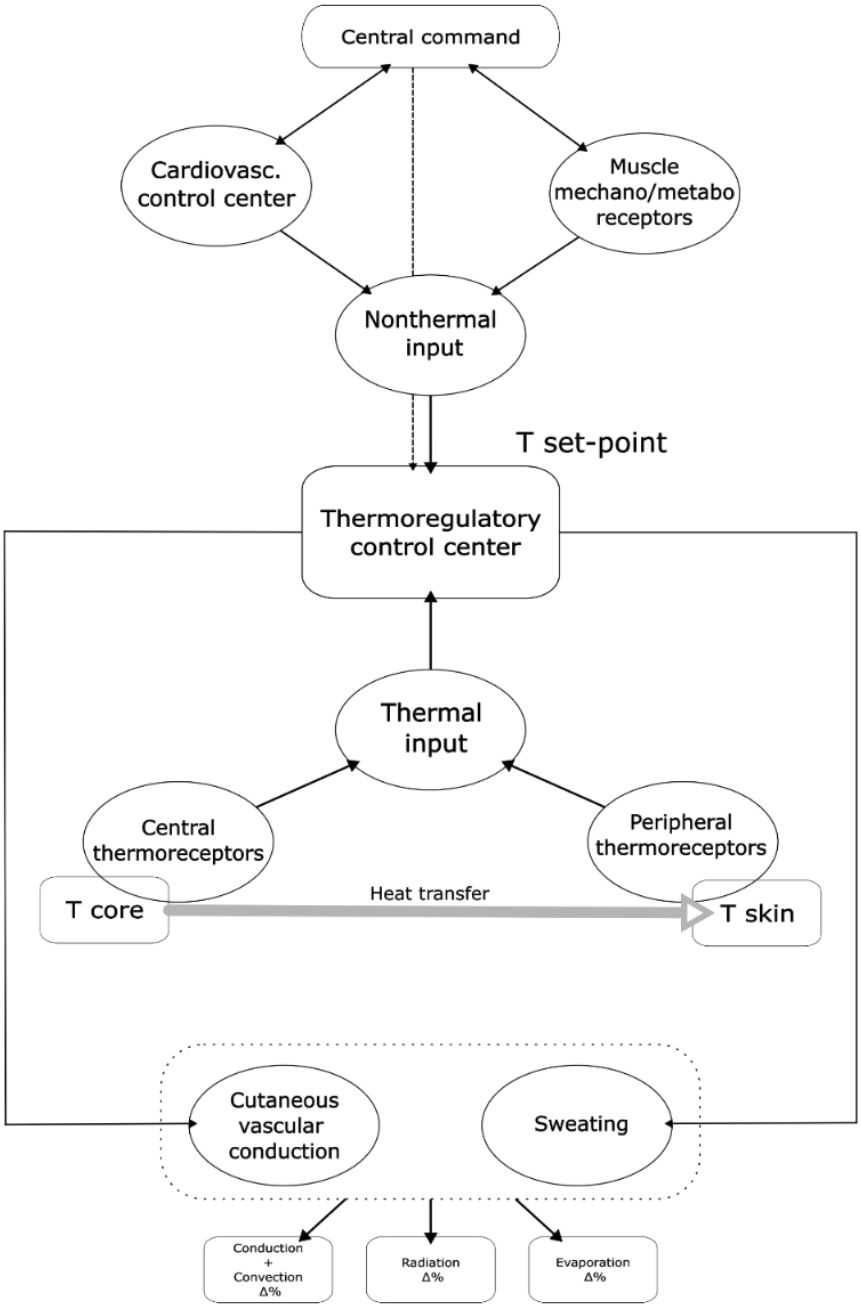


Figure 1. Thermoregulatory mechanisms during exercise.

The thermoregulatory center integrates thermal (from central thermoreceptors sensing changes in core temperature, and peripheral thermoreceptors sensing changes in skin temperature (T)) and non-thermal input, compares the temperature perturbation with the "set-point" value, and activates appropriate reflexes to dissipate excessive heat from the body surface by adjusting the response of cutaneous vascular conductance and sweating, respectively. Temperature gradient determines the quantitative heat transfer from the core to the surface, where heat is dissipated by four physical principles, whose percentages are variable (Δ%) and modified by physiological response according to the quantity of the produced heat and ambient conditions, respectively.

*Evaporation-related heat loss during exercise is increased by sweating*

Another very efficient mechanism of heat elimination is active sweating which is tightly linked to changes in CVC. Each gram of water evaporated from the skin surface removes about 2.5 kJ of heat from the body (1, 5). Sweat glands are innervated by the sympathetic nerve fibers with ACh being a putative transmitter acting concomitantly with other cotransmitters (11, 46); an important role of various aquaporins in the skin has recently been reviewed (47). When fully active, sweat glands can increase their sweating rate up to 30 g sweat/min in response to the body's needs which could account for up to 2-4 L/h during high-intensity exercise in a hot environment (1, 11, 15, 24). Indeed, in a hot environment when the ambient temperature exceeds the core temperature, there is no or even a negative temperature gradient for conductive-convective heat transfer, making evaporation the only way to dissipate heat (1, 2, 4, 24). Yet, besides physiologic adjustments in the sweating rate and composition of sweat (46, 48–50), the efficiency of sweating also depends on environmental factors, predominantly on the humidity (3, 15, 50, 51). Increased humidity can make sweating ineffective when there is no water vapor gradient between the environment and the surface of the body, predisposing to the development of hyperthermia which can be fatal (1, 2).

Increased SkBF and sweating rate persist also far in the recovery period after exercise cessation, depending on the duration and intensity of exercise and the production of heat, until all additional heat is eliminated and the resting temperature steady state is achieved (16, 45, 52–54).

A schematic representation of the mechanisms governing thermoregulation during exercise is depicted in Figure 1.

*How could skin blood flow and sweating be assessed*

The dynamics of skin microcirculation and skin temperature changes during exercise could be traced by using laser Doppler fluxmetry (LDF) (39, 45) and the corresponding skin temperature measurement (Fig. 2), various laser Doppler imaging techniques (55, 56) or ultrasound Doppler flowmetry (UDF) (57), and have been considered to be a more reliable measure of SkBF than plethysmography (58, 59). To obtain a better insight into the particular physiological mechanism behind it, different approaches and algorithms are being developed for the spectral decomposition of the LDF signal (60). Interestingly, glabrous and non-glabrous parts of the skin behave differently during exercise and its recovery (45, 57, 61) (Fig. 2). A strong cross-coupling between glabrous SkBF and core temperature in thermoregulatory function has recently been established based on experimental and modeling data (62). A promising device for tracing sweat rate during exercise as accurately as possible and providing a low-cost device platform to detect other health-relevant biomarkers in the sweat (vapor) as the next-generation sweat sensor for smart healthcare and personalized medicine has been introduced (63).

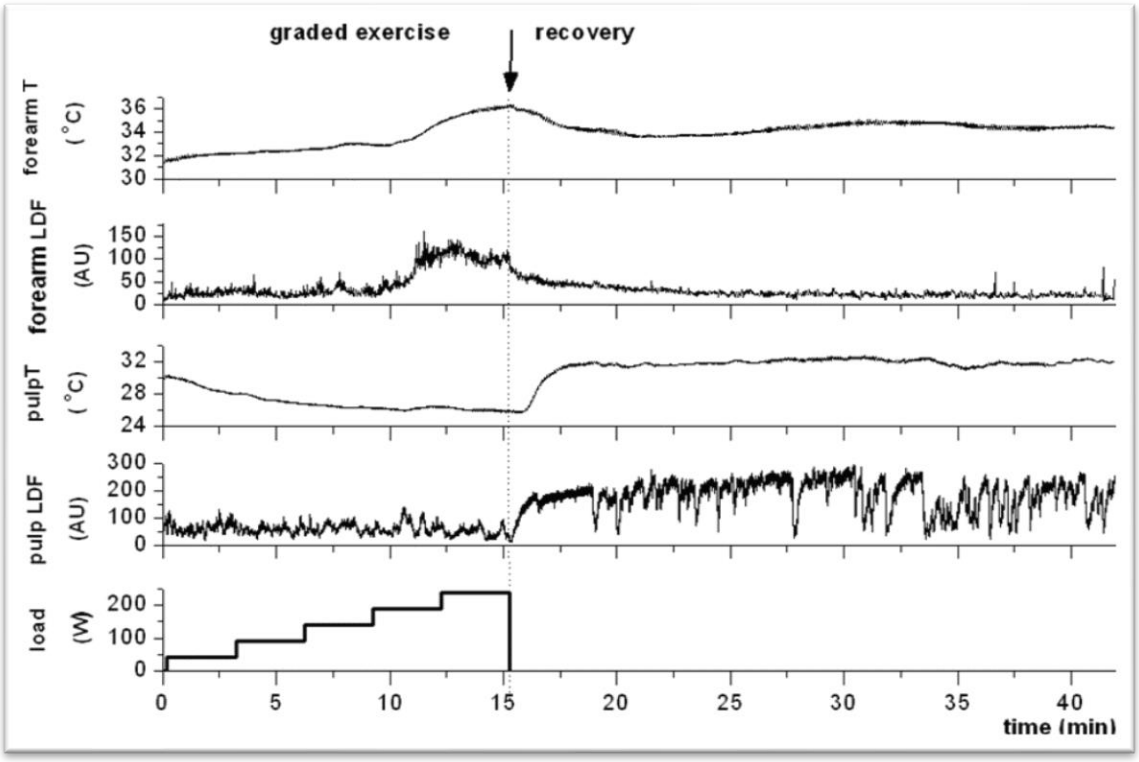


Figure 2. The responses of skin blood flow and the corresponding skin temperature to exercise and its recovery differ between glabrous and non-glabrous areas.

A representative tracing of the laser Doppler flux (LDF), expressed in arbitrary perfusion units (AU) and the corresponding skin temperature (T) in skin microcirculation on the volar forearm (glabrous site) and the finger pulp (non-glabrous site) during graded dynamic exercise and its recovery is shown. Exercise load is expressed in Watts (W). Adapted from (45).

*The main differences between thermoregulation during rest and exercise: a summary*

Apart from thermoreflexes, many opposing non-thermoregulatory reflexes (mechano-, metabo- and baroreflex to list some) are activated during exercise and its recovery to ensure sufficient perfusion of skeletal muscles and the preservation of blood pressure (7, 15, 20, 54, 64–66). Therefore, thermoregulation during exercise importantly differs from that at rest. The most manifest differences are depicted in Fig. 3, representing the relation between the body core temperature and the SkBF. To assess the characteristics of this relation, i.e. the slope of the curve, the body core temperature and the SkBF, potentially with the corresponding skin temperature, should be traced. Core temperature is usually determined by an esophageal thermo-sensor, although other methodological aspects have been addressed (5, 20, 22, 62). Nevertheless, the data should be interpreted cautiously, especially when measuring peripheral skin T to estimate core temperature, which is rather questionable (67). On the other hand, there are much more options for assessing skin temperature; besides the above mentioned, infrared thermography has gained increasing importance; yet, similarly to other methods, it has not been standardized (68, 69).

At the beginning of exercise, an increase of the sympathetic tone throughout the body, presumably due to a central command, enables redistribution of blood flow, increasing CVC and reducing SkBF and thus shifting the starting point toward lower SkBF at unchanged core temperature (Fig. 3) (2, 5, 7, 20, 24). Another distinctive difference is a shift in the threshold to induce active vasodilation and sweating in the skin, toward higher core temperatures (Fig. 3); interestingly, the threshold shift depends on

exercise intensity and is even more pronounced in high-intensity exercise (11, 20, 36). The dependence of the thermoregulatory threshold on the intensity of exercise might partly explain rapid fatigue and exhaustion during intense exercise due to a higher increase in core temperature, which might even lead to hyperthermia (15, 22, 29). The third pronounced difference is the responsiveness of the effectors in the skin: the maximal vasodilation in the skin during exercise comprises only 60% of the maximal vasodilation attained in resting conditions (Fig. 3) (7, 20, 24). Pooling of additional blood in the periphery impedes venous return to the heart, and activates baroreflex, leading to "cardiovascular drift" and heart rate increase that is disproportional to the needs of the skeletal muscles (5, 52, 70).

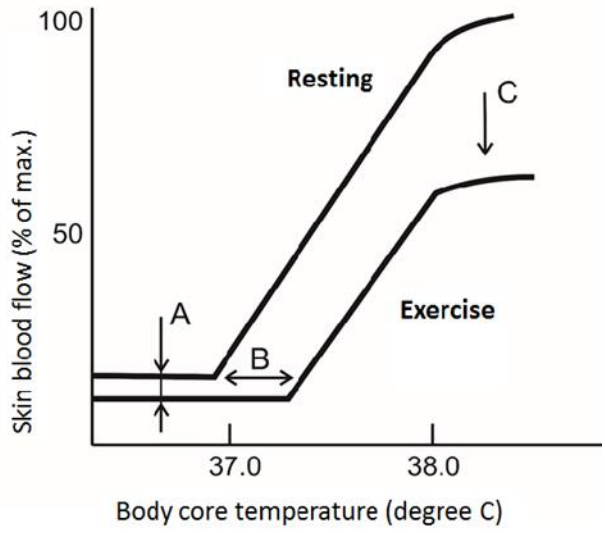


Figure 3. The main differences between thermoregulation during rest and dynamic exercise.

Skin blood flow (SkBF) in relation to body core temperature is shown. The "starting point" is shifted toward lower SkBF at the onset of exercise due to vasoconstriction in the skin (A). The temperature threshold to induce vasodilation is shifted toward a higher core temperature during exercise (B). Maximal vasodilation in the skin is reduced compared to resting conditions (C). Adapted from (4).

In addition, it has been proposed that skin temperature, independently of the core temperature, affects sports performance: maximal oxygen uptake (VO2max) at the same core temperature was shown to be lower at higher skin temperatures than at lower ones (23, 70). Increased skin temperature during exercise reduces the temperature gradient for heat elimination, impeding heat exchange. To overcome the decreased temperature gradient, SkBF is increased on account of diminished blood flow through skeletal muscles. From the above observations on opposing thermo- and non-thermoregulatory reflexes during exercise and its recovery, it is obvious that hyperthermia is more often an issue when exercising than when resting. Interestingly, simplified thermoregulation models of the human body exercising in warm conditions have been proposed considering all the above-mentioned players (62, 71).

## Exercise and thermoregulation in extreme environmental conditions

*Hot environment*

Exercising in a hot environment undoubtedly compromises thermoregulation. Pooling of additional blood in the periphery increases cardiovascular drift in a hot climate. It has been shown that a heart rate increase of 20 beats/min could not compensate for the decreased stroke volume due to the pooling of blood in the skin: cardiac output was about 1.5 L/min lower during heavy exercise in the heat than during comparable exercise in a cool environment (1, 5, 15). Due to insufficient muscle perfusion, the amount of anaerobic metabolism increases, decreasing plasma pH and additionally compromising performance (15, 72).

In addition to the pooling of blood, another problem during prolonged exercise is the loss of water and electrolytes, additionally compromising venous return, and consequently jeopardizing the maintenance of blood pressure. To overcome the threatening hypotension, relative cutaneous vasoconstriction issues, rendering the person more susceptible to developing hyperthermia (1, 5, 24). In addition, the reflex of sweating is reset toward higher core temperatures as water preservation due to

reduced osmolarity surpasses the need to eliminate heat by sweating (20, 73–75). Respectively, a vicious cycle could ensue leading to hyperthermia and/or shock (35). In this respect, sufficient fluid and electrolytes substitution is a prerequisite for sustaining exercise (24, 34, 76). It has been shown that even 1% of body mass reduction due to dehydration strongly impacts sports performance and VO2max (77).

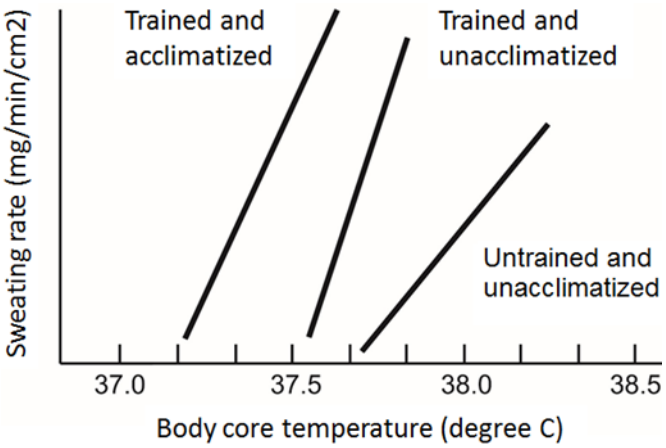


Figure 4. The effect of regular training and acclimatization on thermoregulation

Training and staying in a warm environment for a longer period induce a shift in the temperature threshold for heat elimination (presented as sweating rate) as well as the sensitivity of thermoreflex. Adapted from (81).

When regularly exercising in a warm environment, acclimatization processes take place which improve sports performance: the sensitivity of thermoreflex is reset toward a lower core temperature threshold (Fig. 4), the maximal sweat rate increases and sweat composition changes, becoming more diluted thus helping to preserve body electrolytes (24, 50, 51, 78, 79). In addition, an increase in plasma volume in a longer-term improves circulation and heat exchange (24, 77). All these adaptations improve VO2max and sports performance. Interestingly, similar adaptations have also been shown in endurance elite sportsmen exercising in a thermoneutral environment (Fig. 4) (48, 73, 78–81).

*Cold environment*

On the other hand, exercising in a cold environment is not problematic from the thermoregulatory point of view provided it is not of too long duration or performed in cold water which has a 25-fold higher heat conductivity compared to air (5, 25–27, 82). As long as the body core temperature is maintained in a physiological range, the low ambient temperature does not impact sports performance and VO2max. Yet, cooling of the muscles impacts their functional abilities in terms of decreasing their strength, maximal force, velocity and reaction time (83, 84). Due to vasoconstriction in the skin, the release of free fatty acids from subcutaneous adipose tissue is reduced, limiting the oxidation of fat as fuel for muscle contraction and accordingly increasing the oxidation of carbohydrates. Potential hypothermia results from a drop in core temperature when exercising for a longer time, or insufficiently dressed or performing water sports (26).

Acclimatization to cold induces adaptive mechanisms which enable better preservation of heat on one side and increased production on the other, including shivering and brown fat adipose tissue thermogenesis (2, 25–27).

*Effect of sex and age on thermoregulation during exercise*

An interesting issue to be discussed is the effect of sex on thermoregulation. On one side, centrally mediated mechanisms might affect the response regarding sex, and on the other, sex hormones are known to exert direct peripheral effects on the vascular tone (11, 18, 19, 36, 85). Yet, studies reported controversial results. Most often, the main differences are attributed to the sweating response: the slope of the curve representing sweating rate dependency on the body core temperature was reduced in women compared to men (86, 87) even though women exhibited a greater number of sweat glands (51). The differences in the sweat rate were more pronounced when exercising at higher intensities (86). It seems that the temperature threshold to induce vasodilation in the skin is

higher in women (85). However, a recent meta-analysis has shown that despite increased core T in women in the luteal phase of the menstrual cycle this does not significantly impact thermoregulatory response, at least in terms of sweat rate and skin temperature (19, 88). Finally, it is worth emphasizing that aging also affects the thermoregulatory response to exercise (1).

*Effect of endurance training on thermoregulation during exercise*

Regular training in both men and women induces significant adaptations of the thermoregulatory system, including cutaneous vascular reactivity as well as sweating. The threshold to activate effectors for heat elimination is lower in trained compared to sedentary (64, 77, 81, 89) (Fig. 4). Sweat rate and composition are altered in terms of increased sweat rate at the same exercise intensity (expressed as % VO2max) and more diluted sweat, respectively enabling more efficient electrolytes preservation in sportsmen (51, 78, 80). In addition, sportsmen have been shown to better acclimatize to the heat, which rather than reflecting structural changes has been attributed to functional vessel alterations (79, 90). A part of altered vascular responsiveness might be attributed to increased endothelium-dependent vasodilation (91, 92) which might additionally contribute to beneficial thermoregulatory adaptations.

All these adaptations enable sportsmen better adjustments to physical and thermal stress, reaching higher VO2max values and performing exercise for longer periods, in thermoneutral, as well as in extreme environmental conditions.

## Conclusion

Thermoregulation during exercise differs from that in resting conditions since, during exercise, thermoregulatory reflexes oppose the non-thermoregulatory ones. The opposing reflexes integrate at the level of skin microcirculation which is the main organ for heat elimination. An important issue when exercising is a sufficient substitution of water and electrolytes, especially in a warm and humid environment. Regular physical activity induces several beneficial changes, including better thermoregulatory mechanisms. Yet, exact mechanisms, as well as the most optimal training regime, remain to be determined, and above others, appropriate methods to measure the many players involved in thermoregulation in humans in vivo need to be established.

**Acknowledgement.** None.

## Disclosure

**Funding.** No specific funding was received for this study.

**Competing interests.** None to declare.

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**Author contribution.** Acquisition of data: HL, JŠ
Administrative, technical or logistic support: HL, JŠ
Analysis and interpretation of data: HL, JŠ
Conception and design: HL, JŠ
Critical revision of the article for important intellectual content: HL
Drafting of the article: HL, JŠ
Final approval of the article: HL, JŠ
Guarantor of the study: HL

Review article

# Home-Based Exercise During the Coronavirus Pandemic – A Useful, yet Challenging Treatment Strategy for Improvement of Mental Health, Glycemic Control and COVID-19 Outcomes in Patients With Diabetes

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## Abstract

Lockdown measures to control disease transmission were implemented at the start of the COVID-19 era, worsening the already existing sedentary lifestyle. Reduced physical activity (PA) and unhealthy eating habits have a negative impact on mental health in chronically ill patients, including diabetes patients. Mental illness, on the other hand, encourages a sedentary lifestyle, exacerbating all components of metabolic syndrome. While well-controlled diabetic patients with an HbA1c of less than 7% had a less severe clinical presentation and COVID-19 mortality rates, the favorable effect of PA on immunomodulation and immunoregulation should not be neglected. Given recent data indicating that a sedentary lifestyle is the third independent risk factor for COVID-19 complications and death (after advanced age and organ transplant), including regular PA has never been more vital. Since PA has a major impact on both glycemic control and mental health, implementing structured home-based activity programs could improve glycemic control and psychological well-being, hence positively impacting COVID-19 outcomes.

(Cigrovski Berković M, Ivandić M, Mrzljak A, Cigrovski V, La Grasta Sabolić V, Ormanac K, Sabo D, Omanović Kolarić T, Ružić L, Bilic-Ćurčić I. Home-Based Exercise During the Coronavirus Pandemic – A Useful, yet Challenging Treatment Strategy for Improvement of Mental Health, Glycemic Control and COVID-19 Outcomes in Patients With Diabetes. SEEMEDJ 2023; 7(1); 28-35)

Received: Mar 7, 2023; revised version accepted: Apr 27, 2023; published: Apr 30, 2023

KEYWORDS: physical activity, mental health, diabetes, COVID-19, home-based exercise

## Introduction

Physical activity is defined as any movement produced by skeletal muscles that require energy expenditure (1). It refers to all activities performed while traveling, during leisure time or working hours. Physical activity should be viewed as a continuum, ranging from light to vigorous, while exercise implies planned, structured and repetitive physical activity. Overall, it is crucial for the improvement and/or maintenance of both mental and physical health (2, 3).

Different psychological and physiological mechanisms have been proposed to explain the positive effects of physical activity on mental health, especially on anxiety, depression and stress-related disorders (4, 5, 6). Potential psychological mechanisms include "time out" or distraction from stressors, as well as improved self-efficacy, self-image and confidence, which enhance the sense of control and help to overcome the difficulties faced on a daily basis (7). From the physiological aspect, exercise may induce changes in the hypothalamic-pituitary-adrenal axis modulating stress reactivity (4). It may also boost the levels of endorphins, which help people feel happier and less stressed (8). Besides, the brain-derived neurotrophic factor increases with exercise, thus contributing to the anxiolytic effect of physical activity (9).

It is well known that physical inactivity and sedentary behavior represent the key modifiable factors associated with an increased risk of developing type 2 diabetes (T2D). Nowadays, diabetes is the fastest-growing chronic disease strongly related to rising obesity rates. Regular physical activity constitutes an important part of diabetes/obesity prevention and management. It produces multiple health benefits including enhanced glycemic control, favorable changes in blood lipid levels, reduced low-grade inflammation and improved vascular function (10, 11). Physical activity and exercise also reduce all-cause and diabetes-related mortality in T2D. Mechanisms enabling those benefits are complex and mediated by an increase in glycolipid uptake and utilization, improved insulin sensitivity, optimized body mass index and modulated DNA methylation (12). Recent findings also suggest that exercise induces significant physiological changes in the immune system, including anti-inflammatory cytokine response (12, 13). For people with type 1 diabetes (T1D), physical activity improves cardiovascular fitness, muscle strength and insulin sensitivity (14, 15).

Many patients suffering from chronic diseases have co-morbid mental health conditions (16). Diabetes and mental health issues share a bidirectional association influencing one another in multiple ways. In patients with diabetes, the prevalence of anxiety and depression is significantly higher than in the general population (17, 18). Depression in combination with behavioral and metabolic risk factors increases the risk for type 2 diabetes development, poor glycaemic control and subsequent risk for micro and macrovascular complications. It is important to emphasize that being diagnosed with diabetes represents a strong stressor by itself, requiring a large number of physical and mental adjustments.

The adoption and maintenance of regular physical activity are beneficial for both, diabetes and mental health disorders. However, many patients neither meet their exercise targets, nor adhere to proposed exercise guidelines. Between 25% and 42% of older diabetics meet the physical activity recommendations, while 13–19% are not even likely to be physically active at sufficient levels compared to non-diabetic persons (19). Additionally, more than 60% of patients with T1D remain sedentary (20). People with severe mental illness engage in significantly less vigorous exercise and express greater amounts of sedentary behavior in comparison with healthy controls (21). Their inactivity is predictive of a range of adverse health outcomes including metabolic syndrome and its components (22).

It is reasonable to assume that number of physically inactive people with diabetes and mental health disorders increased during the coronavirus pandemic. Knowledge of commonly perceived barriers to physical

activity, along with psychological factors contributing to these barriers may help in overcoming challenges of sedentarism both in non-pandemic and pandemic COVID-19 times.

## The challenge of physical activity in non-pandemic times

According to current guidelines, people with diabetes should engage in five sessions of moderate aerobic activity weekly (at least 150 min/weekly, i.e. five days per week with no more than two consecutive days between the activity) to maintain the exercise-induced improvements in insulin action. Besides aerobics, resistance training is recommended three times weekly on nonconsecutive days (23). Transcribed into parameters of glucoregulation, a decrease in HbA1c by up to 0.85% can be expected, which matches the reductions seen with some newer antidiabetic agents. So, the intriguing question is why physicians do not prescribe physical activity more persuasively, and why are patients so frequently noncompliant and nonadherent? The assumptions for successful prescription of physical activity imply that medical professionals know how to prescribe, monitor and evaluate the effectiveness of the exercise prescribed (adapting exercise type, frequency, intensity and duration according to patients' individual health status and interests) which is generally not the case. Other known obstacles to physical activity promotion include lack of time and lack of perceived efficacy in changing physical activity behavior in patients (24). The most typical barriers perceived by T2D patients are lack of time, pain and discomfort, being overweight and finding physical activity boring (25). Interestingly, a commonly reported reason for avoiding exercise among overweight T2D females is embarrassment about their appearance. In contrast, in T1D patients, fear of hypoglycemia pops up as the most prevalent reason behind not exercising (26). Patients with diabetes are sedentary, spending too much time viewing TV, which represents an independent risk factor for the increase of all-cause mortality (27). In the case of co-morbid mental health issues, mental health symptoms and tiredness may further complicate exercise performance. For people with severe mental disorders, additional factors that hamper physical activity include medication side effects, complications from obesity and poor physical health, lack of resources and absence of professional support (28).

## COVID-19 pandemic and restrictions related to physical activity

The COVID-19 pandemic imposes challenges for people with diabetes and mental health disorders, tackling their health and well-being. Lockdowns and mobility restrictions impacted the ability to be active during work and leisure time. Many people worked through home-based offices, leaving little or no time for movement and increasing screen time, sedentarism and unhealthy meal plans (29). Moreover, pandemic-driven economic and financial distress distracted people from engaging in physical activity (30) and weakened their motivation to exercise (31). Studies reported direct negative effects on glucoregulation of T1D patients during lockdown (32). Furthermore, high physical inactivity found in most T2D patients before the COVID-19 pandemic additionally worsened during the lockdown (33). At the same time, public health measures such as quarantine, self-isolation and social distancing contributed to the deterioration of mental health and increased stress, anxiety, depression and feelings of isolation (34, 35).

In light of the coronavirus pandemic, the beneficial effects of physical activity on immune function and inflammation can be especially important for people with T2D and mental health issues, as they both have a higher risk of COVID-19 hospitalization and death (36, 37). Also, the favorable impact of physical activity on glycemic control is not negligible because it may help reduce the chance of severe COVID-19 in diabetic patients (38). It is important to point out that only advanced age and a history of organ transplant are stronger independent risk factors for severe COVID-19 outcomes than physical inactivity (39). On the contrary, engaging in various types of exercise is considered to be a coping strategy for mitigating the negative

effects of the coronavirus pandemic on mental health and enhancing general well-being (40).

## Home-based exercise – could it make a difference?

As a part of the COVID-19 social distancing measures, the use of gyms, health clubs and public spaces was either reduced, not recommended, or not permitted, especially for the high-risk populations. The restricted collective activities during the pandemic imposed the importance of physical activity through alternative forms of exercise, especially home-based exercise (HBE). Therefore, knowing the benefits of regular physical activity and exercise on glycemic management and mental health, HBE training emerges as an important approach and coping strategy to promote physical and psychological well-being during the pandemic times.

Several randomized control trials, including people at risk for contracting T2D, patients with T2D and different cardiovascular profiles and women with gestational diabetes mellitus showed health advantages of HBE (primarily moderate-intensity aerobic exercise 3–5 times weekly and less often combined aerobic and resistance exercise), during the time of intervention and follow-up (Table 1). Benefits were, besides glycemic control, seen in lipid profile, body composition, cardiorespiratory fitness and psychological health (41). Patients adherent to the HBE program had a significantly reduced incidence of cardiovascular disease compared to nonadherent patients (42). In addition, none of the studies reported adverse events during the HBE programs or follow-up. Studies provided material about the exercise program, education on diabetes self-management, heart rate monitors and oximeters which helped the participants in safe exercising (43, 44). Unfortunately, no studies assessed the safety and effectiveness of HBE programs in T1D individuals, but with modern technologies such as continuous and flash glucose monitoring and the use of insulin pumps, one can speculate their usefulness and safety during COVID-19 (45).

**Table 1. Selected findings from different studies regarding the effectiveness of various exercise programs**

| Author | Title | Source | Findings |
|---|---|---|---|
| Francesco Cosentino | 2019 ESC Guidelines on diabetes, pre-diabetes and cardiovascular diseases developed in collaboration with the EASD | European Heart Journal | Clinical trials in adults with DM have provided evidence of the HbA1c-lowering value of resistance training, and of an additive benefit of combined aerobic and resistance exercise. |
| S. F. Lee | An investigation and comparison of the effectiveness of different exercise programs in improving glucose metabolism and pancreatic β cell function of type 2 diabetes patients | The International Journal of Clinical Practice | The accumulated million steps group had better glucose metabolism and pancreatic β cell function compared with those in the aerobic exercise group. |
| Jos J Kraal | Clinical and cost-effectiveness of home-based cardiac rehabilitation compared to conventional, center-based cardiac rehabilitation: Results of the FIT@Home study | European Journal of preventive cardiology | Patients in the home-based group were more satisfied with their CR program compared to patients in the center-based group (home-based: 8.7/10, center-based: 8.1/10, p = 0.02). |

The Italian National Association of Athletes with Diabetes (ANIAD) issued a series of daily activities that could be performed home-based, and with an intensity comparable to a one-hour brisk walking with an energy expenditure of 150–200 Kcal (46). The above-mentioned could not only secure the reaching and attainment of recommended physical activity guidelines for people with diabetes, but also enhance access to exercise and its adjustment to patients' lifestyles, daily schedules, and integration with regular home routines, all of which are often depicted by patients as barriers and obstacles for participation in regular physical activity. HBE does not always have to be high-tech and designed on platforms aimed exclusively at diabetes patients. Those narrowly specialized platforms would be best, but their reach could be limited, resulting in a low number of users. The solution might be in general platforms, which give the possibility to choose the location and type of activity. The newest addition is online programs. As the infrastructure already exists, there is a possibility to offer online programs explicitly created for diabetes patients. The collaboration between diabetes centers and such platforms would speed up the online exercise offer by using existing software solutions and a large pool of members.

Nearly all types of physical activity are helpful since both physical exercise and relaxation training can buffer the negative effects of stress on mental health (47). HBE can include aerobic activities such as dancing (35), balance and flexibility training, such as yoga (48), muscle strengthening exercises, such as weightlifting (35), endurance training and others. Participation in physical activities at home has been shown to alleviate a wide range of mental illness symptoms, improve anxiety, mood, social and emotional health (34, 49). Studies have shown that yoga, as an example of indoor physical activity, can reduce perceived stress, enhance emotional control and improve self-efficacy, self-confidence and overall quality of life (50). It can be used alone or in combination with other interventions (48), and it is one of the important measures to prevent or control mental health problems during the pandemic. Exergames, which are exercises based on video games, can encourage younger people to exercise at home, while the internet may further enable social interaction with friends remotely (51).

## Conclusions

Physical inactivity emerges as an important issue with deleterious physical and mental health consequences. In patients with diabetes, regardless of the disease type, regular exercise has well-known and proven benefits on glycemic control and cardiovascular health. Moreover, physical activity can reduce psychological pressure, promote mental health and improve the quality of life. In the context of the COVID-19 pandemic, maintaining regular exercise is especially beneficial, and engaging in alternative modes of physical activity such as HBE can be a key strategy to maintain mental health and physical well-being.

**Acknowledgement.** None.

## Disclosure

**Funding.** No specific funding was received for this study.
**Competing interests.** None to declare.

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**Author contribution.** Acquisition of data: MCB, MI, AM, VC, LGS, KO, DS, TOK, LR, IBC
Administrative, technical or logistic support: MCB, MI, AM, VC, LGS, KO, DS, TOK, LR, IBC
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Provision of study materials or patients: MCB, MI, AM, VC, LGS, KO, DS, TOK, LR, IBC

Original article

# Verification of the Automated ELISA Assay for Hepcidin-25 in Human Serum

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## Abstract

**Introduction:** Hepcidin-25, the bioactive form of hepcidin, is the master protein in regulating iron homeostasis. Serum concentrations, measured by different methods, are often incomparable and complicate results interpretation.

**Materials and Methods:** The aim was to verify the first fully automated enzyme-linked immunosorbent assay (ELISA) method, using the DRG Hybrid XL analyzer (DRG Instruments, Marburg, Germany) standardized against the mass spectrometry method. Intra- (CVi) and inter-assay (CVg) precision and bias were performed using commercially available controls with low (C1) and high (C2) concentrations. The reference interval was verified by analyzing serum samples of 20 healthy males.

**Results:** CVi = 9.1% (C1), 4.5% (C2); CVg = 8.9% (C1), 5.6% (C2); calculated bias was 33% for C1 and 20% for C2, respectively.

**Conclusion:** Verification of the fully automated ELISA method for hepcidin-25 in serum on the DRG Hybrid XL analyzer met the analytical acceptance criteria.

(Rolić T, Mandić S, Lukić I, Horvat V, Banjari I. Verification of the Automated ELISA Assay for Hepcidin-25 in Human Serum. SEEMEDJ 2023; 7(1); 36-41)

Received: Feb 20, 2023; revised version accepted: Mar 20, 2023; published: Apr 30, 2023

KEYWORDS: enzyme-linked immunosorbent assay, hepcidins, homeostasis, iron

## Introduction

Hepcidin-25, the bioactive 25 amino acid peptide is the master iron-regulatory hormone and is predominantly generated in the liver by proteolytic cleavage of prohepcidin at the C-terminus (1). N-terminal removal of hepcidin-25 can result in short peptides of 20-24 amino acids which have reduced activity but can interfere with some immunoassays (2). Hepcidin-25 is the only active form of hepcidin in serum and has the main role in iron status regulation by down-regulating the expression of ferroportin. Hepcidin binds to the cell surface iron transporter – ferroportin – inducing its internalization and degradation (3). The result is increased intracellular iron storage, decreased dietary iron absorption and decreased serum iron concentration (1). In conditions of increased demand for iron, (iron deficiency, hypoxia, anemia and erythropoiesis) hepatocellular hepcidin synthesis decreases (4, 5). Hepcidin deficiency causes hereditary hemochromatosis, characterized by body iron overload that may progress into liver cirrhosis (6). In addition, low hepcidin-25 concentration can be induced in iron-loading anemias and chronic hepatitis C (7, 8). In contrast, hepcidin synthesis is induced by inflammation and infection (9). High hepcidin-25 concentration has been found in iron-refractory iron-deficiency anemia, during infection, chronic kidney disease as well as in cancer (9–11). Therefore, hepcidin-25 serum levels are valuable for identifying and differentiating specific diseases or conditions related to iron homeostasis (7, 10, 12).

So far, two main techniques have been used for serum hepcidin determination: mass spectrometry (MS) and immunochemistry – solid phase enzyme-linked immunosorbent assay (ELISA) (13–15). Immunochemistry assays are commonly used to carry out routine tests in laboratories. However, the ELISA method is not suitable for individual and random-access testing because results provided by different methodologies show high discrepancies.

Analytical method verification is required for providing objective evidence that an analytical procedure meets the requirements suitable for scientific research and routine application in the laboratory (16). In 2016, the first fully automated immunoassay, an important step forward, was introduced to the market. Until then, only manual immunoassays were available.

The aim of this study was to verify in a clinical laboratory the fully automated ELISA method validated by DRG company by MS (results not published) for measuring concentrations of hepcidin-25 in human serum.

## Materials and methods

*Subjects and blood sampling*

Commercially available hepcidin samples were obtained from DRG company (DRG Instruments, LLC (GmbH), Marburg, Germany): C1-normal level (4.6 µg/L) and C2-high level (44.3 µg/L), were used as standards for the verification procedure. Additionally, blood samples from healthy subjects (regular male blood donors aged 18 years or older), were collected in August 2020 at the Department of Transfusion Medicine, Osijek University Hospital, following the guidelines on venipuncture for blood donation. All subjects were previously screened for anemia at the same Department according to the guidelines for blood donors. The exclusion criteria were hemoglobin concentration of less than 135 g/L. Inclusion criteria were iron, ferritin and transferrin levels within the reference range. Indicators of iron status were conducted at the Institute of Clinical Laboratory Diagnostics. Informed consent was signed by all participants. Venipuncture was performed, and blood was collected using tubes (5 mL) (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) without anticoagulants. The tubes were centrifuged for 10 minutes at 2,000 g and the serum was used for measuring the concentration of hepcidin-25.

*Analytical methods*

Analytical verification of the method was performed according to the Clinical and Laboratory Standards Institute (CLSI) guideline EP15-A2 on Method Verification (16). Method verification procedure included the assessment of the accuracy of series (repeatability) and day-to-day (intermediate precision) accuracy, measurement uncertainty and reference interval verification. After reconstituting the lyophilized control samples following the manufacturer's instructions, verification steps were performed. Control standard samples were analyzed in triplicate for five consecutive days and the data were used to calculate both accuracy and variability. The normal reference range was checked from 20 serum samples from healthy blood donors.

Hepcidin-25 concentrations were measured by the automated ELISA method using the DRG Hybrid XL (DRG Instruments, LLC (GmbH), Marburg, Germany) analyzer. The manufacturer declared that the hepcidin-25 ELISA method was standardized against the mass spectrometry method.

All data were calculated using the Excel program: mean value, standard deviation, coefficient of variation and bias according to the formulas provided in Supplement Table 1. Reference interval verification was performed using MedCalc for Windows, version 12.4.0.0 (MedCalc Software, Mariakerke, Belgium). The reference interval provided by the manufacturer was confirmed in serum samples. The distribution of the results was normal, calculated data obtained in Excel were used for the reference interval verification – the interval in which the central 95% values of a healthy subject lie. The used limits of normality: mean ± 1.96 x SD.

## Results

In this research, we performed method verification in terms of intra- and inter-assay precision and bias assessment. Method verification was performed by analyzing two concentration levels of commercial controls. Results are summarized in Table 1. We confirmed the declared inter-assay precision for both concentration levels considering the concentration values. Namely, < 10.7% and < 10.0% for normal (C1) and high (C2) control levels, respectively. Calculated bias was higher in C1 (> 30%) than in C2 (20%).

**Table 1. Coefficients of variation for control samples**

| | CVi | CVg | Total CV | Bias |
|---|---|---|---|---|
| C1 | 9.0% | 8.9% | 11.6% | 33% |
| C2 | 4.5% | 5.6% | 6.7% | 20% |

C1=control sample level 1; C2=control sample level 2; CVi=intraindividual coefficient of variation; CVg=interindividual coefficient of variation; CV=coefficient of variation

The mean hepcidin-25 level in serum samples was 10.2 µg/L (SD 9.9) which is within the normal reference range stated by the manufacturer (DRG Instruments, LLC (GmbH), Marburg, Germany) declared for the 2.5th and 97.5th percentile as of 0.2–34.4 µg/L obtained on 20 healthy male blood donors in the current study.

**Supplemental Table 1: Measured values of control samples during five consecutive days in triplicate**

| 1. Intra- and interassay precision control sample level 1 | | | | | |
|---|---|---|---|---|---|
| Day: | 1 | 2 | 3 | 4 | 5 |
| 1 | 5,25 | 5,95 | 5,87 | 5,70 | 6,92 |
| 2 | 6,25 | 5,90 | 6,13 | 5,93 | 7,08 |
| 3 | 5,38 | 5,20 | 7,54 | 5,47 | 6,29 |
| Mean | 5,63 | 5,68 | 6,51 | 5,70 | 6,76 |
| Sd | 0,54 | 0,42 | 0,90 | 0,23 | 0,42 |
| Sr | 0,55 | | | | |
| CV% | 9,06 | | | | |
| grand mean | 6,06 | | | | |
| Xd - grand mean | 0,19 | 0,14 | 0,21 | 0,13 | 0,50 |
| Sb | 0,54 | | | | |
| CV% | 8,90 | | | | |
| Sl | 0,70 | | | | |
| CV% | 11,56 | | | | |
| **2. Intra- and interassay precision control sample level 2** | | | | | |
| | 1 | 2 | 3 | 4 | 5 |
| 1 | 54,30 | 49,70 | 59,70 | 54,10 | 53,70 |
| 2 | 55,30 | 56,00 | 60,80 | 56,20 | 53,50 |
| 3 | 52,30 | 51,00 | 59,10 | 61,90 | 54,80 |
| Mean | 53,97 | 52,23 | 59,87 | 57,40 | 54,00 |
| Sd | 1,53 | 3,33 | 0,86 | 4,04 | 0,70 |
| Sr | 2,49 | | | | |
| CV% | 4,48 | | | | |
| grand mean | 55,49 | | | | |
| Xd - grand mean | 2,33 | 10,63 | 19,13 | 3,64 | 2,23 |
| Sb | 3,08 | | | | |
| CV% | 5,55 | | | | |
| Sl | 3,69 | | | | |
| CV% | 6,65 | | | | |

SD= standard deviation; CV= coefficient of variation; Sb= standard grand mean deviation; Sr= mean standard deviation; SI= total standard deviation

## Discussion

A study designed by Aune et al. described a framework for optimizing hepcidin measurement and improvements in method standardization (17). Prior to the Aune et al. study, Diepeveen et al. standardized hepcidin methods (18). Both studies proposed an international standard for methods calibration. Aune et al. compared 16 different methods for hepcidin and obtained a mean accuracy of 145% (CI 76–540%), for all methods (17). Interestingly, among 9 MS and 7 ELISA methods, and described here, the ELISA DRG Hybrid XL bioactive hepcidin-25 method, the declared accuracy was 125% (CI 109–147%). Other ELISA methods in the Aune et al. study had similar accuracies with only one method having a higher accuracy (340% (CI 274–540%)). The lack of standardization is reflected by the large variation in results. It is important to note that only hepcidin-25 is the biologically active isoform to be measured, but with ELISA methods there is a certain rate of cross-reactivity

with other hepcidin isoforms, usually declared by the manufacturer.

In the current study, the optimal minimum CV for precision was 2.27% and 1.12%, calculated as CVi x 0.25 pointing out that the ELISA DRG Hybrid XL method has optimal CV when compared to the literature data (< 12.2%) (19).

We must point out that the available data about hepcidin-25 biological variation were obtained on mice and a few human samples, in a study by Murphy et al. (19). The obtained intraindividual biological variation of hepcidin-25 was 48.8% while the inter-individual coefficient of variation was 154.1%. Here described study obtained CVi and CVg around 10%.

The main advantages of the automated ELISA method are straightforward procedure, high sensitivity, ready-to-use reagents, short analysis time (2 hours), small sample volume (160 µL), and the possibility of individual sample analysis compared to MS analysis which is multiplex and delays in results. A significant advantage of this method is its standardization against the MS.

There are limitations to this current study. The DRG company reagent manufacturer did not report the desirable bias or inaccuracy for the method. Also, the DRG company used patient' samples for the initial method validation, while our study verification was performed with commercial control samples so there is a difference in the matrix. In addition, we did not compare the DRG method with other hepcidin-25 methods. For the verification, we used control samples provided by the manufacturer. Therefore, we could not compare our data with the manufacturer's validation data which were conducted on the patient samples. During the verification study, we were not able to perform commercial controls of a third-party manufacturer. Finally, the reference interval verification was performed using only serum samples of healthy males.

## Conclusion

The verification of the fully automated ELISA method for determining hepcidin-25 levels in serum samples performed on the DRG Hybrid XL analyzer showed desirable analytical reproducibility and met all acceptance criteria.

**Acknowledgement.** Hepcidin reagents and supplies were provided free of charge by the limited liability company (GmbH) DRG Diagnostics. All authors do not have any conflicts of interest. The authors would like to thank the local distributor (Croatian) of DRG Diagnostics, Jasika company and Mr. Hrvoje Štimac, for his generous donation, support and contribution, as well as Prof. dr. sc. George Y Wu for detailed revision of the paper.

## Disclosure

**Funding.** No specific funding was received for this study.
**Competing interests.** None to declare.

### List of abbreviations:

MS = mass spectrometry
ELISA = solid phase enzyme-linked immunosorbent assay
C1 = control sample level 1 (normal value)
C2 = control sample level 2 (high value)
CLSI = Clinical and Laboratory Standards Institute
CVi = intraindividual coefficient of variation
CVg = interindividual coefficient of variation
CV = coefficient of variation
SD = standard deviation
CI = confidence intervals

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**Author contribution.** Acquisition of data: TR
Administrative, technical or logistic support: TR
Analysis and interpretation of data: TR
Conception and design: TR
Critical revision of the article for important intellectual content: TR
Drafting of the article: TR
Final approval of the article: TR
Guarantor of the study: TR
Obtaining funding: TR
Provision of study materials or patients: TR

Review article

# Pathophysiological and Diagnostic Aspects of Sarcopenia in Hemodialysis Patients

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## Abstract

Chronic kidney disease and renal replacement therapy, particularly hemodialysis, contribute to the development of negative protein balance and muscle dysfunction in dialysis patients, from the development of protein-energy malnutrition to sarcopenia. Due to multifactorial etiology and complex pathophysiological patterns, sarcopenia has proven to be a significant predictor of cardiovascular events and is associated with a higher risk of overall mortality. Screening methods of chronic kidney patients and patients on hemodialysis who are at higher risk of developing sarcopenia, as well as diagnostic methods for this group of patients are not clearly defined, hence methods used for the general population of elderly patients, especially based on the revised European consensus on definition and diagnosis of sarcopenia of the European Working Group on Sarcopenia in Older People (EWGSOP2), are utilized in this subpopulation as well. Therefore, there is a need to define new biomarkers of sarcopenia such as the existing 24h urine excretion of creatinine, a product of estimated glomerular filtration of cystatin C and creatinine or myostatin and their use in routine work with dialysis patients to identify this condition among them and reduce morbidity and mortality.

(Sitaš Z, Mihaljević D. Pathophysiological and Diagnostic Aspects of Sarcopenia in Hemodialysis Patients. SEEMEDJ 2023; 7(1); 42-54)

Received: Feb 27, 2023; revised version accepted: Mar 27, 2023; published: Apr 30, 2023

KEYWORDS: sarcopenia, hemodialysis, chronic kidney disease, pathophysiology

## Introduction

The word sarcopenia comes from the Greek σάρξ (sarx) which translates to flesh and πενία (penia) meaning loss. It was first introduced in 1989 by Irwin Rosenberg to describe the loss of muscle mass with aging leading to a reduced quality of life as a result of a higher risk of falls, fractures, hospitalizations and ultimately death (1). People suffering from this are at a higher risk of developing adverse outcomes due to bone and mineral disorder as a result of secondary hyperparathyroidism, then the higher rate of depression as a cause of development of anorexia and due to socioeconomic factors, affecting the quality of life that is significantly impaired in dialysis patients. It is estimated that the loss of muscle mass is 1–2% and muscle strength is 1.5–5% per year after the age of 50, primarily the musculature of the lower extremities (2), although muscle volume can be preserved due to myosteatosis and myofibrosis, which will be discussed below. In 1931, Mr. Critchley Macdonald, a British neurologist, was the first to associate aging with a decrease in skeletal muscle mass (3). According to Delmonico et al. in 1,678 subjects after the age of 70, the annual rate of muscle area reduction was 4.9±7.4% in men and 3.2±7.9% in women (4). The prevalence of sarcopenia varies widely in predialysis chronic renal patients of 6–14% (5, 6) and increases in patients on hemodialysis (HD) 4–64% (7, 8), without firm consensus on the prevalence of uremic sarcopenia, although some data suggest the prevalence of uremic myopathy of 50% (9). According to the meta-analysis of Wathanavasin et al., the highest prevalence of sarcopenia is in Europe (29.1%) and the lowest in the US (15.4%) (10). This meta-analysis also showed that sarcopenia is less diagnosed before the hemodialysis procedure (21.5%) compared to after hemodialysis (27.8%), primarily due to volume status, but without statistical significance. Sarcopenia has also been shown to be one of the most important predictors of cardiovascular events (OR 3.80 – 95% CI 1.79-8.09) with a high mortality risk (OR 1.83 – 95% CI 1.40-2.39). Low muscle strength and mass are independent factors of increased mortality in patients on hemodialysis (OR 1.71 – 95% CI 1.20-2.44), all the more so, if they are associated (11).

## Pathophysiology and etiology of sarcopenia

Knowledge of the physiology and histology of skeletal musculature is necessary for understanding the pathophysiology and histopathological changes within chronic kidney disease (CKD) and the uremic state. Skeletal muscles belong to the group of transversely striped muscle tissue made of muscle fibers (actin, myosin), i.e. muscle cells that function as syncytium and are formed by fusion of myoblasts – precursor cells (12). Skeletal muscle stem cells play a key role in muscle regeneration. Various stimuli such as exercise, injury, stretching, etc. can activate these cells that are located between the plasma membrane and the basal lamina leading to asymmetric differentiation into myoblasts responsible for tissue regeneration and pluripotent stem cells responsible for self-renewal. Myoblast differentiation requires primary myogenic regulatory factors Myf5 and MyoD, as well as secondary – myogenin and myogenic regulatory factor 4 – MRF4 (13, 14). Muscle fibers can be classified into three groups according to the isoform of the myosin heavy chain – slow oxidative red fibers (type I), fast oxidative red fibers (type IIA) and fast glycolytic white fibers (type IIX) that were represented in different proportions in skeletal muscles depending on muscle function (15). Various factors are associated with histopathological changes in skeletal muscle in patients with advanced, i.e. terminal chronic kidney disease – especially a condition of chronic inflammation, which shows the replacement of muscle fibers with adipose tissue – myosteatosis, or fibrous tissue – myofibrosis and are a reflection of muscle dysfunction (16). In a study conducted on 60 patients on hemodialysis, the cross-section of muscle fibers was higher compared to the control group, which is explained by the development of interstitial edema in the interdialytic period, but the results also showed reduced oxidative capacity due to dysfunction

of the enzyme succinate dehydrogenase and mitochondrial edema with a reduced network of capillaries (17). Uremic toxins such as indoxyl sulfate, p-crescol and inorganic phosphorus, can impair myogenous differentiation in vitro and promote muscular atrophy in mice with CKD by promoting mitochondrial dysfunction (18, 19).

Development of sarcopenia and etiology of muscle dysfunction of patients on hemodialysis is multifactorial and arises from kidney disease itself, hemodialysis procedure and chronic inflammatory response, which contributes to reduced protein synthesis and increased degradation (20) (see Figure 1).

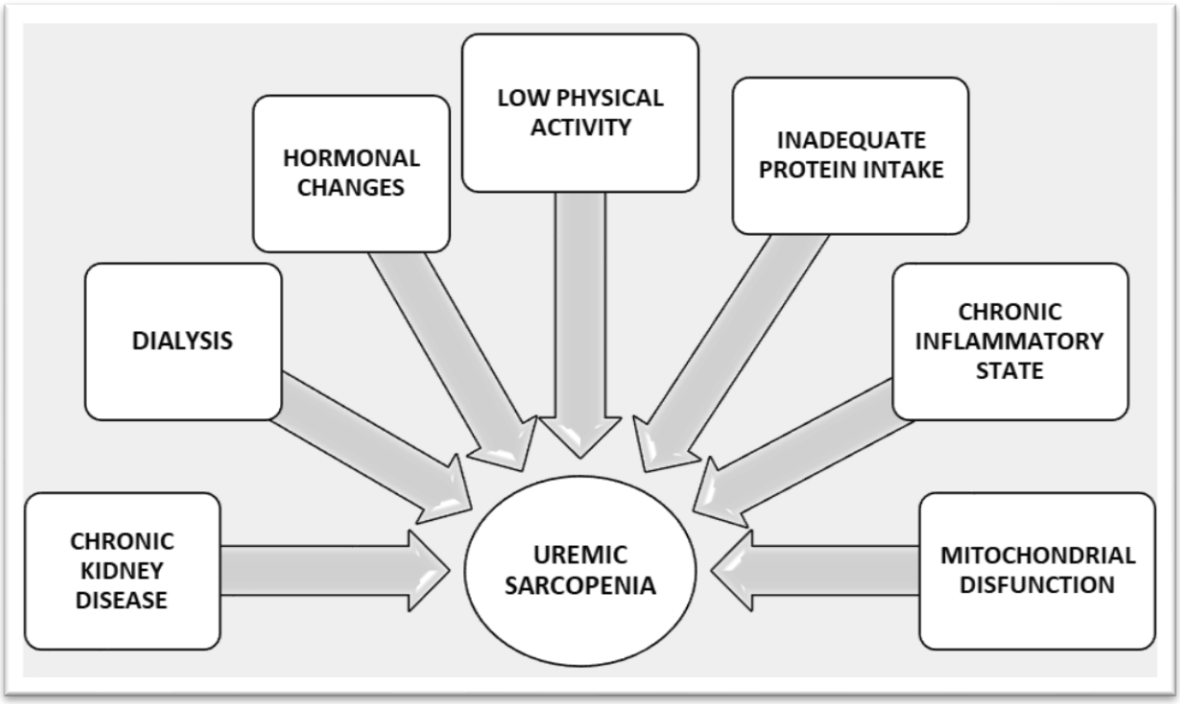


**Figure 1. Etiological factors involved in the development of uremic sarcopenia**

The development of metabolic acidosis in CKD, vitamin D deficiency and insulin resistance also contribute to the negative protein balance. Metabolic acidosis activates caspase-3 and the ubiquitin-proteasome system – two intracellular pathways responsible for protein degradation and promoting insulin resistance and growth hormone resistance (13, 21). 1,25(OH)2 vitamin D as an active form of vitamin D binds to vitamin D receptors in human muscle tissue, especially to C2C12 myoblasts, and causes reduced cell proliferation and promotes myogenic differentiation by MyoD expression and myostatin suppression (22). Vitamin D deficiency also leads to reduced insulin secretion from pancreatic beta cells promoting insulin resistance (23, 7). Insulin-like growth factor 1 (IGF-1) shares a common intracellular pathway in protein synthesis with insulin, activating mTORC1 via PI3K (phosphatidylinositol-3-kinase)/Act leading to phosphorylation of FOXO proteins and inhibiting their translocation into the nucleus. IGF-1 suppresses both Atrogin-1 and MuRF1 (ubiquitin ligase) which antagonizes skeletal muscle catabolism (24, 25). In patients on hemodialysis, the concentration of testosterone is reduced, which leads to the expression of myostatin and alteration of the IGF-1 signaling pathway (26). Myostatin is a protein that negatively affects the growth of muscle tissue through three signaling pathways. Via activin, IIB receptor activates Smad2/Smad3 and dephosphorylation Act thus disrupting the IGF-1 signaling pathway previously specified. It can also inhibit mTORC1 and induce apoptosis via the p38-caspase pathway (27).

The HD procedure also contributes to the negative balance of protein metabolism. During the HD process, a significant loss of proteins and

amino acids (> 5 g) was observed more pronounced at the beginning and end of the procedure itself (28). Patients with CKD, especially patients on HD, have a decreased appetite due to the action of uremic toxins, chronic inflammation and hormonal disorders – primarily by a decrease in ghrelin and neuropeptide Y, and an increase in leptin (29). Chronic inflammatory condition of patients on HD is associated with the use of biocompatible membranes (30) and the role of the gastrointestinal tract or intestinal dysbiosis is increasingly mentioned. Namely, due to dietary restrictions with reduced fiber intake, protein fermentation and exposure to endotoxins that induce the pro-inflammatory state and disrupt the gastrointestinal barrier increases (31, 32). Pro-inflammatory cytokines such as IL-6 and TNF-alpha reduce the suppression of SOCS-3 protein through the activation of caspase-3 and the ubiquitin-proteasome system. IL-6 also stimulates the expression of Stat3 responsible for the expression of myostatin and leads to muscle degradation (33, 34). The production of pro-inflammatory mediators is induced by the formation of advanced glycation end products (AGE) that accumulate in patients with CKD due to reduced renal clearance and increased production (non-enzymatic changes in protein or lipid amino acids that are reduced by exposure to sugars or their metabolites) and lead to oxidative stress, insulin resistance and endothelial dysfunction (35, 36). Yabuuchi et al. showed that the accumulation of AGE in the muscle tissue of mice leads to mitochondrial dysfunction (by reducing the activity of succinate dehydrogenase and PGC1-alpha and reducing the density of the capillary network) (37). Furthermore, AGE can impair muscle function through associated membrane RAGE receptors whose activity reduces the expression of myogenin (38), impairs the distribution of muscle fibers and increases the rigidity of muscle connective tissue (39). These etiological factors contribute to negative protein balance that can eventually lead to the decline of muscle mass, muscle strength and lower physical activity because patients on HD are at a higher risk of developing sarcopenia compared to the general population and these changes occur at an earlier age.

*Diagnosis of sarcopenia and associated conditions*

Skeletal musculature is mostly made of protein and it is the best indicator of protein status. The development of protein-energy malnutrition (PEM) – a term presented in 2007 by the International Society of Renal Nutrition and Metabolism (ISRNM), occurs due to various pathophysiological mechanisms, that include nutritional and metabolic disorders in patients with chronic kidney disease and lead to the development of a state of chronic muscle and adipose tissue catabolism (40, 41). It is associated with chronic inflammation, uremia, anorexia due to decreased appetite, hypoalbuminemia, muscle loss with weight loss or no weight loss and poor clinical outcome. Due to PEM, there is a reduction in protein supplies and energy sources in patients with different CKD stages, leading to a decrease in the functional abilities of the patient. Malnutrition, on the other hand, is an imbalance of energy intake, protein and other nutrients that leads to measurable undesirable effects on tissues and physical functions and can be presented with malnutrition, but also overfeeding (42–45), so we can find it in obese patients too. Such a physiological state can lead to cachexia – a syndrome manifested by severe muscle loss with or without loss of fat tissue (46) and sarcopenia, which, according to the consensus of the EWGSOP2 defines as „a progressive and generalized muscle disorder characterized by low muscle power and reduced quality and quantity of muscle mass“ (47). Quantitative changes are related to the loss of muscle mass and volume, and qualitative to the loss of muscle strength and physical activity (48). In these revised guidelines, the emphasis is on muscle strength because it has a better predictive value for the occurrence of adverse outcomes. If the patient meets the criterion of low muscle strength, it is probably sarcopenia, i.e. presarkopenia, if it meets the second criterion of low muscle quality or quantity, the diagnosis can be confirmed by sarcopenia, and if it meets the third criterion of low physical activity,

sarcopenia is considered severe. According to the International Working Group on Sarcopenia (IWGS), sarcopenia is characterized by low skeletal muscle mass (with an increase in body fat) and decreased muscle function (49). EWGSOP2 consensus provides a broader definition by dividing muscle strength from muscle activity and distinguishes severe sarcopenia.

Sarcopenia can be divided into primary (age-related) when there is no other evident cause and secondary in systemic diseases especially those that can cause inflammatory processes including CKD, conditions of reduced physical activity (sedentary lifestyle, in bedridden patients) and some nutritional factors such as gastrointestinal diseases, malabsorption syndrome, insufficient food intake (9). The main difference between these two groups is that the primary group occurs over a continuous period and is more pronounced after the age of 50, while the secondary is more intense and depends on the conditions that cause it (50). Unlike primary sarcopenia, sarcopenia in chronic kidney patients is characterized by more pronounced protein degradation. EWGSOP2 subcategorizes sarcopenia into acute if it lasts less than 6 months and chronic if it lasts 6 months and more. Such a division places emphasis on periodic assessments of sarcopenia in patients at increased risk (47). In elderly patients, a condition of sarcopenic obesity can be observed, which is presented by reduced muscle mass due to adiposity (51).

For the diagnosis of sarcopenia, a multitude of different tests and methods can be used, the choice of which depends on the patient, the technical availability and the purpose of testing (whether we are examining the progression of the condition or recovery) – see Figure 2.

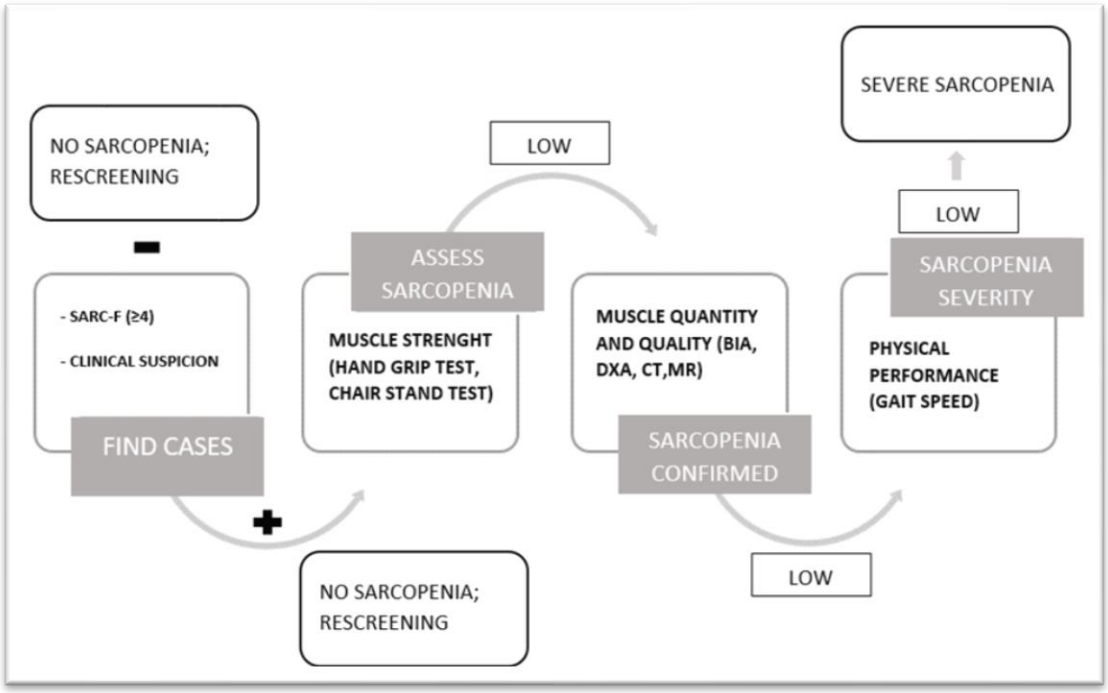


**Figure 2. Sarcopenia diagnostic algorithm – adapted according to EWGSOP2**

Level of strength, assistance with walking, rising from a chair, climbing stairs and falls are criteria in the 5-item questionnaire (SARC-F) as a screening method for patients with sarcopenia. It includes the patient's subjective attitude about the ability to walk, get up from a chair, climb stairs, strength level, and the existence of falls in the past year. The questionnaire has high specificity in predicting low muscle strength and will detect more severe cases as such (52, 53). If the test is positive (SARC-F ≥4), the first criterion – muscle strength – is examined. To test muscle

strength, the strength of the handshake can be measured with a Jamar dynamometer, which is a simple and inexpensive method and correlates with the strength of other body regions, such as the strength of the upper and lower extremities (54, 55) and should be measured before the hemodialysis procedure (56, 57). The meta-analysis by Hwang et al. showed that people with low muscle strength had a 1.88 times higher risk of overall mortality (58). Vogt et al. suggested cut-off values that would suggest higher mortality in patients on HD, <7 kg in women and <22.5 kg in men (59), while according to EWGSOP2, values <16 kg for women and <27 kg for men. In addition to testing the strength of the handshake, the test of getting up from the chair can be used, which predominantly examines the strength of the muscles of the lower extremities (quadriceps). The test measures the time it takes a patient to get out of the chair 5 times without arm support and the time > 15 s is considered significant. If these tests determine reduced muscle strength, it can be said that sarcopenia is probable and confirmed by testing muscle quality or quantity. Muscle quantity can be shown as appendicular muscle mass (AMM), total skeletal muscle mass (SMM) and cross-section of a specific muscle group. Computed tomography (CT) and magnetic resonance imaging (MRI) are considered the gold standards in non-invasive assessment of muscle mass and myosteatosis (60, 61), but they are not widely used due to expensiveness, radiation exposure and necessary logistics. Alternative methods include densitometry (DXA) and bioelectrical impedance analysis (BIA) which can assess the total and appendicular muscle mass and in the case of BIA and nutritional status of patients on HD with better evaluation of intracellular and extracellular fluid (62, 63) which should be done after the HD procedure. The absolute values of SMM and AMM can be modified according to weight (AMM/weight), height square (AMM/height2) or body mass index (AMM/BMI). The cut-off values for these methods are according to EWGSOP2 AMM/height2 <7 kg/m2 for men and <5.5 kg/m2 for women. The method that can be done at the patient's bedside is an ultrasound measurement of the thickness of the quadriceps femoris muscle (rectus femoris and vastus intermedius) which correlates with nutritional status (9). If any method determines low muscle quality or quantity, we can talk about the existence of sarcopenia whose weight is additionally assessed by the analysis of physical activity. Most often, the walking speed test is used. The cut-off value is ≤ 0.8 m/s. The test is performed by measuring time at a distance of 4 m, walking at the usual walking speed (64, 65).

## Diagnostic challenges of sarcopenia in CKD/dialysis patients

Although the diagnostic criteria of sarcopenia are well-defined in the general population, there is no consensus on the diagnosis of uremic sarcopenia in dialysis patients. Most studies apply definitions relating to the elderly population leading to the heterogeneity of the results of the prevalence of sarcopenia in the dialysis population as previously stated. There is a risk of underestimating the prevalence of low muscle mass if in obese patients SMM is modified according to the square of height and in such patients, there is a better modification according to body mass index (66). Furthermore, several studies have shown that low muscle mass in dialysis patients is not associated with increased mortality unlike low muscle strength and low muscle activity defined by a weak hand-grip test and slow gait speed (67, 68), and using this approach overlooks patients only with low muscle strength. This is supported by the KDOQI (Kidney Disease Outcomes Quality Initiative) guidelines according to which hand grip strength is useful in assessing protein energy wasting because the use of BIA and DEXA is determined by volume status, so patients who are in overvolemia or with early changes in muscle structure would be misdiagnosed (69). Fahal et al. (70) examined the contractile properties and muscular strength of dialysis patients with muscle quadriceps biopsy. The main difference between this group of patients and the control was poor muscle relaxation, which can affect muscle strength independently of muscle mass, which is supported by the fact

that 45% of patients had type I and 40% of type II muscle fibers atrophy and fibers type II were significantly lower in malnourished patients, which again shows that muscle mass is not the only determinant of muscle strength. Pereira et al. (71) define sarcopenia in CKD patients treated conservatively by reduced muscle function (hand grip strength – 30% percentile of the reference population), reduced muscle mass (measuring mid-arm muscle circumference) and reduced skeletal musculature index according to bioimpedance analysis (<6.76 kg/m2 in women and < 10.76 kg/m2 in men). Furthermore, if low muscle mass is confirmed, patients may be in pronounced muscle wasting. Therefore, in the assessment of sarcopenia in dialysis patients the focus should be on the components of low muscle strength and physical activity and such patients should be encouraged to change their lifestyle habits, diet and exercise for the preservation of muscle mass and muscle function.

In addition to the aforementioned tests for diagnosis and monitoring of patients with sarcopenia, there was a need to define certain biomarkers for early detection of sarcopenia in patients with CKD. As an endogenous metabolite of skeletal muscle, the determination of creatinine excretion in 24h urine is justified in the assessment of muscle mass (72, 73) with a lack of adequate collection of all-day urine in certain patients. This problem was attempted to be solved by determining a new sarcopenia index – the product of the estimated glomerular filtration of cystatin C and creatinine (74). In the study of Lin et al., this index was independently associated with muscle strength, mass and walking speed in patients with CKD (75). Measurement of serum concentration of myostatin as a muscle consumption biomarker was also considered but with conflicting results of studies due to the influence of age, gender, inflammatory conditions, physical activity and metabolic syndrome. Myostatin values are generally higher in patients with CKD, i.e. patients on HD with greater muscle strength and muscle mass compared to healthy individuals (27, 76).

## Conclusions

Chronic kidney disease and renal replacement therapy, particularly hemodialysis, contribute to the multifactorial etiology of sarcopenia negative protein-energy balance and increased mortality of chronic kidney patients. Adequate screening systems and diagnostic methods are crucial in the early recognition of patients at elevated risk. Diagnostic tests and methods in the assessment of sarcopenia should be adapted to the conditions in which they are performed, patient groups and systematic monitoring. In conditions of unavailability of tests and methods adapted to dialysis patients, existing methods of assessing muscle strength, muscle quality and quantity, and physical activity can be used according to the EWGSOP2 group of experts with a tendency to find new biomarkers of sarcopenia. Due to the complex pathophysiology of protein catabolism, it is expected to find biomarker panels and their application in the stratification of heterogeneous groups of patients such as patients with CKD with the aim of adequate action and prevention of premature morbidity and mortality. The emphasis should be put on the fact that sarcopenia is a modifiable risk factor for adverse outcomes in CKD patients, using preventive nutritional measures based on data obtained by existing diagnostic methods and tests, so it should be further studied and adequately diagnosed and treated.

**Acknowledgement.** None.

## Disclosure

**Funding.** No specific funding was received for this study.
**Competing interests.** None to declare.

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**Author contribution.** Acquisition of data: ZŠ, DM
Administrative, technical or logistic support: ZŠ, DM
Analysis and interpretation of data: ZŠ, DM
Conception and design: ZŠ, DM
Critical revision of the article for important intellectual content: ZŠ, DM
Drafting of the article: ZŠ, DM
Final approval of the article: ZŠ, DM
Guarantor of the study: ZŠ, DM

Review article

# Computer Vision Solutions for Range of Motion Assessment

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## Abstract

Joint range of motion (ROM) is an important indicator of physical functionality and musculoskeletal health. In sports, athletes require adequate levels of joint mobility to minimize the risk of injuries and maximize performance, while in rehabilitation, restoring joint ROM is essential for faster recovery and improved physical function. Traditional methods for measuring ROM include goniometry, inclinometry and visual estimation; all of which are limited in accuracy due to the subjective nature of the assessment. With the rapid development of technology, new systems based on computer vision are continuously introduced as a possible solution for more objective and accurate measurements of the range of motion. Therefore, this article aimed to evaluate novel computer vision-based systems based on their accuracy and practical applicability for a range of motion assessment. The review covers a variety of systems, including motion-capture systems (2D and 3D cameras), RGB-Depth cameras, commercial software systems and smartphone apps. Furthermore, this article also highlights the potential limitations of these systems and explores their potential future applications in sports and rehabilitation.

(Aleksic J*. Computer Vision Solutions for Range of Motion Assessment. SEEMEDJ 2023; 7(1); 55-66)

Received: Mar 29, 2023; revised version accepted: Apr 17, 2023; published: Apr 30, 2023

KEYWORDS: joint range of motion, rehabilitation, computer vision system, machine learning, motion capture

## Introduction

Joint range of motion (ROM) is an important indicator of physical functionality and musculoskeletal health (44). It plays a vital role in the sports field, as athletes require adequate levels of joint mobility to reduce the risk of injuries and maximize their performance (57, 12). Similarly, restoring the joint range of motion in rehabilitation is essential for faster recovery and better physical functionality (16, 32, 68). Therefore, assessment of the joint range of motion is necessary for determining the limitations in joint mobility and creating treatment or training plans that can effectively enhance joint functionality, reduce the risk of injury and maintain optimal levels of performance (40, 51).

Common traditional methods of measuring the range of motion in physical therapy include using goniometers and inclinometers, as well as visual estimation through various mobility tests (38, 52). The goniometer is widely considered the standard tool (i.e. the golden standard) for evaluating a range of motion in clinical settings (52). This tool measures the angle of joints by positioning the arms of the goniometer along the joint's axis. To obtain a more comprehensive and accurate assessment of the joint range of motion, goniometers are often used in conjunction with inclinometers; instruments that measure the position of body segments (38, 52). Finally, a method that is used when specialized tools such as goniometers and inclinometers are not available is a visual estimation of joint mobility during various flexibility tests. This method can provide a quick estimation of the joint range of motion; however, it greatly depends on the rater's ability to visually determine the degree of motion (23).

While all of these methods can provide some degree of accuracy, their estimation is subjective as it may vary depending on the observer's perception and experience or placement of the tool (1). Developments in technology have now opened the possibility of using computer vision for a more objective range of motion assessment in sports and rehabilitation. Computer vision (CV) is a new technology that employs several techniques such as pattern recognition, machine learning and image processing, to extract meaningful information from visual data. This technology can automate many tasks that require subjective assessment, potentially making them more efficient and accurate (35). Current computer vision-based systems have mainly been developed for entertainment purposes (i.e. the gaming industry), although there have been many successful attempts to apply these systems in sports and rehabilitation (48). A common issue with these systems is achieving accurate pose-estimation results, which has limited their use in a range of motion assessment (7).

The rapid advancements of this technology continually introduce new possible solutions, highlighting the importance of constantly assessing the reliability and validity of these novel systems. Therefore, this study aims to present an up-to-date review of currently available computer vision-based systems, focusing on their reliability and validity in range of motion assessment, as well as their practical applicability.

### Computer Vision-Based Range of Motion Solutions

The use of computer vision-based systems offers several advantages over the aforementioned traditional range of motion assessment methods: 1) computer vision-based systems eliminate the need for physical contact with the patient, making the assessment more comfortable and efficient (35); 2) these systems can capture a large amount of data in a short period, therefore providing detailed and objective quantitative measurements (56); 3) moreover, computer vision systems can track a range of motion both in real-time and over a longer period, which enables trainers and clinicians to monitor progress and adjust training or treatment plans accordingly (60).

This paragraph will discuss some of the most commonly used computer vision systems for a

range of motion assessment, including motion capture cameras (i.e. 2D, 3D and RGB-Depth cameras), commercial software systems and smartphone apps (Table 1).

**Table 1. Computer vision systems used for range of motion assessment**

| Technology | Device / App | Studies (authors) | ROM assessed | Reliability/ validity | Price |
|---|---|---|---|---|---|
| **Marker-based 3D systems** | Vicon; Qualisys | Faber et al. (2009); Inokuchi et al., 2015; Me et al., 1998 | shoulder, neck, lower extremities | ICC= 0.78-0.98<br>r = 0.779–0.863<br>(compared to CROM device) | >$10,000 |
| **Markerless 3D systems** | DARI Motion; The Captury | Cabarkapa et al., 2022; Fleisig et al., 2022; Harsted et al., 2019 | hip, knee and ankle; shoulder and elbow | ICC= 0.64-0.92<br>r=0.74-0.99<br>(compared to Vicon) | >$10,000 |
| **RGB-Depth cameras** | Kinect | Beshara et al., 2020, 2021; Cai et al., 2019; Hawi et al., 2014; Mortazavi et al., 2018; Özsoy et al., 2022; Zulkarnain et al., 2017 | shoulder and lower extremities | ICC=0.62-0.98<br>r= 0.73-0.97<br>(compared to Vicon) | ~$399 |
| **Commercial software systems** | Kinetisense | Macaulay, 2017 | shoulder and hip; elbow/wrist knee/ankle | ICC= 0.85-0.96<br>ICC= 0.61-0.69 | >$1,000 |
| **Smartphone apps** | Goniometer Pro | Pourahmadi et al., 2016; Wellmon et al., 2016 | wrist and shoulder | ICC= 0.79-0.82<br>r≥ 0.80<br>(compared to goniometer and inclinometer) | ~$9.99 |
| **Combination of systems** | MIRA software + Kinect | Wilson et al., 2017 | shoulder | r= 0.96-0.99<br>(compared to Vicon) | >$1,000 |

## Motion-capture (MoCap) camera systems

Motion capture (MoCap) cameras are advanced systems that capture and record movement in two-dimensional or three-dimensional spaces. Some of these systems require the placement of passive markers on the body which reflect infrared light emitted by the cameras (31). In contrast, other systems can automatically detect anatomical markers through depth-of-field sensors or deep-learning-based algorithms (7). Regardless of the need for active or passive marker placement, motion-capture systems have the potential to be used for a range of motion assessment as they can track the movement of anatomical segments of the body. However, the accuracy of measurements can vary depending on the motion-capture technology integrated with the system (7). Based on previous research, some of the most common motion-capture systems that are used for a range of motion assessment include:

2D and 3D systems. These systems use high-speed cameras and specialized software to track the position and movement of the body in a two-dimensional or three-dimensional space (31). The main difference between these two systems is that 2D cameras can track objects and record movement along a two-dimensional axis (i.e. height and width), while 3D cameras also include a third dimension (i.e. depth of field) (63). To estimate the position of body segments and human motion, 2D systems use the techniques of direct regression to identify key points on the body or heat maps to represent the probability of a joint being located at a particular position (9, 70). However, it is important to acknowledge that 2D systems lack the precision and accuracy of 3D motion capture systems, as they do not include the dimension of depth, which is important for a more comprehensive range of motion assessment (i.e. measuring rotation) (15, 63). Therefore, marker-based 3D motion capture systems are highly regarded as the preferred method for a range of motion assessment (6). These systems use marker-based or markerless pose estimation techniques to capture and analyze movement (4, 34).

Marker-based systems (i.e. Vicon and Qualisys) capture the position of markers that reflect the infrared light emitted from the cameras (30). When these markers are placed on specific anatomical landmarks on the body, this reveals the position and orientation of each marker in a three-dimensional space and allows the system to precisely determine the joint orientation (34). Normally, time-of-flight (TOF), triangulation techniques and machine-learning algorithms are used to calculate the position of each marker and estimate human motion with more precision and detail in real-time (31). A crucial step in this process is targeted marker placement around the joint segments of the body (34), which can greatly affect the accuracy of data obtained from the cameras. However, this is usually a time-consuming process that is not very practical for collecting data outside laboratory settings (4, 5).

This poses a significant advantage of markerless systems (i.e. DARI Motion and Captury) as they greatly reduce the time required to prepare the subject for testing and facilitate data collection on the field (4, 5). Previous studies have demonstrated good to excellent reliability (ICC>0.80) of DARI Motion (DARI Motion, Overland Park, KS, USA) (10) in measuring the range of motion related to squat exercise (4, 5). However, only one study directly compared the accuracy of this system to a marker-based system, specifically in relation to baseball pitching-related range of motion (14). The results of this study showed that while the internal consistency of joint angle measurements was good (ICC=0.64–0.92), the magnitudes of angle measurements differed between systems for up to 16 degrees (14). Another study compared the validity of the Captury (The Captury GmbH, Saarbrüken, Germany) (65) markerless system to Vicon (i.e. the golden standard) and found strong correlations for all range of motion measurements related to squat (r=0.74–0.99) and jump exercise (r=0.63–0.98) (19).

While markerless systems certainly show promising results and better practicality of use, marker-based systems are still widely considered the golden standard in human motion analysis (19). Moreover, the cost of both

marker-based and markerless systems (~$10,000–$300,000 price per unit) remains a significant obstacle to their broad implementation in sports and rehabilitation (63).

RGB-Depth camera systems are innovative, low-cost solutions that combine the technology of 3D motion analysis with the practicality of use. These systems integrate depth-of-field sensors, which can calculate the distance of each point from the camera and create a three-dimensional representation of the model (22). The techniques for determining the dimension of depth may vary between camera models. Some systems use a narrow-baseline binocular stereo vision technique to estimate the depth dimension by gathering multiple 2D captures (i.e. PointGrey Bumblebee and Stereolabs Zed camera) (22). While other, more advanced systems use time-of-flight (TOF) and infrared (IR) technology to determine the depth of field by calculating the time needed for light to travel between two points (i.e. Microsoft Azure Kinect) (7, 62). These systems also implement machine learning algorithms to estimate the position of joints and track human motion without the need for the placement of reflective markers (47).

Although systems such as Kinect (Microsoft Corp., Redmond, WA, USA) were originally designed for gaming and virtual reality purposes, researchers have identified their potential for implementation in sports and rehabilitation due to their markerless pose estimation technology (2). Previous studies found moderate-to-good intra- and inter-rater reliability (ICC=0.62–0.99) for shoulder range of motion measurements (2, 3, 8, 20, 21, 36, 49, 55, 73). At the same time, validity varied from poor to excellent for active shoulder range of motion compared to a video motion capture system (r=0.53) (59), lateral photographs (r= 0.33–0.79) (42) and Vicon 3D motion capture system (r=0.73–0.97) (6). Similarly, good to excellent agreement was found between Kinect and Vicon for lower extremities flexion and extension measurements (24, 33), while poor agreement was found for rotational movements (33).

These results certainly highlight the vast potential of RGB-Depth cameras being used for a range of motion assessment in the future; moreover, considering their portable, lightweight design and affordable price (~$399 per unit), these systems pose a much more accessible option for widespread use. However, considering the lack of studies and varied results, further research is needed to better assess their accuracy before fully adopting these systems in sports and rehabilitation.

## Commercial software systems

Commercial software systems based on computer vision are becoming increasingly popular in sports and rehabilitation. These systems use advanced statistical algorithms and deep-learning frameworks, which are able to interpret and predict visual data, recognize anatomical segments and analyze human motion (50). One of the main advantages of these systems is that they can be paired with various types of video-capturing devices (i.e. standard video cameras or motion-capture cameras) to provide enhanced precision and more detailed measurements of human movement (41). Some of these systems may also include their proprietary sensors or cameras (i.e. Vicon Nexus) developed to work seamlessly with the software (37).

Given the increasing number of commercial software systems available on the market, this review will specifically focus on software systems developed or researched for a range of motion assessment. These systems include:

*Vicon Nexus* (VICON, Oxford Metrics Ltd., Oxford, UK) is the gold standard in advanced kinematic data analysis (37). This software processes signals from reflective markers captured with Vicon 3D motion-capture cameras, allowing Vicon Nexus to create a 3D representation of the model, which can be viewed and analyzed from any angle (64). The software also provides various tools for measuring joint angles and calculating complex kinematic and kinetic data (i.e. force, velocity, acceleration and inverse kinematics).

*Kinetisense* (Kinetisense Inc.) is a software designed to upgrade the default algorithm included in the Microsoft software developer kit by offering an advanced range of motion algorithm. This software can be paired with Microsoft Kinect systems for kinematic analysis (39). Although there is a lack of studies assessing the accuracy of this software, one study showed good reliability of Kinetisense in measuring shoulder and hip range of motion (ICC=0.85–0.96) and moderate reliability in measuring elbow/wrist and knee/ankle range of motion (ICC=0.61–0.69) (39).

*Theia3D* (Theia Markerless Inc.) is a commercial software package that offers a markerless motion-capture solution for a range of motion assessments, among many other types of activities. This software can be integrated with 2D cameras to capture movement and then biomechanically analyze human motion based on computer vision and machine-learning algorithms. This software calculates the 3D coordinates of each anatomical segment by estimating their 2D locations on each frame and then recreates a 3D model of the body (29).

*iPi Mocap Studio software* (iPi Soft LLC) is a computer vision-based software that can be paired with 2D or RGB-Depth cameras to extract spatiotemporal information, track movement and automatically pinpoint up to 16 anatomical markers at a time (33). Previous studies found that iPi Mocap Studio software can be used as a valid tool for measuring the hip and knee range of motion in the sagittal and frontal planes (33).

*Medical Interactive Recovery Assistant* (MIRA; MIRA Rehab Ltd., London, UK) is a software platform originally designed for exergaming and telerehabilitation purposes to help patients recover faster from injuries. This software integrates a range of motion measurement tools and requires depth-sensing cameras (i.e. Kinect) to capture and analyze movement data in order to help therapists assess patients virtually (72). A study by Wilson et al. (2017) evaluated the validity of MIRA software paired with a Kinect camera for shoulder range of motion assessment. It showed very strong correlation results between measurements obtained from MIRA+Kinect (r=0.96–0.99) and Vicon 3D motion capture system (72).

Despite the numerous advantages of these systems, their cost (>$1,000–10,000) remains a significant obstacle against their potential widespread use for a range of motion assessment in sports and rehabilitation. While low-cost or free alternatives are available in the form of open-source software (i.e. OpenPose, OpenCam and Free MoCap) or smartphone apps, their accuracy may be inferior to those of a commercial system. Still, it is worth noting that such alternatives are also available on the market for a range of motion assessment.

## Smartphone apps

With the advancements in smartphone technology, smartphone apps have emerged as a potentially more affordable option for assessing joint range of motion and identifying joint asymmetry (27, 28). Newer models of smartphones are usually equipped with high-performance motion sensors such as gyroscopes, accelerometers and magnetometers which could potentially be used to assess joint mobility (54). Compared to most previously mentioned methods, smartphone apps are usually cost-effective and easily available to most people who own a smartphone, making them a convenient option for practitioners. There are numerous free or low-cost apps for a range of motion assessment that smartphone users can easily download; however, the accuracy of measurements taken by these apps can differ significantly from one app to another (17, 45, 58, 61). Some apps that have already been studied for their reliability and validity in measuring range of motion include:

*ROMcam* is a relatively new app that utilizes 2D web cameras and OpenPose (GitHub, San Francisco, California, USA) free library based on machine learning models in order to track and detect the 2D key points of human anatomical segments (60). Although initial studies have

found good reliability and validity of this app for pose-estimation assessment, more research is needed to fully evaluate the scope of its potential for a range of motion assessment in sports and rehabilitation (60).

*iPhone® Compass app* (Apple Inc., California, USA). A study by Furness et al. (2018) examined the potential of using the Compass app, which is pre-integrated into the iPhone's basic software package, to measure the thoracic rotation range of motion (17). The assessment was performed by positioning an iPhone firmly against the T1-T2 levels of the participant's back during active thoracic rotation. Results showed good to excellent inter-rater reliability (ICC=0.72–0.89) and concurrent validity ($r=0.835$, $p<0.001$) compared to the goniometer (17).

*Goniometer Pro* (Digiflex Labs, Skien, Norway) is an app designed to act as a dual-axis goniometer and bubble inclinometer. Based on previous research, this app showed good-excellent reliability (ICC=0.79–0.82) in measuring active wrist range of motion (58) and shoulder range of motion (11), as well as good concurrent validity ($r\geq0.80$) compared to a universal goniometer (58) and inclinometer (71). Another study also showed excellent reliability of this app (ICC=0.995–1.000) in measuring angular changes that normally happen during the range of motion assessment (71).

Most apps for a range of motion assessment rely on the smartphone camera or built-in motion sensors (i.e. accelerometer, inclinometer, etc.) for data collection (54). Therefore, the limitations of using apps to assess ROM greatly depend on the smartphone model; for instance, older smartphones may not have the technology to accurately measure the range of motion. Moreover, the battery capacity of older models may be degraded, which could result in the phone shutting down during or prior to data collection, and lead to the loss of important information (58).

Additionally, smartphone sensors are company-manufactured and cannot be calibrated by the user, which can also be problematic for older smartphone models that do not have well-developed sensor technology (17). Practitioners should also be aware that the accuracy of measurements can originate from the app itself or the experience of the rater; therefore, while smartphone apps offer a convenient and accessible option for joint ROM assessment, these limitations should be taken into consideration when interpreting the results.

## Discussion

With the rapid development of technology, new solutions for a range of motion assessment are introduced regularly. This study aimed to offer insight into novel computer vision-based systems that can potentially be used for a range of motion assessments in sports and rehabilitation fields. As traditional tools for a range of motion assessment (i.e. goniometer and inclinometer) are often limited in their accuracy due to the subjective nature of the assessment (1), novel computer vision-based systems can provide more objective and precise measurements, as well as more detailed information about joint kinematics (2, 4, 11). These systems include motion-capture cameras (i.e. 2D and 3D cameras), RGB-Depth cameras (i.e. Kinect), commercial software systems and smartphone apps.

While 3D systems (i.e. Vicon and Qualisys) are unmatched in their precision and are widely considered the golden standard for kinematic analysis, their high cost and robust design limit their practicality and widespread use in sports and rehabilitation (4, 63). Therefore, RGB-Depth camera systems, commercial software systems and smartphone apps pose a much more feasible solution for assessing a range of motion in a practical setting (2). Based on studies evaluating the validity of these novel systems compared to what is considered the golden standard (i.e. 3D motion capture system or goniometer), RGB-Depth cameras (i.e. Kinect, Microsoft Corp., Redmond, WA, USA) stand out as the most promising option for a range of motion assessment in practical settings, especially when paired with commercial software systems or smartphone apps (39, 72).

More specifically, Kinect paired with MIRA software (MIRA Rehab Ltd., London, UK) shows excellent validity of shoulder range of motion measurements (r=0.96–0.99) compared to Vicon (72). In addition to that, smartphone apps such as Goniometer Pro (Digiflex Labs) and Compass app (Apple Inc., California, United States) also show good validity results (r≥0.70) for a certain range of motion measurements (i.e. shoulder and wrist ROM) (11, 17). However, further research is needed to better assess the accuracy of these systems in automatic pose estimation, which is crucial for measuring the range of motion (5).

The major benefit of RGB-Depth camera systems is that they can easily be mounted in any environment without requiring highly specialized knowledge to operate them (64). Moreover, they can be paired with smartphone apps (i.e. Goniometer Pro) or commercially-available and open-source software systems in order to obtain more precise and field-specific information (39, 72). A possible practical application of these systems in sports and rehabilitation includes remote and real-time monitoring of a range of motion changes during exercise. In sports, this can help trainers track various performance parameters that are important for movement efficiency and injury prevention. While in rehabilitation, this can enable a more objective assessment of the range of motion, as well as facilitate remote sessions for clients who cannot attend in-person appointments. However, further research is required to better assess the possibilities of using these systems in such a way.

## Conclusions

Overall, motion capture systems based on computer vision have the potential to significantly improve the range of motion assessment compared to traditional methods such as goniometry, inclinometry and visual estimation. These systems provide more objective and accurate measurements of the range of motion and offer the possibility of real-time or remote feedback, as well as tracking changes in joint kinematics over time. However, as each system mentioned in this review has its advantages and limitations, it is difficult to determine which system could best replace traditional methods used for a range of motion assessment. As these systems continue to develop and become more accessible to the general public, they may become the standard for assessing a range of motion in the future. However, more research is needed to fully assess their accuracy and potential before implementing them in the field of sports and rehabilitation.

**Acknowledgement.** None.

## Disclosure

**Funding.** No specific funding was received for this study.

**Competing interests.** None to declare.

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**Author contribution.** single author article

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We would like to thank our reviewers in 2022:

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